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Status of Myocardial Function in Irreversible Hemorrhagic Shock

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STATUS OF MYOCARDIAL FUNCTION IN IRREVERSIBLE HEMORRHAGIC SHOCK

by

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BIOGRAPHY

Bernell Coleman was born April 26, 1929. He was graduated from elementary school in 1944 and from high school in 1948. He attended Alcorn College and the University of Chicago. He received the Bachelor of Science degree in June, 1952.

Mr. Coleman was employed as a Research Assistant in the Department of Pathology, University of Kansas Medical Center from 1952 to 1953. He served with the Army Security Agency from 1953 to 1956. He was a Research Assistant in the Department of Biochemistry, University of Chicago from 1956 to 1957 and in Cancer Research at Hines VA Hospital from 1957 to 1959.

Bernell Coleman entered the Graduate School of the University of Illinois Professional College, Chicago, Illinois, in September, 1959. He was a University Fellow from 1959 to 1960. He transferred to the Graduate School of Loyola University of Chicago in September, 1960, where he was a Teaching Assistant in the Department of Physiology from 1960 to 1961. He was a United States Public Health Service Predoctoral Fellow from 1961 to 1963. He is a member of the following societies: AAAS, Sigma Xi, APS.

List of Publications:

1. V.V. GLAVIANO and B. COLEMAN, Depletion of myocardial norepinephrine in hemorrhagic hypotension, Fed. Proc. 20: 116, 1961.

2. V.V. GLAVIANO and B. COLEMAN, Myocardial depletion of norepinephrine in hemorrhagic hypotension, Proc. Soc. Exper. Biol. and Med. 107: 761, 1961.

3. B. COLEMAN and V.V. GLAVIANO, Myocardial composition in irreversible hemorrhagic shock, Fed. Proc. 21: 119, 1962.

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INTRODUCTION

Several investigators have offered suggestive evidence which indicates that "myocardial depression" is the precipitating factor for the eventual and progressive course of circulatory failure in hemorrhagic shock. Cardiodynamic studies have shown that the heart in shock exhibits an increase in right atrial pressure, a decrease in contractile force as well as a decrease in the velocity of systolic ejection. In addition, studies involving cardiac metabolism have also indicated that the heart in shock exhibits a negative pyruvate extraction during both the oligemic and hypervolemic stages of hemorrhagic shock. The infusion of blood fails to correct this alteration of metabolism initiated during the oligemic phase. This metabolic alteration would indicate that myocardial hypoxia may be an important factor in the production of myocardial "depression". That is to say, failure of the myocardium in irreversible hemorrhagic shock could result from an anoxic condition caused by an inadequate coronary flow.

In an attempt to further explore the "state of myocardial depression", the work for this dissertation was designed to study the water and electrolyte alterations in the myocardium during irreversible hemorrhagic shock. The electrolytes chosen to ascertain the status of myocardial function were Na^{+}, K^{+}, Mg^{++}, Ca^{++},

and Cl^-. These electrolytes were chosen on the following premises: (1) the distribution of sodium and potassium across the cell membrane is such that the concentration of Na^+ is highest in the extracellular compartment, whereas K^+ is present in high concentrations inside the cell. The maintenance of this ionic distribution is dependent upon the metabolic processes of the individual cells. The energy of metabolism is involved in re-establishing the normal electrolyte gradient following the action potential. In myocardial failure, because of a default in metabolism, sodium and potassium tend to move according to their respective concentration gradients. The outcome of such electrolyte shift is a reversal of the normal ionic distribution leading to edema of cardiac muscle. The presence of cardiac edema is consistent with the concept that the movement of water and sodium is in the same direction; (2) calcium is thought to be involved in the coupling mechanism between electrical excitation and mechanical contraction, (3) magnesium is involved in many enzymatic processes, such as ATPase and hexokinase reactions, and (4) chloride was used as a measurement of extracellular space. The measurement of extracellular space becomes significant when fluctuations in water content can be expected to occur as in myocardial failure.

Since water and electrolyte disturbances in blood from hemorrhagic shock have been repeatedly confirmed, it was considered important to assess these

alterations in cardiac muscle at a cellular level. There is considerable information on water and electrolyte changes of the myocardium in proven cases of congestive heart failure and in myocardial ischemia, but a complete lack of information concerning these changes in the heart of animals in hemorrhagic shock.

The general significance of the present study may lie not only in the description of the myocardial water and electrolyte alteration in hemorrhagic shock, but also in providing factual information concerning the changes that occur in the electrolyte gradient across the cell membrane. This is especially important in considering the role of potassium in the eventual outcome of hemorrhagic shock. As an adjunct to the major hypothesis of this study, experiments will be described involving the biophysical action of l-norepinephrine on the heart in shock. In addition, the results of cardiodynamic studies of the heart in shock demonstrate that the pumping action of the heart is not impaired until minutes before the animal succumbs to ventricular fibrillation. From these experimental observations, it is my contention that myocardial failure occurs only as a terminal event in irreversible hemorrhagic shock.

CHAPTER I

REVIEW OF THE LITERATURE

A. EARLY LITERATURE

The origin of the word "choc" has been attributed to Le Dran (1743), who used the term to designate the act of collision rather than the resulting functional damage. However, it was left for Guthrie (1815) to demonstrate an understanding of the pathogenesis of shock when he counseled postponement of operations until the "alarm and shock" had subsided. In discussing gunshot wounds of the Crimean War, Cooper (1838) stated that many wounded soldiers had died without significant loss of blood, severe pain, or serious injury. He wrote: "surgeons were in the habit of saying men died of shock without asking themselves what they meant by the term." By the middle of the nineteenth century it was recognized by Paget (1862) that caution must be used in making diagnosis of shock. He emphasized that every form of instantaneous death or circulatory failure following surgical operations is not necessarily due to shock. In this way the diagnosis came gradually to be based on a definite group of signs and symptoms which ultimately lead to a downward course.

In 1870, Fischer pointed out that patients who developed shock after trauma and surgical operations manifest many similar signs. He postulated that

stasis occurred in the abdominal vessels owing to reflex paralysis of the vasomotor nervous system. According to Fischer, the heart receives and pumps less blood, arterial pressure falls, and somatic structures suffer from the reduction in blood volume. He held that the reduction in circulating blood volume accounts not only for the pallor and coldness of the skin, but also for the impairment of reflexes. By far, the most comprehensive analysis of the shock problem in the nineteenth century was made by Groenigen (1859). He seriously attempted to employ the scientific method in applying the existing physiological knowledge to the interpretation of shock. After careful examination of the available evidence he concluded that: (1) vagal depression of the heart cannot explain the circulatory signs in shock, and (2) depression of blood pressure does not account for the impairment of reflexes. His analysis led him to conclude that shock is due to fatigue or exhaustion of the spinal cord and medulla. These changes resulted from intensive stimulation of sensory nerves or from direct damage to central nervous structures. The circulatory failure due to vasodilatation was regarded as only part of the general syndrome produced by central nervous exhaustion.

Toward the end of the nineteenth century accumulation of physiological knowledge concerning the control of the circulation served to focus attention on circulatory failure as the important feature of shock. Paralysis or inhibition of

the vasomotor center, pooling of blood in abdominal vessels, and hypotension were regarded as sequential events.

Crile (1899) published the first report on experimental shock. He produced shock in various ways, and since blood pressure could be quickly and temporarily restored by intravenous infusion, he concluded that peripheral circulatory failure occurs since the capacity of the heart to pump blood was not affected. Crile agreed with Fischer that failure of arterial pressure was the primary cause of shock. Failure of the heart, respiration and motor activity were considered as secondary factors for causing circulatory failure.

A major attempt to elucidate the subject of shock experimentally was made by Howell (1903). He produced shock by limb or intestinal trauma and venous occlusion. This author concluded that shock may be either of cardiac or vascular origin. Once an interest had been created, physiologists began to test the various theories that had been advanced to explain the circulatory failure of shock. Porter (1903, 1908) demonstrated that substantial depression of arterial pressure could still be induced by stimulation of the depressor nerve in rabbits eight hours after intestinal trauma. Since such depressor effects can only operate through the vasomotor center, it would seem that this center was functioning in shock. Seelig and Lyon (1909, 1910) and later Githens et al, (1918) found that venous flow from a femoral vein still augmented after section

of the sciatic nerve in animals in an advanced state of shock. These investigators interpreted their finding to indicate that the arterioles remain under nervous control for the entire course of shock. It was observed by Mann (1914) that unilaterally innervated vessels of the dog's tongue, rabbit's ear, and kitten's paw appeared constricted during shock when compared to the contralaterally denervated vessels. These observations repeatedly confirmed, rendered untenable the hypothesis that shock is initiated or perpetuated by failure of the vasomotor center. Furthermore, investigators were unsuccessful in attempts to produce shock by prolonged stimulation or traumatizing somatic nerve trunks alone (Porter, 1908; Mann, 1914; Janeway and Ewing, 1914). However, it was found by Guthrie (1917) and Wiggers (1918) that such stimulation appears to facilitate induction of shock by otherwise ineffective trauma and hemorrhage.

Henderson (1908) improved the technique for recording volume curves of the ventricles. On the basis of studies made by 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 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 CO_2 by hyperventilation. However, in subsequent papers, Henderson (1921, 1938) abandoned this theory in favor of a theory of venous atonia or venous dilatation.

B. PERIPHERAL CIRCULATORY FAILURE

Several different theories were proposed to explain peripheral circulatory failure in shock. Dale and Richards (1918) gave impetus to the concept that capillary damage was the causal factor. They claimed that capillary rather than arteriolar dilatation caused by histamine was sufficient to cause the circulatory signs of shock. Later, Dale, Laidlaw, and Richards (1919) reported that histamine injection induced not only the circulatory characteristics of shock, but other alterations such as oligemia and hemoconcentration. Erlanger and Gasser (1919), who confirmed the claim that shock can be produced by epinephrine, believed that it is brought on by an intense and prolonged constriction causing asphyxia of tissues. Gesell (1919), and later Gesell and Moyle (1922), in

studying the effects of hemorrhage and tissue trauma, found that the reduction in nutrient flow to the salivary glands and muscles is much greater than can be attributed to changes in arterial pressure. They inferred that compensatory vasoconstriction develops reflexively in order to maintain an adequate flow of blood through the "vital organs", such as the heart and brain. Gasser and Erlanger and Meek (1919) also found that reduction in circulating blood volume, hemoconcentration, and in exceptional cases concentration of plasma, occurred in all forms of shock. They listed the factors causing shock from plasma loss as follows: (1) transudation of plasma, (2) dilation of capillaries and veins, and subsequent slowing of the circulation with rise of capillary pressure, (3) packing of corpuscles into capillaries and veins, particularly in the intestinal mucosa, and (4) loss of fluid or hemorrhage into the intestinal lumen. The concept gradually developed that loss of plasma with hemoconcentration was an important factor in the production of shock in ways other than hemorrhage.

In 1921 Macleod discovered the presence of lactic acid in blood in advanced states of shock. The possibility that acidosis was more than a contributory factor was seriously studied and rejected. 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).

At that time the best evidence available indicated the myocardium to be 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 circulating blood and the capacity of the vascular system. It remained undecided whether an increased capacity produced through dilatation of minute vessels or a reduction in blood volume by hemorrhage or trauma was chiefly concerned.

During the First World War attempts were made to apply fundamental physiology to treatment of shock. Previous to this time the only blood substitutes were isotonic sodium chloride and glucose solutions. The use of colloidal solutions as blood substitutes were based on the observations of Starling (1896, 1899). He demonstrated that colloids exert a small but measurable osmotic pressure and thereby perform an important function within the vascular system. Of the various colloids tried, the addition of gum acacia appeared to yield the best results (Bayliss, 1919). However, after its widespread use in battle casualties, the introduction of colloidal solutions to replace blood transfusions was not accepted and found in some cases even to be harmful (Wiggers, 1949).

Subsequent to the First World War, interest developed in the possible role of capillary activity in the pathogenesis of shock. 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 his collaborators presented evidence which indicated that: (1) the reduction in effective blood volume and the changes in blood chemistry after adrenalectomy were similar to those of hemorrhage, (2) animals in a profound state of shock could be revived by injections of adrenal cortical extracts, (3) adrenalectomized animals regained their normal resistance if previously protected with large doses of cortical extracts, (4) adrenalectomized animals withstand less trauma and hemorrhage, and (5) shock could not be produced by standard methods in normal animals which were pretreated with cortical hormone (Swingle et al, 1936). From earlier studies, Swingle and colleagues (1934) concluded that changes in cellular permeability with shifts of water and electrolytes and reduction in circulatory 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. During this period the possibility that histamine was responsible for the circulatory failure of shock received less experimental support.

Experiments involving plasma water and electrolyte determinations have shown that blood potassium can increase under a variety of conditions. These conditions generally involve excessive loss of sodium and water such as hemorrhage, trauma, intestinal obstruction and intraperitoneal injection of glucose (Zwemer and Scudder, 1938). These investigators advanced the concept that potassium released by cell injury represents the elusive toxic agent in all types of shock. Studies soon revealed that the rise of potassium occurs in the blood only in the late stages of shock. Reports by Bisgard et al (1938), and Fenn (1940) indicated the 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. Large doses of potassium causes 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). Similar electrocardiographic changes have not been found in the course of shock.

Moon (1938, 1942) restudied the gross histological changes in death following shock and allied states in man. His conclusion was that a definite pathological picture of shock exists which is highly suggestive of generalized capillary damage. Moon supported the views of Scudder (1940) that hemocon-

centration constitutes a pathognomonic sign of shock because it is the result of general capillary damage which causes leakage of plasma that results in the reduction of plasma volume. However, investigation by Gregerson and Root (1947) demonstrated that hemoconcentration and progressive decrease in blood volume are not essential features of shock. Blood volume determinations before and at various times after injury indicated that the decrease in volume occurred at the time of the injury. From then on for several hours, during which the signs of shock developed and the animal either succumbed or began to recover, the blood volume, plasma protein concentration, and the hematocrit value remained essentially unchanged. In addition, Holmes and Painter (1947) performed tissue analyses of injured and uninjured tissues. Their results indicated that fluid was being absorbed by the blood stream from uninjured tissues at a rate sufficient to compensate for the gradual leakage into the injured region. The significance of this observation in obvious, for it demonstrated that the normal compensatory dilution mechanism is not deranged in shock as postulated by the capillary leakage concept.

C. INVOLVEMENT OF SPECIFIC ORGANS

Several reports have attempted to assess the role played by specific organs in the development of an irreversible circulatory state.

1. Adrenal Gland

It was considered possible that the adrenal medulla might become overactive through reflex stimulation and thus become exhausted. On the other hand, Erlanger and Gasser (1919) confirmed the claim that shock can be produced by epinephrine administration. They believed that it is brought on by an intense and prolonged constriction causing asphyxia of tissues. The studies by Swingle and associates (1933) suggested that exhaustion of the adrenal cortex may be involved. Many of the manifestations of adrenalectomy results in changes in cell membrane permeability. In 1938, they renounced this idea and concluded that the absence of adrenal cortex hormones causes capillary atony, dilatation, and stasis, which in turn results in increased capillary permeability.

2. Liver

Whipple and his associates (1920) believed that the hypoproteinemia of shock was of paramount importance which they attributed to the failure of the liver to replace plasma proteins. This concept was weakened by the investigation of Amberson et al (1934), who removed all but the last traces of normal blood and replaced it with a hemoglobin-Ringer solution. In subsequent work (Sanbury et al, 1936) they reduced plasma protein to a level of 0.05 to 0.15 mg per cent by repeated bleedings and reinjection of washed cells mixed with Ringer's solution containing 6 per cent gum acacia. Edema never developed in

cats and only rarely in dogs. Shock was never observed in these animals. It was concluded that plasma proteins have only one physical function, that is, to maintain the viscosity and colloidal osmotic pressure of plasma.

It is conceivable that the detoxifying function of the liver is impaired in shock with the result that substances which are toxic for the heart and circulation gain access to the blood stream. It is even conceivable that the vasodepressor substances arising in the liver (Chambers and Zweifach, 1947) represent loss of detoxifying function rather than the formation of a new product. On the other hand, 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 is 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 consists 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 plays 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 suggests

that mechanisms are 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, favors mesenteric pooling. It was observed in the reported experiments that most dogs dying in irreversible hemorrhagic shock consistently exhibited a severe bloody diarrhea.

3. Heart

For the last several years 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. Kohlestaedt and Page (1944) noticed that after an initial diminution in the size of the heart, progressive bleeding led to an increase in both the systolic and diastolic size of the heart. Apparently, the stroke volume first diminished, while the residual volume gradually increased. 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 inadequate coronary circulation.

Studies involving coronary blood flow were carried out by Opdyke and Foreman (1947). 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 re-examined this question in dogs with hemorrhagic shock. They 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. The authors claimed that irreversible shock may be due to acute cardiac failure. No significant changes in the peripheral circulation of these animals were detected.

Sarnoff et al (1954) showed that after varying periods of hypotension, first the left followed by the right ventricles exhibited evidence of myocardial failure. This showed itself as a rise in atrial pressure (more marked on the left side), a grossly observable cardiac dilatation, and a decrease in the vigor and rapidity of arterial systole. They were able to reverse the elevated left atrial pressure and cardiac dilatation by augmenting left main coronary artery flow by perfusion from a donor dog, while maintaining the same degree of hypotension. It was concluded that myocardial failure in hemorrhagic shock was consequent upon an insufficient coronary flow.

Melcher and 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. The mechanism by which these changes occur is unknown; however, anoxemia was suggested as the underlying cause. Similar changes in the myocardium in hemorrhagic and tourniquet shock were noted by Mylon et al (1944). Master et al (1950) also found pathologic changes in the heart muscle; these changes were rarely seen in the right ventricle.

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 normovolemic phase. The metabolic changes apparent during the oligemic phase persisted during post-infusion shock. The blood levels of pyruvate 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 Kohlstaedt and Page (1944), by Crowell and Guyton (1961, 1962), and by Sarnoff et al (1954).

Serious myocardial impairment of a dog's heart in shock was further noted on a cellular basis by Strawitz and Hift (1956). They observed that cardiac mitochondria in irreversible hemorrhagic shock underwent a change in size and shape. In addition to structural alterations of mitochondria, impaired activity of the hexokinase system was also suggested. The significance of the latter finding has been questioned on the basis of the paucity of data and the small differences observed (Levenson et al, 1961). In further studies with heart mitochondria isolated from similarly hemorrhaged dogs, Hift and Strawitz (1958) attempted to obtain evidence for the presence of an abnormal component or loss

of a normal constituent. They found that heart mitochondria of shocked dogs had increased amounts of phosphate and that some of their proteins (intramitochondrial) became more readily extractable with water. Since in both papers by these workers the shed blood was apparently not replaced, there seems to be no basis for their statement that their "dogs were subjected to irreversible hemorrhagic shock". Packer et al (1958) studied the phosphorylation processes in mitochondria prepared from heart of rats subjected to various types of shock. They claim to have found a decreased synthesis of ATP and lowered P/O ratios with unchanged ATPase activity in heart mitochondria in traumatic shock. They reported that after drum shock, the electrolyte composition of heart mitochondria showed an increase of total phosphorus and potassium, a decrease in sodium, and only slight difference in calcium and magnesium. Since increased phosphate content, they cite, may result in decreased efficiency of phosphorylation (Packer et al, 1958), they concluded that the "electrolyte changes appear to be responsible for the structural and functional changes in mitochondria of animals in shock". However, inspection of their electrolyte data (the only data for which P values are given) indicates that only the increase in total phosphorus was statistically significant ($P = 0.05$), all other electrolyte differences had identical P values of 0.10. Aldridge and Stoner (1960) dismiss the observations of Packer et al, because "the statistical evaluation of their published data is not possible".

D. WATER AND ELECTROLYTIC DISTURBANCES

Considerable attention has been given to the effect of hemorrhagic shock on plasma electrolytes and metabolic substrates. From the numerous papers published in this area of investigation, the findings of Root and his co-workers (1947) have been repeatedly confirmed. They observed for either hemorrhagic or traumatic shock the following alterations of plasma constituents: an increase in the levels of pyruvate, sulphate, phosphate, lactate, potassium, and magnesium ions, with a concurrent decrease in blood pH, arterial carbon dioxide content and bicarbonate levels. Little or no change was found in plasma water, chloride, calcium and sodium ions. These workers specifically indicated that the elevations in plasma potassium and magnesium were terminal events.

Although a distinction should be made between the effects of the injury per se and those caused by shock, this is not always possible because the effects of each are incompletely known and changes often blend together. Sudden serious disturbances in water, electrolytes and plasma proteins may result directly from the initial injury, independently of shock and not as a consequence of it. It has long been known that the early movement of blood constituents into, adjacent to, and from the injured area may lead to fatal functional depletion of water, electrolytes and proteins; if adequate replacement therapy is provided in time, death may be averted. Millican (1960) has pointed out that the possible

role of hormones in the production and regulation of the swelling at the site of trauma has not been clarified. The extent of extracellular and intracellular volume shifts is not definitely known since these estimates are based on inadequate measurements. In particular quantitative electrolyte and fluid changes of specific organs and tissue are incompletely known; most of the available information deals with changes in skin and muscle.

Tabor and Rosenthal (1945) made quantitative measurements of the total changes in water, sodium and potassium occurring in the hind legs of mice following the application of tourniquets. In this form of shock there was a gain in sodium 33 per cent greater than could be accounted for by the fluid increase. There was also a corresponding decrease in potassium suggesting a Na^+-K^+ interchange. These electrolyte shifts occur not only in injured areas, but also generally in areas distant from the primary injury, and may result from tissue hypoxia in shock. Fuhrman (1960) has raised the question whether this systemic response depends on a metabolic failure of the sodium "pump". Plasma potassium concentration may also rise as a result of cellular release of potassium associated with the net breakdown of tissue protein and glycogen. As K^+ leaves the cells, Na^+ and H^+ enter, and possibly also basic amino acids, e.g., lysine (Fuhrman, 1960). The total Na^+ accumulated in the injured area of untreated mice was equivalent to the entire Na^+ in the circulating blood, or approximately

25 per cent of the total extracellular fluid. Changes of similar magnitude have been reported in mice, rats, rabbits and dogs (Fox and Keston, 1945; Fuhrman and Cresmon, 1951 a, b; Walser and Bodenlo, 1954; Holmes and Painter, 1947).

Experimental as well as clinical evidence has indicated that loss of water does not by itself usually result in circulatory failure; significant salt depletion is required with water depletion. This point was demonstrated by the experiments of Darrow and Yannet (1935) and Elkinton, et al (1946). Salt depletion was produced in dogs by intraperitoneal injection of glucose solutions, followed by withdrawal of the accumulated fluid 4 - 6 hrs. later. Water depletion was produced by intravenous injection of urea or glucose solutions to cause diuresis. When the salt content of the body remained unchanged and the water content was decreased, plasma volume dropped proportionately to the extracellular fluid volume with no plasma protein depletion. The animals remain vigorous and healthy in contrast to those suffering from salt depletion. The latter condition induces a reduction in plasma protein, which is explained vaguely by its segregation "somewhere in the body through a disturbance of dynamic protein equilibrium". Animals so affected pass into a shock-like state. Similar results were obtained from intramuscular injections in dogs by Davis (1949). Ashworth and Kregel (1942) examined the changes in partition of water and extracellular electrolytes in hemorrhagic and traumatic shock. They concluded that water

passes from the extracellular spaces into cells after hemorrhage but in the reverse direction in traumatic shock.

E. THE ROLE OF POTASSIUM

The change in plasma potassium deserves special consideration, since it has been the subject of considerable investigation during the last thirty years. Several investigators have reported the release of K^+ into blood after hemorrhage (Kerr, 1926; Thaler, 1935-6), muscle trauma (Rabboni, 1934), arterial occlusion (Baetjer, 1935), or asphyxia (Fenn et al, 1939). There are other reports of higher K^+ concentration in the edema fluid of injured tissues than in the plasma (Manery and Solandt, 1942-3; Ricca et al, 1945; Underhill and Fisk, 1930; Zwemer and Pike, 1938). Zwemer and Scudder (1938) postulated K^+ to be a toxic factor in shock based on the K^+ blood levels observed in shocked animals and normal animals intoxicated with K^+ and also the large increases in K^+ in the blood in the terminal phase of shock. Neither Brues and his associates (1945) nor Root and his colleagues (1947) found very significant electrolyte changes in blood during advanced stages of hemorrhagic and traumatic shock. Water, chloride, sodium, and calcium were not altered; magnesium and potassium increased only slightly until the terminal stage when a sharp rise was manifested. Similar findings have been reported by other investigators (Holmes, 1947; Manery and

Solandt, 1942-3; Richards, 1944; Scudder et al, 1939; Winkler and Hoff, 1943). Winkler and Hoff (1943) investigated the role of potassium as a cause of death in traumatic shock. They concluded that serum potassium does not reach levels previously found necessary to produce electrocardiographic changes (10 mEq/L). Miller (1943) found an increase in potassium both in skeletal muscle and serum after scalding and hemorrhage, and offered the suggestion that this may be due to loss of muscle protoplasm without an equivalent loss of potassium. Though the hyperpotassemia is usually quantitatively small during shock, the untreated animal may become increasingly sensitive to extracellular potassium. Concentrations sufficient to cause death may be encountered as terminal events. Tabor and Rosenthal (1945) found that the tourniquet-shocked mice died from an injection of K^+ equal to one eighth of the lethal dose for a normal mouse. A similar increased sensitivity has been reported for the dog by Davis (1949) and in the tourniquet-shocked rat by Ravin et al (1954).

Although the blood levels of K^+ in terminal stages of shock seldom reach values attained in normal animals killed by infusion of potassium, the possibility is suggested that death may occur at a lower level of potassium in the shocked animal highly sensitive to intoxication than in the normal animal. Evidence in support of this possibility was provided by Tabor and Rosenthal (1945). They compared the serum K^+ of (a) rabbits in terminal stages of shock, (b)

shock rabbits killed acutely by K^+ administration and (c) normal rabbits killed acutely by K^+ administration. They found these values to be: 12.1, 13.2 and 16.6 mEq/L of serium for (a), (b), and (c) respectively.

It has been reported that uninjured tissues are able to adjust to the increase concentrations of K^+ which results from the release of K^+ from injured areas. Darrow and Engel (1945) studied liver electrolytes and water in moribund rats. In one series of experiments they noted a 10 to 25 per cent reduction in liver potassium with an increase in sodium after massive hemorrhages. These results suggested a loss of intracellular water and potassium. In another series in which repeated hemorrhages were used, they found a 50 per cent increase in liver potassium and 50 per cent decrease in sodium. The entry of potassium was explained on the basis of a breakdown of the hepatic cell permeability. In addition, liver water was also elevated. Several investigators have reported similar results from uninjured muscle, pancreas, and red cells following hemorrhage or muscle trauma in dogs, rats and mice (Brues et al, 1945; Fox and Baer, 1947; Fox et al, 1953). Clark and Cleghorn (1942) have been the only investigators to report increases in the K^+ concentration of cardiac muscle following hemorrhage. However, their data can be criticized on several very important points. First of all, the number of animals on which they analyzed for K^+ in cardiac muscle was very small. They claimed that the decision to include the

heart data was made at the last minute. The second and most important criticism is that they did not have their own controls. Instead they compared their shock data with the control data that was in the literature at the time.

The toxic cardiovascular actions of K^+ have usually been studied by injection of KCl solutions. The reactions consist of hyperpnea, slowing of the heart, decrease in systolic discharge, and fall in blood pressure. Muscular weakness, loss of reflexes, and inhibition of central nervous functions also occur. Excess potassium in small concentrations affects the rhythm and conductivity of the heart before its dynamic functions are altered. This is manifested by changes in the S-T segment and T wave of electrocardiograms and later by alterations in QRS complexes. In isolated heart small additions of potassium to the perfusion fluid increase and somewhat larger concentrations decrease excitability and contractility.

Winkler et al, (1938) found that concentrations of 5 - 7 mEq/L are required to produce the earliest evidence of toxic actions in electrocardiograms. Cardiac arrest occurs at concentrations of 14 to 16 mEq/L. Decline of arterial pressure does not become evident until serum potassium increases to approximately 10 mEq/L (Winkler and Hoff, 1943). The toxic effects of potassium may be accentuated by hyponatremia, hypocalcemia, or acidosis, all of which may be seen in shock. Millican (1960) raised the question whether potassium may

adversely affect organs other than the heart. Also, the participation of magnesium in the eventual outcome of shock has been completely overlooked. This ion is consistently and significantly elevated in irreversible hemorrhagic shock.

CHAPTER II

MATERIALS AND METHODS

A. ANESTHESIA AND SURGERY

Fifty-eight mongrel dogs that appeared in good health were employed in this study. The dogs were anesthetized with the intravenous administration of sodium pentobarbital (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 continuously with a mercury manometer. The left femoral artery and vein were isolated and cannulated for bleeding and for reinfusion of blood respectively. To render the blood incoagulable, an initial dose of 5 mg/kg of heparin (sodium salt) was administered by vein. One half of the initial dose was administered every hour during the course of the experiment.

The dogs were divided into three groups. The first group consisted of 25 dogs which served as controls. The second group numbered 16 dogs that were subjected to irreversible hemorrhagic shock. A third group, comprised of 17 dogs was subjected to the same shock procedure for group 2 dogs, however, these animals received 2.5 μg/kg/min of l-norepinephrine (total dose 500 μg of

Levophed base) following blood replacement. The rate of l-norepinephrine infusion maintained mean blood pressure at 100 to 120 mm Hg.

The principle of inducing shock was essentially that of Wiggers (1950). Mean blood pressure was lowered by rapid arterial bleeding to a level of 40 mm Hg. This pressure was maintained for 4 hours either by withdrawing or transfusing small quantities of blood. The hypotensive period was followed by complete reinfusion of the blood remaining in the reservoir. After a transitory pressor effect of the transfusion, eighty-five per cent of the animals demonstrated a gradual decline in blood pressure. When postinfusion blood pressure declined to 60 - 50 mm Hg, the animal was placed on artificial respiration, the chest was opened in the 5th interspace, and approximately 1 - 3 gm samples of left ventricular muscle were excised from the apex of the beating heart. After the epicardial fat was removed, the tissue was immediately washed in triple-distilled water and placed in a canister containing liquid nitrogen. The tissue which was not immediately analyzed was wrapped tightly in aluminum foil and stored at -20° C.

B. CHEMICAL DETERMINATIONS

Samples of left ventricular muscle were analyzed in all dogs (controls, shock, and shock plus l-norepinephrine) for Na^{+}, K^{+}, Mg^{++}, Ca^{++}, Cl^{-} and

water content. Control arterial blood samples were obtained from the femoral artery of all dogs, and a second blood sample was taken from dogs in groups 2 and 3 just prior to opening the chest. Plasma obtained from these blood samples were analyzed for Na^{+}, K^{+}, Mg^{++}, Ca^{++}, Cl^{-} and water content.

The procedure for determining water content of cardiac muscle was as follows: one gram samples of fresh tissue were weighed after they had been dissected free of visible fat and blotted on filter paper to remove surface blood. The samples were then dried to constant weight (48 hrs) at 60 - 65° C. Percentage fat-free dry weight was measured by reweighing the dried sample after double extraction with diethyl ether according to the method of Lowry and Hastings (1942). For the determination of sodium, potassium, magnesium and chloride, 1 - 2 gms carefully weighed samples of minced frozen tissue were homogenized for 10 min in a Potter-Elvejhem tissue grinder containing 5 ml of triple distelled water. The tissue dilution was 1:15, with the additional water used in the rinse (3 rinses). These homogenates were handled according to the following schema:

Scheme for Processing Homogenates of Frozen Heart Muscle

Na^+, K^+, Cl^-

5 ml tissue homogenate + 5 ml 1.5 N HNO_3

Centrifuge for 10 min. at 1200 g for supernatant fraction

Cl^-
1 ml supernatant titrated directly for chloride determination

K^+
1 ml supernatant + 2.5 ml 30 mEq/100 ml lithium sulfate diluted to 25 ml with water for potassium determination

Na^+
3 ml supernatant + 2.5 ml 30 mEq/100 ml lithium sulfate diluted to 25 ml with water for sodium determination

Mg^{++}

5 ml tissue homogenate + 5 ml water

Mg^{++}
3 ml tissue homogenate + 7 ml 5% trichloroacetic acid. Let stand at least 30 min and filter through dense textured double acid washed filter paper. Magnesium determined on filtrate by colorimetry.

Chloride was measured by an argento-amperometric titration procedure based on the method of Carr (1951), with an Aminco-Cotlive chloridometer. Sodium and potassium determinations were made on a Process and Instruments (Model 1B) flame photometer, using lithium as an internal standard. The use of the internal standard allows for greater stability and, therefore, greater accuracy in analysis can be achieved by adding lithium to the solution being analyzed. The added lithium is introduced so as to always yield the same concentration in every

solution being analyzed. The light output from the flame is collected by two optical systems, one provided with a lithium filter, the other with a sodium (or potassium) filter. These two beams are allowed to fall on two separate barrier layer photocells (the Li^+ cell and the Na^+- K^+ cell). The output of the Li^+ cell is then balanced against the output of the Na^+ (K^+) cell using the galvanometer as a null point meter. In this way, any factors causing fluctuation in flame intensity or photocell output other than directly due to the concentration of the Na^+, K^+, Li^+ in the flame will affect both photocells almost equally. Since only the ratio of the outputs is measured, these fluctuations tend to cancel or balance out. This instrument has a reproducibility of 1.0 per cent and is linear over the range of 0 to 100 microequivalents per 100 cc of solution. Magnesium was determined by a modification of the Titan yellow colorimeter methods (Garner, 1946; Orange and Rhein, 1951).

Calcium was determined directly from the deproteinized solution of the tissue with 10% trichloroacetic acid according to the micro-complexometric method of Mori (1959). This method employs EDTA as the titrant and calcein as an indicator (Gilbert and Mc Gann, 1958). The method is based on the metal chelating property of EDTA, and utilizes the fact that above a pH of 12, EDTA chelates calcium more easily than it does magnesium. Therefore, in adding EDTA to a solution of magnesium and calcium, (plus any other cations equally

well or more easily chelated by EDTA than calcium), a calcium ion indicator changes color at the precise point at which only magnesium remains unchelated, if the pH is greater than 12.

Samples of plasma were analyzed for concentration of water, chloride, sodium, potassium and magnesium by essentially the same methods as used for tissue analysis. Plasma calcium was determined by the method of Kovacs and Tarnoky (1961). Control experiments were run to determine the affects of heparin and sodium pentobarbital on plasma electrolytes. These were found not to be significantly altered by the procedure. In addition, recoveries from adding electrolytes to muscle and plasma samples, prior to extraction, averaged 97 per cent.

C. CARDIODYNAMIC STUDIES

Another group consisting of 6 dogs was employed in a study of the dynamics of cardiac function in irreversible hemorrhagic shock. This study was undertaken in collaboration with Joseph Traxler, a sophomore medical and graduate student in the Department of Physiology. The shock procedure was essentially the same as previously described, except that the chests of these animals were opened for cardiac catheterization. The dogs were placed on their right sides and ribs 1 through 5 were cut approximately 1 cm from the sternum and

retracted. This procedure made possible the catheterization of all four chambers of the heart. The ventricles were catheterized with rigid No. 8 radiopaque catheters, 5 - 6 cm long connected to 20 gauge needles. Polyethylene tubing (I. D. 0. 034") connected to 18 gauge needles were used in atrial catheterization. The cannulae were in turn connected to Statham P23AC pressure transducers. The distance between the opening of the superior vena cava to the right atrium to the center of the transducer dome was carefully measured and corrections made for this column of fluid. Clotting was prevented by the use of heparinized saline in the catheters and frequent flushing of the catheters was performed during the course of the experiment. Pressures were recorded prior to hemorrhage and at 30 min intervals throughout the oligemic and postinfusion stages of hemorrhagic shock. Cardiac pressure pulses were recorded with a Model 5 Grass polygraph. Paper speeds of 25 mm/sec and 2. 5 mm/sec were employed for all recording periods. Mean arterial blood pressure was continuously monitored on a mercury monometer connected to the left common carotid artery.

D. PRESENTATION OF DATA

The most acceptable practice of reporting the results of tissue analysis is in terms of a stable reference base. The tissue constituent chosen as a base should be unaffected by such variable tissue substrates as water, fat and

collagen materials. Fat-free non-collagen dry tissue (FFNCDT) fulfills most of the requirements for a stable reference base. An almost equally stable reference base for most purposes is the concept of fat-free dry tissue (FFDT) which has been employed in this work since cardiac muscle is practically devoid of collagens. In addition, normal heart muscle removed acutely is not expected to have increases in collagens in contrast to chronically injured muscle. Expression of data per unit weight of dry tissue is especially important when shifts in water occur as could be expected in a state of hemorrhagic shock. Attempts to compare normal muscle with edematous muscle on the basis of fresh tissue weight may have given misleading or meaningless results.

Derivations of extracellular and intracellular concentrations of electrolytes and water were attempted only on those dogs where both chloride and potassium were determined in cardiac muscle and plasma. In making these calculations it was assumed that electrolytes were distributed between plasma water and interstitial water according to a Gibbs-Donnan equilibrium. A Donnan factor for monovalent ions of 0.98 for the myocardium was used on the basis of the finding of Drinker et al (1940) of a protein concentration of 4% in cardiac lymph. Based on averages of experimental values cited by Manery (1954), ratios of 0.739 and 0.894 for myocardial calcium and magnesium were used respectively. Interstitial water was derived by assuming that sodium is largely restricted to

the extracellular compartment. As a result, potassium and chloride are distributed between intracellular and interstitial water in such a way that intracellular chloride concentration closely approximates the concentrations of potassium in interstitial water (Robertson and Peyser, 1951).

On the basis of these assumptions the following relationships were expressed for cardiac muscle:

$$[K]_E = [K]_P \times 0.98 \quad (1)$$

$$[Cl]_E = [Cl]_P / 0.98 \quad (2)$$

$$[Ca]_E = (\sqrt{[Ca]_P} \times 0.739)^2 \quad (3)$$

$$[Mg]_E = (\sqrt{[Mg]_P} \times 0.894)^2 \quad (4)$$

$$(H_2O)_E = \frac{1000\,(Cl)_T - [K]_E\,(H_2O)_T}{[Cl]_E - [K]_E} \quad (5)$$

$$(H_2O)_I = (H_2O)_T - (H_2O)_E \quad (6)$$

$$(K)_I = \frac{(K)_T [K]_E \times (H_2O)_E}{1000} \quad (7)$$

$$[K]_I = \frac{1000 (K)_I}{(H_2O)_I} \quad (8)$$

Brackets in these equations refer to concentration in milliequivalents per kilogrm of water, parentheses refer to milliequivalents (grams of water) per 100 gm FFDT. The subscripts E, I, T, and P refer to extracellular phase, intracellular phase, total tissue, and plasma respectively.

The membrane potentials of the heart muscle were calculated by the Nernst equation:

$$E_m = \frac{RT}{F} \ln \frac{[K]_I}{[K]_E}$$

E_m is the potential difference between the outside and inside of the muscle fiber, R is the gas constant (8.314 joules/degree), T the absolute temperature (273° A), and F is in faradays (96,500 coulombs).

CHAPTER III

EXPERIMENTAL RESULTS

Following the reinfusion of the total bled volume, eighty-five per cent of the dogs (28 out of 33) showed the progressive decline in arterial blood pressure which is characteristic of irreversible hemorrhagic shock. Those animals receiving l-norepinephrine, after postinfusion blood pressure had declined to 60 mm Hg, maintained a normal pressure during the infusion. However, with the completion of the infusion there was a precipitous fall in blood pressure to 60 - 50 mm Hg.

A. WATER AND ELECTROLYTES

The myocardial concentrations of water and electrolytes in the control dogs are in general agreement with those reported by other investigators using fat-free dry myocardium as the reference base (Benson, et al, 1956). In calculating the intracellular and extracellular volumes, it was assumed that extracellular K^+ closely approximates the concentration of Cl^- in the intracellular compartment. Therefore, these quantities differ by approximately 10 per cent

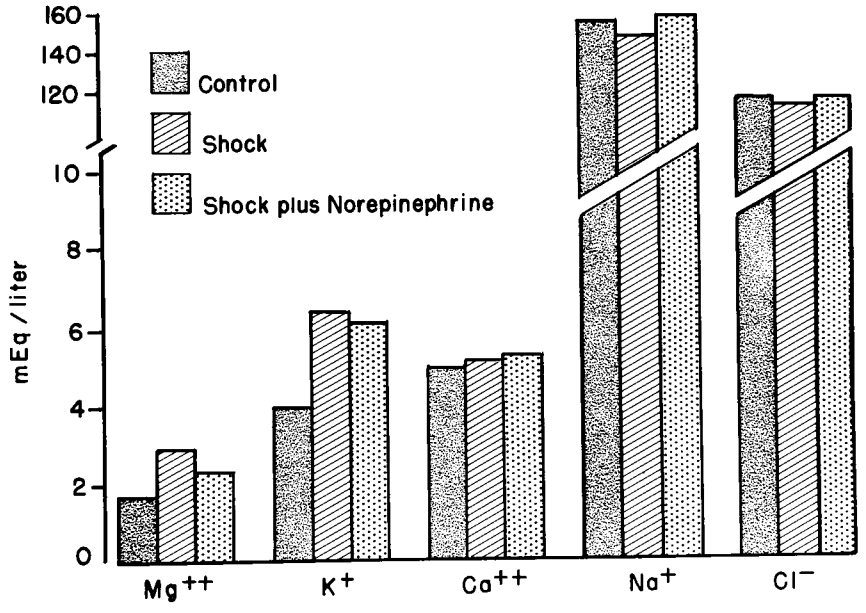
from results reported by Lowry and Hastings (1942). These authors derived intracellular and extracellular water on the basis of Cl^- being restricted purely to the extracellular compartment.

1. Plasma Water and Electrolytes

Figure 1 compares the changes that occurred in plasma electrolyte concentrations of 25 normal dogs to 16 dogs in irreversible hemorrhagic shock. The alterations of plasma electrolytes were similar to those reported by other investigators (Gregerson, 1946; Root, et al, 1947). Plasma potassium and magnesium concentrations were significantly elevated in hemorrhagic shock. Control levels that averaged 4.13 and 1.55 mEq/L, increased to 6.65 mEq/L ($P < 0.001$) and 2.84 mEq/L ($P < 0.001$) respectively. Tables I and V show that the plasma potassium and magnesium levels were elevated in all animals subjected to the procedure of hemorrhagic shock as compared to the average control. It is difficult to assess the contribution of hemolyzed red cells to the elevation of plasma magnesium since the occurrence of hemolysis in blood samples was minimal. It has been suggested that the calcium and magnesium gradients across cell membranes are metabolically dependent; a situation similar to sodium and potassium. In normal cells, magnesium is the primary intracellular divalent cation, whereas calcium is confined principally to the extracellular compartment. However,

FIGURE 1

Plasma Electrolyte Concentrations in Control Dogs, Dogs in Hemorrhagic Shock and Shocked Dogs Receiving l-norepinephrine Infusions



Upper Part of Scale Refers Only to Na^+ and Cl^- Concentrations

under conditions of generalized tissue hypoxia as it can be expected to occur in shock, cells might be expected to lose magnesium and gain calcium because of increases in membrane permeability. Manery (1954) has made the observation that the cellular magnesium content is not easily altered; it remained unchanged even though plasma magnesium fell to half of the normal value. Therefore, the source of elevated plasma magnesium under the conditions of the experiments reported cannot be ascertained. In dogs the plasma potassium alterations are more easily explained. Since the concentration of potassium in the dogs red cells is small, hemolysis does not become an important factor to consider. Under conditions of generalized tissue hypoxia, as it occurs in hemorrhagic shock, plasma potassium concentration may rise as a result of the breakdown of tissue protein and glycogen (Manery, 1954; Fuhrman, 1960). As potassium leaves the cells, sodium and hydrogen enter, and possibly also basic amino acids, e.g., lysine (Fuhrman, 1960).

Plasma sodium, chloride and water content showed a slight decrease, while calcium in plasma was slightly elevated. The analytical results for plasma sodium in these two groups of dogs are presented in Table III, chloride in Table IX, water in Table XI, and calcium in Table VII. The tabulated data show that the levels of sodium (av. 147.59 mEq/L), chloride (av. 113.62 mEq/L), calcium (av. 5.05 mEq/L), and water content (av. 913 gm/L) in the shocked animals do not differ significantly from their respective control groups.

The infusion of norepinephrine in the shocked animals did not correct the altered plasma electrolyte pattern (fig. 1). Magnesium levels were slightly reduced (2.84 to 2.27 mEq/L, $P < 0.4$). Likewise, potassium levels also showed a slight reduction (6.65 to 6.06 mEq/L, $P < 0.20$). The reduction for both cations in the shocked animal receiving norepinephrine did not differ significantly from the shock group(Tables II and VI). Sodium (156.50 mEq/L), chloride (116.27 mEq/L) and water content (919 gm/L) returned to near control values, while calcium (5.25 mEq/L) exhibited a further slight increase. Data obtained from plasma sodium analyses are presented in Table IV, those for chloride in Table X, water, Table XII, and calcium, Table VIII.

2. Left Ventricular Water and Electrolytes

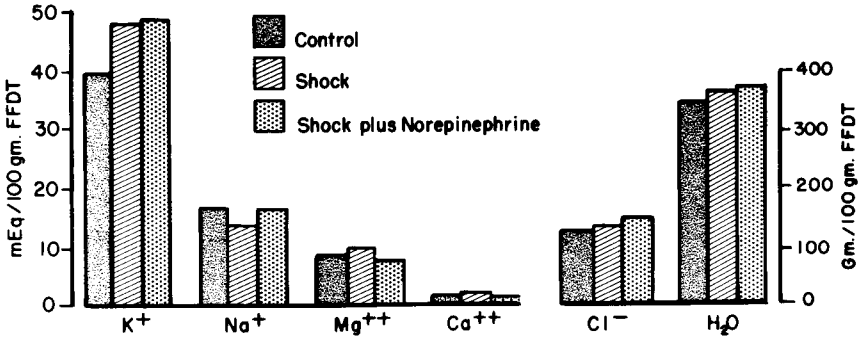
The data obtained from the analyses of cardiac muscle are expressed on the basis of fat-free dry tissue. Expression of data per unit weight of dry tissue is particularly important when shifts in water can be expected as in states of shock. In making the correction for fat it was assumed that neutral fat was an inactive diluent of cardiac muscle.

The summary of data in figure 2 shows that the concentration of potassium in Group 2 was significantly elevated. When levels of potassium in the myocardium of the control and shocked animals were compared, the difference

FIGURE 2

Water and Electrolyte Content in

Left Ventricular Muscle of the Three Experimental Groups



Scale to the left of diagram refers to left ventricular electrolytes expressed in mEq/100 gm of fat-free dry tissue (FFDT). The scale to the right refers only to left ventricular water content in grams per 100 gm fat-free dry tissue (FFDT).

between the two averages was statistically significant ($P < 0.001$). That the increase in myocardial potassium concentration is real and does not represent a shift in water, is evident from the insignificant change in the water content of cardiac muscle given to the right of the figure. Table I shows that the increase in total tissue potassium in left ventricular muscle, of the shocked animal, represents an increase in the concentration of this ion solely in the interstitial $[K]_E$ compartment. On the other hand, the change in intracellular $[K]_I$ was insignificant ($P < 0.40$) as compared to the control intracellular $[K]_I$. The elevated interstitial potassium concentration may serve as a basis for the electrocardiographic changes observed by Wiggers (1930) in dogs subjected to hemorrhagic shock.

Associated with the increases in muscle potassium were decreases in the sodium content of cardiac muscle (fig. 2). There was a reduction in the sodium concentration of both the interstitial $[Na]_E$ and intracellular $[Na]_I$ compartment (Table III). However, the changes in interstitial sodium were quantitatively greater than the changes in intracellular sodium. These changes in the sodium and potassium concentrations confirm the general contention that there is an inverse relationship between the movement of these two cations across the cell membrane.

The observed alterations in the sodium and potassium content of cardiac

muscle are contrary to what one would expect to find if there were a severe degree of myocardial hypoxia in hemorrhagic shock. The proponents of the "myocardial depression" theory of irreversibility believe the loss of myocardial propulsive power to be due to an inadequacy of coronary blood flow which in turn leads to hypoxia of cardiac muscle (Edwards, et al, 1954; Sarnoff, et al, 1954; Wiggers, 1945, 1947). Russell et al (1961) have shown that ischemic cardiac muscle loses potassium and gains sodium and water. Similar results have been reported by Benson et al (1956) for hearts in congestive failure. Since the myocardium of the animals in hemorrhagic shock exhibited a net gain in muscle potassium, the suggestion that myocardial hypoxia precipitates the irreversible state seems unjustifiable. Figure 2 includes average values of magnesium (8.55 mEq/100 gm), calcium (1.12 mEq/100 gm), chloride (12.4 mEq/100 gm), and water (344 gm/100 gm). These parameters all show a tendency to increase in the myocardium of the shocked animals (Group 2). However, as can be seen from Tables V, VII, IX, and XI, these changes were not significantly different from the control group of dogs.

The infusion of norepinephrine following circulatory failure had no detectable influence on the total potassium concentration in cardiac muscle. On the other hand, sodium (16.2 mEq/100 gm, FFDT), magnesium (7.58 mEq/100 gm, FFDT), and calcium (1.01 mEq/100 gm, FFDT) returned to near control levels.

Chloride (14.9 mEq/100 gm, FFDT), and water 373 gm/100 gm, FFDT) showed a further slight increase following norepinephrine infusion. These effects of norepinephrine on the electrolyte balance of cardiac muscle do not lend themselves to an easy explanation. Bulbring (1960) has suggested that the effect of norepinephrine on the movement of electrolytes is secondary to its effects on energy metabolism. There is, however, the possibility that norepinephrine has a direct effect on membrane permeability. The proof of this concept must await further experimental evidence.

B. DERIVED INTERSTITIAL AND INTRACELLULAR COMPARTMENTS

In order to ascertain what proportion of water and/or electrolytes entered the cells and whether there had been a change in the concentrations of electrolytes in the cell water, it was necessary to assume some basis for calculating the space and its composition. Benson et al (1956) makes the assumption that electrolytes in cardiac muscle are distributed between serum water and interstitial water according to a Gibbs-Donnan equilibrium. The Donnan factors for monovalent and divalent ions for the myocardium were based on the finding of a protein concentration of 4 per cent in cardiac lymph. It is further assumed that sodium is generally restricted to the extracellular phase and that as a result, potassium and chloride are distributed between intracellular and interstitial

water in such a way that intracellular chloride closely approximates the concentration of potassium in the interstitial water (Robertson and Peyser, 1951).

With determination of the interstitial volume of water, it was possible to calculate other pertinent data. The following values were calculated only for those animals on which chloride determinations were made; the concentration of interstitial and cellular water associated with 100 gm of dry muscle fibers and the concentration of electrolytes in a kilogram of cellular H_2O. The derived interstitial and intracellular spaces and the concentrations of potassium therein are shown in figure 3. It should be noted that the volume of intracellular (275 gm/100 gm, FFDT) and interstitial (83 gm/100 gm, FFDT) compartments in the left ventricle of the shocked dogs did not differ significantly from the controls. However, when norepinephrine (2.5 μg/kg/min) was administered in hemorrhagic shock, the interstitial volume increased 87 to 110 gm/100 gm, FFDT) while the intracellular space remained unchanged.

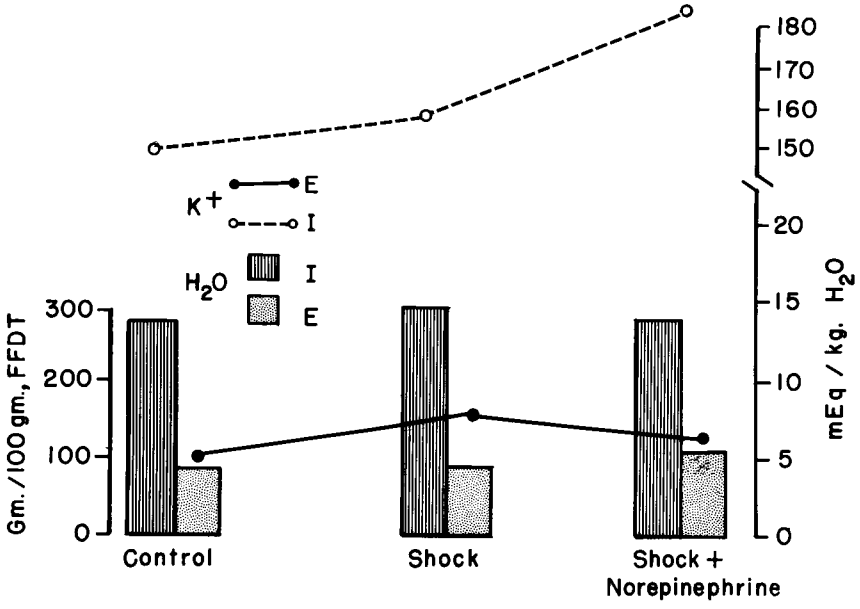
The scale on the right of the diagram (figure 3) represents the concentration of potassium in mEq/kg of water. The broken line at the top represents intracellular potassium. The lower solid line represents interstitial potassium. The most striking and consistent change was observed in the interstitial potassium concentration which increased in all shock experiments. The intracellular potassium concentration in the shock group of dogs did not differ from the controls (av. 159 and 151 mEq/kg of water, respectively).

When norepinephrine was administered to dogs in hemorrhagic shock following the reinfusion of the bled volume, interstitial potassium concentration was reduced from 7.52 to 6.23 mEq/kg of water. Concurrent with the reduction in interstitial potassium, the concentration of potassium in the intracellular compartment was elevated from 159 mEq to 183 mEq/kg of cellular water. This diagram has several significant features which are as follows: (1) the fact that the volume of the interstitial compartment in the shocked animals did not change, coupled with the significant increase in interstitial potassium, would suggest that the excess potassium ions now occupy the space that was once occupied by sodium. As given in figure 1 the increase in total tissue potassium (21 per cent) was quantitatively the same as the reduction in total tissue sodium (20 per cent). The slight discrepancy between the two changes can be accounted for by the slight increases in the concentrations of the divalent ions, magnesium and calcium. (2) Norepinephrine treatment of the shocked animals seemed to exert a stablizing influence on the cardiac cell by increasing the intracellular potassium concentration. This observation contrasts with that of Robertson and Peyser (1951), who found a reduction in the intracellular potassium concentration of cardiac muscle, following massive infusions (500 μg/kg) of epinephrine in the normal cats.

Although the role of epinephrine in electrolyte and water metabolism is

FIGURE 3

Interstitial and Intracellular Volumes and Interstitial and Intracellular Potassium Concentrations in Left Ventricular Muscle of Control, Shock and Shock Animals Receiving l-norepinephrine Infusions



The scale on the left refers only to the bar graph. The units are grams of intracellular (H_2O ▥$_I$) and interstitial (H_2O □$_E$) water per 100 gm fat-free dry tissue (FFDT). The scale to the right represents the concentration of K^+ in mEq/kg of water. Broken line refers to intracellular K^+ and the solid line indicates interstitial K^+.

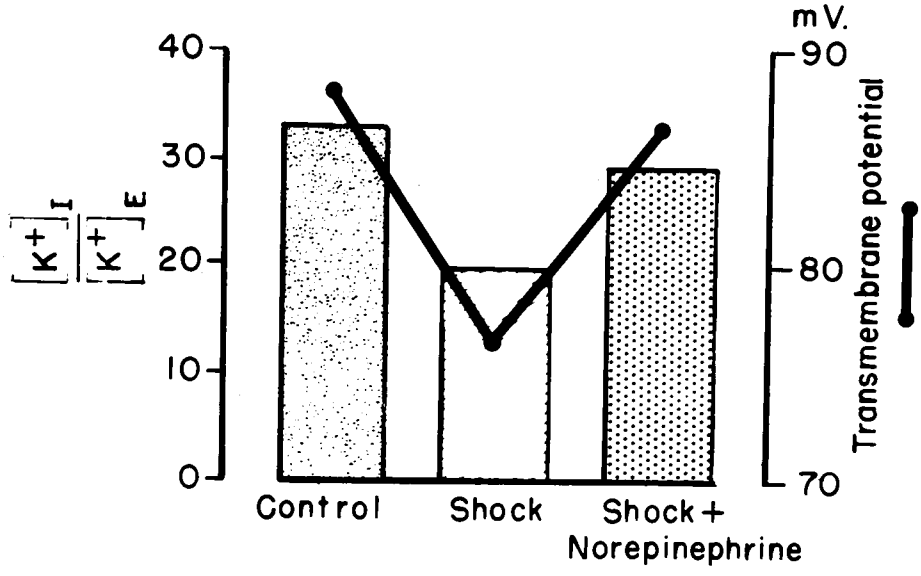
overshadowed by that of the hormones of the adrenal cortex, observations have been reported which indicate that the medullary hormones are intimately involved. Fenn (1940) has reviewed investigation of the loss of potassium from the liver perfused with epinephrine, an observation which was originally reported by D'Silva (1936). Hebb and Nimmo-Smith (1946) reported a similar loss of potassium from lungs perfused with epinephrine. On the other hand, epinephrine has been reported to increase the cellular potassium concentrations of muscle (Fenn, 1940). Similar results have been reported for the effects of norepinephrine on the electrolyte balance of cardiac muscle (Ellis, 1959; Bulbring, 1960).

C. CALCULATED MEMBRANE POTENTIAL OF THE LEFT VENTRICLE

The calculated average membrane potential for left ventricular muscle are given in figure 4. The mean value of 88.2 $\pm$ 0.94 mV, in the control dogs, compares well with the experimentally determined value for resting potential of single beating dog and cat ventricular fibers, measured with microelectrodes by Hoffman and Suckling (1952). Figure 4 also includes the ratios of intracellular to interstitial potassium for the three groups of dogs. This parameter is represented by the bar graph, and the scale to the right of the figure. The ratio of intracellular/interstitial potassium showed a profound reduction in the shocked animals, which returned to near control level following the administration of norepinephrine.

FIGURE 4

Intracellular/Interstitial Potassium Ratios and Calculated Transmembrane Potentials of Left Ventricular Muscle in the Three Groups of Dogs



The scale on the left is a plot of the ratio of intracellular to interstitial K^+ (represented by the bar graph). The scale to the right refers to the heavy black line which is a plot of the resting transmembrane potential of the left ventricle.

The calculated ventricular membrane potential decreased from 88.2 ± 0.94 mV in the control group to 77.2 ± 1.87 mV ($P < 0.001$) in hemorrhagic shock. The lowered myocardial membrane potential could contribute to the increased excitability that is often observed in late hemorrhagic shock. When norepinephrine was administered to the shocked animals, the calculated resting membrane potential returned to 85.9 ± 2.54 mV, or near the control level.

The derived membrane potential for each animal, where sufficient information (intracellular and interstitial potassium concentrations) was available for the calculations, is presented in Table XIII. Only dog No. 55 of the shocked group had a calculated ventricular membrane potential that was close to the average control value. The other seven animals in this group had myocardial membrane potentials that were considerably less than any of the dogs in the control series. With the exception of dogs 47, 48, and 54, the myocardial membrane potentials in the shocked dogs that received norepinephrine infusions were equal to those in the control group.

On the basis of these data it would appear that a lowered myocardial membrane potential and therefore greater excitability, is a rather constant finding in irreversible hemorrhagic shock. The infusion of norepinephrine, in small doses, may have a beneficial effect in that it promotes the entrance of potassium into the muscle cell (Bulbring, 1960). This may inhibit the production of ventricular ectopic beats by increasing the $[\ K\ ]_I / [\ K\ ]_E$ gradient.

The results of these electrolyte studies give no indication of myocardial failure, commonly referred to as "myocardial depression", as a precipitating factor in the production of irreversible hemorrhagic shock. This does not rule out the possibility that the contractile proteins are in some way deranged as a result of prolonged hemorrhagic hypotension. The evidence that the coronary circulation is seriously curtailed during the oligemic phase of hemorrhagic shock is rather convincing (Edwards, et al, 1954; Opdyke and Foreman, 1947; Sarnoff, et al, 1954). However, there are conflicting opinions as to what happens to coronary flow after reinfusion of whole blood (Opdyke and Foreman, 1947; Edwards, et al, 1954). It is possible that there is a derangement of some area of metabolism during the oligemic state which is not corrected by blood transfusion. The critical biochemical measurement most probably has not been made.

TABLE I

Potassium Concentration in Plasma and Cardiac Muscle of Control Dogs (Group 1) and Dogs in Irreversible Hemorrhagic Shock (Group 2)

Group No. 1					Group No. 2				
Exp. No.	$(K^+)_T$	$[K^+]_I$	$[K^+]_E$	$[K^+]_P$	Exp. No.	$(K^+)_T$	$[K^+]_I$	$[K^+]_E$	$[K^+]_P$
25	40.6	146	4.15	3.90	33	45.6	149	8.01	7.51
26	39.7	150	4.12	3.90	34	45.9	133	8.02	7.51
27	36.2	133	4.80	4.54	40	48.1	163	6.58	6.15
28	40.9	173	4.70	4.45	44	55.5	171	8.72	8.05
29	43.7	146	4.40	4.18	51	53.2	167	9.83	9.18
30	43.6	158	4.60	4.30	52	38.8	161	6.24	5.84
49	44.9	147	4.41	4.23	53	36.7	149	6.73	6.22
50	43.5	152	5.28	4.99	55	58.0	179	6.03	5.57
2	33.7	---	----	3.55	1	45.2	---	----	5.30
4	40.1	---	----	4.15	3	46.0	---	----	6.82
5	37.3	---	----	3.42	11	49.8	---	----	9.83
7	35.6	---	----	4.55	14	40.7	---	----	6.44
9	39.4	---	----	4.05	15	50.7	---	----	4.93
13	38.6	---	----	4.10	16	48.5	---	----	4.52
17	38.8	---	----	3.99	19	53.2	---	----	5.82
18	36.7	---	----	3.71					
Mean	39.6	151	4.56	4.13		47.7	159	7.52	6.65
S.E.	0.81	3.81	0.13	0.10		1.55	5.12	0.48	0.39

$(K^+)_T$ = mEq. potassium/100 gm fat-free dry muscle

$[K^+]_I$ = mEq. potassium/kg cell water

$[K^+]_E$ = mEq. potassium/kg interstitial water

$[K^+]_P$ = mEq. potassium/L plasma

TABLE II

Potassium Concentration in Plasma and Cardiac Muscle in Hemorrhagic Shock (Group 2) and Shocked Animals Receiving l-norepinephrine Infusion (Group 3)

	Group No. 2					Group No. 3			
Exp. No.	$(K^+)_T$	$[K^+]_I$	$[K^+]_E$	$[K^+]_P$	Exp. No.	$(K^+)_T$	$[K^+]_I$	$[K^+]_E$	$[K^+]_P$
33	45.6	149	8.01	7.51	35	49.8	181	4.70	4.40
34	45.9	133	8.02	7.51	37	45.4	---	----	6.85
40	48.1	163	6.58	6.15	42	41.6	170	5.70	4.77
44	55.5	171	8.72	8.05	46	47.8	193	5.02	4.68
51	53.2	167	9.83	9.18	47	58.0	195	7.98	7.45
52	38.8	161	6.24	5.84	48	54.0	190	8.20	7.70
53	36.7	149	6.73	6.22	54	51.8	187	7.78	7.32
55	58.0	179	6.03	5.57	56	44.2	---	----	7.20
1	45.2	---	----	5.30	57	56.7	181	6.50	6.12
3	46.0	---	----	6.82	58	50.0	170	4.56	4.23
11	49.8	---	----	9.83	6	41.7	---	----	6.35
14	40.7	---	----	6.44	8	47.2	---	----	6.18
15	50.7	---	----	4.93	20	44.3	---	----	5.97
16	48.5	---	----	4.52	21	42.3	---	----	4.13
19	53.2	---	----	5.82	22	44.7	---	----	5.17
					23	49.8	---	----	6.43
					24	50.0	---	----	7.45
Mean	47.7	159	7.52	6.65		48.2	183	6.23	6.06
S.E.	1.55	5.12	0.48	0.39		1.21	3.41	0.41	0.30

$(K^+)_T$ = mEq. potassium/100 gm fat-free dry muscle

$[K^+]_I$ = mEq. potassium/kg cell water

$[K^+]_E$ = mEq. potassium/kg interstitial water

$[K^+]_P$ = mEq. potassium/L plasma

TABLE III

Sodium Concentration in Plasma and Cardiac Muscle of Control Dogs (Group 1) and Dogs in Irreversible Hemorrhagic Shock (Group 2)

Group No. 1					Group No. 2				
Exp. No.	$(Na^+)_T$	$[Na^+]_I$	$[Na^+]_E$	$[Na^+]_P$	Exp. No.	$(Na^+)_T$	$[Na^+]_I$	$[Na^+]_E$	$[Na^+]_P$
25	17.0	0.47	175.76	165.00	33	13.8	19.60	164.40	154.00
26	17.2	10.80	181.97	171.75	34	13.7	8.24	162.66	152.20
27	14.8	8.77	182.95	171.75	40	13.6	5.65	158.60	148.25
28	16.1	0.00	178.96	168.00	44	14.0	1.25	170.36	157.50
29	20.9	16.16	190.35	180.25	51	10.8	0.00	157.17	146.75
30	17.8	16.06	191.80	180.25	52	10.6	0.00	172.15	161.08
49	15.3	0.00	160.58	150.75	53	11.6	0.00	168.95	155.70
50	13.1	2.87	146.21	138.15	55	16.6	2.50	161.60	149.40
2	20.2	-----	------	165.75	3	17.1	-----	------	139.75
4	19.4	-----	------	181.25	10	9.4	-----	------	139.25
5	19.7	-----	------	171.50	11	8.1	-----	------	134.00
7	14.6	-----	------	148.50	14	9.5	-----	------	117.40
9	13.9	-----	------	138.25	15	13.9	-----	------	146.75
12	16.8	-----	------	155.40	16	16.6	-----	------	152.93
13	12.9	-----	------	156.50	19	18.6	-----	------	158.83
17	12.9	-----	------	147.98					
18	15.7	-----	------	137.93					
Mean	16.4	6.89	176.06	160.53		13.2	4.02	164.49	147.59
S.E.	0.63	2.08	2.5.47	7.08		0.81	1.85	1.95	2.92

$(Na^+)_T$ = mEq. sodium/100 gm fat-free dry muscle

$[Na^+]_I$ = mEq. sodium/kg cell water

$[Na^+]_E$ = mEq. sodium/kg interstitial water

$[Na^+]_P$ = mEq. sodium/L plasma

TABLE IV

Sodium Concentration of Plasma and Cardiac Muscle in Hemorrhagic Shock (Group 2) and Shocked Animals Receiving l-norepinephrine Infusion (Group 3)

Group No. 2					Group No. 3				
Exp. No.	$(Na^+)_T$	$[Na^+]_I$	$[Na^+]_E$	$[Na^+]_P$	Exp. No.	$(Na^+)_T$	$[Na^+]_I$	$[Na^+]_E$	$[Na^+]_P$
33	13.8	14.60	164.40	154.00	35	18.8	0.73	169.27	159.25
34	13.7	8.24	162.66	152.20	37	19.7	6.82	159.61	153.75
40	13.6	5.65	158.60	148.25	42	14.6	0.00	171.13	161.00
44	14.0	1.25	170.36	157.50	46	22.7	0.00	214.68	200.00
51	10.8	0.00	157.17	146.75	47	17.0	8.47	188.50	176.00
52	10.6	0.00	172.15	161.08	48	12.4	0.00	159.78	150.00
53	11.6	0.00	168.95	155.70	54	13.9	0.00	148.15	139.23
55	16.6	2.50	161.60	149.40	56	11.2	0.00	150.13	138.33
3	17.1	-----	------	139.75	8	14.2	----	------	177.50
10	9.4	-----	------	139.25	20	20.3	----	------	143.53
11	8.1	-----	------	134.00	21	18.5	----	------	134.08
14	9.5	-----	------	117.40	22	15.5	----	------	146.23
15	13.9	-----	------	146.75	23	14.0	----	------	149.23
16	16.6	-----	------	152.93	24	13.7	----	------	162.80
19	18.6	-----	------	158.83					
Mean	13.2	4.02	164.49	147.59		16.2	2.00	170.18	156.50
S.E.	0.81	1.85	1.95	2.92		0.90	1.24		

$(Na^+)_T$ = mEq. sodium/100 gm fat-free dry muscle

$[Na^+]_I$ = mEq. sodium/kg cell water

$[Na^+]_E$ = mEq. sodium/kg interstitial water

$[Na^+]_P$ = mEq. sodium/L plasma

TABLE V

Magnesium Concentration in Plasma and Cardiac Muscle of Control Dogs (Group 1) and Dogs in Irreversible Hemorrhagic Shock (Group 2)

Group No. 1					Group No. 2				
Exp. No.	$(Mg^{++})_T$	$[Mg^{++}]_I$	$[Mg^{++}]_E$	$[Mg^{++}]_P$	Exp. No.	$(Mg^{++})_T$	$[Mg^{++}]_I$	$[Mg^{++}]_E$	$[Mg^{++}]_P$
36	6.70	27.80	1.25	1.45	33	11.8	38.40	2.34	2.70
38	7.80	29.42	1.54	1.77	34	12.0	34.82	2.40	2.75
39	8.10	28.98	1.30	1.48	40	7.4	24.76	1.96	2.26
41	8.50	30.95	1.45	1.68	44	7.3	22.09	2.28	2.60
43	8.70	33.12	1.12	1.30	51	6.3	19.68	1.80	2.07
45	5.70	23.60	1.04	1.20	52	7.2	28.78	3.80	4.38
2	7.80	-----	----	1.53	53	11.8	47.76	2.34	2.64
4	9.30	-----	----	1.78	55	7.5	22.37	3.42	3.88
5	11.40	-----	----	1.75	3	8.5	-----	----	2.67
7	8.30	-----	----	1.37	10	9.2	-----	----	3.00
9	8.40	-----	----	1.64	11	8.7	-----	----	2.83
12	7.30	-----	----	1.68	14	9.4	-----	----	2.13
13	10.20	-----	----	1.43	15	10.3	-----	----	4.00
17	10.60	-----	----	1.55	16	8.9	-----	----	2.43
18	9.40	-----	----	1.62	19	9.6	-----	----	2.22
Mean	8.55	29.98	1.29	1.55		9.06	29.83	2.54	2.84
S.E.	0.39	1.47	0.08	0.04		0.46	3.45	0.25	0.18

$(Mg^{++})_T$ = mEq. magnesium/100 gm fat-free dry muscle

$[Mg^{++}]_I$ = mEq. magnesium/kg cell water

$[Mg^{++}]_E$ = mEq. magnesium/kg interstitial water

$[Mg^{++}]_P$ = mEq. magnesium/L plasma

TABLE VI

Magnesium Concentration in Plasma and Cardiac Muscle in Hemorrhagic Shock (Group 2) and Shocked Animals Receiving l-norepinephrine Infusion (Group 3)

Group No. 2					Group No. 3				
Exp. No.	$(Mg^{++})_T$	$[Mg^{++}]_I$	$[Mg^{++}]_E$	$[Mg^{++}]_P$	Exp. No.	$(Mg^{++})_T$	$[Mg^{++}]_I$	$[Mg^{++}]_E$	$[Mg^{++}]_P$
33	11.8	38.40	2.34	2.70	35	7.40	26.34	1.90	2.18
34	12.0	34.82	2.40	2.75	37	8.10	23.59	1.72	2.02
40	7.4	24.76	1.96	2.26	42	6.30	25.61	1.69	1.94
44	7.3	22.09	2.28	2.60	46	6.10	24.34	1.44	1.64
51	6.3	19.68	1.80	2.07	47	7.90	26.34	1.93	2.20
52	7.2	28.78	3.80	4.38	48	5.30	18.28	1.74	2.01
53	11.8	47.76	2.34	2.64	54	12.30	44.10	2.43	2.80
55	7.4	22.37	3.42	3.88	56	6.80	22.40	2.82	3.19
3	8.5	-----	----	2.67	6	9.10	-----	----	2.67
10	9.2	-----	----	3.00	8	9.50	-----	----	2.34
11	8.7	-----	----	2.83	20	7.80	-----	----	2.13
14	9.4	-----	----	2.13	21	8.00	-----	----	2.33
15	10.3	-----	----	4.00	22	9.30	-----	----	2.20
16	8.9	-----	----	2.43	23	9.30	-----	----	2.27
19	9.6	-----	----	2.22	24	8.50	-----	----	2.17
Mean	9.06	29.83	2.54	2.84		7.58	26.38	1.96	2.27
S.E.	0.46	3.45	0.25	0.18		0.39			0.10

$(Mg^{++})_T$ = mEq. magnesium/100 gm fat-free dry muscle

$[Mg^{++}]_I$ = mEq. magnesium/kg cell water

$[Mg^{++}]_E$ = mEq. magnesium/kg interstitial water

$[Mg^{++}]_P$ = mEq. magnesium/L plasma

TABLE VII

Calcium Concentration in Plasma and Cardiac Muscle of Control Dogs (Group 1) and Dogs in Irreversible Hemorrhagic Shock (Group 2)

Group No. 1					Group No. 2				
Exp. No.	$(Ca^{++})_T$	$[Ca^{++}]_I$	$[Ca^{++}]_E$	$[Ca^{++}]_P$	Exp. No.	$(Ca^{++})_T$	$[Ca^{++}]_I$	$[Ca^{++}]_E$	$[Ca^{++}]_P$
29	1.41	4.01	3.17	5.40	33	1.32	3.75	3.10	5.20
30	1.19	3.58	2.96	5.00	34	1.42	3.53	3.35	5.60
31	1.18	3.55	3.17	5.40	40	1.01	2.71	2.99	5.00
32	0.99	2.78	2.92	5.00	44	1.17	2.91	3.03	5.00
36	1.16	3.88	2.72	4.60	51	1.05	2.59	2.99	5.00
38	1.04	2.82	2.82	4.80	52	0.98	2.83	2.95	5.00
39	1.24	----	----	4.20	53	1.54	4.98	2.76	4.60
41	1.00	2.66	2.72	4.60	55	1.25	2.97	3.03	5.00
43	0.74	1.81	2.76	4.60					
45	1.27	4.39	2.62	4.40					
Mean	1.12	3.28	2.87	4.80		1.22	3.28	3.03	5.05
S.E.	0.06	0.27	0.07	0.13		0.07	0.28	0.04	0.09

$(Ca^{++})_T$ = mEq. calcium/100 gm fat-free dry muscle

$[Ca^{++}]_I$ = mEq. calcium/kg cell water

$[Ca^{++}]_E$ = mEq. calcium/kg interstitial water

$[Ca^{++}]_P$ = mEq. calcium/L plasma

TABLE VIII

Calcium Concentration in Plasma and Cardiac Muscle in Hemorrhagic Shock (Group 2) and Shocked Animals Receiving l-norepinephrine Infusion (Group 3)

Exp. No.	$(Ca^{++})_T$	$[Ca^{++}]_I$	$[Ca^{++}]_E$	$[Ca^{++}]_P$	Exp. No.	$(Ca^{++})_T$	$[Ca^{++}]_I$	$[Ca^{++}]_E$	$[Ca^{++}]_P$
33	1.32	3.75	3.10	5.20	35	1.36	3.85	2.82	4.80
34	1.42	3.53	3.35	5.60	37	1.25	2.58	2.56	4.40
40	1.01	2.71	2.99	5.00	42	0.85	2.22	3.53	6.00
44	1.17	2.91	3.03	5.00	46	1.17	2.99	3.13	5.20
51	1.05	2.59	2.99	5.00	47	0.77	1.76	3.24	5.40
52	0.98	2.83	2.95	5.00	48	0.71	1.15	3.35	5.60
53	1.54	4.98	2.76	4.60	54	0.96	1.99	2.96	5.00
55	1.25	2.97	3.03	5.00	56	1.01	2.36	3.39	5.60
Mean	1.22	3.28	3.03	5.05		1.01	2.36	3.12	5.25
S.E.	0.07	0.28	0.04	0.09		0.08			0.18

$(Ca^{++})_T$ = mEq. calcium/100 gm fat-free dry muscle

$[Ca^{++}]_I$ = mEq. calcium/kg cell water

$[Ca^{++}]_E$ = mEq. calcium/kg interstitial water

$[Ca^{++}]_P$ = mEq. calcium/L plasma

TABLE IX

Chloride Concentration in Plasma and Cardiac Muscle of Control Dogs (Group 1) and Dogs in Irreversible Hemorrhagic Shock (Group 2)

Group No. 1				Group No. 2			
Exp. No.	$(Cl^-)_T$	$[Cl^-]_E$	$[Cl^-]_P$	Exp. No.	$(Cl^-)_T$	$[Cl^-]_E$	$[Cl^-]_P$
25	12.3	126.70	114.23	33	9.8	128.40	115.61
26	11.4	131.20	118.95	34	11.1	124.70	112.14
27	9.9	126.70	114.23	40	13.0	148.00	132.83
28	12.8	127.80	115.28	44	13.3	130.70	116.07
29	10.1	128.90	117.48	51	13.5	127.96	114.74
30	10.3	129.80	117.21	52	13.9	119.03	106.97
31	10.9	127.26	115.61	53	17.4	124.13	110.08
32	11.3	127.58	116.15	55	13.0	113.19	100.50
36	12.7	128.00	116.16				
38	15.7	128.36	116.10				
41	14.3	126.80	114.57				
43	14.9	134.69	121.04				
45	12.8	132.36	118.42				
49	15.2	128.20	115.54				
50	11.8	122.50	111.09				
Mean	12.4	128.46	116.14		13.4	127.01	113.62
S.E.	0.49		0.60		0.78		3.30

$(Cl^-)_T$ = mEq. chloride/100 gm fat-free dry muscle

$[Cl^-]_E$ = mEq. chloride/kg interstitial water

$[Cl^-]_P$ = mEq. chloride/L plasma

TABLE X

Chloride Concentration in Plasma and Cardiac Muscle in Hemorrhagic Shock (Group 2) and Shocked Animals Receiving l-norepinephrine Infusion (Group 3)

Group No. 2				Group No. 3			
Exp. No.	$(Cl^-)_T$	$[Cl^-]_E$	$[Cl^-]_P$	Exp. No.	$(Cl^-)_T$	$[Cl^-]_E$	$[Cl^-]_P$
33	9.8	128.40	115.61	35	16.7	140.20	126.70
34	11.1	124.70	112.14	37	18.9	128.00	117.50
40	13.0	148.00	132.83	42	13.8	139.70	126.80
44	13.3	130.70	116.07	46	18.8	126.20	112.92
51	13.5	127.96	114.74	47	11.8	127.30	114.23
52	13.9	119.03	106.97	48	18.0	135.00	121.73
53	17.4	124.13	110.08	54	19.2	120.52	108.78
55	13.0	113.19	100.50	56	13.3	118.83	105.15
				57	13.2	129.90	117.50
				58	16.4	125.40	111.87
				20	14.0	------	------
				21	14.1	------	------
				22	13.4	------	------
				23	10.8	------	------
				24	11.3	------	------
Mean	13.4	127.01	113.62		14.9	129.11	116.27
S.E.	0.78		3.30				

$(Cl^-)_T$ = mEq. chloride/100 gm fat-free dry muscle

$[Cl^-]_E$ = mEq. chloride/kg interstitial water

$[Cl^-]_P$ = mEq. chloride/L plasma

TABLE XI

Water Content of Plasma and Cardiac Muscle of Control Dogs (Group 1) and Dogs in Irreversible Hemorrhagic Shock (Group 2)

Group No. 1					Group No. 2				
Exp. No.	$(H_2O)_T$	$(H_2O)_I$	$(H_2O)_E$	$[H_2O]_P$	Exp. No.	$(H_2O)_T$	$(H_2O)_I$	$(H_2O)_E$	$[H_2O]_P$
25	372	276	96	920	33	361	304	57	918
26	341	262	79	925	34	407	340	67	917
27	337	269	68	920	40	367	292	75	916
28	326	234	92	920	44	400	320	80	906
29	365	297	68	928	51	393	313	80	915
30	344	274	70	921	52	341	237	104	917
49	410	302	108	920	53	365	241	124	905
50	367	283	84	926	55	418	320	98	906
2	312	---	--	925	1	339	---	--	---
4	331	---	--	925	3	374	---	--	---
5	324	---	--	925	10	329	---	--	---
7	356	---	--	926	11	352	---	--	---
9	333	---	--	937	14	315	---	--	---
12	320	---	--	921	15	348	---	--	---
13	329	---	--	---	16	326	---	--	---
17	331	---	--	---	19	355	---	--	---
18	342	---	--	---					
Mean	344	275	83	924		362	296	87	913
S.E.	5.86	7.52	5.20	1.24		7.54	13.3	7.7	2.03

$(H_2O)_T$ = gm water/100 gm fat-free dry muscle

$(H_2O)_I$ = gm intracellular water/100 gm fat-free dry muscle

$(H_2O)_E$ = gm interstitial water/100 gm fat-free dry muscle

$[H_2O]_P$ = gm water/kg plasma

TABLE XII

Water Content of Plasma and Cardiac Muscle in Hemorrhagic Shock (Group 2) and Shocked Animals Receiving l-norepinephrine Infusion (Group 3)

Exp. No.	$(H_2O)_T$	$(H_2O)_I$	$(H_2O)_E$	$[H_2O]_P$	Exp. No.	$(H_2O)_T$	$(H_2O)_I$	$(H_2O)_E$	$[H_2O]_P$
33	361	304	57	918	35	383	273	110	922
34	407	340	67	917	37	446	316	130	944
40	367	292	75	916	42	329	239	90	921
44	400	320	80	906	46	383	244	139	913
51	393	313	80	915	47	372	295	77	915
52	341	237	104	917	48	395	279	116	920
53	365	241	124	905	54	413	271	142	921
55	418	320	98	906	56	385	292	93	903
1	339	---	---	---	57	381	294	87	923
3	374	---	---	---	58	410	290	120	910
10	329	---	---	---	6	403	---	---	---
11	352	---	---	---	8	359	---	---	---
14	315	---	---	---	20	341	---	---	---
15	348	---	---	---	21	341	---	---	---
16	326	---	---	---	22	342	---	---	---
19	355	---	---	---	23	337	---	---	---
					24	327	---	---	---
Mean	362	296	87	913		373	279	110	919
S.E.	7.54	13.3	7.7	2.03					

$(H_2O)_T$ = gm water/100 gm fat-free dry muscle

$(H_2O)_I$ = gm intracellular water/100 gm fat-free dry muscle

$(H_2O)_E$ = gm interstitial water/100 gm fat-free dry muscle

$[H_2O]_P$ = gm water/kg plasma

TABLE XIII

Calculated Resting Membrane Potential (mV)

Exp. No.	Control	Exp. No.	Shock	Exp. No.	Shock and *NE
25	89.7	33	73.6	35	92.0
26	90.5	34	70.7	42	88.5
27	83.7	40	80.9	46	91.9
28	90.8	44	76.0	47	80.5
29	88.2	51	71.3	48	79.2
30	89.1	52	81.9	54	80.1
49	89.3	53	78.0	57	83.8
50	84.6	55	85.0	58	91.9
Mean	88.20		77.20		85.90
S.E.	0.94		1.84		2.02

* NE = Norepinephrine

	P values
Control vs. Shock	< 0.001
Shock vs. Shock and NE	< 0.01
Control vs. Shock and NE	< 0.40

D. CARDIODYNAMIC STUDIES

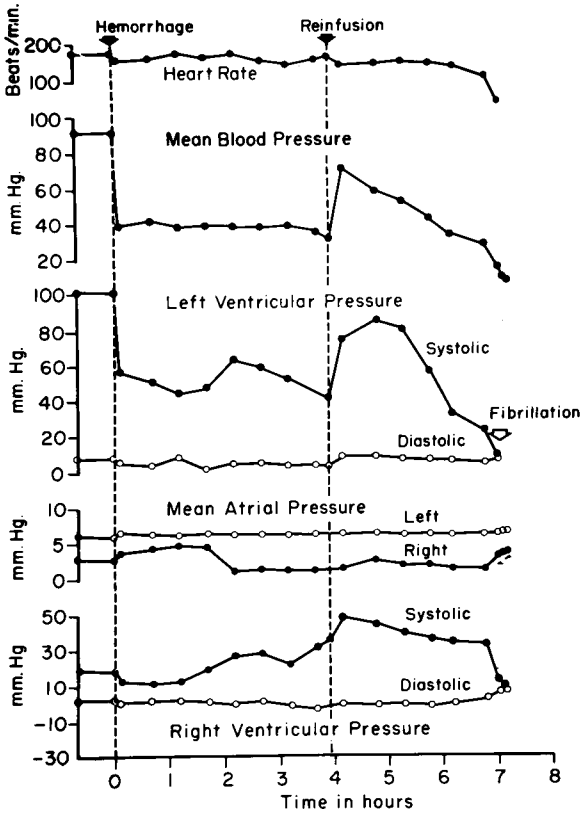
Six experiments were conducted for the purpose of confirming the conclusion on the absence of myocardial failure in the transition from reversible to irreversible hemorrhagic shock. Any evidence of cardiac failure associated with the production of an irreversible state should be reflected in the intracardiac pressure recordings. The data obtained from one experiment (Dog No. 6) is plotted in figure 5. A vertical analysis of this figure shows that immediately following a severe hemorrhage a reduction occurs in: (1) mean arterial blood pressure, (2) mean left and right atrial pressures, and (3) left and right ventricular systolic and diastolic pressures. These changes are obviously the result of a reduced venous return resulting from a decrease in circulating blood volume. Mean atrial pressures and ventricular end-diastolic pressures remained at a level below control, and at no point during the course of eligemia did pressures in these chambers rise above control. These observations indicate that there is no disparity between cardiac output and venous return. When the total bled volume was reinfused, mean arterial blood pressure and intracardiac pressures returned to near control levels. The infusion of whole blood was only of temporary benefit as was evident by the gradual and progressive decline in all pressures during the postinfusion period. The

systemic blood pressure continued to decline up to the point where ventricular fibrillation supervened. This terminal event lends support to the concept that the elevation in interstitial potassium could be an important factor to consider in the eventual outcome of irreversible hemorrhagic shock. It should also be noted from figure 5 that approximately 30 minutes before the animal succumbed to ventricular fibrillation there was a slight rise in mean atrial and ventricular end-diastolic pressures. However, this event occurred only after irreversibility had become evident. The results of this cardiodynamic study adds further evidence against the concept that "myocardial depression" is the precipitating factor responsible for the circulatory failure in prolonged hemorrhagic hypotension.

Figure 6 is a polygraph record of cardiac pressure pulses obtained from Dog No. 4. The oscillations in the left atrial pressure pulse represent an undamped system. However, since the interest of this investigation was in mean atrial pressure, this recording difficulty does not detract from the validity of the interpretations. These records were taken during the control period, in late oligemic shock (hypotension) and immediately after complete reinfusion of the total bled volume (postinfusion). Additional records were taken 45 minutes and 3 hours after reinfusion. Mean atrial pressures and ventricular end-diastolic pressures were considerably lower than control during the oligemic state (Table XIV). The mean arterial blood pressure at this time was 52 mm Hg. Reinfusion

FIGURE 5

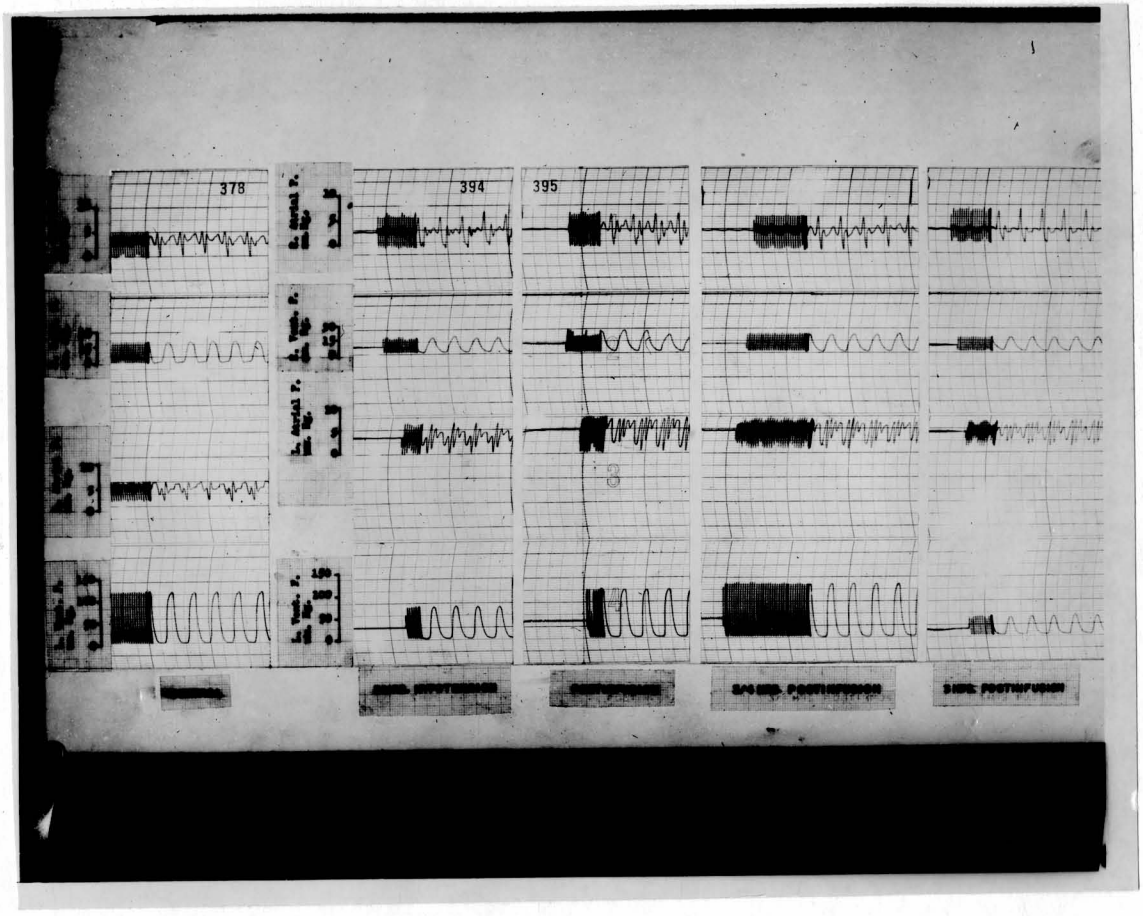
Heart Rate, Mean Blood Pressure, and Intracardiac Pressures Recorded During a Typical Hemorrhagic Shock Experiment



of whole blood caused an elevation in mean blood pressure and pressures recorded from the chambers of the heart. The pressure increases caused by the infusion of blood lasted for approximately 90 minutes. This animal replapsed into irreversibility as evidenced by the gradual decline in both mean arterial and intracardiac pressures. The 5th section of figure 6 was recorded 3 hours after the replacement of the total bled volume (3 hours postinfusion). It can be noted that even though the circulating blood volume is theoretically greater than normal, all cardiac pressures are considerably below normal. This animal died 20 minutes after this record was taken of what appeared to be cardiac arrest.

FIGURE 6

Polygraph Recording of a Typical Hemorrhagic Shock Experiment (Exp. No. 4) Recorded During the Control Period, After 3 hrs Hypotension, Immediately After Complete Reinfusion, 45 mins After Reinfusion and 3 hrs After Reinfusion of the Total Bled Volume



LEGEND FOR TABLE XIV

MBP	Mean Blood Pressure
LVSP	Left Ventricular Systolic Pressure
LVDP	Left Ventricular Diastolic Pressure
MLAP	Mean Left Atrial Pressure
MRAP	Mean Right Atrial Pressure
RVSP	Right Ventricular Systolic Pressure
RVDP	Right Ventricular Diastolic Pressure
HR	Heart Rate

TABLE XIV

Mean Blood Pressure, Heart Rate and Cardiac Chamber Pressures in Dogs in Irreversible Hemorrhagic Shock

Exp. No. 1

TIME (min)	MBP (mm Hg)	LVSP (mm Hg)	LVDP (mm Hg)	MLAP (mm Hg)	MRAP (mm Hg)	RVSP (mm Hg)	RVDP (mm Hg)	HR (beats/min)
0	90	94.0	2.6	3.80	2.79	12.3	1.2	138
55	40	61.0	0.0	1.50	2.20	19.1	2.3	128
85	40	60.0	0.0	1.50	1.74	20.0	0.0	126
115	40	61.5	0.0	1.00	2.25	20.3	2.4	132
160	40	70.0	0.0	1.00	2.30	20.2	2.4	120
180	53	69.0	0.0	2.30	2.30	25.0	0.0	132
210	53	70.5	0.0	1.30	2.74	25.0	0.9	126
243	45	67.5	3.0	1.27	5.70	20.3	0.0	132
274	57	81.0	0.0	1.80	5.70	15.4	1.1	114
280	---------- FIBRILLATION ----------							

TABLE XIV
continued

Exp. No. 2

TIME (min)	MBP (mm Hg)	LVSP (mm Hg)	LVDP (mm Hg)	MLAP (mm Hg)	MRAP (mm Hg)	RVSP (mm Hg)	RVDP (mm Hg)	HR (beats/min)
0	80	123.0	0.7	5.75	0.80	24.8	2.05	132
5	36	76.0	2.3	0.70	0.50	clot	clot	150
30	40	84.0	5.2	1.20	1.00	20.8	3.30	180
60	40	76.0	5.2	0.65	0.75	20.8	4.60	180
90	40	80.7	2.3	1.20	2.00	22.3	4.60	180
120	40	87.0	2.3	1.20	2.50	22.3	3.30	180
150	40	87.0	2.3	0.64	2.50	29.6	2.20	210
180	34	76.0	2.3	0.70	3.75	27.2	2.20	210
210	40	88.0	3.7	0.40	1.75	39.0	3.30	190
270	28	64.0	5.9	4.65	1.75	28.4	5.00	150
279	46	96.0	6.0	7.00	1.50	34.6	4.50	150
295	36	88.5	8.2	5.40	0.50	29.6	5.80	150
312	33	78.8	2.3	7.00	0.50	28.4	2.10	150
323	28	75.7	1.5	6.50	0.50	27.0	1.00	150
346	---------- FIBRILLATION ----------							

TABLE XIV
continued

Exp. No. 3

TIME (min)	MBP (mm Hg)	LVSP (mm Hg)	LVDP (mm Hg)	MLAP (mm Hg)	MRAP (mm Hg)	RVSP (mm Hg)	RVDP (mm Hg)	HR (beats/min)
0	92	135.1	5.10	3.35	3.05	30.3	1.35	210
4	40	58.1	-0.80	1.49	2.30	10.1	0.00	180
11	40	55.1	5.10	0.95	2.05	10.1	-1.15	186
39	43	57.1	5.10	1.45	2.55	16.3	1.35	180
69	40	61.1	-0.75	0.95	2.55	15.1	1.35	168
99	45	64.4	-6.60	0.68	3.30	20.1	0.10	168
129	40	64.9	-0.78	0.43	3.05	18.9	-1.15	168
158	47	60.1	-3.72	-0.10	3.30	22.6	-1.15	168
191	41	72.7	-3.60	-1.41	4.30	41.4	-6.15	186
219	39	108.4	2.16	-0.62	5.55	52.6	-4.90	202
234	34	81.6	-3.72	-0.62	5.05	33.9	-3.65	186
256	65	113.9	-11.76	-2.80	4.80	53.9	-1.15	234
289	---------- FIBRILLATION ----------							

TABLE XIV
continued

Exp. No. 4

TIME (min)	MBP (mm Hg)	LVSP (mm Hg)	LVDP (mm Hg)	MLAP (mm Hg)	MRAP (mm Hg)	RVSP (mm Hg)	RVDP (mm Hg)	HR (beats/min)
0	97	129.7	5.7	5.07	3.20	20.8	4.80	162
12	40	67.7	5.7	5.07	3.45	14.6	3.30	180
49	40	clot	clot	3.20	1.45	clot	clot	195
79	40	clot	clot	2.04	2.55	19.5	0.15	180
116	38	58.7	2.7	3.22	2.70	13.3	3.30	180
139	35	52.9	2.8	2.91	1.45	15.8	3.30	173
169	52	73.7	0.20	3.50	2.80	17.6	2.67	170
189	70	119.0	2.75	4.80	3.20	23.3	3.30	162
199	80	120.7	2.75	4.54	3.50	18.3	2.67	158
219	88	135.7	2.70	5.07	3.20	20.8	3.20	162
234	89	124.7	1.30	4.70	2.45	19.6	3.05	168
264	83	116.9	1.30	4.02	2.32	15.9	2.05	165
294	64	94.2	1.00	2.70	1.83	21.4	-3.20	168
299	60	76.7	2.70	3.50	3.20	23.3	2.00	168
324	50	77.3	-1.70	3.22	2.58	21.1	-0.40	168
354	48	73.7	-0.20	3.75	2.45	20.8	-0.45	174
384	47	64.7	1.00	3.48	2.70	23.3	-0.45	168
414	40	47.0	-0.20	2.48	2.83	24.5	0.80	168
428	30	19.0	14.6	5.60	2.45	15.8	3.90	165
433	---------- CARDIAC ARREST ----------							

TABLE XIV
continued

Exp. No. 6

TIME (min)	MBP (mm Hg)	LVSP (mm Hg)	LVDP (mm Hg)	MLAP (mm Hg)	MRAP (mm Hg)	RVSP (mm Hg)	RVDP (mm Hg)	HR (beats/min)
0	93	101.5	7.5	6.3	2.7	19.0	2.0	180
9	40	57.0	6.5	6.6	3.7	13.0	1.0	165
45	43	51.5	3.5	6.2	4.2	12.0	2.0	165
76	40	45.5	7.5	6.2	4.7	13.0	2.0	180
105	40	48.5	1.5	6.3	4.5	18.0	1.0	174
135	40	63.5	5.0	6.2	1.2	26.0	-1.0	180
165	40	59.5	5.0	6.2	1.2	28.0	1.0	165
195	41	54.0	3.5	6.2	1.2	22.0	-2.0	150
225	37	45.5	3.5	6.2	1.0	31.0	-3.1	165
255	73	75.0	9.0	6.4	2.2	48.0	-1.0	150
290	60	86.0	9.0	6.3	2.5	44.0	-2.0	155
320	54	80.5	6.5	6.2	1.9	39.0	-1.0	155
350	45	56.5	6.5	6.2	1.7	36.0	-1.0	150
375	35	31.5	6.5	6.2	1.2	24.0	-1.0	145
411	29	23.0	5.5	6.2	1.2	22.0	1.0	115
425	16	9.0	6.5	6.4	3.5	13.0	4.4	42
430	---------- CARDIAC ARREST ----------							

TABLE XIV
continued

Exp. No. 9

TIME (min)	MBP (mm Hg)	LVSP (mm Hg)	LVDP (mm Hg)	MLAP (mm Hg)	MRAP (mm Hg)	RVSP (mm Hg)	RVDP (mm Hg)	HR (beats/min)
0	120	124.1	3.2	6.8	2.5	21.9	3.20	210
7	40	59.1	3.2	6.8	2.0	13.2	2.90	180
37	41	42.6	1.7	6.7	1.5	13.2	2.90	198
67	39	41.6	1.7	6.7	1.6	13.2	1.30	208
97	40	48.6	0.0	6.7	1.5	15.6	1.60	200
127	40	47.5	-2.8	6.7	1.2	15.6	0.64	192
162	42	32.6	1.4	6.7	1.2	19.4	1.90	175
187	66	85.1	1.7	6.8	2.0	23.1	1.20	138
213	47	56.3	3.2	6.7	1.5	20.9	1.40	150
225	28	35.6	3.2	6.8	2.0	13.4	1.40	126
230	20	29.1	2.0	6.8	3.1	10.9	1.40	102
238	---------- FIBRILLATION ----------							

CHAPTER IV

DISCUSSION

The purpose of this investigation was to assess the role of myocardial depression (deterioration in myocardial expulsive power) as a factor responsible for the irreversible phase of hemorrhagic shock. The hypothesis that myocardial depression is the precipitating factor in initiating irreversibility was based primarily on the findings of Wiggers (1942, 1945, 1947). Dogs in experimental hemorrhagic shock were observed to exhibit an increase in right atrial pressure, a decrease in force of ventricular contraction and a decrease velocity of systolic ejection. These cardiodynamic findings were based on cardiac pressure and volume curves. Wiggers specified that although the evidence suggested that myocardial depression is a frequent and important complication it might not be a factor in all cases of shock. The severity of myocardial depression would be conditioned by the preshock condition of the myocardium and coronary blood vessels.

In the present study, it was decided that if myocardial depression were a significant contributor to the development of irreversibility, it would cause abnormal distribution of electrolytes in cardiac muscle. Benson et al (1956)

produced cardiac failure in dogs by pulmonary stenosis and tricuspid insufficiency. They found increases in intracellular sodium, chloride and water content of heart muscle. The changes in intracellular potassium, magnesium and calcium were insignificant. On the other hand, Russell et al (1961) produced myocardial ischemia by a two-stage ligation of the anterior descending coronary artery. The ischemic areas in the hearts of these dogs showed increases in sodium and water and a decrease in the potassium content. This shift in electrolyte pattern has been attributed to a failure of the "sodium pump" resulting from inadequate energy metabolism. Therefore, in myocardial failure or ischemia of cardiac muscle, movement of ions is according to their respective concentration gradients. Obviously ionic movement under these conditions represents a change in membrane permeability.

The results of the study reported here give no indication of myocardial membrane permeability changes as a result of hemorrhagic hypotension. Samples of left ventricular muscle taken after post infusion blood pressure had declined to 60 - 50 mm Hg showed an increase in total tissue potassium per unit fat-free dry weight. Concurrent with the increase in muscle potassium the sodium content was correspondingly reduced. Similarly, there was no significant cardiac edema. These findings render untenable the hypothesis that myocardial

failure precipitates the irreversible state of hemorrhagic shock. It should be pointed out that these muscle samples were taken only after circulatory failure had supervened, which was evident by the gradual and progressive decline in arterial blood pressure. This decline in postinfusion blood pressure indicated that these animals were refractory to the replacement of whole blood. If there were any significant degree of myocardial hypoxia at this time, the water and electrolyte pattern should be altered in such a way that muscle potassium would decrease. At the same time, myocardial sodium and water content should rise. The end result of such an alteration in water and electrolytes would bring about a state of cardiac edema. This phenomenon was not observed in these experiments. Therefore, on the basis of the water and electrolyte data, it can be said that for the shock state studied in these experiments, at the time circulatory failure supervened there were no indications of myocardial failure.

The elevated plasma potassium and magnesium in hemorrhagic shock is in accord with previous reports (Gregersen, 1946; Root et al, 1947). However, the elevations in the plasma levels of these cations have been reported to be terminal events. The present experiments have demonstrated that plasma potassium and magnesium are significantly elevated several hours before the animal dies of cardiorespiratory failure.

The source of the elevated potassium and magnesium in the blood plasma of shocked animals is undoubtedly hypoxic tissues. Both of these cations are primarily confined to the intracellular compartment by metabolic processes. When there is a reduction in nutrient blood flow to specific tissues, as occurs in hemorrhagic shock, metabolic processes are limited. The ultimate outcome of inadequate energy metabolism is the loss of cellular integrity. Components that are actively transported against their concentration gradients under normal conditions, will now move passively in the opposite direction. In addition to the effects of generalized tissue hypoxia on potassium liberation, catecholamines also play a significant role. These substances are markedly elevated in hemorrhagic shock (Manger, et al, 1957; Watts and Bragg, 1957, Glaviano, et al, 1960). It has been shown by Freeman (1933, 1941) that epinephrine infusions or excitation of the sympathetic nervous system will produce shock in cats. These influences, whatever else they may do, operate to release potassium from the liver and possibly also from the intestine (Shoemaker and Finder, 1961). Certainly, in the present study, the experimental condition was appropriate for excitation of the sympathetic nervous system and attendant catecholamine liberation. On the basis of the experimental work quoted, this might be expected to lead to shock and loss of potassium from the liver.

Whether the potassium lost from the liver and intestine of the shocked dogs in this study accounted for the increased plasma level cannot be said with certainty. There was a rise in plasma potassium of 2.52 mEq/L (an increase of 61 per cent). Even though there was minimal hemolysis of red cells, this is not an important factor to consider. The concentration of potassium in this component of the dog's blood is very low.

The source of the elevated potassium in cardiac muscle is probably the same as for plasma, since the distribution of electrolytes between plasma and interstitial fluid is according to the Gibbs-Donnan equilibrium. In confirmation of this hypothesis Brooks, et al (1955) reported that either infusion of epinephrine or stimulation of the hepatic nerve resulted in a decreased myocardial threshold as measured by microelectrodes. The changes in threshold correlated well with the plasma level of potassium. The calculated resting membrane potentials in the present experiments agree with this observation.

In assessing the importance of elevated plasma potassium in shock, the rate of increase should be considered; this probably is more important than the actual level attained. The investigation of Schamp (1941) showed that the amount of injected potassium, which Winkler, et al (1938) reported to be lethal for normal dogs, can be greatly exceeded if the infusion is made slowly. Furthermore, such dogs will withstand for several days an elevation of plasma

potassium to a level equal to, or greater than, that seen except terminally, in shock. Obviously potassium is not the sole factor in shock. It is possible, however, that an increased level of potassium in the blood is more important physiologically when there is a reduction in the circulating blood volume, as in hemorrhagic shock. It may contribute to the failure of the animal to mobilize fluid from the interstitial spaces into the blood stream by an effect on capillary function. In addition, there is evidence which suggests that shocked animals are more sensitive to potassium intoxication than normal animals (Tabor and Rosenthal, 1945). While potassium death in normal animals is attributed to cardiac effects, other loci of action cannot be excluded. The toxic effects of potassium may be accentuated by fluid loss, hyponatremia, hypocalemia, or acidosis, all of which are seen in shock. Fluid loss, sodium loss and potassium liberation are known to be interdependent.

The role of magnesium in fatal shock has not been investigated. This electrolyte is consistently elevated in the plasma of all animals in hemorrhagic shock. It has been claimed that magnesium salts reduce the excitability of the heart and therefore serves to suppress ventricular ectopic beats. The main cardiovascular effect of magnesium salts is to dilate arterioles, thus reducing the systemic blood pressure. Magnesium may also dilate veins in much the same way as do nitrites. This could lead to a reduction in venous return as a result

of pooling of blood in relaxed venous reservoirs. In addition, magnesium b blocks myoneural junction transmission by a curare-like effect, which will ultimately stop respiration. The level of magnesium which has respiratory influences is considerably less than that causing cardiovascular effect. The pooling of blood in the venous system and the respiratory effects of magnesium could be important considerations in the eventual outcome of hemorrhagic shock.

The results of intracellular electrolyte concentrations must be regarded as first approximations. Their validity depends not only on the accuracy of the analytical procedures, but also on the validity of the assumptions made in the derivations. If chloride ions enter the intracellular space under abnormal conditions, one might conclude that other components of the extracellular compartment enter it as well in the approximate proportions in which they exist in the extracellular fluid compartment. If, on the other hand, the term "chloride space" is substituted for "extracellular fluid compartment" in describing the fluid mass in which chloride is distributed according to the Boyle-Conway theory (Boyle and Conway, 1941) of a modified Gibbs-Donnan equilibrium, this difficulty is removed. The term extracellular fluid compartment or interstitial fluid was chosen with this provision in mind. The term does not necessarily describe a compartment in the strict anatomical sense.

The calculations based on the stated assumptions demonstrated that the increase in total tissue potassium $(K^+)_T$ in the hearts of shocked animals represents an increase in the interstitial concentration of this ion $[\ K^+\ ]_E$. The intracellular concentration of potassium $[\ K^+\ ]_I$ was essentially unchanged. The result of this interstitial increase would be a lowered $[\ K^+\ ]_I\ /[\ K^+\ ]_E$ gradient which in turn would lead to a decreased transmembrane potential and therefore greater excitability. Such a heart would be more prone to develop ectopic foci, which could lead to ventricular fibrillation as the ultimate outcome of hemorrhagic shock. This statement is not meant to imply that the elevated potassium in the muscle of the left ventricle is instrumental in the transition from the reversible to the irreversible state, but that it could be a complicating factor in the fatal outcome of hemorrhagic shock. It was pointed by Hajdu (1953) that maximum change in tension on contraction depends on intrafiber $Na^+ + K^+$ content. Above a certain limit of internal $Na^+ + K^+$, decreased tension developed. Lower contents resulted in decreased relaxation and eventual contracture. The total intrafiber $Na^+ + K^+$ content in the shocked animals, of this present study, was essentially the same as in the controls.

Experiments that are currently in progress indicate that the functional sympathetic nervous supply to the heart is impaired in hemorrhagic shock. This probably means that the normal sympathetic transmitter (norepinephrine)

that is available for physiological functions is exhausted as a result of the prolonged period of hypotension. In addition, Ellis (1959) and Bulbring (1960) have suggested that the infusion of exogenous norepinephrine promotes the entrance of potassium and the loss of sodium from cardiac muscle. On the basis of this information, it was decided to study the effects of l-norepinephrine infusion in reversing the observed myocardial electrolyte alterations in hemorrhagic shock. The results showed that norepinephrine had no detectable effects on total myocardial potassium content. However, the concentrations of sodium, chloride and water were slightly elevated, while that of calcium and magnesium showed a tendency tc decline. When these changes were compartmentalized, it was found that the volume of the interstitial space had increased following norpinephrine infusion, even though the intracellular space remained unchanged. The intracellular potassium concentration was elevated under these conditions, while the intracellular sodium content of cardiac muscle was further decreased. The norepinephrine results were interpreted as an effect on an area of metabolism that controls the influx of potassium and the efflux of sodium. Although the metabolic effects of norepinephrine has been completely overshadowed by that of epinephrine, Mayer and Moran (1959) have demonstrated that the two hormones are equipotent in activating myocardial glycogen phosphorylase, The activation of this enzyme and the subsequent glycogen breakdown would provide

additional energy for the operation of the "sodium pump", the function of the pump being to move sodium and potassium against their respective concentration gradients. Bulbring (1960) has suggested that, in addition to its metabolic effects, norepinephrine may have a direct effect on membrane permeability which involves the movement of sodium and potassium. The present data permit no conclusion concerning the mechanism for the increase in intracellular potassium of cardiac muscle following norepinephrine infusion. The potassium shift is apparently real since there was no change in the intracellular fluid volume. The result of such a change in the intracellular potassium concentration may be of benefit to the animal in shock by stabilizing the myocardial cell membrane.

Additional confirmatory evidence against the concept that myocardial depression is a precipitating factor in irreversible hemorrhagic shock has come from a cardiodynamic study. Pressures recorded from the four chambers of the heart throughout the oligemic and postinfusion stages of hemorrhagic shock have shown that arterial pressure and ventricular end-diastolic pressure declined simultaneously after reinfusion of the bled volume. Also there is no increase in central venous pressure until minutes before the animals died in ventricular fibrillation. If the decline in arterial blood pressure resulted from a reduction in ventricular propulsive power rather than a reduction in venous return,

the central venous pressure and end-diastolic pressure should rise. However, since arterial, central venous and ventricular end-diastolic pressures declined at the same time, circulatory failure must be a result of inadequate venous return. The reduction in venous return could be caused by expansion of the vascular capacity, e.g., by a reduction in venomotor tone (Alexander, 1955). The reduction in venous return may also result from the loss of fluid to extravascular compartments because of damage to capillary membranes. The only evidence of capillary damage, in these experiments, was the presence of a bloody diarrhea in late hemorrhagic shock. Selkurt, et al, (1947) stated that the elevation of the portal/arterial pressure ratio is thought to play an important role in mesenteric pooling by a back pressure effect, and that increase in hepatic resistance is the initiating cause. These investigators made the observation that the behavior of the mesenteric circulation suggest that mechanisms are operative which favor sequestration or pooling of blood in mesenteric vessels. This pooling and sequestration of blood could contribute to the irreversibility which characterizes the standard hemorrhagic shock procedure. A similar explanation might account for the bloody diarrhea observed in the reported experiments. On the other hand, the occurrence of this phenomenon may have resulted from the release of endotoxins into the systemic circulation (Fine, et al, 1959). The release of endotoxins has been attributed to the metabolic activity

of certain anaerobes that flourish under conditions of intestinal hypoxia. Although it has been known for several years, it was not appreciated that a primary effect of endotoxins was changed vascular activity. The changed vascular activity has been reported as alternating vasospasm and vasodilatation of small vessels (Spink, 1960). This phenomenon was associated with increased permeability and edema. Mice injected with endotoxins consistently exhibited a mucoid and bloody diarrhea. Conversely, mice pretreated with cortisone were protected against the lethal action of endotoxins. Treated animals remained in a good state of health, exhibiting no bloody diarrhea, and no changes in intestinal wall (Spink and Anderson, 1954).

A more likely explanation for the circulatory failure in hemorrhagic shock is an increase in the size of the vascular capacity. Vasodilator substances have been reported to be released from the gut due to the reduction in blood flow to this organ. The reduction in intestinal blood flow appears to be due both to a loss of circulating blood volume and to an increased secretion of catecholamines leading to severe visceral vasoconstriction (Longerbeam, et al, 1962). The redistribution of blood flow to certain "critical areas", i.e., the cerebral and coronary circulations, is a protective mechanism against shock, since it serves to maintain vital functions in the face of severe stress. The peculiar vulnerability of the dog's intestine is undoubtedly due to the anatomical

arrangement of its circulation. Since the portal vein in this specie has been demonstrated to be innervated by a sympathetic nervous supply, the increased intestinal resistance appears to be a baroceptor response to a reduced systemic blood pressure. Therefore, the stagnant hypoxia could lead to ischemic necrosis and damage to intestinal capillary membranes. In addition, Chambers and Zweifach (1947) demonstrated a vasodepressor substance arising in the liver. These substances could conceivably exert their influence on either the venous or the arterial side of the circulation to produce vasodilatation. Data obtained from the cardiodynamic study, in the present series, indicate that venous return is seriously curtailed in postinfusion shock. The pumping action of the heart appears unimpaired until just before the animal succumbs to cardiorespiratory failure. When the arterial pressure falls below a critical level as a result of an inadequate venous return, the heart apparently fails because of a deficit of nutrient blood flow to the myocardium.

CHAPTER V

SUMMARY

Left ventricular muscle and plasma analyses for sodium, potassium, magnesium, calcium, chloride and water content have been made on dogs in irreversible hemorrhagic shock. Similar analyses were performed on normal controls and shocked dogs receiving l-norepinephrine infusions. The following significant changes were found:

a) Increases in potassium and magnesium were observed in the plasma of all dogs in hemorrhagic shock; an increase in potassium and a decrease in sodium was found in left ventricular muscle.

b) Calculations of intracellular and interstitial fluid compartments indicated that the alterations in the electrolyte content of cardiac muscle represent changes solely in the interstitial compartment.

c) l-norepinephrine infusions in the shocked animal caused an elevation in myocardial sodium and chloride concentrations; the intracellular potassium content under these conditions was significantly elevated while the interstitial concentration was essentially unchanged.

The increase in the concentration of potassium and magnesium in plasma of the shocked animals was ascribed to generalized tissue hypoxia. In addition, catecholamines undoubtedly play a significant role in the elevation of plasma potassium through their effects on liver glycogenolysis. The breakdown of glycogen and the subsequent release of glucose is associated with an equivalent release of potassium into the circulating blood. The consequence of an increased interstitial potassium concentration would be a lowered transmembrane potential and therefore greater excitability of cardiac muscle. The increased interstitial potassium could conceivably lead to ventricular fibrillation through the development of ectopic foci.

The effect of l-norepinephrine infusions on myocardial electrolyte distribution was interpreted as an influence on an area of metabolism involving the influx of potassium and the efflux of sodium. It is suggested that norepinephrine may be of benefit to an animal in hemorrhagic shock by stabilizing the myocardial cell membrane.

The importance of increased plasma potassium and magnesium in hemorrhagic shock is discussed and it is concluded that, while these elevated cation concentrations cannot be the sole factors in shock, in association with a reduced blood volume they may have a deleterious effect. The role of potassium in the production of ventricular fibrillation, and that of magnesium in

vasodilation are considered. While potassium death in normal animals is attributed to cardiac effects, other loci of action cannot be excluded. Three factors exist in shock which are interdependent. These are fluid loss, sodium loss and potassium liberation. Although the magnitude of each of these factors may be sufficient to produce death, their combined effects may augment one another and profoundly influence mortality in shock.

The cardiodynamic study has shown that myocardial failure is not causally related to the transition from a reversible to an irreversible state of hemorrhagic shock. The pumping action of the heart appears to be unimpaired as evidenced by the absence of increased mean atrial and ventricular end-diastolic pressures prior to the development of irreversibility. The fact that the heart could adjust its output to the increased venous return in the early postinfusion period would suggest that the function of the myocardium was not depressed. The heart apparently fails in late postinfusion shock, but this happens only after systemic blood pressure falls to very low levels.

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APPROVAL SHEET

The dissertation submitted by Bernell Coleman has been read and approved by five members of the faculty of the Graduate School.

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

The dissertation is therefore accepted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

January 14, 1964
Date

Vincent V Clavisano
Signature of Advisor