Glycolytic Metabolism of the Dog's Myocardium in Hemorrhagic Shock

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PUBLICATIONS


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CHAPTER I

INTRODUCTION

The state of hemorrhagic shock is a phenomena to which investigators have been unable to attribute a single basic cause. Hemodynamic changes and metabolic disturbances have been demonstrated in almost all organs of the body. The heart has recently been regarded as a major site responsible for the progressive deterioration of the circulation in shock. In addition to peripheral vascular failure, Wiggers (1950) postulated that "myocardial depression" was a precipitating factor in initiating the irreversibility of hemorrhagic shock. Dogs in hemorrhagic shock were observed to undergo an increase in right atrial pressure, a decrease in force of ventricular contraction and a decreased velocity of systolic ejection. From his cardiodynamic studies, Wiggers and his group concluded that deterioration of myocardial expulsive power contributed to the progressive circulatory failure of oligemic shock, and that the various compensatory mechanisms which tend to maintain the coronary circulation were not sufficient to spare the myocardium.

Based on the work of Wiggers and coworkers, investigators observed that the heart in hemorrhagic shock possesses an altered metabolism. Experiments reported from various laboratories suggested that the
heart in shock has an abnormal pattern of carbohydrate metabolism. This abnormal pattern was demonstrated in a negative myocardial extraction of pyruvate and an impaired lactate and glucose extraction (Hackel, 1955). These results indicated that the changes in myocardial metabolism during hemorrhagic shock are similar to generalized tissue anoxia from a decrease in blood flow (Bing, 1954).

Glycolysis is the major pathway for the degradation of carbohydrates to lactate in heart muscle. In this process, glycogen is degraded anaerobically to pyruvate which may be reduced to form lactate (West, 1963). Phosphorylase activity is the rate limiting enzyme in the initial breakdown of glycogen (Cori, 1945, 1951). It was believed that a study of these substances would indicate the extent of anaerobic glycolysis in myocardial muscle and blood. In a portion of the dogs used in this work, coronary sinus flow and oxygen content were measured in order to follow the changes in consumption of lactate, pyruvate and oxygen. Upon determination of these levels it was possible to show that the metabolic changes occurring during the state of anoxia cannot be loosely applied to the shock state. Glycolytic metabolism in shock does not reflect the severe conditions normally seen under conditions of low oxygen content or decreased coronary blood flow to the myocardium (Huckabee, 1961).

Lactate and pyruvate levels in the blood in hemorrhagic shock have been confirmed previously, but it was also considered advantageous to assess the alterations of these metabolites in cardiac muscle. Since these two substrates are being supplied as well as produced by the heart,
the speculations that blood levels, arterial and venous, reflect the levels in tissue (Huckabee, 1961) became opened to question.
CHAPTER II

REVIEW OF LITERATURE

A. General History of Shock

From the earliest recognition of the shock syndrome, attempts to define it have been almost as difficult as to determine its mechanisms. The definition by Gross in 1872 of shock as "a manifestation of a rude unhinging of the machinery of life," offered some idea of the difficulties involved (Davis, 1949). Le Dran (1743) is credited with using the word "choo" for the first time, but he used it to designate the act of collision rather than the resulting functional damage. It is highly probable that a transfer in meaning from that of a violent collision to that of the resulting bodily effects grew insidiously in hospital parlance long before the word had become elegant enough to use in written reports. By the middle of the 19th century it was used in medical parlance in approximately the modern sense.

During the descriptive period in the early 1800's, it was recognized that the development of shock was not necessarily related to the degree of trauma or to the local damage inflicted and that the prognosis cannot be based on the severity of the initial reactions. By the middle of the 19th century it was recognized by Paget (1862) and von Nussbaum
(1877) that caution must be used in making a diagnosis of shock. Both emphasized that every form of instantaneous death or circulatory failure following surgical operations was not due to shock. In this way the diagnosis of shock came gradually to be based on the occurrence of a definite syndrome leading to a downward course which is not explained by well-known reactions or necropsy findings.

In view of the inadequate state of physiological knowledge during the 19th century, it is not surprising that the sudden collapse of vital processes attributable to no apparent pathological state remained unknown (Morris, 1867). The profound apathy, reduced sensibilities, extreme motor weakness, reduced cerebration and impaired reflexes suggested a primary default of the nervous system; whereas the feeble heart action, weak and rapid pulse, soft arteries, and pallor pointed toward cardiac failure. It is possible that additional confusion resulted when Marshall Hall (1850) applied the term shock to the nervous depression which occurs immediately after transection of the spinal cord or an equivalent injury by accident.

Meanwhile, progress was being made in the understanding of the circulation which was to prove of basic importance. In 1825, the Weber brothers demonstrated that the pulse traveled as a wave and was not synchronous in all arteries, and in 1945 they discovered that the vagus nerves were not motor but inhibitory to the heart. The kymograph was invented by Ludwig in 1847, and mean arterial pressure in animals was then recorded for the first time. The velocity of nerve impulses was
measured by Helmholtz in 1850. During the 1850's, Claude Bernard, Brown-Sequard, and Schiff presented proof for the existence of vasomotor nerves, the mechanisms and functions of which were further studied by von Cyon and Ludwig in 1866 (Wiggers, 1950).

The most comprehensive analysis of the nineteenth century was made by Groeningen (1885). This author seriously attempted to apply existing physiological knowledge to the interpretation of shock. After a careful consideration of evidence, he concluded that vagal depression of the heart cannot explain the circulatory signs, and that depression of blood pressure does not account for the reduction of motility and sensibility or the impairment of reflexes. His analysis led to the conclusion that shock was due to fatigue or exhaustion of the spinal cord and medulla which results from intensive stimulation of sensory nerves from direct concussion of central nervous structures. The circulatory failure due to vasodilation was regarded as only a part of the general picture produced by central nervous system exhaustion.

The experimental era began with the extensive investigations of Crile, the first of which was published in 1899. Crile produced shock in various ways, and since blood pressure could be quickly and temporarily restored by intravenous infusions, he concluded that the capacity of the heart to pump blood was not affected and that, therefore, peripheral circulatory failure must occur. The next major attempt to elucidate the subject experimentally was made by Howell (1903), who concluded that shock may be of either cardiac or vascular origin; he endorsed the use of alkali
therapy and commented on the difficulty of producing shock experimentally with any degree of constancy.

Physiologists were not slow to test the various theories that had been advanced to explain the circulatory failure of shock. Porter (1903) demonstrated first that substantial depression of arterial pressure can still be induced by stimulation of the depressor nerve in rabbits eight hours after intestinal traumatization. He concluded that, since such depressor effects can operate only through the vasomotor center, it would seem clear that this center is not exhausted in shock. Seelig and Lyon (1909), and later Githers and his associates (1918), found that venous flow from a femoral vein was still augmented after section of a sciatic nerve in animals in an advanced state of shock. These observations rendered untenable the hypothesis that shock is initiated by failure of the vasomotor center and indicated that the arterioles remain under nervous control until the terminal period.

Henderson (1908) improved the technique for recording volume curves of the ventricles and with this method he concluded that a decrease in venous return was the chief factor for the reduction of cardiac output and arterial pressure. In 1910, when he formulated his concept of the venopressor mechanism, he wrote: "venous pressure is, so to speak, the fulcrum of the circulation... shock, as surgeons use the word, is due to failure of the fulcrum. Because of the diminished venous supply, the heart is not adequately distended and filled during diastole, hence the picture of a failing heart is revealed by the weak peripheral pulse. For
the same reason, arterial pressure ultimately sinks in spite of intense activity (not because of failure) in the vasomotor nervous system, and in spite of contraction (not because of relaxation) of the arterioles."

Henderson believed that the reduction of venous return in all forms of shock was caused by acapnia; the loss of CO₂ by hyperventilation. However, in subsequent papers, Henderson (1921, 1938) abandoned this theory in favor of a theory of venous atonia or venous dilatation.

During the First World War the interest among physiologists rose sharply, some of whom joined with surgeons in the battle zones of France in order to utilize the extraordinary opportunities presented by the war for studying shock in man. Interest became focused chiefly on the locus and nature of the peripheral vascular changes. The greatest impetus to the idea that capillary damage is the cause of peripheral circulatory failure was given by the studies of Dale and Richards (1918), who claimed that histamine caused a fall of arterial pressure by action on the capillaries rather than arterioles. Later (1919), Dale, Laidlaw and Richards reported that histamine injection induced not only the circulatory characteristics of shock, but other alterations such as oligemia and hemoconcentration. The theory that changes in capillary size and permeability constituted major factors in shock was thus launched and in subsequent years became a favored view of investigators.

The alleged findings that the epinephrine content of blood is increased and that shock can be experimentally produced by continuous intravenous injections of epinephrine suggested that excessive liberation of
this hormone by the adrenal medulla might be a factor. Erlanger and Gasser (1919), who confirmed the claim that shock can be produced by epinephrine, believed that it was brought on by an intense and prolonged constriction causing asphyxia of tissues. Gesell (1919), and Gesell and Moyla (1922), in studying the effects of hemorrhage and tissue trauma, found that the reduction in nutrient flow to the salivary glands and muscles was much greater than can be attributed to changes in arterial pressure. They inferred that compensatory vasoconstriction developed reflexly in order to maintain an adequate flow of blood through the vital organs, such as the heart and brain. However, this throttled blood flow through many tissues and, if prolonged, led to capillary stasis, clumping of corpuscles, blocking of venules, and loss of fluid from the vascular system.

It was observed quite early during World War I, in both clinical and experimental shock, that the alkali reserve of the blood was reduced (Cannon, 1917; Guthrie, 1918; McEllroy, 1918; Penfield, 1919; Erlanger and Gasser, 1919). It was the consensus that this represented a fixed-acid acidosis following the reduction of oxygen supply to the tissues with subsequent liberation of metabolic acids. Macleod (1921) discovered the presence of an elevated lactic acid in terminal shock. The possibility that acidosis was more than a contributory factor was seriously studied, but it was decided in the negative. Experiments revealed that injections of acid in sufficient amounts to cause a marked reduction in alkali reserve did not produce experimental shock (Dale and Richards, 1918; Gesell, 1919). Henderson and Gaggard (1918, 1920, 1921) continued to give the reduction in
alkali reserve a different interpretation. They held that reduction of $\text{CO}_2$
by deep breathing was the primary event and that this caused alkali reserve,
and the latter could account for the oligemia and hemoconcentration. This
concept was not widely accepted, however, for a number of reasons: 1) The
degree of hyperventilation required was greater than that found in experi-
mental or clinical forms of shock; 2) prolonged hyperventilation induced
reflexly by nerve stimulation did not produce shock (Kann, 1914; Guthrie,
1918; Wiggers, 1918); and 3) excessive ventilation produced circulatory
failure by mechanical effects on the pulmonary circuit regardless of
whether $\text{CO}_2$ was used (Janeway and Ewing, 1914; Dale and Evans, 1922).

At that time, the best evidence available indicated that the myocardium was unaffected. In addition, the activity of the vasomotor center
appeared to be increased. The concept was accepted that shock develops
whenever a disparity occurs between the capacity of the volume of circu-
lating blood and the capacity of the vascular system. It remained unde-
cided whether an increased capacity produced through dilatation of minute
vessels or a reduction in blood volume by hemorrhage or trauma was chiefly
concerned.

Subsequent to the First World War, the possible role of capillary
activity in the pathogenesis of shock was considered. Deficiency of the
adrenal cortex was also thought to play a dominant role in the development
of the shock syndrome. Many investigators noted that adrenalectomized
animals died from a state of circulatory failure resembling the circulatory
failure of shock. Swingle and colleagues (1934) concluded that changes in
cellular permeability with shifts of water and electrolytes and reduction in circulatory blood volume constituted the basic disturbances. In later studies (1938) they found that minor states of injury or small losses of blood caused shock in adrenalectomized dogs, with slight disturbances in water and electrolyte balance. Swingle and collaborators were of the opinion that circulatory failure was induced by lack of an adrenal cortex hormone which was necessary for the maintenance of capillary tone.

Zwemer and Scudder (1938) advanced the concept that potassium released by cell injury represented the elusive toxic agent in all types of shock. Studies soon revealed that the rise of potassium occurred in the blood only in the late stages of shock. Reports by Bisgard et al (1938), and Fenn (1940) indicated a lack of correlation between the blood level of potassium and the severity of circulatory failure. In addition, intravenous injections of potassium failed to produce the characteristic circulatory failure of shock, although large doses of potassium caused cardiac arrhythmia and abnormal conduction (Wiggers, 1930; Winkler et al, 1939). Alterations in T wave and the S-T segment of the electrocardiogram developed when plasma concentration reached approximately 14 mEq/L (Winkler et al, 1939). Later (Manrique and Pasternack, 1946), similar electrocardiographic changes were found in the course of shock.

Many investigators have attempted to assess the role played by specific organs in the development of an irreversible circulatory state. As noted previously, the studies of Swingle and associates (1933) suggested that exhaustion of the adrenal cortex may be involved. In 1938, they re-
nounced this idea and concluded that the absence of adrenal cortical hormones caused capillary atony, dilation and stasis, which in turn resulted in increased capillary permeability.

It is conceivable (Chambers and Zweifach, 1947) that the detoxifying function of the liver was impaired in shock with the result that substances which are toxic for the heart and circulation gain access to the blood stream. Fine and his associates (1947) were unable to demonstrate vasodepressor substances in the blood of unanesthetized dogs in a state of shock. Reinhard et al (1948) found that the liver was not crucially involved either in the development of hemorrhagic shock or in the temporary recovery which follows transfusion of withdrawn blood. Wiggers (1950) suggested that the chief contributing influence of the liver on the development of shock in dogs consisted in the persistence of an extreme state of resistance to portal blood flow, thus reducing venous return.

Selkurt et al (1947) stated that elevation of the portal/arterial pressure ratio played an important role in the mesenteric pooling of blood. This change was postulated to result from an increase in hepatic vascular resistance. These investigators observed that the behavior of the mesenteric circulation suggested that mechanisms were operative which favor sequestration or pooling of blood in mesenteric vessels in irreversible hemorrhagic shock. They also noted that, in some animals, vascular resistance progressively declined during the hypotensive period, which combined with an elevated portal/arterial pressure ratio, favored mesenteric pooling. It was observed in the reported experiments that most dogs dying in irre-
versible hemorrhagic shock consistently exhibited a severe bloody diarrhea.

B. The Heart in Shock

The early workers in the field of shock believed that cardiac failure was an important factor in the circulatory failure. Howell (1903) suggested that exhaustion of the vagus center occurred, and Boise (1914), noting by means of the myocardiograph the decrease in the cardiac volume, concluded that "cardiac spasm" or "cardiac tetanus" developed and was a potent cause of the fall in blood pressure. These conclusions were not verified by later investigators and, for a time, the concept of cardiac failure in shock was abandoned. For the last two decades, however, failure of the heart to pump blood has been implicated as an important factor in the pathogenesis of irreversible shock. In 1942, Wiggers and Werle noticed that, during the terminal stages of hemorrhagic shock, effective atrial pressure rose while arterial pressure fell. This was interpreted as signifying myocardial failure. Kondo and Katz (1945) produced shock by venous occlusion of the hind limbs and found a constant decline in heart size which was explained as the result of diminution in venous return. Wiggers (1945, 1947) concluded that deterioration of myocardial expulsive power in shock contributed to the progressive circulatory failure of oligemic shock. Since various compensating mechanisms which tend to maintain adequate coronary circulation were not sufficient to spare the myocardium, he was of the opinion that "myocardial depression" contributed to the redevelopment of circulatory failure when transfusions were given after the development
of an irreversible state. From these observations, it appeared that depression of myocardial function during shock may have resulted from an inadequate coronary circulation.

Opdyke and Foreman (1947) carried out studies involving coronary blood flow. Their work showed that, during the period of hemorrhagic hypotension, coronary flow was seriously curtailed, providing ample opportunity for myocardial changes. Immediately following reinfusion, the coronary flow rose significantly above control. Recently, Crowell and Guyton (1961, 1962) have studied particularly the period of time during which the animal passed from a reversible state of shock to the irreversible phase. They found no significant changes in oxygen consumption, cardiac output, or peripheral resistance during this phase of transition. However, during this period, left atrial pressure began to rise until the death of the animal. No significant changes in the peripheral circulation of these animals were detected. These authors claimed that irreversible shock may be due to acute cardiac failure.

Sarnoff et al (1954) showed that, after varying periods of hypotension, first the left followed by the right ventricles exhibited evidence of myocardial failure. They observed a marked rise in left atrial pressure, a slight rise in right atrial pressure, cardiac dilatation and a decrease in the vigor and rapidity of arterial systole. By augmenting the left coronary artery flow while maintaining the hypotension, they were able to reverse the left atrial pressure. They concluded that myocardial failure in hemorrhagic shock was due to insufficient coronary flow.
Wolcott (1951) made the observation that dogs subjected to prolonged shock induced by a variety of techniques were found to exhibit areas of fatty infiltration and necrosis of cardiac muscle when sacrificed after clinical recovery from the shock state. Edwards et al (1954) found that during hemorrhagic shock there was a fall in cardiac output and stroke volume during the oligemic and normovolemic phases. In contrast to the observations of Opdyke, it was found that coronary flow and myocardial oxygen consumption remained below control levels during the oligemic phase and lactate remained significantly elevated above control values. These changes are in line with those observed during general tissue hypoxia. In many of the animals the myocardial balance of pyruvate which had become negative during the hypovolemic phase of shock remained negative during the post-infusion phase. Apparently, the infusion of blood had failed to correct the metabolic disturbance initiated during the oligemic phase. This observation indicates that myocardial hypoxia may be an important factor in the production of myocardial depression described by Wiggers (1942, 1945, 1947), by Crowell and Guyton (1961, 1962), and by Sarnoff et al (1954).

C. Protein and Lipid Metabolism

In shock, hypercatabolism with loss of nitrogen, potassium, sulfates, phosphates and uric acid has been observed (Cuthbertson, 1960). At first, the urea in blood increases, but later it decreases owing to insufficient formation by the anoxic liver (von Slyke, 1944). Accordingly, the quantity of amino acids in the blood increases, not only because of
excessive protein breakdown, but also because of reduced deamination (Engel, 1952). The catabolism also results in liberation of polypeptides from tissue protein and of hyaluronates from the mucopolysaccharide fractions with a consequent increase in capillary permeability (Cuthbertson, 1960). Synthesis of peptides and proteins (Engel, 1952) is probably also inhibited, corresponding to a diminution in the plasma albumins, whereas the globulins and fibrinogen may increase. However, according to the results of experimental research by Hift and Strawitz (1961) on advanced hemorrhagic shock in the dog, the hepatic mitochondria may show an increase in the protein content itself and in the functional capacity in vitro despite the extreme gravity of the general condition. As far as lipid metabolism is concerned, in hemorrhagic and tourniquet shock, hyperlipemia seems to be frequent with an increase in neutral fats, lipoproteins and phospholipids in the blood (Spitzen, 1955). In the liver, the accumulation of lipids may be parallel to the fall in glycogen (Masoro and Pelts, 1958). According to their in vitro studies carried out on the liver of rats exposed to cold and fasting, there is insufficient oxidation of acetate and fatty acids proportionate to the loss of glycogen and reversible to normal after the addition of glucose. It also appears that the synthesis of fatty acids is depressed owing to a deficiency of ATP and TPNH.

D. Carbohydrate Metabolism in Shock

Carbohydrates provide precursors for the formation of amino acids, lipids and nucleic acids and, most importantly, are responsible for
about 30% of the production of the energy required for cardiac work. It is readily apparent why the role of the intermediary metabolism of carbohydrate in shock has long occupied the attention and thoughts of many shock investigators.

In 1877, Claude Bernard noted that hemorrhage leads to hyperglycemia (Robertson, 1935). As a rule the blood sugar reaches a maximum in about 30 minutes and continues at a high plateau for 3 or 4 hours. The magnitude of the hyperglycemia seems to be roughly proportional to the size of the hemorrhage and the rate at which blood is drawn (Scott, 1914). It also seems clear that the hyperglycemia results from accelerated glycogenolysis in the liver, for the glucose concentration of hepatic venous blood exceeds that in the blood stream and a rise can be prevented by clamping the portal vein (Robertson, 1935). Aub and Wu (1920) detected a definite and marked increase in blood sugar in well-advanced states of traumatic shock. The inference that hyperglycemia, once induced, is maintained almost to the terminal stage has been repeatedly affirmed in the study of various types of experimental shock (Westerfeld et al, 1944).

The utilization of glucose as reflected by the lactate and pyruvate levels in the blood has been noted in the past. That these levels are elevated in various forms of shock is well established. The metabolism of carbohydrate may be aerobic or anaerobic. The aerobic form normally predominates and proceeds by way of the condensation of pyruvic acid and oxaloacetic acid which is the first step of the tricarboxylic acid cycle of Krebs (1943) resulting in the formation of water and carbon dioxide.
The anerobic form takes place when an insufficient supply of oxygen is present and proceeds to the production of lactic acid, the final step in the formation of which is the interaction of pyruvic acid with reduced di-phosphopyridine nucleotide. In shock there occurs a transformation from an aerobic to an anerobic form of carbohydrate metabolism (Davis, 1949). Beatty (1945) showed that in hemorrhagic shock in dogs there occurred an initial increase in the blood concentrations of lactate and pyruvate with little change in the lactate/pyruvate ratio from the normal. In the terminal states of shock, however, the blood lactate levels increased greatly without a corresponding increase in pyruvate. This resulted in a high lactate/pyruvate ratio.

According to Davis (1949), the accumulation of lactic acid in the blood is not altogether disadvantageous to the survival of the organism in shock. Normally the greater part of the lactic acid is used by the liver to form glycogen. In shock this may take place to a small extent or not at all. However, lactic acid can be utilized by the brain (Ashford and Holmes, 1931) and by the heart (Evans, 1933). It is possible that the heart may utilize lactic acid in preference to other forms of fuel (Evans and his co-workers, 1933).

The glycogen content of the tissues in shock has been studied by LePage (1946). This investigator observed that the glycogen content of the kidney, like that of the liver, was greatly reduced but that appreciable amounts of glycogen still were present in the brain, heart and muscles in terminal hemorrhagic shock in rats. The glycogen changes in the liver in
tourniquet shock were reversible until the late stages (Haist and Hamilton, 1944) after which the changes became irreversible. To what extent is the irreversibility characteristic of shock related to the disturbance of carbohydrate metabolism? Seligman and his associates (1947) concluded that, in hemorrhagic shock in dogs, irreversibility to blood transfusion was not due to inability of the liver to metabolize lactic and pyruvic acids. Similarly, it was pointed out by Burdette and Wilhelmi (1946) that in irreversible shock in rats the retardation of the oxygen consumption by heart muscle slices was not caused by impaired ability of the heart muscle to oxidize pyruvate.

Despite the attempts of many investigators, the extent to which the anoxia that accompanies shock is responsible for the alterations in carbohydrate metabolism is not exactly known. However, it is known that anoxia in rats does accelerate the process of glycogenolysis (Lewis and his associates, 1942). Acute severe anoxia in rats decreased the glycogen content of the liver to a point comparable with that present in shocked rats (Haist and Hamilton, 1944). The rate of glycogenesis is reduced in acute anoxia, a phenomenon which was noted by Haist and Hamilton (1944) and by Middlesworth (1946). There is a decreased sugar tolerance as evidenced by the occurrence of hyperglycemia and glycosuria when glucose was fed to rats suffering from acute anoxia (Middlesworth, 1946). Finally, the accumulation of glycogen in the liver and heart is less in acute anoxia than in nonanoxic animals when both groups are fed glucose. It is evident, therefore, that the action of acute anoxia (unaccompanied by circulatory
failure) on carbohydrate metabolism resembles that of shock and it is probable that the oxygen lack which accompanies shock is responsible in part, at least, for the changes in carbohydrate metabolism (Davis, 1949).

After hemorrhage, adrenalectomized dogs have an elevation of blood lactate and pyruvate, as do intact animals, but the blood glucose level falls; unlike the response of normal dogs, blood lactate and pyruvate remain elevated after transfusion, possibly because of the absence of corticoids and the gluconeogenesis which they support. Continuous intravenous infusion of epinephrine into normal dogs results in changes of blood lactic and pyruvic acids that are not as marked as those occurring after hemorrhagic shock (Seligman et al, 1947). These observations suggest that the alterations of blood lactic and pyruvic acids in hemorrhagic shock are not solely attributable to epinephrine or corticosteroids, and are not necessarily correlated with the blood glucose level.

Later studies of dogs in hemorrhagic shock demonstrated that the carbohydrate metabolism of the myocardium, in particular, was altered. Hackel and Goodale (1955) studied myocardial metabolism of 12 dogs during control periods and after production of hemorrhagic shock. They noted that the most striking abnormality found was negative myocardial extraction of pyruvate. The myocardial arterial-venous difference of lactate was maintained in shock, but it was in the face of a markedly increased arterial level, so that the percent extraction ratio (A-V/A) of lactate during shock was significantly decreased. Myocardial oxygen consumption did not change significantly from the normal animal.
Edwards, Siegel and Bing (1954) attributed the change in myocardial metabolism to anoxia. They noted that, during hemorrhagic shock in dogs, coronary blood flow diminished during the oligemic and normovolemic states. As a result of failure of oxygen extraction to increase, myocardial oxygen usage declined. In the hypovolemic phase of shock, arterial blood levels of glucose rose, but the rise in glucose extraction was statistically not significant. However, myocardial pyruvate extraction by the heart also declined and coronary arterio-venous differences frequently were found to be negative. Blood lactate rose to very high levels, presumably as a result of anaerobic glycolysis in other tissues. Total myocardial extraction of lactate was actually higher during control periods, but not as high as one would have expected in a fully oxygenated heart. After reinfusion of blood, coronary blood flow and myocardial oxygen consumption continued to be low and the extraction of glucose, pyruvate and lactate remained impaired. The changes in myocardial metabolism during hemorrhagic shock were therefore, they concluded, not isolated occurrences but followed the general pattern of myocardial anoxia resulting from a diminished coronary blood flow. A more recent work from this laboratory (Bing and Ramos, 1962) approached the problem of myocardial anoxia in shock with different techniques. Estimations were made of the redox potentials of the myocardium from differences in redox potentials of coronary vein and arterial blood. In the anoxic heart the changes in redox potential between coronary vein and arterial blood, expressed as delta $E_n$, were found to be negative. The values for the difference in oxidation-reduction potential
between coronary vein and arterial blood of dogs in hemorrhagic shock were shown to be positive. In many instances from control the delta $E_h$ actually increased in oligemic phase, demonstrating that myocardial anoxia does not develop during the oligemic phases despite the fall in myocardial oxygen consumption.
CHAPTER III

MATERIALS AND METHODS

A. Surgery

Randomly selected mongrel dogs appearing in good health were
anesthetized by intravenous (or intraperitoneal) injection of sodium pento-
barbital (32.5 mg/kg). A tracheotomy was performed from a midline neck
incision and the left common carotid artery was isolated and cannulated for
monitoring mean blood pressure with a mercury manometer. A polyethylene
tube was inserted into the isolated left femoral artery for bleeding and
the left femoral vein was cannulated for the reinfusion of blood.

The animal was artificially ventilated with a positive pressure
pump and the chest of the animal was opened in the left 5th interspace.
Ribs 2, 3, 4, 5 and 6 were cut and the lungs were retracted and held in
place with saline-soaked gauze. A pericardial cardle was prepared and a
cannula was inserted into the coronary sinus via the isolated left external
jugular vein. A thread was looped around the coronary sinus with a needle
and the tip of the cannula was tied at the ostia of the sinus. Blood from
the coronary sinus was returned to the right external jugular vein, which
had interposed a "T" for measuring flow directly with a graduate and a stop
watch. Venous blood samples were collected from this circuit, while arter-
ial samples were taken from the femoral artery for the analysis of lactate, pyruvate and oxygen levels. An initial dose of 5 mg/kg of heparin (sodium salt) was administered by vein to prevent clotting. One-half of the initial dose was administered every hour during the course of the experiment.

The dogs employed in this study were divided into two groups. One group of dogs served as controls, while the second group was subjected to hemorrhagic shock. Hemorrhagic shock was induced by rapid arterial bleeding (25 cc/min) to a mean blood pressure level of 40 mm Hg. This pressure was maintained for 3 to 4 hours by either withdrawing or transfusing small quantities of blood. When it became evident that the animal could not maintain a blood pressure of 40 mm Hg, the total bled volume (at room temperature) was reinfused at a rate of 10 cc/min. After a 10 minute period of equilibrium a sample of left ventricular muscle (lateral apical portion) was excised from the beating heart. Approximately 500 mg of this tissue was placed in versene solution for phosphorylase analysis. The remaining portion of muscle was frozen in liquid nitrogen for the analysis of lactate, pyruvate and glycogen.

B. Chemical Determinations

1. Lactate

Duplicate samples of left ventricular muscle in all dogs (control and shock) were analyzed for lactate. Approximately 200 mg of the frozen muscle was weighed and then homogenized in a Potter-Elvejhem tissue grinder immersed in ice and H₂O and containing 5 ml of cold 0.6 N perchloric acid.
An additional 2 cc of perchloric acid was used to rinse the homogenizer. The homogenate was centrifuged at 4°C at 3000 rpms for 10 minutes and the supernatant was analyzed by the method of Barker and Summerson (1941). Anaerobic arterial and coronary sinus blood samples from all dogs were also analyzed for lactate. One cc of blood was added to 9 cc of ice cold 10% trichloracetic acid solution in an ice water bath, shaken, and centrifuged in the cold. The supernatant then was treated in the same manner as the tissue supernatant.

2. Pyruvate

One gram of left ventricular muscle which had been frozen in liquid nitrogen was homogenized in 5 cc of 0.6 N perchloric acid and 2 cc of perchloric acid were used as a wash. Supernatants of blood samples were prepared for analysis by mixing 5 cc of blood and 5 cc of perchloric acid. The tissue and blood preparations were centrifuged and the supernatants were analyzed for pyruvate content by the method of Friedman and Haugen (1943). Control blood levels were also analyzed using an enzyme method based on the measured changes in light absorbancy of NADH₂ (Buecher, 1964). All samples were run in duplicate for both methods.

3. Glycogen

Frozen left ventricular muscle samples weighing from 50 to 100 mg from control and shock dogs were immersed in 1 cc of 30% KOH solution in glass weighing bottles. After determining the accurate weight of the samples, they were digested by heating in a water bath at 100°C for 20 minutes
and then analyzed in triplicate according to Seifert (1949).

4. Oxygen

Anaerobic blood samples for the analysis of oxygen content were obtained in control and shock dogs. The arterial sample was taken from the femoral artery and the venous sample from the coronary sinus-jugular vein circuit. Five cc syringes were lightly lubricated with paraffin oil. Blood was then withdrawn from the animal and a small amount of mercury was drawn into the syringe for later mixing of the sample. The needle end of the syringe was inserted into a rubber stopper and placed in an ice bath. The method of Roughton and Scholander (1943) was used for analysis of oxygen content.

5. Phosphorylase Activity

The method of Cori (1956) for determining phosphorylase activity was adapted for the analysis of cardiac muscle in the dog. The principle of the analysis is based on the following equation:

\[
\text{Glycogen} + P_{O_4} \xrightleftharpoons{\text{Phosphorylase}} \text{Glucose - 1 - P}_{O_4}
\]

The concentration of reactants in cells appears to be such that an increase in phosphorylase activity gives rise to a breakdown of glycogen rather than synthesis. "In vitro" the reaction can be driven to the left with adenosine-5-P\textsubscript{O}_4. As phosphorylase activity increases, the level of phosphate (inorganic) increases.
The enzyme phosphorylase occurs in muscle in an active and inactive form, referred to as phosphorylase "a" and "b", respectively. The distinction is based on the fact that only the "a" form is active by itself, while the "b" form requires adenosine-5'-P0₄ for activity in a concentration which is far above that known to occur in muscle. The level of "a" and the total level of phosphorylase "a + b" are expressed as Cori units (Cori, 1943). The percentage activity of the enzyme is expressed as the ratio \( \frac{a}{a+b} \times 100 \).

The following chemicals were used in the analysis:

1. Versene solution: 0.001 M Ethylenedinitrilo-tetraacetic acid disodium salt, Eastman Organic Chemicals, Lot #14A and 0.02 M Sodium Floride, Mallinokrodt, pH 6.8.

2. Dowex AG I-X4 (100 - 200 mesh, high porosity) ion exchange resin, chloride form, Cal Biochem, Lot #36072

3. Glycogen, 4%, Matheson Coleman and Bell

4. Glucose-1-phosphate dipotassium salt, 0.064 M, Nutritional Biochemicals Corp., Control #6493

5. Adenosine 5' Phosphoric Acid, 0.004 M in Glucose-1-P0₄ solution, General Biochemicals, Lot #49444

6. Cysteine HCl, 0.06 M, Matheson Coleman and Bell, Lot # 5, freshly neutralized to pH 6.8

7. Sodium Glycerophosphate (30% H₂O), 0.08 M, Matheson Coleman and Bell, pH 6.8

About 500 mg of left ventricle was excised from the beating
heart, rinsed with triple distilled water, and immediately placed in a homogenizer containing 5 cc of ice cold versene solution (0.001 M EDTA-0.02 M NaF) at a pH of 6.8. The tissue was homogenized and 10 ml of the versene solution was used as a wash. The final total volume of the medium was 30 ml/g tissue. The extract was centrifuged at 3500 rpm for 10 minutes at 4°C. About 250 mg of washed Dowex resin (Haugaard, 1961) was added to 5 ml of the supernatant. After shaking, the sample was centrifuged at 4°C for 10 minutes. Five ml of the 4% glycogen solution was also treated in a similar manner with the resin.

An aliquot of the resin-treated supernatant was diluted 3-fold with freshly mixed cysteine-glycerophosphate buffer (pH 6.8). When this diluted extract was warmed to 30°C, 0.1 ml aliquots were added to 5 graduated centrifuge tubes containing 0.05 ml warm (30°C) resin-treated glycogen solution. This mixture was incubated for 20 minutes. One of the tubes served as a blank. The reaction was started by adding 0.05 ml of glucose-1-phosphate (pH 6.8) solution to two of the incubated tubes. To the remaining two tubes a Glucose-1-phosphate – adenosine 5' phosphoric acid solution (pH 6.8) was added. The tubes were incubated for 5 minutes and then 7 ml of 0.125 N H₂SO₄ was added to stop the reaction. The Fiske-Subbarow method (1925) was used to determine the inorganic phosphate levels. The small amount of inorganic phosphate present in the enzyme was determined by the blank consisting of enzyme plus glycogen.
CHAPTER IV

RESULTS

In order to compare levels of blood and myocardial substrates it was deemed necessary to express values in common units. Therefore, all blood values are given in mM/Kg of blood and values of substrates obtained in heart muscle are expressed in mM/Kg of wet tissue. Coronary sinus blood flow was measured directly from the circuit described. Values obtained were in cc/min/100 g of left ventricle and were converted to grams of blood/min/Kg of left ventricle. The weight of the left ventricle was calculated using the equation of Herman (1926):

\[ \text{Wt. of left ventricle} = 0.00369 \times \text{body weight} \]

A. Oxygen and Coronary Flow

The arterial content of oxygen in control dogs, as shown in Table I, was 20.0 cc/100 cc of blood \( \pm 0.7 \) while oxygen content of coronary sinus samples averaged 5.7 cc/100 cc \( \pm 0.8 \), resulting in an average arteriovenous difference of 14.3 cc/100 cc \( \pm 0.8 \). This agrees well with published data of Gregg (1950). In the shock dogs, oxygen content in blood taken from the femoral artery averaged 20.4 cc/100 cc of blood \( \pm 1.3 \), giving an A-V difference of 12.1 cc/100 cc \( \pm 0.6 \).
As shown in Table I, coronary sinus flow was 77.2 cc/min/100 g ± 4.6 of left ventricular muscle in control dogs and 73.8 cc/min/100 g ± 6.6 in animals subjected to hemorrhagic shock. Oxygen uptake dropped from 11.1 cc/min/100 g ± 1.01 to 8.8 cc/min/100 g ± 0.8 (p > .05) in the shock dog. This statistically insignificant drop is due to both a slight decrease in coronary sinus flow and a drop in arteriovenous difference from 14.3 cc/100 cc to 12.1 cc/100 cc (p < .05). In the work from Bing's laboratory (Edwards, 1954), values for oxygen consumption in shock (7.3 cc/min/100 g) are significantly lower than control levels (9.8 cc/min/100 g). These values are due only to a decrease in coronary sinus flow (88 to 61 cc/min/100 g) during the normovolemic state of shock. Bing obtained no change in arteriovenous difference during the shock state. It should be pointed out that Bing used the nitrous oxide method for measuring coronary blood flow, which is often criticized because of wide variation in results. There is also a possibility that the time at which the measurements were taken in the two studies might play an important role. In the reported experiments, a time interval of 10 minutes after blood reinfusion was allowed before taking the flow measurements. No specific time was given by the other laboratory.

B. Phosphorylase Activity

The methods described by various investigators for the analysis of phosphorylase activity vary considerably. One of the major problems in this analysis is to prevent the breakdown of ATP which causes increased
levels of activity. Best results were obtained from the method described in the previous section. The results shown in Table II demonstrate the variations obtained with different preparations of cardiac muscle. The tissue was not frozen because it was found that freezing caused artificially high levels of activity due to extensive breakdown of ATP, which in turn resulted in high concentrations of AMP. The resin treatment was found to markedly decrease high values of phosphorylase activity by removing AMP. These results have been substantiated by Haugaard (1961).

Under control conditions, 5.74 units ± 0.34 of phosphorylase "a" were found in myocardial tissue; in shock, the level decreased slightly to 4.72 units ± 0.55. The total number of phosphorylase units "a + b" remained essentially unchanged with 13.1 units in control dogs and 13.8 units in shock dogs. The levels of phosphorylase activity in normovolemic dogs, as shown in Table III, averaged 34.2 ± 2.3 (p < .01). Bing (1962) has postulated myocardial anoxia and has stated that he would expect higher levels of phosphorylase activity in shock. In addition to anoxic conditions, it is also known that phosphorylase activity is increased through the mediation of catecholamines (Cori, 1956). Since the blood levels of epinephrine and norepinephrine are elevated during shock (Walton, 1959), this would give further reason to postulate that the phosphorylase activity would increase. Bing and coworkers (1961) also speculated previously, that accumulations of lactic acid lower the pH of tissue, depressing phosphorylase "a" content. In the reported experiments, the increase of lactic acid in both blood and tissue during shock is substantiated. In
an attempt to confirm the evidence that pH lowers phosphorylase activity, lactic acid was infused in one dog. The pH of the blood dropped from 7.60 to 7.15 after an infusion of 300 cc of 0.1 M lactic acid. The level of the phosphorylase activity in the myocardium was 34.3%, agreeing closely with levels found in shock.

C. Glycogen

In Table V the myocardial concentrations of glycogen in control dogs averaging 36.3 mM/Kg + 2.2 are within the magnitude reported by other laboratories (Michal, 1959). The levels of glycogen during shock averaged 47.8 mM/Kg + 2.5, a level significantly higher than control (p < .01). The tissue levels in hemorrhagic shock in the dog have not previously been reported, but it was suggested by Bing (1962) that during shock there would be a fall in myocardial glycogen. In states of myocardial anoxia, such as ventricular tachycardia and fibrillation, there is a decrease in myocardial glycogen levels and these investigators claimed that sufficient information is available to project these findings in hemorrhagic shock.

D. Lactate

The values obtained for the arterial and venous levels of lactate in both control and shock dogs, as seen in Tables IV a and IV b, agree with those obtained by previous investigators (Edwards, 1954). In the control dogs, an average level of 1.67 mM/Kg + 0.18 was found in the arterial blood, while coronary sinus blood dropped to an average of 1.05 mM/Kg +
0.09. In normovolemia, the levels were 9.99 mM/Kg ± 1.06 and 9.31 mM/Kg ± 1.24 in the arterial and coronary sinus blood, respectively. Under control conditions, an average A-V difference of 0.62 mM/Kg ± 0.13 was obtained, but in hemorrhagic shock the values were widely scattered. Two out of 8 dogs showed negative A-V differences during shock; 6 out of 8 values were positive, ranging from 0.11 to 6.54 mM/Kg.

It has been known for many years that the heart is one of the organs which, under normal conditions, actively extracts lactate from the blood. It has been shown that lactate can be utilized directly by the heart. Under shock conditions, Bing (1954) states that a rise in arterial lactate concentration was accompanied by a statistically significant increase in myocardial lactate arteriovenous difference. However, because these results were accompanied by a fall in coronary flow, the myocardial uptake of lactate which he found remained close to control levels. In this work it is shown that the myocardial uptake of lactate in 7 out of 8 dogs decreases during shock.

Levels of lactic acid in the dog myocardium in shock have not been previously reported. A level of 2.58 mM/Kg ± 0.23 was found in control dogs, while in the shock state the level rose to 6.94 mM/Kg ± 0.80. As shown in Tables IV and V, the implications of these results are that the tissue level of a substrate cannot be predicted from the arterial or venous level since substantially higher values were found in cardiac muscle.

Figure 1 shows the relationship between the blood lactate and tissue lactate of a typical control dog and a typical dog in hemorrhagic
shock. Myocardial lactate levels were always found to be higher than arterial levels and venous levels under control conditions. A positive A-V difference was found in all control dogs. In shock, the level of lactate increased in cardiac muscle in all cases, but the blood levels in 6 out of 8 cases increased to a greater extent. The A-V differences vary widely in shock, but are usually less than control. Tissue levels under control or shock conditions do not reflect levels found in the blood, thus questioning Huckabee's (1961) contention that the process of extraction of lactate from blood is by simple diffusion.

E. Pyruvate

The arterial and venous levels of pyruvate agree with those found by other investigators using the same method (Edwards, Siegel, and Bing, 1954). As shown in Table VI, the arterial level of pyruvate was found to be $0.13 \text{mM/Kg} \pm 0.02$ and the coronary sinus blood level was $0.14 \text{mM/Kg} \pm 0.02$. The arteriovenous differences were found to be slightly negative in most cases, although positive values were obtained for some dogs. The average in the paper just mentioned is slightly positive. However, Edwards also obtained values with zero arteriovenous difference, which could be construed as a possible negative value. Krasnow and Gorlin (1962) have shown negative arteriovenous differences of pyruvate in the dog.

To test the validity of this analysis, the more recent lactic dehydrogenase method of Bauer (1956) was used on arterial and venous control samples. Values obtained were $0.087 \text{mM/Kg}$ and $0.093 \text{mM/Kg}$ for arter-
ial and venous samples, respectively. These values are approximately 30% lower than the Freidmann method, because it is known that the ketone substances such as α-keto-glutrate interfere with the analysis by the Freidmann method. Using this analysis, slightly negative to slightly positive arteriovenous differences were also obtained. Although this enzyme method possibly gives the more accurate levels of pyruvate, the Freidmann method was used as a comparison to work from other laboratories. This was also necessitated by the fact that it was impossible to obtain pyruvate levels in tissue using the enzyme method. The results were scattered and recovery studies warranted its omission. Good recovery was obtained in tissue studies with the Freidmann method. The values in four determinations ranged from 93 to 112% recovery. Since it was felt that an accurate comparison between blood and tissue levels of the metabolite was essential for this study, the Freidmann method was used, bearing in mind that the levels obtained could be about 15 to 30% too high.

There is essentially no uptake of pyruvate by the myocardium either in the control or shock state, as shown in Table VI. Edward's results (1954) were similar to these, +0.22 mgm/min/100g under control conditions and -0.08 mgm/min/100g in shock. He obtained a positive extraction of pyruvate in the control animal but his values are widely scattered. Despite the essentially negative pyruvate uptake, the tissue levels of pyruvate increased from 0.21 mM/Kg ± 0.02 in the control to 0.33 mM/Kg ± 0.04 (p = < .025) in the shock animals. Tissue levels of pyruvate have not previously been determined in the myocardium.
Figure 2 shows the myocardial tissue and blood levels of pyruvate in a typical control dog and a typical dog in hemorrhagic shock. Tissue levels increased under shock conditions while A-V differences remained relatively the same. In 4 out of 6 control dogs the A-V difference was slightly negative; in shock, 3 out of 5 were slightly positive.

A criterion used to determine the state of anoxia of a tissue is to calculate what is called the "excess lactate." Huckabee (1961) has proposed this convention as a means of determining the relative degree of anoxia in a tissue. Calculations are made using the arterial and venous levels of the substrates, lactate and pyruvate, according to the following equation:

$$\Delta XL = (L_v - L_a) - (P_v - P_a)(L_a/P_a)$$

In Table VII, the "excess lactate" levels in the dogs used were calculated. The calculations show that all values are negative, except one which is essentially zero. Thus there is no "excess lactate" in control or shock dogs.
CHAPTER V

DISCUSSION

Since Wiggers (1942) first suggested that myocardial depression might be one of the precipitating factors for the eventual and progressive course of circulatory failure in shock, many laboratories have been concerned with finding the cause of this deterioration of myocardial expulsive power. His cardiodynamic studies exhibited an increase in right atrial pressure, a decrease in contractile force and velocity of systolic ejection. These observations led to an interest in studying the metabolism of the heart in order to explain these cardiodynamic alterations in shock. Previous to the work of Wiggers, little if any work had been done in this area of metabolism. Edward, Seigel and Bing (1954) noted that, during the normovolemic stage of hemorrhagic shock, the coronary blood flow and oxygen consumption were lower than the control state and that the extraction of glucose, pyruvate and lactate was impaired. These investigators claimed that the changes in myocardial metabolism they observed were not isolated occurrences, but followed the general pattern of myocardial anoxia resulting from a decrease in coronary blood flow.

Glycogen is synthesized from blood glucose through the uridine diphosphate glucose pathway (Leloir, 1957). Glycogen breakdown to glucose—
1-phosphate is accomplished in the presence of phosphorylase. When the activity of this enzyme is increased, the breakdown of glycogen is accelerated. Elevation of catecholamine levels, as under stress conditions, causes the activity of this enzyme to increase substantially. Therefore, when the heart is subjected to an anoxic stress, the levels of glycogen decrease considerably (Michal, 1959), most likely through the action of liberated catecholamines from the adrenal medulla.

In the normal heart, as in skeletal muscle, anaerobic glycolytic metabolism involves the breakdown of glycogen to pyruvate. The intermediate substrates are utilized for energy production through the citric acid cycle, or they are reduced to lactic acid as an end-product. The myocardium is capable of extracting lactate from the blood for energy production through pyruvate. In a state of myocardial anoxia, as in other organs, there is an excessive production of lactic acid, resulting in an increase in lactate/pyruvate ratio of the cell. The lactate/pyruvate system seems suitable to represent the intracellular redox status, since both substrates are accepted as being highly diffusable and since the production of lactate appears not to be an intermediate but a final metabolic step (Buechehr, 1958). Two methods for measuring cardiac anoxia are Huckabee's "excess lactate" determination and the measurement of redox potentials.

Analysis of cardiac muscle for lactate, pyruvate, glycogen and phosphorylase activity for this report demonstrated that the myocardium in shock cannot be compared to the myocardium during severe anoxia. In this report, all of the values obtained for both blood and cardiac muscle under
shock conditions are representative of the normovolemic stage of shock. This period of shock was chosen to study these tissue constituents because it was necessary not to have changes occurring which are due to hypovolemia, but specifically resulting from 3 to 4 hours of previous hypotension. Since blood was reinfused and 10 minutes was allowed for equilibration, it was felt that the hemodynamic changes due to a low circulating blood volume were eliminated. At this point, it was shown in these experiments that the coronary sinus flow values returned to near normal and, therefore, the normal supply of nutrients (Tables IV, V and VI) and oxygen (Table I) were available to the myocardium.

Bing's laboratory (Klarwein, 1961) showed that, during anoxia due to ventricular or atrial fibrillation, the levels of glycogen in the myocardium were markedly decreased. The depletion of this important substrate was found to occur concurrently with the rise in phosphorylase activity. In shock, glycogen levels did not decrease; the values reported are higher than control (Table V). Bing never actually recorded the levels of glycogen in shock, but he speculated that they would reflect a state of anoxia. Migone (1962) studied rabbits in traumatic shock and found that the glycogen content of all muscles, especially the injured ones, was low but that the opposite occurred in the heart. Phosphorylase activity was suggested by Bing (1962) to be high in shock, but the levels are actually lower than control (Table III). The significant decrease from 44% in control to 34% in shock represents a substantial loss of phosphorylase activity and further affirms the glycogen levels found in these experiments. According to
Migone (1962), the increase in myocardial glycogen in traumatic shock may be due to pyruvate and lactates released from the muscular lesions to blood. He also proposed that, since a hyperglycemia of hepatic origin is present in early shock, the increased blood glucose would contribute to glycogen buildup in the heart. The brain, too, except during the final stages of shock, maintains its capacity to utilize glucose, even showing an increase in hexokinase activity and a normal content of energy-rich phosphate compounds (Kovach and Fanio, 1960). However, while the brain and the heart still retain these functional capacities, most of the other tissues show a general reduction in their utilization of glucose.

The levels of catecholamines may intervene in changes observed in glycogenolysis because peripheral blood levels of these substances are elevated in shock. During the later phases of shock, regulation of the glycogen content in the liver and myocardium was not modified by adrenal medullectomy (Stoner, 1958), thus depletion of these substances could be suspected. It has been shown that there is catecholamine depletion in shock in the cardiac muscle of the rabbit (Glaviano, 1961; Coleman, 1963). This further substantiates the decrease in phosphorylase activity found in shock, since catecholamines activate phosphorylase. Stoner and Threlfall (1960) observed that the initial metabolic changes appear in some organs in the early or intermediate stages of shock and stated that these changes seem to depend on hormonal factors rather than circulatory disorders.

The pH of the blood in hemorrhagic shock is an important factor to consider. According to Root et al (1947), the pH of the blood of dogs
in hemorrhagic shock was 6.90 to 7.20. In the reported experiment, a pre-
liminary check of the pH in blood of 4 dogs in shock was found to be 7.1.
Since hemorrhagic shock results in a condition of marked acidosis, a de-
crease in phosphorylase activity could be expected (Klarwein, 1961) from
the increased levels of lactic acid. It seems reasonable to assume that
the problem of acidosis in shock plays a significant role in cardiac meta-
bolism. Since each enzyme performs at an optimum pH, it would be logical
to assume that severe acidosis would affect, in general, enzyme performance
in the process of cardiac metabolism.

The results of this work give evidence that the heart in shock is
not embarrassed in the same manner as the heart in severe anoxia. However,
it is not possible to state that the heart possesses a normal oxidative
metabolism, because the data does show that lactate is present in increased
amounts of the myocardium (Table V). The experiments give evidence that
oxygen is available to the heart and that it is taken up to a substantial
degree during normovolemia; although control levels of oxygen uptake were
never reached. The same arterial levels of oxygen were reported in control
and shock dogs, but the arteriovenous difference in shock is less than con-
tral. Despite the reinfusion of blood, the cardiac output and blood pres-
sure never reach control levels, so the work performed by the heart remains
necessarily decreased. Thus, it would seem that oxygen is available to the
heart, but due to the decrease in external work, the uptake declines. It
should be pointed out that the drop in oxygen uptake of 20% during normo-
volemic shock does not render the heart sufficiently anoxic to result
in positive values for "excess lactate," Huckabee's (1961) criterion for anoxia in the myocardium. Despite Bing's finding on a decrease in coronary flow and oxygen consumption in early papers, one of his recent works (1962) questions the presence of anoxia in the myocardium of the shocked animal. In this work, estimations were made of the redox potentials of the myocardium from determining differences in redox potentials of coronary vein and arterial blood, in a limited number of animals. In the anoxic heart, the changes in redox potential between coronary vein and arterial blood, expressed as delta $E_h$, were found to be negative. The values for the difference in oxidation-reduction potential between coronary vein and arterial blood of dogs in hemorrhagic shock were shown to be positive, as were control values. In many instances, from control the delta $E_h$ actually increased in the oligemic phases despite the fall in myocardial oxygen consumption. During normovolemia the values remained positive but were close to or lower than control level. Thus, equating the conditions existing in the myocardium of the dog in anoxia offers a distorted picture of the true state of cardiac metabolism in hemorrhagic shock. While the metabolism of the dog's heart in shock is distinctly changed, the underlying mechanism still remains to be adequately described.

The relationship between tissue levels of a substrate and blood levels has been a topic of speculation for years. Huckabee (1961) has analyzed the levels of pyruvate and lactate in red blood cells and in plasma, and from these results has postulated that these substrates pass into the tissue by simple diffusion and that the level of these substrates
in tissue is thus necessarily lower than blood levels. In this laboratory, we have determined, for the first time, the myocardial levels of these substrates from which one can speculate as to the relationship between tissue and blood levels of a substrate and also attempt to understand the changes which occur during hemorrhagic shock. Under control conditions, the levels of these metabolites were always found to be higher in cardiac muscle than in the blood. This could imply that lactate and pyruvate do not pass into the tissue merely by diffusion, but that some active process is occurring whereby these substrates are extracted from the blood. Under shock conditions the levels of pyruvate in tissue are also higher than blood levels, implying an increased production by the tissue or an increased formation from lactate. In the case of lactate, the tissue levels increase substantially in shock but the blood levels in most cases increase to an even greater extent as shown in Figure 1, resulting in a condition opposite to control with the blood levels being higher than cardiac muscle levels. The heart in shock has a higher lactate level than control. This could be attributed to several causes, namely, 1) increased rate of glycogen breakdown or glucose, 2) diffusion into the tissue from the blood, or 3) non-utilization of lactate in heart muscle. The levels of glycogen in heart muscle are shown in this report to increase in shock, and Bing (1954) has shown that in shock there is an impaired extraction of glucose by the heart so the build-up of lactate cannot be due to increased breakdown of glycogen or glucose; furthermore, there is a decrease in blood sugar in late shock. Since the level of lactate in cardiac muscle in shock is usually lower than
in blood, there could, in this case, be diffusion from the blood to the heart. Regardless of the mechanism, the fact remains that tissue levels do not reflect blood levels of a substrate, so the postulation of the passage of lactate or pyruvate into the tissue by simple diffusion cannot be valid. The status of metabolism of the cell must play the important role.
CHAPTER VI

SUMMARY

Left ventricular muscle has been analyzed for glycogen, lactate and pyruvate content and phosphorylase activity in dogs in hemorrhagic shock. Arterial and coronary sinus blood samples from these dogs have been analyzed for lactate, pyruvate and oxygen content. Measurement of the coronary sinus flow was also determined. The following changes were noted:

1. Increases in glycogen content of left ventricular muscle were shown in dogs in hemorrhagic shock; the phosphorylase activity of cardiac muscle showed a decrease from control levels. The increases in glycogen content of left ventricular muscle and the decrease in phosphorylase activity were ascribed to the altered metabolism found during shock. Opposite conditions exist during severe anoxia and thus equating results found in anoxic states with those existing in shock was refuted.

2. Blood levels of lactate and pyruvate increased in the shock state. Increases in these substrates were not as pronounced in cardiac muscle. Calculations of the level of anoxia in the myocardium by Huckabee's concept of "excess lactate", using the
lactate and pyruvate levels of the blood, gave negative results.

3. Oxygen uptake was found to decrease slightly in shock while the coronary sinus flow remained close to control conditions. The decreased uptake was due to the small decrease in the extraction of oxygen.

4. Tissue levels of lactate and pyruvate of cardiac muscle in the control and shock state do not reflect the levels in arterial or coronary sinus blood. It was demonstrated that the passage of lactate and pyruvate from the blood to the tissue in the normal state cannot be by simple diffusion and a suggestion was made that some "active" process was involved.
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TABLE I

Oxygen Content in Blood of Control and Shock Dogs

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<tr>
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<th>Arterial*</th>
<th>Venous*</th>
<th>A-V Diff.</th>
<th>CSF*</th>
<th>Myocardial Uptake</th>
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<td>(cc/100cc)</td>
<td>(cc/100cc)</td>
<td>(cc/100cc)</td>
<td>(cc/min/100g)</td>
<td>(cc/min/100g)</td>
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<th>CSF*</th>
<th>Myocardial Uptake</th>
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* Values are under STP conditions

* Coronary Sinus Flow
TABLE II

% Phosphorylase Activity in the Myocardium of Control Dogs

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<th>No Freezing Resin Treatment</th>
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<td>56.3</td>
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TABLE III

Phosphorylase Activity in Myocardial Tissue

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<th></th>
<th></th>
<th></th>
</tr>
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<td>Units-Total (a + b)</td>
<td>% (\frac{a}{a+b})</td>
<td>Units-a</td>
<td>Units-Total (a + b)</td>
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<td>3.00</td>
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* Levels are given at Cori units
### TABLE IV a

**Lactate Concentration in Blood of Control Dogs**

<table>
<thead>
<tr>
<th>#</th>
<th>Weight (Kg)</th>
<th>Arterial (mM/Kg)</th>
<th>Venous (mM/Kg)</th>
<th>A-V Diff. (mM/Kg)</th>
<th>CSF* (g/min/Kg)</th>
<th>Myocardial Uptake (mM/min/Kg)</th>
</tr>
</thead>
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<tr>
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<td>0.87</td>
<td>0.23</td>
<td>643</td>
<td>0.147</td>
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<td>16.5</td>
<td>1.74</td>
<td>0.92</td>
<td>0.82</td>
<td>829</td>
<td>0.679</td>
</tr>
<tr>
<td>3</td>
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<td>0.39</td>
<td>724</td>
<td>0.282</td>
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<td>3.12</td>
<td>1.30</td>
<td>1.82</td>
<td>776</td>
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<td>18.0</td>
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<td>1.79</td>
<td>1.17</td>
<td>855</td>
<td>1.000</td>
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<td>787</td>
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<td>0.41</td>
<td>1094</td>
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<td>0.03</td>
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<td>0.73</td>
<td>0.89</td>
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<td>784</td>
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<td>1.12</td>
<td>1.15</td>
<td>1049</td>
<td>1.206</td>
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</table>

Mean 16.9  | 1.67  | 1.05  | 0.62  | 821
S.E.+  --   | 0.18  | 0.09  | 0.13  | 34

*Coronary Sinus Flow (g/min/Kg of left ventricle)*
TABLE IV b

Lactate Concentration in Blood of Shock Dogs

<table>
<thead>
<tr>
<th>#</th>
<th>Weight (Kg)</th>
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<th>Venous (mM/Kg)</th>
<th>A-V Diff. (mM/Kg)</th>
<th>CSF* (g/min/Kg)</th>
<th>Myocardial Uptake (mM/min/Kg)</th>
</tr>
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<tr>
<td>1</td>
<td>19.5</td>
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* Coronary Sinus Flow
TABLE V

Cardiac Muscle Concentrations

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<th>Pyruvate (mM/Kg)</th>
<th>Glycogen (mM/Kg)</th>
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<td>Control</td>
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<td>3.79</td>
<td>.34</td>
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<td>2.95</td>
<td>4.70</td>
<td>.21</td>
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<td>12.17</td>
<td>.13</td>
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<td>.27</td>
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P = <.001  P = <.025  P = <.01
**TABLE VI**

**Pyruvate Concentration in Blood**

**of Control and Shock Dogs**

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<tr>
<th>#</th>
<th>Arterial (mM/Kg)</th>
<th>Venous (mM/Kg)</th>
<th>A-V Diff. (mM/Kg)</th>
<th>CSF Uptake (g/min/Kg)</th>
<th>Myocardial Uptake (mM/min/Kg)</th>
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<tr>
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<td>-0.01</td>
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<td>776</td>
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<td>+0.03</td>
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<tr>
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<th>Venous (mM/Kg)</th>
<th>A-V Diff. (mM/Kg)</th>
<th>CSF Uptake (g/min/Kg)</th>
<th>Myocardial Uptake (mM/min/Kg)</th>
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<td>+0.00</td>
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<tr>
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<td>.03</td>
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</table>

*Weights of dogs are shown in Table IV.*
TABLE VII

"Excess Lactate" Calculations

Method of Huckabee

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<tr>
<th>Dog #</th>
<th>$L_v - L_a$</th>
<th>$P_v - P_a$</th>
<th>$L_a/P_v$</th>
<th>$(P_v - P_a)L_a/P_a$</th>
<th>$\Delta XL$</th>
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</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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$L_a$ = arterial lactate concentration

$L_v$ = coronary sinus lactate concentration

$P_a$ = arterial pyruvate concentration

$P_v$ = coronary sinus pyruvate concentration

$\Delta XL$ = "excess lactate"
The figure demonstrates the level of lactate passing through the left ventricle at the recorded coronary sinus flow under control and shock conditions.
Control

Arterial 0.11 mM/Kg

Left Ventricle 0.27 mM/Kg

Venous 0.12 mM/Kg

Coronary Sinus Flow (829 g/min/kg)

Shock

Arterial 0.23 mM/Kg

Left Ventricle 0.38 mM/Kg

Venous 0.22 mM/Kg

Coronary Sinus Flow (825 g/min/kg)

FIGURE 2

MYOCARDIAL TISSUE AND BLOOD LEVELS OF PYRUVATE IN A CONTROL DOG AND A DOG IN HEMORRHAGIC SHOCK

The figure demonstrates the level of pyruvate passing through the left ventricle at the recorded coronary sinus flow under control and shock conditions.
APPROVAL SHEET

The thesis submitted by Joan Mary Loersching has been read and approved by three members of the Department of Physiology.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form, and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

Jan 18, 1965

Date

Signature of Advisor

Vincent V. Lawrence