VAScular smooth muscle and red cell sodium and potassium in
haemorrhagic shock measured by lithium substitution analysis

by

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to the required standard

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A new method of measuring intracellular Na and K using Li substitution was applied to a study of vascular smooth muscle and red cell Na changes in haemorrhagic shock. A rat haemorrhagic shock model was used. Controlled haemorrhage was allowed with a syringe reservoir and the arterial blood pressure was maintained at 30 mm Hg.

In a pilot study, using 20 rats, the plasma Na and plasma K were monitored. A fall in plasma Na and a rise in plasma K were observed. Both returned towards normal following retransfusion and recovery for one hour.

In vascular smooth muscle, significant changes in both cell Na and K occurred following a 2 hour period of haemorrhagic shock. The vascular smooth muscle cell Na in control animals was 27.0±1.5 mEq/kg dry weight and 42.7±1.4 mEq/kg dry weight in the shocked animals (P<0.001). The cell K was 127.8±6.0 in the control animals and 74.7±4.2 in the shocked animals.

In red cell studies, significant increases in red cell Na were found. The red cell Na in controls was 7.09±0.29 mEq/litre cells, whilst in the shocked animals the red cell Na was 8.26±0.33 mEq/litre cells (P<0.025). This was associated with a small but not statistically significant fall in red cell K.

In both sets of experiments, the plasma Na and K were monitored and similar changes to those of the pilot study were found. Following retransfusion and recovery for 1 hour in the vascular tissue study and 2 hours in the red cell study, no significant recovery of cellular Na or K occurred.
The results of these studies are consistent with a significant impairment of cell membrane function in haemorrhagic shock. The importance of both normal vascular responses and red cell function following severe haemorrhage is obvious. The fact that both may be impaired may have important implications in relation to the treatment and prognosis of haemorrhagic shock.
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INTRODUCTION

A major source of controversy concerning the subject of shock has centred around an acceptable definition. The reason for this difficulty has been a lack of understanding of the nature of this syndrome. Messmer defined shock as follows:

Shock is a syndrome characterized by an acute reduction in the nutritive blood supply to the vital tissues, associated with a disproportion between oxygen supply and demand and inadequate elimination of acid metabolites from the tissue. As a result of this haemodynamic disturbance, functional and structural changes take place in the organs affected.

The classification of Blalock has become widely accepted. He divided shock into four categories: 1. Haematologic (oligaemic), 2. Neurogenic, 3. Vasogenic, 4. Cardiogenic. The neurogenic and vasogenic types of shock can be correlated with regard to a change in resistance of the vessels as the primary cause; the cardiogenic type involves failure of the heart as a pump, and the haematologic type results from a decreased blood volume due to a loss of fluid from any of the fluid compartments of the body. The common denominator of the above four categories is a state of reduced flow to the vital (and non vital) organs of the body. This work of Blalock's was a milestone in the understanding of hypovolaemic shock. He ended many years of futile attempts by experimental workers to find a toxic agent to explain the clinical syndrome of hypovolaemic shock.

The four categories of shock may be brought under one definition at a cellular level, and shock may be defined as a failure of cell metabolism caused by poor capillary perfusion. With this definition in
mind, those interested in the underlying mechanisms of haemorrhagic shock have studied the abnormalities of cell function in this syndrome in great detail. They have used many different methods of study and have looked at both morphological and biochemical changes occurring at the cellular level.
ORIGIN OF THE TERM "SHOCK"

The word shock as described previously is used to define a clinical syndrome. The exact origin of the term as used to define a state occurring after some gross injury or insult to the body is not known. According to Wiggers, 66 Le Dran 45 in 1743 was the first to use the word "choc", but he used it to refer to a state of collision. The change to its present day clinical meaning probably occurred insidiously over many years. By the mid nineteenth century, the word was widely used in a context similar to its present usage. Although obvious and severe trauma was recognised as a cause of shock initially, it was not until later that it was realised by Latta 43 that the onset of shock could follow other severe illnesses such as cholera. It became widely believed that a toxic agent was responsible for both traumatic and non-traumatic shock. Subsequently, the term shock became overused and was applied to a wide variety of unrelated clinical states, including syncope, hysterical illness, and often any severe generalised illness. In 1862, Sir James Paget 53 cautioned against the excessive use of the term and slowly the term evolved to its present day meaning.

EARLY CONCEPTS OF PATHOPHYSIOLOGY

Early concepts of the aetiology of shock centred around neurogenic or cardiogenic mechanisms. In 1850, Hall 34 described spinal shock following cord transection and further confused attempts to define shock. In the late nineteenth century, many investigators were studying the
physiology of circulation and gradually there evolved an understanding of the effects of hypovolaemia. Blum,\(^9\) in 1876, concluded that reflex vagal action resulting in impaired cardiac function was responsible for the state of shock. Crile\(^{16}\) is credited with the first significant experimental work on the subject of shock. He concluded that failure of arterial pressure was the primary cause of shock and that cardiac, respiratory, and neurological failures were related to this primary abnormality. About this time, many workers began studying the various types of shock syndrome, both clinically and experimentally. In 1910, Henderson\(^{35}\) 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 system and in spite of contraction (not because of relaxation) of the arterioles.

His clear understanding of the role of poor venous return in the pathophysiology of shock is obvious from his writings. Over the next twenty years or so, including the period embracing World War I, a great deal of effort went into the search for a humoral agent, which might be the major factor in the causation of shock. Some considered that histamine was such an agent. Swingle\(^{61}\) performed experimental studies on adrenalectomised animals and concluded that circulatory failure in shock was related to changes in cellular permeability with shifts in water and electrolytes and reduction in circulatory volume.
MODERN CONCEPTS OF THE PATHOPHYSIOLOGY OF SHOCK

Recently, Messmer has reviewed the pathophysiology of shock. He has stressed that an inadequate supply of nutrients to the tissues and an alteration of transcapillary gradients for water soluble metabolites and ions occurs in shock. These metabolites accumulate in the extracapillary space. The manifestations of shock in various organs have been studied in detail. Particular attention has been focused on the abnormalities in cerebral, pulmonary, renal, and gastro-intestinal systems. The pathological changes which occur in shock are widespread and ultimately all organs are involved, but to different degrees depending on the local supply of tissue nutrients. The cardiac output is distributed in a disproportionate way in both low and high cardiac output shock. Acute low cardiac output, with a decreased venous return, as occurs in hypovolaemia, causes a reflex sympathetico-adrenal reaction, mediated largely through baroreceptors in the aorta and carotid sinus. The blood flow is particularly decreased in regions with a high concentration of alpha receptors including skin, kidneys, and splanchnic bed. The coronary and cerebral circulations, which lack alpha receptors, are spared at the expense of these other systems. Thus the cardiac output is redistributed and the arterial blood pressure is stabilised, at least temporarily. In high output shock, as occurs in severe sepsis or severe trauma without fluid loss, peripheral arteriovenous shunting occurs and although the cardiac output is raised, there is a functional decrease in blood flow and supply of nutrients. In both types of shock an increase in blood viscosity may occur with erythrocyte aggregations in the postcapillary venules. This results in further impairment of the microcirculation.
FUNCTIONAL ABNORMALITIES OF ORGANS IN SHOCK

Decreased blood flow and metabolic depression of various organs in post traumatic shock was studied in detail during the Korean war. A fall in blood Na and a rise in K was shown. The peripheral eosinophil count falls due to adrenal cortex activity. An early rise in serum bilirubin and increased bromsulphthain retention are indicators of liver function disturbance. Muscle metabolism is increased with an increase in urinary creatine. Evidence of insulin resistance is indicated by a diabetic type glucose tolerance curve and a flattened insulin tolerance curve. Decreased glomerular filtration and urine output with an increased blood urea nitrogen occur early and both gastric and salivary secretions are decreased.

Decreased coronary blood flow may contribute to the fall in cardiac output due to myocardial depression. The entity of "shock lung" is observed with severe multiple trauma and with severe sepsis. Whilst a great deal is now known about this syndrome its exact aetiology remains unknown. Low blood flow itself is not thought to be a major primary factor in producing this syndrome.

There is a complex endocrine response in response to most types of shock. The adrenal glucocorticoids and mineralocorticoids, the adrenal medullary catechol amines, angiotensin, antidiuretic hormone are all secreted in increased amount in shock.

The metabolic responses are widespread and non-specific. Essentially, the impaired supply of nutrients, including oxygen, and the accumulation of metabolites due to the poor microcirculation result in widespread abnormalities. Because of the hypoxia, energy rich phosphate synthesis is decreased and anaerobic metabolism occurs.
CELL ABNORMALITIES IN SHOCK

Cell metabolic products, including $\text{H}^+$ ions, lactate, and ketones accumulate. The increased ADH and aldosterone cause further oliguria and Na retention with K loss occurs. Many of the local tissue changes cannot be detected in the peripheral blood because of the microcirculatory disturbance. These local changes include cell organelle (lysosome and mitochondria) and cell membrane damage. These local reactions are, in themselves, damaging and the local release of lysosomal enzymes combined with a poor supply of nutrients and hypoxia result in further damage. A vicious circle occurs and a continuous and increasing deterioration of the microcirculation occurs leading to further cell damage and ultimately to cell disruption.

The metabolic and biochemical changes in the cells of different organs during shock have been studied by many different techniques. Such studies have added considerably to our knowledge of the pathophysiology of shock.

Cell function studies in shock have included those concerned with morphology, lysosomal enzymes, cyclic AMP, Nucleic acid synthesis, ATPase activity, and cation transport changes. The tissues used in these studies include liver, kidney, skeletal muscle, myocardium, red cells, connective tissue. In this study we are concerned with changes in cellular Na and K which occur in haemorrhagic shock. The basic physiology of Na and K distribution and current literature on cell Na and K changes in shock will therefore be reviewed.
The approximate distribution of electrolytes in the various body compartments are shown in figure 1. The values given for the cell electrolyte concentrations vary slightly in different tissues. It is seen that Na and Cl are mainly extracellular, whereas K and PO₄ are mainly intracellular. It is also notable that the interstitial fluid has a very low protein content compared with the cell and plasma concentrations. The barriers which separate these compartments are one of the main reasons for the different compositions seen. The forces responsible for water and electrolyte movement across these barriers (i.e. the capillary wall and the cell membrane) are diffusion, solvent drag, active transport, exocytosis and endocytosis. The particular roles of each of these forces are described in standard texts.

The cell membrane separates the cellular and interstitial fluid and is responsible for the different concentrations of ions and water in the two phases. The inside of a cell has a negative potential relative to the exterior. This resting membrane potential varies in different tissues, from -10mV to -100mV. The membrane permeability is a very important barrier mechanism. Thus, cell membranes are relatively impermeable to large organic anions, including proteins. They are quite freely permeable to K⁺ and Cl⁻, but less so to Na⁺. Thus, K⁺ permeability is 50 to 100 times the Na⁺ permeability. Whether or not membranes possess pores which determine these differences in permeability is unknown. In any case, membranes behave as if they have pores of about 0.7nm diameter. The difference between Na and K may be related to the fact that when hydrated the Na⁺ ion is larger than the K⁺ ion. Forces
acting across cell membranes on various ions can be examined and analysed. The tendency of Cl to diffuse along its concentration gradient into cells is balanced by the electrical gradient caused by the negative intracellular charge. The membrane potential at which equilibrium occurs is called the equilibrium potential and can be calculated from the Nernst equation. Na has a special status with regard to diffusion across membranes. With the system as described, Na has both electrical and concentration gradients tending to cause Na to enter cells. However, the cell Na concentration remains low due to active transport of Na out of the cell and K into the cell. This is achieved by means of a Na-K pump mechanism, located in the cell membrane and deriving its energy source from ATP. Na and K transport are coupled in a variable ratio. The Na-K pump activity is proportional to the Na concentration inside cells. The pump may be electrogenic if it extrudes more Na molecules than it takes in K, i.e. by increasing the 'coupling ratio'. The pump is inhibited by cardiac glycosides and by metabolic poisons which prevent ATP formation. The pump is also very temperature sensitive and Na-K pump activity is inhibited by low temperatures. The above knowledge is applied in experimental studies of the nature of the pump mechanism. Na-K ATPase, a large lipoprotein with a molecular weight of 670,000, is the enzyme which hydrolysates ATP to ADP in order to produce the energy required to drive the pump. This enzyme requires Mg for activity. The negative membrane potential described above depends on the nature of the membrane and the distribution of ions across the membrane. The Na-K pump is also related to cell volume maintenance. If active Na transport is inhibited it will diffuse into the cell and water would follow against the osmotic gradient. The evidence for the above pump mechanism has
been reviewed by Glynn. The role of ATP as an energy source has been suspected for many years, but proof was lacking until experiments on giant squid nerve fibres which involved the intracellular injection of ATP were performed. Further proof was obtained by experiments using red blood cells.

SODIUM, POTASSIUM AND WATER DISTRIBUTION IN SHOCK

Flear studied patients undergoing major surgery and performed Rectus Abdominis biopsies at various stages throughout the procedure. He measured the tissue levels of Na, K, and water and concluded that muscle as a tissue gained water, sodium and Cl and lost K during the procedure. Hagberg performed measurements on tissue fluid and plasma electrolytes in dogs subjected to haemorrhagic shock and found greatly increased levels of tissue fluid K with slight increases in plasma K and he suggested loss of intracellular K to interstitial fluid as the likely cause. The same worker performed in vivo muscle biopsies of single isolated muscle fibres and using X-Ray fluorescence microanalysis measured the intracellular K levels. He found a 26 per cent fall in intracellular K using this technique and concluded that this loss was induced by some cell membrane damage occurring in haemorrhagic shock. He did comment, however, that the cell membrane was traumatised during isolation of the single muscle fibre.

Data based on indirect methods using Cl as an extracellular tag have been criticised because of the tendency of Cl to enter the cell. Johnson and Tucker used Na₂SO₄ as an extracellular tag and took biopsies from the Rectus Abdominis muscle of dogs subjected to haemorrh-
hagic shock. They found evidence of a rise in water, K and Na in plasma measurements and a fall in intracellular Na and a rise in intracellular K. These results were inexplicable even by the authors themselves. They therefore performed more experiments using deltoid biopsies and obtained further equivocal results. They concluded that their extracellular tagewas unreliable in shock.

Shires et al. used ultramicroelectrodes to monitor cell membrane function in shock. Using baboons, subjected to severe haemorrhage, they monitored the transmembrane potential difference in skeletal muscle cells impaled with Ling electrodes. They also analysed the fluid and electrolyte content of muscle samples. They found increased levels of intracellular Na in association with sustained muscle membrane depolarisation. They noted that these changes were reversed on resuscitation of the animal. Similarly, with prolonged and severe haemorrhagic shock in dogs they found that the resting membrane potential of muscle decreased from -90mV to -60mV. With this change in potential difference and according to the Cl space, it was calculated from the Nernst equation that Na had entered the cell and K had left. The interstitial space around the muscle was analysed with a micropipette and high K levels up to 18 mEq/l were found. Trunkey, in similar studies using baboons, came to similar conclusions and showed a 40 per cent reduction in amplitude of the action potential and a prolongation of both the depolarisation and repolarisation times. Following resuscitation, amplitude and depolarisation time recovered, but the repolarisation time remained prolonged for up to 10 days, indicating that some impairment of cell membrane function may persist for this long period.

Coleman investigated the electrolyte changes in cardiac muscle of
dogs during haemorrhagic shock. He measured the total Na and K levels and concluded that there was no significant loss of intracellular K.

Brand\textsuperscript{10} performed similar studies and found similar results. Based on these studies, they concluded that a failure of the transport mechanism for Na and K in heart muscle with a consequent deficit in myocardial energy metabolism was not a major factor in the pathogenesis of irreversible shock.

Essiet and Stähl\textsuperscript{21} studied water and electrolyte changes in skeletal muscle, connective tissue and kidney in rats subjected to haemorrhagic shock and surgical trauma. They observed a balanced movement of water, Na and K in roughly normal proportions into relatively acellular connective tissue, but in the more cellular renal and muscle tissue examined they found that Na levels were increased significantly more than water and K. They postulated an Na-K pump failure as the mechanism involved.

Studies of changes in red cell cations during shock have been carried out in both monkeys and humans. Curreri\textsuperscript{18} observed elevated cell Na levels in patients suffering from burn shock and also showed that the red cell Na level returned to normal levels as the patient's clinical condition improved. Johnson and Baggett\textsuperscript{39} measured red cell Na and K in monkeys subjected to varying degrees of haemorrhagic shock. They found that in prolonged haemorrhagic shock there was a movement of Na into red cells and K outwards. Cunningham\textsuperscript{17} carried out similar studies on the red cells of humans using normal volunteer controls and patients in varying degrees of shock. They found also that in severe or prolonged shock, there was a significant rise in cell Na.

Liver cell function has been widely studied by workers in this field. Sayeed and colleagues\textsuperscript{57} have investigated in detail the ability of the
liver to support active ion transport and maintain normal cell volume in haemorrhagic shock. Using a rat experimental model, they showed that in slices of liver tissue there was a 100 per cent increase in Na in late shock and also a significant loss of K. The same workers have demonstrated in other experiments that liver, muscle and kidney ATP concentrations are decreased in shock. Based on this they have used ATP-MgCl₂ for the treatment of experimental shock.¹³

In addition to metabolic studies, Baue⁶ described the morphological changes in liver tissue in haemorrhagic shock. Holden³⁶ also studied the morphological changes in greater detail.

Sayeed⁵⁶ has studied active Na and K transport and ATP levels in the lung in rats subjected to haemorrhagic shock. He found that the energy dependent transport of Na and K in the lung was not altered. He postulated that direct utilisation of atmospheric oxygen in the lung might allow maintenance of cellular energy levels during the low flow state which occurs. The same worker had previously shown that mitochondrial function in the lung was not deficient in haemorrhagic shock.

Miller⁴⁹ studied the metabolic changes in the brain in shocked rats and showed only very minor changes. He suggested that selective compensatory cardiovascular adjustments were responsible for preserving normality in this tissue.
LITHIUM SUBSTITUTION ANALYSIS OF Na AND K IN VASCULAR SMOOTH MUSCLE IN THE RAT

Although the vasogenic type of shock was one of the major categories as classified by Blalock⁸ and despite the importance of the vascular homeostatic mechanisms in shock, very little is known about the changes which occur in vascular tissue during shock. The role of Na in the regulation of vascular smooth muscle tension and the importance of reciprocal movements of Na⁺ and K⁺ in association with acute vasoconstriction have been discussed by Friedman and Friedman.²⁶ The cell Na and K changes in vascular smooth muscle have not been previously studied during haemorrhagic shock. Until recently, there was no simple method available for the measurement of cell Na and K in vascular smooth muscle. Whilst indirect methods, based on the use of extracellular space markers have been used, the accuracy of such methods depends on the reliability of extracellular fluid volume measurements.⁴⁰ In studying vascular smooth muscle cell Na and K there are special problems related to the paracellular matrix. This matrix binds ions within its network and also limits the reliability of extracellular space markers, which are unable to gain access to this space. Friedman et al.²⁷,²⁸ have recently studied this problem and have devised a method based on lithium (Li⁺) substitution, which has been used to measure vascular smooth muscle cell Na and K in both normal and hypertensive states. Lithium was chosen because of its monovalent nature and because the affinity of chondroitin sulphate (which forms the major part of the polyanionic gel of blood vessels) is greater for Li than for Na throughout the physiological range. The principle of this method is based on the fact that Li does not replace cell Na or K
at 2°C, but exchanges readily with extracellular Na at all temperatures. In his studies, Friedman showed that there was a diffusion barrier preventing free exchange between Na and Li and that cell disruption removed the barrier and that the exchange was metabolically regulated. He showed that on incubating an artery in a physiological saline solution, in which Na is replaced by Li at 2°C for 30 minutes, the extracellular Na was replaced by Li, leaving the cell Na unaffected (Figure 2).

LITHIUM SUBSTITUTION ANALYSIS OF RED CELL Na AND K

In other studies Friedman et al. have measured red cell Na and K using Li substitution analysis. They showed that Li was unable to cross the red cell membrane in either direction at low temperatures and used the method to study the red cell Na and K changes in hypertensive rats.

Other workers have used MgCl₂ to wash out extracellular Na in order to measure the red cell Na concentration in haemorrhagic shock. However, the use of MgCl₂ for this purpose is undesirable, because its different ionic strength may affect the result and because of its known effects on ATP metabolism. It has been shown that Mg in vivo and in vitro inhibits the deamination and dephosphorylation of ATP. It has also been shown that bivalent ions, including Mg²⁺ play an important role in the structural integrity of the cell membrane.

For these reasons the Li substitution method offers theoretical advantages for red cells Na and K measurements.
The purpose of this study was as follows:

1. In a pilot study, to verify the validity of a rat haemorrhagic shock model and to measure the plasma sodium (Na) and potassium (K) changes occurring in haemorrhagic shock.

2. To apply a new ion exchange method, based on lithium (Li) substitution, to the measurement of vascular smooth muscle and red cell Na and K in haemorrhagic shock.

3. To add to our understanding of the changes in cell function which occur in haemorrhagic shock.
MATERIALS AND METHODS

HAEMORRHAGIC SHOCK MODEL (Figure 3)

Adult male albino rats weighing 300 to 400 G were anaesthetised with an intraperitoneal injection of 4-5mg sodium pentobarbital per 100 gm body weight. (The solution contained 15mg Na pentobarbital per ml.) After anaesthesia was obtained (10-15min) the rat was placed in the supine position and the hind legs were restrained loosely using a rubber band. Rectal temperature was monitored during the experiment. The forelegs were not restrained. A 2cm incision was made in each groin and both femoral arteries were exposed and dissected free. Both arteries were cannulated with polyethylene tubing (P.E.50). The cannulae were tied in place with fine black silk. The distal end of one tube was attached to a 3-way stopcock and a plastic syringe was attached to permit bleeding into the syringe and the taking of blood samples. The other tube was used to monitor arterial blood pressure via a transducer and polygraph recorder. After cannulation, 250 units of heparin were given intrararterially through one of the cannulae. An initial blood sample (0.1ml) was obtained and bleeding was initiated by allowing controlled haemorrhage into the syringe until the mean arterial blood pressure was reduced to 30-35mm Hg. This degree of hypotension usually occurred after the removal of 8-9ml of blood in 2-3 minutes. The blood pressure was then maintained at 30mm Hg for the desired length of the experiment by the removal or addition of small amounts of blood as necessary. Following the defined period of hypovolaemia, the animal was retransfused at 1ml per minute. Rectal temperature and syringe volume were recorded at 5 minute intervals.
The body temperature was kept above 35°C by the use of a warming lamp when necessary.

For each animal, the average bleeding volume was recorded and the estimated blood volume was calculated from the body weight. All animals were resuscitated at the end of the procedure and were returned to the cage, where they were allowed food and water ad lib as soon as they were awake. If an animal survived more than 48 hours it was considered a survivor. The overall mortality for a 1 hour period of hypovolaemia at 30±35 mm Hg was 20 per cent.

PLASMA SODIUM AND POTASSIUM MEASUREMENTS

All plasma Na and K measurements were made on 0.1ml samples of blood taken at intervals throughout the experiment. A glass electrode method, as devised by Friedman et al.²,³ was used for estimating the Na and K levels. This method uses cation sensitive glass electrodes and is not only fast and accurate, but can be used with very small blood samples.

As a pilot study a series of 20 rats were subjected to a 1 hour period of haemorrhagic shock, following which each animal was retransfused and allowed to recover for 1 hour. A pre-shock blood sample was taken and further samples were taken at 10 min, 30 min, 1 hour and finally 1 hour after retransfusion.

CELL SODIUM AND POTASSIUM IN VASCULAR SMOOTH MUSCLE

A series of 30 rats was used in this experiment. Ten paired rats were bled to an arterial pressure of 30mm Hg as described above. This pressure was maintained for a 2 hour period. One of the pair was then
retransfused and allowed to recover for 1 hour. Ten anaesthetised, but unshocked rats were used as controls. The glass electrode method described previously was used to monitor the plasma Na and K levels at 30 min intervals.

With the rat in the supine position, the rat tail artery was exposed by incising skin and fascia through a longitudinal ventral incision. The entire length of the artery was exposed by gentle dissection and the collateral vessels were divided (Figure 4). The artery was transected at the base of the tail and at the distal end. The artery was then divided into equal halves, placed between 2 filter papers and gently pressed to remove blood from the lumen. The arteries were placed in a physiological salt solution in which Na had been replaced by LiCl at 2°C, for 45 minutes. The artery was then blotted dry between 2 filter papers and weighed. It was processed by dessication, defatting with ether and was re-weighed prior to extraction for 7 days with 0.75 Nitric acid. Cation measurements were made using atomic absorption ion analysis. The composition of the physiological solutions used for incubation are shown in Table I.

RED CELL SODIUM AND POTASSIUM

In this experiment, 11 rats were subjected to haemorrhagic shock for a 2 hour period. Eleven control animals were anaesthetised and their femoral arteries were cannulated, but they were not bled. These controls were alternated with the test animals in pairs. Again the plasma Na and K were monitored during the experiment.

Blood samples of 2.0ml were taken from each of the experimental
animals at the end of the 2 hour period of haemorrhagic shock. Following this, the shocked animals were retransfused with the reservoir blood and allowed to recover for 2 hours and a 2.0ml blood sample was again taken. Each sample was washed twice in normal Kreb's solution at 2.0°C. The cells were allowed to stand, with occasional stirring, for 30 minutes. The suspended cells were then centrifuged at 1550 rpm for 5 minutes, and 0.1ml samples of precipitated cells were transferred to 5ml of distilled water to which 5ml of 10 per cent trichloracetic acid were added to precipitate the proteins. The Na and K were then measured by atomic absorption spectrophotometry.

Similarly, red cell Na and K measurements were made on 2.0ml samples from the controls.
RESULTS

PILOT STUDY

After one hour of hypotension at 30mm Hg, there was a small decrease in plasma Na, from a mean pre-operative value of 141±1.0 mEq/litre to 138±1.0 mEq/litre (Table II). At the same time, plasma K rose from 4.7±0.1 mEq/litre to 6.0±0.2 mEq/litre. Following retransfusion and recovery for 1 hour, the plasma Na returned to its pre-shock level of 141±0.3 mEq/litre, and the plasma K fell to 5.2±0.2 mEq/litre. These results are summarised in figure 5. The overall mortality of this degree of shock was 20 per cent within the 48 hour period following retransfusion.

CELL SODIUM AND POTASSIUM IN VASCULAR SMOOTH MUSCLE

In vascular smooth muscle, significant changes in cell Na and K occurred following the 2 hour period of haemorrhagic shock (Table III). The mean cell Na in the control animals was 27.0±1.5 mEq/kg dry weight whilst in shocked animals the cell Na was 42.7±1.4 mEq/kg dry weight (p<0.001). Cell K was 127.8±6.0 mEq/kg dry weight in controls and 74.7±4.2 mEq/kg dry weight in the shocked animals (p<0.001). On retransfusion and recovery for 1 hour, cell Na was 43.1±2.0 mEq/kg dry weight and cell K was 81.9±4.3 mEq/kg dry weight. It was not possible to correlate the degree of cell Na or K changes with the overall mortality, which was 25 per cent. Figure 6 is a histogram which summarises the findings in this experiment.
RED CELL SODIUM AND POTASSIUM

The results of red cell Na and K changes are summarised in Table IV. Red cell Na in control animals was 7.09±0.29 mEq/litre cells and in the shocked animals cell Na was 8.26±0.33 mEq/litre cells (p<0.025). Red cell K in control animals was 118.67±2.08 and in the shocked animals it was 115.87±2.11. Following retransfusion and recovery for 2 hours there was no significant changes in either red cell Na (8.22±0.28) or in red cell K (115.43±2.06).

In both the vascular smooth muscle and red cell experiments there were significant plasma Na and K changes, as summarised in Tables III and IV. These findings simply confirmed those of the pilot study. It was interesting that in both experiments there was a persisting abnormality of cell Na changes after retransfusion, whilst the plasma Na and K both returned towards normal.
THE EXPERIMENTAL SHOCK MODEL

Haemorrhagic shock has been widely studied in the experimental laboratory, with the use of a canine model subjected to the Wiggers procedure or a modification of his model. Bacalzo et al., in experiments using male albino rats, devised a standardised rat model. They analysed several critical factors, such as the role of bleeding volumes, haemodynamic levels and duration of hypotension. They concluded that the preferred model for haemorrhagic shock in rats was based on allowing bleeding into an adjustable reservoir to induce sustained hypotension. The rat haemorrhagic shock model was also studied by Butcher et al., who summarised its advantages as follows: (1) The reproducability of experiments was greater in rats of a pure inbred strain than in mixed pedigree dogs available in most laboratories. (2) The time required to perform similar experiments was less in the rat. (3) Experiments in a given number of rats were less expensive than in the same number of dogs.

For these reasons, the rat model was used in the present study, and the procedure used was similar to that of Bacalzo and Butcher.

METHODS OF CELL SODIUM AND POTASSIUM MEASUREMENT

The methods used for the measurement of cell Na and K in this study are based on the observation that the entry of Li into cells is blocked at low temperatures.

In vascular tissue there is an extensive paracellular matrix surrounding the vascular smooth muscle cells. Previous methods of
measuring cell Na and K in this tissue have been based on the use of extracellular markers, which are unreliable because of their limited access to this paracellular space. Li, a monovalent ion, has a higher affinity than Na for chondroitin sulphate, which is a major component of the polyanionic gel of the paracellular matrix. It was therefore chosen by Friedman as the basis of the method he devised for measuring cell Na and K in vascular smooth muscle. He showed that Li does not enter cells at low temperatures and this allows the extracellular Na to be readily exchanged for Li at 2°C, leaving the intracellular Na intact. Cell Na and K could then be estimated simply as the residual after incubation in a physiological saline solution in which Na had been replaced by Li. Friedman has used this method to measure cell Na in vascular smooth muscle in normal and in hypertensive states.

The method has also been modified to measure red cell Na and K. In these studies it was shown that Li does not cross the red cell membrane in either direction at low temperatures and in applying this method it was shown that there was increased permeability to both Li and Na in hypertensive rats.

EARLY STUDIES OF SODIUM AND POTASSIUM IN SHOCK

Early experimental workers had shown that the blood K increased in a variety of clinical conditions, including haemorrhage, traumatic shock, severe gastrointestinal disorders and following intraperitoneal injections of glucose. Zwemer and Scudder believed that K released by cell injury was the elusive toxic agent responsible for shock. However, experiments in which the physiological effects of intravenous K injection
were studied failed to substantiate the theory. Large doses simply caused arhythmias and not a shock like state. It was also noted that such arhythmias were not a feature of clinical shock.

Wiggers, in his monograph on shock, stated: "... a marked degree of salt depletion can induce all the signs of shock independently of trauma or haemorrhage, and lesser degrees may aid the production of shock from trauma and other causes."

In 1940, Fenn showed that K left muscle cells after haemorrhage and returned after blood or saline was transfused. Price also noted that the plasma K increased during terminal shock and also noted a slight increase in plasma Na and Cl. Clarke and Cleghorn were also able to demonstrate a rise in K in shock, but did not find any change in Na or Cl. They also noted a fall in K in the liver of rats subjected to severe haemorrhage. Miller found increased K levels in both skeletal muscle and in serum after scalding and haemorrhage and believed that this was due to loss of muscle protoplasm without an equivalent loss in K.

CELL SODIUM AND POTASSIUM CHANGES IN HAEMORRHAGIC SHOCK

Tissues used in studies of cell electrolyte changes in shock have included liver, kidney, skeletal muscle, cardiac muscle, red cells, and connective tissue. Although no one has studied smooth muscle Na and K changes in shock, studies on skeletal muscle have shown consistently that there is a gain in Na and a loss of K in haemorrhagic shock. Flear, in clinical studies of patients undergoing major surgical procedures, and in experimental studies in which he performed microanalysis of single muscle fibres, has noted an increase
in Na concentration in muscle. In association with these findings he found a 26 per cent fall in cell K in the isolated muscle fibre microanalysis. Many workers have reported similar findings,21,32,62 the exception being Johnson and Tucker38 who used Na2SO4 as an extracellular marker and studied the plasma Na and the cell Na and K in the rectus abdominis muscle of dogs subjected to haemorrhagic shock. They found an increased Na and K in plasma and a decreased cell Na with an increased cell K. They were unable to explain these results, however, and concluded that their extracellular tag was not reliable in shock.

Coleman and Glaviano15 and Brand10 were unable to find any change in cell K in cardiac muscle during haemorrhagic shock. They concluded that a failure of the Na and K transport mechanisms with a consequent failure of myocardial efficiency was not a major factor in the pathogenesis of haemorrhagic shock.

The studies of cell Na and K changes in vascular smooth muscle performed in the present study have shown that these cells gain Na and lose K in haemorrhagic shock. This fact may have important implications with regard to the pathogenesis and treatment of haemorrhagic shock.

The importance of Na and K movements in the regulation of vascular tension have been reviewed by Friedman and Friedman.26 In view of the role of Na and K in the regulation of vascular responses it is likely that a significant change in the transmembrane distribution of these ions, as shown in the present study, might result in impairment of the usual vascular homeostatic mechanisms which occur following severe haemorrhage.

Alterations in red cell Na concentration has been shown to occur in a number of different clinical conditions. Welt et al.63 reported that red cell Na was raised in patients with uraemia. The same worker noted
similar changes in cancer and severe burns. Johnson and Baggett studied red cell fluid and electrolyte changes during haemorrhagic shock in the monkey. In these studies, they measured red cell Na and K after washing the cells in isotonic MgCl\(_2\). They found a small rise in cell Na and a fall in cell K. They also found a significant rise in plasma K and a small, but statistically insignificant rise in plasma Na. Cunningham et al. carried out studies on patients in varying degrees of shock. They noted a marked increase in red cell Na in severe shock. They used a similar method to that of Johnson and Baggett and found that red cell Na in severely shocked patients was 17.0±5.8 mEq/litre cells, whilst in controls it was only 7.5±2.1 mEq/litre cells. They noted that the magnitude of cell Na increase was a function of the severity and duration of shock and could be well correlated with changes in the clinical course when sequential measurements were made.

In the present study we have confirmed that the red cell Na does rise in haemorrhagic shock. The Li method of red cell Na and K measurement used in this study has two potential advantages over MgCl\(_2\) washing of red cells. First, MgCl\(_2\) has the disadvantage that its different ionic strength may affect the results. Second, it may influence the Na-K pump through its known effects on ATP. Green and Stoner have shown that both in vivo and in vitro Mg inhibits the deamination and dephosphorylation of ATP. MgCl\(_2\) has been used in studies concerned with the therapy of haemorrhagic shock because of these effects on ATP metabolism. The fact that the red cell membrane appears unable to maintain a normal cell Na concentration may have important implications in relation to red cell function in shock.

The fall in plasma Na and the rise in plasma K noted in the present
study were expected in view of the cell changes found. The rise in plasma K has been a consistent finding in many studies, but there have been studies where an increased plasma Na was found. On retransfusing the animals it was noted that after a 2 hour period of recovery, the plasma Na and K recover, whilst the cell Na and K do not. This was the case in both the vascular tissue and red cell studies. Thus, the fact that the plasma Na and K have recovered does not necessarily mean that the cell membrane dysfunction has recovered. These findings are consistent with those of Kenney and Randall, who described long term changes in plasma volume and cellular electrolytes following haemorrhagic shock in dogs. Thus, the impairment in cell membrane function responsible for the cell Na and K changes in shock appears to persist for some time after retransfusion, even though this may not be reflected by the plasma Na and K levels.

THERAPEUTIC IMPLICATIONS OF CELL SODIUM AND POTASSIUM CHANGES IN SHOCK

The apparently widespread changes in cellular and extracellular Na and K in haemorrhagic shock have possible therapeutic implications. Some early workers, such as Amberson, regards the effects of isotonic saline infusions as harmful. Others disagreed and supported the use of saline solutions in shock. More recently, experimental studies in rats have confirmed that increased survival is achieved when electrolyte solutions (such as normal saline or Ringer's lactate) are used to supplement blood transfusions. These studies have shown that in severely shocked rats survival is doubled when blood transfusion is supplemented by Ringer's lactate infusions. Shires has been one of the strongest
advocates of the use of balanced salt solutions in the treatment of shock. Using tagged Na$_2$SO$_4$ he noted a decrease in extracellular fluid space following severe haemorrhage, which was not reversed by the replacement of shed blood alone. He concluded that there was a functional deficit in the extracellular fluid compartment with haemorrhage and that electrolyte was required with blood for proper treatment. The advantages of saline solutions either alone or in combination with other infusions in the management of shock might be explained by changes in transmembrane distribution of Na and K which occur in shock. As Na enters cells and K leaves, a vicious circle is set up, whereby the cell membrane and the Na-K pump might suffer further damage. Attempts to maintain the normal ratios of ions across cell membranes by the infusion of appropriate electrolyte solutions seems a logical step to take in an effort to stop this vicious circle.

The arguments in favour of electrolyte solutions for early resuscitation have been discussed in detail by Moss.$^{52}$ He quoted the following paragraph from W. Arbuthnot Lane,$^{42}$ written in 1891, and thought to be the first documented use of salt solution in the treatment of haemorrhage:

It came to my knowledge but a few weeks ago that our late lamented colleague, Dr. Wooldridge, had, shortly before his death, been making experiments on transfusion in animals, by means of which he was able to show that the usually accepted views as to the inutility of introducing saline solutions into the circulatory system to replace blood lost in severe haemorrhage were absolutely false. He found that by the injection of a sufficiently large quantity of saline solution into the vessels of a dog which had lost enough blood to result in death he was able at once to restore the animal to activity and health. He thereby showed that after an animal had sustained a loss of blood sufficient to terminate its life, there was left in the blood vascular system enough haemoglobin to sustain life, if only enough fluid be added to keep it in circulation.
Moss further reviewed the known clinical experience and trials using saline solutions and provided evidence that there was an increased requirement for sodium during shock. He based this need partly on evidence for the contraction of the interstitial space and the adsorption of Na and water on interstitial collagen during shock. He noted that the use of saline was not associated with the development of pulmonary oedema. He also described the trials carried out by the United States Navy unit in Da Nang, which showed that massive infusions of saline (12 litres) in addition to blood replacement in massively injured patients resulted in improved survival.

The other therapeutic implications of the changes in cell Na and K found relate to the use of more specific agents such as membrane stabilising drugs (e.g. steroids) and Na-K pump stimulators (ATP-MgCl₂). The use of high dose steroid therapy in shock is at present undergoing extensive clinical investigation. Based on studies which have shown depleted ATP levels in shock and on the theory that the changes in cell Na and K found in shock may be due to impairment of the Na-K pump, attempts have been made to treat shock with infusions of ATP-MgCl₂ in addition to volume replacement. Chaudry et al. have shown that this treatment is beneficial in experimental studies and have shown that there is improved survival in rats treated with such infusions.

The above discussion of the therapeutic implications of the cell Na and K changes relates to the generalised change in cell Na and K found in haemorrhagic shock.

In relation to the present study, in which the changes in vascular smooth muscle and red cell Na and K have been studied, there are some more specific implications which have been mentioned previously. Thus,
the possible impairment of vascular reactivity resulting from the changes in vascular smooth muscle and the impaired red cell function which may result from the red cell Na changes may significantly affect the course and prognosis of haemorrhagic shock.
SUMMARY AND CONCLUSIONS

The effects of haemorrhagic shock on the changes in cellular and extracellular (plasma) Na and K were studied in the rat. Shock was induced by allowing controlled haemorrhage into a syringe reservoir, until the arterial pressure was 30mm Hg, and this pressure was maintained for a defined period by the removal or addition of blood as necessary. Cell Na and K changes in vascular smooth muscle and in red cells were studied by an Li substitution method. This method is based on the fact that Li does not enter cells at low temperatures. The plasma Na and K were measured by a glass electrode method.

After a 2 hour period of haemorrhagic shock, there was a significant rise in cellular Na in both vascular smooth muscle and in red cells. There was a corresponding fall in cell K in vascular smooth muscle, but only a small fall in red cell K. Plasma Na fell and plasma K rose during haemorrhagic shock.

By interfering with the normal vascular homeostatic mechanisms which occur following severe haemorrhage, and by impairment of red cell function, the disturbed cell membrane function demonstrated in these studies may have important implications with regard to the treatment and prognosis of haemorrhagic shock.
TABLE I

The Compositions of Physiological Salt Solutions (mM),
Aerated with 95% O₂, 5% CO₂ (pH 7.4±0.1 at 37°C)

<table>
<thead>
<tr>
<th>Solution</th>
<th>Na</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>Cl</th>
<th>HCO₃⁻</th>
<th>HPO₄²⁻</th>
<th>Li</th>
<th>CO₃⁻</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norm (PSS)</td>
<td>141.2</td>
<td>5</td>
<td>1.7</td>
<td>1.2</td>
<td>123.4</td>
<td>25</td>
<td>1.2</td>
<td>-</td>
<td>-</td>
<td>11</td>
</tr>
<tr>
<td>Li Subst. (LiPSS)</td>
<td>-</td>
<td>5</td>
<td>1.7</td>
<td>1.2</td>
<td>123.4</td>
<td>-</td>
<td>1.2</td>
<td>141.2</td>
<td>25</td>
<td>11</td>
</tr>
</tbody>
</table>
TABLE II

Plasma Na\(^+\) and K\(^+\) Changes in Haemorrhagic Shock

All values expressed in mEq per litre ± S.E. of the mean

<table>
<thead>
<tr>
<th>TIME (min)</th>
<th>Na</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Shock</td>
<td>141±1.0</td>
<td>4.7±0.1</td>
</tr>
<tr>
<td>100</td>
<td>140.4±0.7</td>
<td>5.4±0.2</td>
</tr>
<tr>
<td>30</td>
<td>139.0±0.6</td>
<td>5.2±0.2</td>
</tr>
<tr>
<td>60</td>
<td>138±1.0</td>
<td>6.0±0.2</td>
</tr>
<tr>
<td>120 (recovery)</td>
<td>141±0.3</td>
<td>5.2±0.2</td>
</tr>
</tbody>
</table>
TABLE III

Intracellular and Plasma Na and K Measurements
in Vascular Smooth Muscle

<table>
<thead>
<tr>
<th></th>
<th>Control (10)</th>
<th>Shock (10)</th>
<th>Shock Plus Recovery (10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell Na</td>
<td>27.0±1.5</td>
<td>42.7±1.4</td>
<td>43.1±2.0</td>
</tr>
<tr>
<td>Cell K</td>
<td>127.8±6.0</td>
<td>74.7±4.2</td>
<td>81.9±4.3</td>
</tr>
<tr>
<td>Plasma Na</td>
<td>141.0±1.0</td>
<td>137.0±1.0</td>
<td>140.0±0.4</td>
</tr>
<tr>
<td>Plasma K</td>
<td>4.8±0.1</td>
<td>6.1±0.2</td>
<td>5.3±0.2</td>
</tr>
</tbody>
</table>

(Cell Na and K values in mEq/kg dry weight, Plasma Na and K in mEq/l ± S.E. of the mean.)
### TABLE IV

Red Cell and Plasma Na and K in Haemorrhagic Shock

<table>
<thead>
<tr>
<th></th>
<th>Control (11)</th>
<th>Shock (11)</th>
<th>Recovery (11)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Red Cell Na</strong> (mEq/1 cells)</td>
<td>7.09±0.29</td>
<td>8.26±0.33*</td>
<td>8.22±0.28ns</td>
</tr>
<tr>
<td><strong>Red Cell K</strong> (mEq/1 cells)</td>
<td>118.67±2.08</td>
<td>115.87±2.11ns</td>
<td>115.43±2.06ns</td>
</tr>
<tr>
<td><strong>Plasma Na</strong> (mEq/litre)</td>
<td>140.5±1.1</td>
<td>136.0±1.2*</td>
<td>141.2±0.8**</td>
</tr>
<tr>
<td><strong>Plasma K</strong> (mEq/litre)</td>
<td>4.6±0.1</td>
<td>5.9±0.1**</td>
<td>4.7±0.1**</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.

* *p 0.025

** *p 0.005

ns = not significant
Fig. 1 Note that the Na and Cl are mainly extracellular, whereas K and \( \text{PO}_4 \) are mainly intracellular. The barriers separating these compartments are responsible for the different compositions. (From Leaf and Newburghi Significance of Body Fluids in Clinical Medicine, 2nd Ed. Thomas, 1955).
Fig. 2 Diagramatic representation of Li replacing Na in the extracellular space, including the paracellular matrix, following incubation in LiPSS at 2°C.
Fig. 3 Rat Haemorrhagic Shock Model. Both femoral arteries are cannulated, one being connected to a transducer and polygraph recorder to monitor the arterial blood pressure and the other to a syringe to allow bleeding.
Fig. 4 The entire length of the rat tail artery is exposed by incising skin and fascia, and after dividing collateral vessels it is transected at its base and distal end.
Fig. 5. Extracellular (Plasma) Na and K Changes. The slight fall in plasma Na coincides with a marked rise in plasma K. Following retransfusion and recovery for one hour, both tend to return towards the control levels.
Fig. 6 Intracellular Na and K Changes in Vascular Smooth Muscle. There is a significant rise in cell Na in the shocked animals, with no significant recovery after retransfusion and recovery for one hour. There is a corresponding fall in cell K, which also fails to recover within that period.


42. Lane, W.A. A surgical tribute to Dr. Wooldridge. Lancet 2:620, 1891.


45. Le Dran, H.P. A treatise, or reflections drawn from practice on gunshot wounds (translated). London, Clark, 1743.


