THE RELATIONSHIP BETWEEN CARDIOVASCULAR CHANGES AND INTESTINAL ABSORPTION OF SALTS AND WATER IN THE CANINE ILEUM FOLLOWING HEMORRHAGE

A Thesis

Presented to

The Faculty of The Department of Biology

University of Houston

In Partial Fullfilment

Of The Requirements For The Degree

MASTER OF SCIENCE

---

by

Robert W. Dillon

August , 1969

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THE RELATIONSHIP BETWEEN CARDIOVASCULAR CHANGES AND INTESTINAL ABSORPTION OF SALTS AND WATER IN THE CANINE ILEUM FOLLOWING HEMORRHAGE

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ABSTRACT

Experiments were undertaken to measure the regional blood flow and blood pressure in the canine ileum, and to determine the unidirectional fluxes of sodium and water, and net flux of 3-0-$^{14}CH_3$-glucose. An eight-inch segment of terminal ileum was isolated with its nerve and blood supply intact. The gut was filled with an isosmotic solution of NaCl and $MgSO_4$, containing ^{22}Na, $^{3}H_2O$, 3-0-$^{14}CH_3$-glucose, and phenol red as a poorly absorbed volume indicator. A branch of the mesenteric vein draining the segment was cannulated with a Jelco IV catheter. Normally the blood was allowed to flow back into the femoral vein. Blood flow was estimated by collecting blood into a graduate for one minute. Transmural potentials were determined with a high impedance electrometer. Samples were taken at fifteen minute intervals through gut cannulae. Tracer activity was measured by liquid scintillation counting. Unidirectional fluxes were calculated by the method of Berger and Steele.

The animals were hemorrhaged 20% of the estimated blood volume. Following removal of the blood there was a significant decrease ($P<0.01$) in blood flow and blood pressure. There was a significant increase ($P<0.05$) in sodium absorbed. The increase was due to a significant decrease in flux into the lumen, and not flux out of the lumen. There was a significant decrease in water influx and efflux, but no change in the volume absorbed.

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I.

INTRODUCTION

INTRODUCTION

The primary function of the gastrointestinal tract is the absorption of ingested material. The process of absorption can be defined as the movement of molecules from the intestinal lumen into the extracellular fluid, principally lymph and blood.

The most important transfer phenomena involved in gastrointestinal absorption are passive and facilitated diffusion, solvent drag and active transport. One, or a combination of several of these mechanisms account for the movement of water, electrolytes, carbohydrates, and amino acids from the lumen.

Historically, the process of diffusion was the first mechanism elucidated, and deviations from this process led to the discovery of other phenomena (Wilson, 1962). The mechanism of simple or passive diffusion is defined as the movement of charged or uncharged molecules down their electrochemical gradients. The rate of diffusion is linearly related to the existing concentration gradients. Hans Ussing (1949) measured the bidirectional flux rates of an ion across epithelial tissue, and established a mathematical criteria for passive diffusion. A second method for quantitatively determining if a molecule is passively distributed is by application of the Nernst equation. This method involves the measurement of the serosal to mucosal electrochemical potential of an equilibrated ion. If this value coincides with the calculated Nernst ratio, then one can assume that the

molecule moves by passive diffusion.

Facilitated diffusion accounts for movement of molecules which would not be expected to cross the lipid membrane down a concentration gradient. The concept of facilitated diffusion was introduced by Danielli (1954). This type of molecular movement postulates the carrier to which the molecule becomes affixed, and is moved across the membrane where it is released. Characteristics of facilitated diffusion are: a high temperature coefficient, a relative degree of substrate specificity, no requirement for metabolic energy, and inhibition by related substrates. Danielli postulated the existence of carrier mediated diffusion to explain a system which reached equilibrium down its concentration gradient at a rate above that of passive diffusion, but did not require metabolic energy (Danielli, 1954). Active transport and facilitated diffusion can be distinguished from each other because in contrast to the latter, the former is an energy requiring process.

A third mechanism by which material is transported from the lumen to the extracellular fluid is that of solvent drag. This phenomenon, as described by Koefoed-Johnson and Ussing (1953), accounts for the movement of solute in a solvent stream through aqueous filled pores in the plasma membrane. The water diffuses across a membrane from a region of higher water activity to an area of lower water activity. The bulk water flow through the aqueous channels "drags" smaller solutes with it (Ussing and Anderson, 1955).

Probably, the most significant transport mechanism present in epithelium is active transport. It is by this method that all cells within an organism maintain their proper intracellular composition. The process of active transport requires energy, is substrate specific, is inhibited by metabolic inhibitors, and most significant, it is capable of concentrating material against a concentration gradient. Although the characteristics of active transport were known for some time, many investigators sought a strict definition of this concept. Early criteria for active transport designated it as any process which was inconsistent with the concept of simple diffusion. Rosenberg (1954) found that definition untenable, and proposed that an active transport process is a mechanism which brings about net accumulation against an electrochemical potential. Curran and Schultz (1968) indicate one major weakness in Rosenberg's definition. They indicate that the Rosenberg definition considers all processes active which can concentrate matter against the electrochemical potential, even those which are seen in non-living systems. More recently, Jardetzky and Snell (1960) and Kedem (1961) have applied non-equilibrium thermodynamics in an attempt to define active transport. However, all of the authors indicate their definitions are purely theoretical, and have no practical application. Schoffeniels (1967) described a more useful definition which is accepted by many investigators. He defines an active transport system as a metabolically linked process, capable of transferring a molecule up its concentration and potential gradient.

In recent years much work has been conducted on the application of the previously defined mechanisms of molecular transport to the understanding of intestinal absorption. There appears to be a mutually dependent relationship for the transport of electrolytes, water, and sugars. Although there is an interdependence among these molecules in relation to their transport, much research has been done upon the individual ions and sugars, in order to elucidate the mechanism of transfer of the specific molecules.

Early beliefs held that the intestinal mucosa was freely permeable to substances in the direction from the lumen to the blood. Ingraham and Visscher (1938) showed that sodium could be absorbed against a very high concentration gradient. Visscher and his co-workers (1945) disproved the tenet that the mucosa is permeable only in one direction by means of radioactive tracers. Ussing and Anderson (1955), employing a short circuit technique, showed that the ratio of unidirectional fluxes of sodium was greater than calculated by the Ussing equation, indicating active transport of that ion.

That sodium is actively transported is a well established fact. Recent investigations initiated by Riklis and Quastel (1958), and further pursued by other investigators (Clarkson and Rothstein, 1960; Csaky, 1960; Crane et al., 1961), have shown that sugar transport is dependent upon sodium. The coupling of these two molecules will be discussed later.

Movement of sodium across the intestinal epithelia may occur, in part, by solvent drag. Anderson and Ussing (1957) have shown that net water movement across the gut can result in a translocation of sodium from the mucosa to serosa against its concentration gradient. Additional data supporting the entrainment of solute in bulk solvent flow were obtained by Hakim and Lifson (1964), who showed that the movement of water from the mucosa to serosa, and in the reverse direction, had a profound effect upon urea transport. However, Curran and Solomon (1957) and Green et al. (1962) observed no change in unidirectional fluxes of sodium following abolishment of net water flow. Both groups of investigators believe the role of solvent drag is not significant in net sodium transport.

The method by which chloride is absorbed from the intestine is presently being investigated. Rabinovitch (1927), and Verzar and McDougall (1936) observed the absorption of chloride from a solution which was hypotonic to the plasma. Subsequent investigations by Ingraham and Visscher (1936) indicated chloride was absorbed against a concentration gradient, and they described this movement as "chloride impoverishment." More recently, Curran and Solomon (1957) and others (Kinney and Code, 1964), have demonstrated active chloride uptake in rat and dog ileum *in vivo*. From these data, it appears that chloride is actively transported *in vivo*.

Contrary to the results observed *in vivo*, the data accumulated *in vitro* indicate that chloride transfer is by passive diffusion. Investigations

using the rat intestine indicated chloride was translocated from the mucosa to the blood via passive diffusion (Clarkson et al., 1961). Similar conclusions concerning the mechanism of chloride transfer were obtained (Schultz et al., 1964), using the short circuit technique and measuring chloride fluxes. Measured Cl^- fluxes coincided with the calculated ratios, indicating that chloride flux could be attributed to passive diffusion.

Finally, investigators (Bucher et al., 1944; Parsons 1956) have observed an increase in luminal bicarbonate concentration concomitant with a decrease in chloride concentration. Thus, there is the possibility for the existence of an anion exchange mechanism which may account for chloride absorption.

The direction and mechanism by which potassium moves across the gut is subject to considerable controversy. The results of Code et al., (1960) paralleled those of earlier investigators (D'Agostino et al., 1953; Cooperstein and Brockman, 1959), all of whom found the potassium concentration increased in the lumen when filled with Tyrode's solution. Additionally, these investigators observed that with a decrease in sodium concentration there was a reciprocal increase in potassium ions. Phillips and Code (1966) found the luminal potassium concentration influences its own rate and direction of movement. When the concentration is less than 5 m Eq/l potassium was secreted, while at greater luminal concentrations there was net potassium movement out of the lumen. The mechanism for potassium movement

appears to be passive diffusion.

The pH of the small intestine is variable, and each segment has a pH characteristic to that region. The jejunum has been shown to be on the acid side, while alkalinity predominates in the ileum (Parsons, 1956). The pH gradients in the intestine appear to be directly related to the pCO_2 and bicarbonate concentration. The jejunum is capable of absorbing bicarbonate, but the ileum characteristically secretes it, perhaps as has already been discussed, as a counterion in a mechanism for chloride exchange (Parsons, 1956; Wilson and Kazyak, 1957). The rate of bicarbonate secretion increases following hemorrhage, and it has been suggested that lactic acid is secreted by epithelial cells into the blood and displaces CO_2 (Hamilton et al., 1968). It is known that following hemorrhage there is an increase in anaerobic metabolism and an increase in lactate levels. If there is a displacement of CO_2 by lactate, then this could account for the post-hemorrhage rise in bicarbonate. The transport of bicarbonate is still under investigation, and no definite mechanism has yet emerged.

The mechanism involved in water absorption from the intestine has been studied extensively for many years. As early as 1901, Reid observed net water movement from the mucosal to serosal surface across rabbit ileum in vitro. Numerous investigators have observed the movement of water from isotonic and hypertonic solutions instilled into the gut. Ingraham et al., (1938) postulated the "fluid circuit" theory, in which water moves across

the intestine in two separate channels. The concentration of solute need not be identical in each stream, and from this they concluded that sodium absorption is the result of movement within solvent streams of different sodium concentrations. However, according to Schultz and Curran (1968), this theory is not wholly tenable.

Several authors have explained water absorption on the basis of active transport. However, two theories have emerged which explain water transport against adverse activity gradients on the basis of active solute transport.

Curran and Solomon (1957) observed that water absorption in the rat intestine was closely related to sodium transport. Curran (1960) later explained this phenomenon on the basis of his "double membrane" theory. According to this hypothesis, a solute is actively transported across a membrane from one compartment into another compartment which is confined on all sides. The properties of the membranes are such that an effective osmotic pressure is created in the closed compartment and water flows into it down its activity gradient. The increase in hydrostatic pressure in the second compartment induces the movement across a second, freely permeable membrane. Thus, water can flow from an area of low activity to a region of high water activity. Essential in Curran's hypothesis are the characteristics of the membranes. The first membrane must permit the formation of an osmotic gradient (it therefore has a high Staverman reflection coefficient), and

the second membrane must be incapable of distinguishing between solute and solvent, (the reflection coefficient is near zero).

Diamond (1967) modified the "double membrane" theory, and has postulated the "standing gradient" osmotic flow of water. He theorized there is a channel closed at one end (mucosa), and open at the other end (serosa), rather than Curran's three compartment model. With the use of the electron microscope, Diamond was able to see the existence of the channels in the toad gallbladder. The standing gradient theory assumes that solutes are actively transported across the membrane at the lateral cell membrane into the channel, creating a local osmotic gradient which induces the flow of water into the channel. Water diffuses from the mucosa across the apical cell membrane and through the cytoplasm into the aqueous pore. As the solute passes down the length of the tube dilution occurs and the osmolarity decreases resulting in a reduced rate of water absorption. Thus, water can move from a region of low osmotic pressure to one of high activity because of local gradients created within a channel. These two theories support the belief that water transport in the intestine is a passive process which is closely linked to active solute transport.

The mechanism for sugar transport is believed to be located in the brush border of the intestinal epithelium (Crane and Mandelstom, 1960; McDougal et al., 1960). The actual site of interaction of the sugars with

the absorptive process is not known as yet.

One postulated mechanism for sugar transport across the gut is Crane's mobile carrier theory. On the basis of kinetic studies, it is assumed that the carrier can move freely within the membrane, and has different affinities for sugar on either side of the membrane. There is a specific requirement for sodium in order for sugar transport to be optimum (Riklis and Quastel, 1959). Crane believes sodium attaches to a binding site on the carrier, and collects in the cell down its concentration gradient, while sugar also moves inward and attached to a second binding site. Following release of sugar and sodium, the latter is actively pumped out of the cell, thus maintaining ionic balance in the cell.

The cell membrane structure is such that it has a high electrical resistivity of about a thousand ohm.cm^2. Consequently, there is a separation of charges, with a resulting potential difference. The origin of the bioelectric potential is presumed to be a result of the selective permeability of the membrane, which results in an unequal distribution of ions.

The relationship between transmural potentials and ionic movement was first reported by Ussing and Anderson (1955). They employed the short circuit technique, and determined the electrical potential resulting from the active transport of sodium ions.

Not all potentials generated are due solely to active sodium transport,

although that is the most common type. Other processes are involved in creating an electrical gradient, and additional types of potentials are the diffusion and streaming potentials.

The nature of the electrogenic pump is such that sodium (but not chloride) may be actively transferred across the membrane, setting up a separation of charge and therefore, a potential. Assuming chloride is permeable to the membrane, a potential could still be generated if the net sodium movement is greater than chloride transfer. Evidence has been presented by Koefoed-Johnson and Ussing (1953) that the Na pump is not electrogenic by itself, but rather has a requirement for potassium in order to operate optimally. They believe the net potential is the difference between the voltage generated by active sodium influx, and potassium diffusion outward across the frog skin.

A diffusion potential is created as a result of active transport of both sodium and chloride at equal concentrations, and sodium passively diffusing back, thereby creating a concentration and potential difference.

A third type of electrical potential is that due to an osmotic gradient (Schoffeniels, 1967). The streaming potential which is created results from water flowing through negatively charged pores within the membrane toward a hyperosmotic region. The water flow pulls with it sodium ions and thus creates a concentration and electrical gradient across the membrane. The

negative charge within the pores presents an energy barrier to chloride ions, and they consequently remain on the side of low water activity.

Thus, by measuring transmural potentials, and the concentration of electrolytes and water, it is possible to infer what mechanism is involved in ionic transfer. Since the bioelectric potential is the result of ionic separation, a change in magnitude or polarity of a potential is an indication of ion transport.

The pathologic condition of blood loss, either internally or externally, is referred to as hemorrhage. There are numerous cardiovascular, neural and hormonal responses following hemorrhage, and all attempt to help the system reattain equilibrium. There is an immediate decrease in circulating blood volume, which causes a decline in venous return, cardiac output, arterial pressure and regional blood flow. One of the most significant compensatory responses is that of maintaining adequate blood flow through vital organs, such as the brain and heart. There is increased sympathetic activity, and a concomitant post-hemorrhage release of acetylcholine. The action of cholinergic and adrenergic fibers causes a decreased capacity of the venous system, and an increase in pressure for a given blood volume. There is a marked decrease in venous capacity in the splanchnic region, resulting in a reduction in blood flow (Gregg, 1962). As a consequence of decreased oxygen delivery (blood flow X arterial $[O_2]$) to the viscera, there

is a shift in metabolism to anaerobiosis, and an increased concentration of lactate and pyruvate (Abel et al., 1965). The accumulation of acid metabolites tends to counteract sympathetic vasoconstriction, and with time there is a slight increase in blood flow to the splanchnic region.

The elevated sympatheticoadrenal activity after hemorrhage initiates compensatory changes within the heart itself. Following acute hypovolemia there is an increase in both inotropic and chronotropic effects upon the heart. Thus, there is a tendency to maintain cardiac output.

Post-hemorrhage release of epinephrine and norepinephrine is accompanied by the liberation of aldosterone and antidiuretic hormone. The latter two hormones increase the rate of salt and water reabsorption respectively, in the kidney. Levitan and Ingelfinger (1965) have indicated that aldosterone increases sodium absorption in the colon as well as the kidney. Recent work by Soergel et al., (1968) has shown that ADH causes an increase in net water flux in the ileum, despite its known vasoconstrictor effect.

In addition to compensation for decreased blood volume by an elevated peripheral resistance, there are responses which restore the circulating blood volume towards pre-hemorrhage levels. There is a shift of fluid from the interstitial space into the vascular system. Due to the fluid shift, one typically finds a decreased hematocrit following hemorrhage. Additional responses which restore fluid balance are observed in the marked reduction of

kidney functions, and consequently, a decreased glomerular filtration rate (Gregg, 1962).

During the past twenty years investigators have been concerned with the measurement of regional blood flow, and correlating it to organ functions. Blood flow to the small intestine has been measured by several investigators, and the values vary from 0.2 ml/min./gm (Selkurt et al.,(1947) to 1.38 ml/min./gm (Geber, 1960) with an accepted mean value of 0.5 ml/min./gm gut weight. Factors such as the type of instrument used, amount of handling of the gut, and extent of vasoconstriction affect blood flow. The process of cannulation itself causes vasoconstriction; however, this effect is short lived. (Grim, 1963). Increased circulating levels of epinephrine, norepinephrine and ADH all mimic the effects of splanchnic nerve stimulation (McMichael, 1932; Clark, 1934). MacLean (1956) and other investigators have observed an initial reduction in blood flow to the intestine following epinephrine injection, with a subsequent increase of blood flow with time. Thus, hemorrhage or any stressful situation which causes sympathetic discharge or adrenal medullary hormone release, will cause a local ischemia within the intestine.

The effect of reduced blood flow to the small intestine and the ensuing anoxia was investigated by Van Liere et al., (1938) who found absorption of sodium chloride from physiological saline solutions greater following

hemorrhage than during control periods. They also observed that the volume of distilled water absorbed from the gut lumen of hemorrhaged animals was less than that absorbed by normal animals. Finally, when an isosmotic solution of sodium chloride and sodium sulfate was placed in the lumen the rate of absorption of water and salts was constant for control and hemorrhaged animals (Van Liere et al., 1947).

Varro and his co-workers (1965) studied intestinal absorption during ischemia, and produced the decreased blood flow by mesenteric artery occlusion. They did not observe any change in absorption, and concluded there was a compensatory mechanism in effect. They postulated the existence of a myogenic reflex which was stimulated by decreased arterial pressure. The reflex response was arteriolar dilation. Therefore, the intestine could maintain a constant rate of absorption in the presence of decreased blood flow.

Grim, Lee and Visscher (1955) observed the flux rate of heavy water to be dependent upon blood flow; however, the authors indicated it was impossible at that time to draw a definite conclusion.

The sequence of responses within the cardiovascular system after hemorrhage are well established; changes which occur post-hemorrhage are designed to maintain adequate circulation to vital organs. However, what is the physiology or pathology of the organs from which blood has been shunted?

Prolonged anoxia is known to induce morphological changes in the intestine (Bounous, 1965). The following research was conducted to comprehend more fully the participation of the intestine in the general countervailing reactions subsequent to acute hemorrhage.

II.

STATEMENT OF THE PROBLEM

STATEMENT OF THE PROBLEM

One of the primary functions of the intestine is to absorb solutes and water. In view of this fact, it was of interest to see if the gut could respond to the decreased blood volume following hemorrhage by increasing its normal rate of absorption of salts and water.

The research to be presented was conducted to more fully comprehend the role of gut absorption in the generalized responses following hemorrhage.

III

METHODS AND MATERIALS

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ANIMALS

Dogs whose weights ranged from 13 to 18 kilograms were obtained from Baylor University School of Medicine. The animals were dewormed and maintained for one week. The dogs were fasted for 24 hours before the experiment with water allowed ad libitum. The animals were anesthetized with sodium pentobarbital (30 mg/kg) intravenously, and then transported to the University of Houston.

SURGICAL PROCEDURES

After exposure of the femoral artery and vein in each hind limb, a polyethylene cannula (Intramedic, P.E. 240) was inserted into the left femoral artery. This cannula was attached to a Bourdon type Pressure transducer and an ink writing polygraph (E&M, Physiograph). The left femoral vein was cannulated with polyethylene tubing, at the free end of which was attached a three way metal valve (Luer-Lock). The trachea was cannulated to facilitate respiration. All incisions were carried out with a cautery knife (National).

An eight inch midline incision was made, and a segment of terminal ileum with its nerve and blood supply intact was isolated. The ileum was identified by the presence of the ileocolic artery, located on the anti-mesenteric border of the gut. The intestine was flushed with about one liter of saline, followed by 500 ml of an isosmotic $MgSO_4$-NaCl solution. Both

solutions were maintained at 38° C. Two rubber plugs were placed in the ends of the cut intestine. One of the plugs contained a tube attached to a two-way valve through which samples could be removed, and a salt bridge composed of KCl (1M) - agar (4%).

A terminal branch of the superior mesenteric vein draining the isolated ileal segment was isolated and cannulated. The cannula (Jelco, I.V.) was attached to the three-way valve, and the blood allowed to flow back into the femoral vein. The valve could be switched so that blood flowed into a graduated centrifuge tube for one minute, and the blood flow could be determined. Heparin (150U/kg) was injected intravenously every thirty minutes to prevent clotting of the blood in the mesenteric vein cannula.

PROTOCOL

The intestinal segment with its nerve and blood supply intact was flushed and filled (15 ml to 20 ml depending upon the size) with an isosmotic solution of $MgSO_4$-NaCl (1:1, v/v), which contained radioactive sodium-22, carbon-14 3-0-methyl glucose and tritiated water. Phenol red, a poorly absorbed molecule, was present as a volume indicator. The intestinal segment was treated the same in each period. The three periods were control, post-hemorrhage, and post-reinfusion.

After the control period, the animal was hemorrhaged 20% of its total blood volume, estimated originally to be eight per cent of the body weight.

Blood was removed through an indwelling catheter placed in the right femoral artery. Collected blood was heparinized (1 U/ml), and placed in a metabollic shaker at 38° C to keep it well aerated. After the post-hemorrhage period, the blood was reinfused over a 15 minute period from a burette attached to an indwelling catheter. The use of indwelling catheters to hemorrhage and to reinfuse, allowed normal blood flow to be maintained in the cannulated hind limb.

The gut was placed in a lucite trough, submerged in warm saline, and covered with gauze and a heating pad. Temperature in the trough was monitored by an electronic thermometer (Tri-R Instruments).

Transmural potentials were determined by means of the KCl-agar electrode within the gut which was connected to a calomel electrode by means of a 1M KCl solution. A second calomel electrode placed in the trough completed the circuit to a high impedance electrometer (Kiethly). The 1M KCl-4% Agar salt bridge and the calomel reference electrode were equilibrated in 1M NaCl overnight. The electrode potentials were less than 0.5 mV, and were checked at the beginning and end of the experiment.

Samples (1.5 - 2.0 ml) were withdrawn from the gut at fifteen minute intervals for one hour. At the end of the period the remaining solution was drained and its volume noted. The segment was flushed with isosmotic $MgSO_4$-NaCl, and the flush saved for later analysis. Usually 15 to 20 ml

were placed in the gut, filling it to capacity without causing undue stretch.

ANALYTICAL PROCEDURES

Of the samples collected, 0.2 ml was collected anaerobically for the immediate determination of pH and pCO_2 (pH meter, Instrumentation Laboratory). The bicarbonate concentration was calculated using the Henderson-Hasselbalch equation, employing a solubility coefficient of 3×10^{-2} mM bicarbonate per mm Hg pCO_2 (Handbook of Physics and Chemistry). A 0.1 ml sample was used to determine the sodium and potassium concentrations by flame photometry (Eppendorf). Another 0.1 ml sample was used to determine the chloride concentration with a Cotlove chloridimeter (Buchler Instruments). A 0.2 ml sample was used for determining osmotic pressure by a freezing point depression osmometer (Precision Instruments). Phenol red concentration was obtained by placing a 0.1 ml sample in 5 ml of water, and developing color with a pH 11.8 NaOH buffer. The tubes were centrifuged to remove the precipitated proteins, and subsequently read at 560 mu in a spectrophotometer (Bauch & Lomb, Spectronic 20). The samples were acidified with a pH 2.2 acetic acid buffer, read at 560 mu, and the optical densities subtracted from the previous readings. This procedure was carried out in order to account for any dissolved hemoglobin which might have been in the samples, and could increase the apparent optical density of the phenol red. Any color present at the acidic pH was not measurable. The flushes were read

similarly, and used to account for the phenol red left in the gut at the end of each period. Phenol red recovery was 95.9% ± 0.77 (S.E.). The remaining 4.1% can be accounted for by residual phenol red trapped in the crypts, and inaccuracy in sampling.

Another 0.1 ml sample was employed for counting Na-22, 3-O-$C^{14}H_3$ Glucose and tritiated water. The sample was dissolved in 10 ml of scintillation cocktail consisting of 8.5 ml of toluene, 1.5 ml of solublizer (Bio-Solv, Beckman Instruments), and 6 grams per liter of 2,5-diphenyloxazole (P.P.O.). Counting was carried out on a Beckman L.S. 150 Scintillation counter. Samples were corrected for quenching using quench curves generated with plasma and phenol red (8 mg%) in isosmotic $MgSO_4$-NaCl. Spillover was corrected for by suitable equations and correction factors obtained from the quench curves. These calculations were carried out on an SDS 1014 computer, by a program in the Fortran IV language.

Unidirectional fluxes of water and sodium were calculated in both directions for all animals by the method of Berger and Steele (1958), and determined by computer.

The specific protocol was as follows: The first hour consisted of a control period. After the gut was flushed, the animal was hemorrhaged 20% of its estimated blood volume, the gut filled, and a second hour period was conducted. At the end of the experimental (hemorrhage) period the gut was

flushed, and the warm, heparinized blood reinfused via the femoral vein. The gut was filled, and the final (recovery) period was carried out. During the entire experiment blood pressure was monitored continuously. Temperature in the trough was monitured during the three periods. Samples were drawn from the gut at fifteen minute intervals to determine the unidirectional fluxes. Blood samples obtained from the mesenteric vein were also analyzed for radioactive tracers and ion concentrations.

STATISTICAL ANALYSIS

The relationship between variables was computed by the least squared regression method. Statistical analysis of significance between parameters was calculated by analysis of variance (ANOV). Comparisons were made between the control versus the experimental period and between the control and hemorrhage period versus the recovery period. These comparisons are independent as required by ANOV. All calculations were carried out by computer.

IV.

RESULTS

RESULTS

After an initial increase in blood flow (Table 1) following the first fifteen minutes, there is a relatively constant flow rate. Blood flow to the segment of ileum was determined by the amount of flow per minute in the cannulated vein, multiplied by the same generation blood vessels in the segment. The product was then divided by wet gut weight, giving total blood flow per gram of gut. Blood flow, subsequent to hemorrhage, decreased by more than 50%, to a value of 0.274 ml/min/gm wet weight from time 15 to 30 minutes. The reduction of blood flow in Period II is significant at the 1% level. After reinfusion of blood, there was an increase in blood flow to the gut. After an initially elevated flow rate, flow declined to a steady value. While blood flow in Period III was above hemorrhage, it never reattained control values. The rise in blood flow during post-hemorrhage over experimental was lacking significance at the 5% level, indicating a prolonged but slight effect of hemorrhage on recovery blood flow.

Mean blood pressure is presented in Table 1. Decreased blood pressure observed during the second Period is significantly lower than control ($P < 0.01$). The significant reduction in blood pressure following hemorrhage is a typical response. After the withdrawn blood was reinfused, blood pressure rose to a maximum of 122 mm Hg, but never reached control values. Although blood pressure following reinfusion was lower than control values,

CARDIOVASCULAR PARAMETERS

TABLE I

TIME (min)

PERIOD	NO. OF DOGS	0 - 15	15 - 30	30 - 45	45 - 60
			Blood Flow (ml/min/gm wet weight)		
I	5	0.58 (0.11)	0.65 (0.18)	0.65 (0.13)	0.63 (0.13)
II**	5	0.27 (0.06)	0.27 (0.07)	0.27 (0.08)	0.29 (0.08)
III	5	0.44 (0.09)	0.38 (0.09)	0.33 (0.07)	0.33 (0.06)
			Mean Blood Pressure (mm Hg)		
I	5	151 (9)	150 (9)	149 (7)	145 (7)
II**	5	79 (9)	81 (10)	87 (10)	87 (10)
III	5	122 (10)	120 (9)	116 (11)	117 (12)

Values are Mean ± (S.E.M.)
** $P<0.01$ When II is compared with I

the difference is not significant when compared to both the control and post-hemorrhage period.

Luminal water influx (Table 2) is at a maximum during the first fifteen minutes of the control period, after which it decreases and becomes constant.

Water influx during recovery is greater than that occuring during hemorrhage, but is less than control values. The influx is not significantly lower when compared to both hemorrhage and control.

Water efflux (flux into blood) during Period I is constant. Efflux decreases slightly following hemorrhage, reaching a minimum of 0.010 ml/min/gm wet weight between 15 and 45 minutes. There is a significant difference between water efflux during control and hemorrhage. The values for water efflux during recovery parallel the first two periods. There was no significant difference in water efflux between period III and the first two periods.

Water efflux and influx are correlated at the 1% levels during control, experimental and recovery. This would indicate an exchange-diffusion like phenomenon with respect to water.

Water absorption (Table 3) is the difference between efflux minus influx multiplied by fifteen minutes. Negative values indicate secretion of water into the lumen. The direction of water movement during control was into the lumen, although the amount was small. Despite net absorption of water

WATER FLUXES

TABLE 2

TIME (min)

PERIOD	NO. OF DOGS	0 - 15	15 - 30	30 - 45	45 - 60
			Water Influx (ml/min/gm wet weight)		
I	5	0.021 (0.002)	0.015 (0.003)	0.015 (0.004)	0.013 (0.001)
II**	5	0.013 (0.001)	0.012 (0.000)	0.008 (0.001)	0.012 (0.005)
III	5	0.011 (0.001)	0.012 (0.001)	0.010 (0.001)	0.014 (0.004)
			Water Efflux (ml/min/gm wet weight)		
I	5	0.015 (0.002)	0.015 (0.003)	0.013 (0.004)	0.014 (0.002)
II*	5	0.013 (0.002)	0.010 (0.001)	0.010 (0.002)	0.013 (0.005)
III	5	0.011 (0.001)	0.010 (0.002)	0.008 (0.002)	0.015 (0.004)
			Water Absorbed (ml/min/gm wet weight)		
I	5	-0.088 (0.032)	0.008 (0.040)	-0.030 (0.015)	0.015 (0.019)
II	5	0.000 (0.017)	-0.024 (0.014)	0.022 (0.020)	0.019 (0.009)
III	5	-0.004 (0.007)	-0.033 (0.019)	-0.020 (0.021)	0.017 (0.015)

Values are Mean ± (S.E.M.)

* $P < 0.05$ When II is compared with I

** $P < 0.01$ When II is compared with I

LUMINAL ELECTROLYTE CONCENTRATIONS

TABLE 3

PERIOD	NO. OF DOGS	TIME (min) 0	15	30	45	60
				Na (mEq/l)		
I	5	80.0 (2.6)	79.3 (2.5)	73.2 (2.2)	67.8 (3.5)	63.7 (3.5)
II**	5	78.8 (1.6)	75.6 (1.9)	68.8 (3.7)	60.6 (5.8)	52.9 (7.7)
III	5	78.8 (2.0)	76.2 (0.6)	72.3 (3.3)	66.3 (6.6)	61.5 (7.7)
				Cl (mEq/l)		
I	5	76.2 (1.0)	72.9 (2.9)	59.7 (4.9)	52.2 (5.5)	42.0 (8.2)
II	5	74.3 (4.0)	67.9 (4.5)	59.5 (6.1)	51.9 (6.8)	45.6 (8.0)
III	5	77.4 (1.1)	70.9 (1.8)	66.4 (4.1)	57.1 (5.5)	49.5 (7.6)
				K (mEq/l)		
I	5	0.7 (0.4)	2.3 (0.7)	2.8 (0.5)	3.3 (0.5)	3.9 (0.6)
II	5	0.6 (0.3)	1.2 (0.3)	2.1 (0.5)	2.6 (0.6)	3.2 (0.8)
III	5	0.5 (0.2)	1.4 (0.4)	2.2 (0.6)	3.1 (0.7)	4.2 (1.0)

Values are Mean ± (S.E.M.)
** $P < 0.05$ When II is compared with I

during the experimental period, the increase was not statistically significant. Total water movement during recovery was into the lumen; however, the amount was not significantly different when compared with Periods I and II.

Sodium concentration (Table 3) decreased with time during all three periods; a greater decrease was observed during the period of hemorrhage. The difference in sodium concentration between Periods I and II was significant at the 5% level.

Sodium influx (Table 4) is initially at a maximum in Period I during time 0 - 15 and then declines. Sodium influx during the experimental period decreases from its highest value during the first fifteen minutes, to its lowest value during the last sampling interval. Despite the initially high influx of sodium during Period II, the total influx is significantly less during hemorrhage. During recovery, the sodium influx increases to approximate control values.

Sodium efflux (Table 4), during the control period, remains relatively constant. Sodium efflux, following hemorrhage, has its maximum value during the first sampling interval, and decreases throughout the period having its lowest value during the last sample interval. There are no significant differences among the three periods.

Data for sodium absorbed appears in Table 4. The large amount of sodium secreted during the first fifteen minutes of control is attributable to

SODIUM FLUXES

TABLE 4

TIME (min)

PERIOD	NO. OF DOGS	0 - 15	15 - 30	30 - 45	45 - 60
			Na Influx (uEq/min/gm wet weight)		
I	5	1.26 (0.32)	0.49 (0.14)	0.62 (0.10)	0.55 (0.15)
II**	5	0.76 (0.05)	0.63 (0.07)	0.34 (0.10)	0.25 (0.12)
III	5	0.60 (0.09)	0.77 (0.12)	0.51 (0.17)	0.44 (0.06)
			Na Efflux (uEq/min/gm wet weight)		
I	5	0.81 (0.17)	0.84 (0.19)	0.72 (0.14)	0.77 (0.14)
II	5	0.92 (0.13)	0.82 (0.17)	0.72 (0.10)	0.54 (0.08)
III	5	0.70 (0.13)	0.76 (0.14)	0.57 (0.17)	0.62 (0.10)
			Na Absorbed (uEq/15 min/gm wet weight)		
I	5	-6.76 (4.08)	5.16 (4.59)	1.47 (0.89)	3.24 (1.43)
II**	5	2.38 (1.57)	2.89 (2.73)	5.75 (2.42)	4.34 (1.21)
III	5	1.50 (1.41)	-0.24 (3.21)	0.85 (2.14)	3.32 (1.29)

Values are Mean ± (S.E.M.)
** $P < 0.05$ When II is compared with I

the high influx rate during the corresponding time interval of the first period. Following the initial secretion, there is net absorption during the remainder of the period. Sodium is consistently absorbed during the experimental period, with the greatest absorption occurring from time 30 to 45 minutes. The total sodium absorbed was significantly greater ($P<0.05$) during hemorrhage than control. Sodium absorption declined to levels below both control and hemorrhage after reinfusion of the blood.

Sodium and water absorption were correlated at the 1% level during all periods.

Chloride concentration (Table 3) decreases during the first period. Similarly, the luminal chloride concentration declined during the experimental period. There is no significant difference in chloride concentration between these two periods. Intraluminal chloride is at a higher concentration during recovery as compared with experimental or control, but was not significantly elevated over the first two periods.

Chloride absorption (Table 5) has a pattern similar to sodium absorption (Table 4), with initial secretion during the control period, followed by net absorption. Chloride absorption also parallels sodium absorption during the second period, both being maximally absorbed from 30 to 45 minutes. Net sodium and chloride absorption are significantly correlated at the 1% level in all three periods.

ELECTROLYTES AND SUGAR ABSORBED OR SECRETED

TABLE 5

TIME (min)

PERIOD	NO. OF DOGS	0 - 15	15 - 30	30 - 45	45 - 60
			Chloride Absorbed (uEq/15 min/gm wet weight)		
I	5	-4.32 (4.51)	10.27 (6.18)	3.31 (0.94)	6.33 (1.71)
II	5	4.60 (1.92)	4.09 (3.13)	5.12 (2.14)	3.70 (0.74)
III	5	4.63 (2.05)	4.86 (2.48)	4.15 (1.39)	4.16 (1.47)
			Potassium Secreted (uEq/15 min/gm wet weight)		
I	5	1.46 (0.41)	0.24 (0.29)	0.49 (0.14)	0.30 (0.14)
II	5	0.45 (0.07)	0.66 (0.18)	0.28 (0.18)	0.31 (0.19)
III	5	0.66 (0.23)	0.59 (0.16)	0.66 (0.19)	0.58 (0.20)
			Net Flux 3-O-CH_3Glucose (Mumoles/min/gm wet weight)		
I	5	0.051 (0.009)	0.039 (0.006)	0.024 (0.005)	0.023 (0.004)
II	5	0.045 (0.009)	0.035 (0.006)	0.025 (0.007)	0.018 (0.003)
III	5	0.040 (0.004)	0.029 (0.008)	0.021 (0.008)	0.028 (0.009)
			Bicarbonate Secreted (umoles/15 min/gm wet weight)		
I	5	2.13 (0.20)	2.36 (0.53)	2.54 (0.81)	2.18 (0.54)
II	5	2.04 (0.60)	2.57 (0.39)	1.78 (0.27)	0.59 (0.37)
III	5	2.29 (0.20)	1.94 (0.39)	2.98 (0.36)	1.26 (0.27)

Values are Mean ± (S.E.M.)

in Table 3, and the amount of potassium secreted is given in Table 4. Initially, there is no potassium present in the solution within the lumen, therefore the low values. The amount in the lumen increases due to characteristic secretion of potassium by the ileum. There was no significant change in potassium concentration during any of the three periods.

Data for3-O-methyl glucose are presented in Table 5. The flux rates are almost identical between periods, and there is no significant change during control, experimental or recovery periods.

Partial pressure of CO_2 is measured as mm of Hg above atmospheric pressure. Table 6 presents the data for pCO_2. All the time O samples read less than 10 because the fluid placed in the gut has the same pCO_2 as in the atmosphere. Ten mm Hg is the lower limit of detection and the instrument Partial pressure of CO_2 increases to a maximum at the end of each period. The pCO_2 decrease following hemorrhage was not significant.

Bicarbonate secretion (Table 5) as calculated from the Henderson-Hasselbalch equation is constant throughout the control period. Bicarbonate secretion during the control period is relatively constant, but following hemorrhage increases to a maximum between 15 and 30 minutes. There were no significant differences among the three periods with respect to bicarbonate secretion.

Table 6 presents the data for luminal pH. The intestine becomes more

LUMINAL $_pH$, $_pCO_2$, OSMOTIC PRESSURE AND POTENTIAL

TABLE 6

TIME (min)

PERIOD	NO. OF DOGS	0	15	30	45	60
				$_pH$		
I	5	6.75 (0.10)	7.07 (0.06)	7.18 (0.07)	7.21 (0.06)	7.28 (0.07)
II	5	6.84 (0.08)	7.03 (0.05)	7.09 (0.06)	7.12 (0.06)	7.15 (0.06)
III	5	6.68 (0.08)	7.01 (0.07)	7.10 (0.05)	7.12 (0.05)	7.20 (0.08)
				$_pCO_2$ (mm Hg)		
I	5	10 (0)	23 (5)	32 (8)	41 (10)	52 (9)
II	5	10 (0)	16 (5)	27 (5)	36 (6)	38 (7)
III	5	10 (0)	17 (3)	22 (4)	32 (4)	35 (5)
				Osmotic Pressure (m-Osm/kg H_2O)		
I	5	306 (3)	304 (1)	301 (2)	297 (2)	297 (2)
II *	5	317 (4)	309 (4)	304 (5)	304 (4)	305 (3)
III **	5	318 (4)	315 (5)	314 (3)	313 (3)	307 (4)
				Potential (mV)		
I	5	-1.64 (6.95)	-1.84 (6.95)	-0.96 (7.24)	-2.58 (7.12)	-4.34 (5.72)
II	5	1.64 (6.72)	0.62 (8.19)	0.44 (9.39)	1.54 (10.88)	1.50 (10.88)
III	5	-0.48 (7.80)	-1.06 (6.64)	-1.99 (6.64)	-0.62 (7.28)	0.66 (7.29)

Values are Mean ± (S.E.M.)

* $P<0.05$ When II is compared with I

** $P<0.01$ When II is compared with I + II

Potential was taken with serosa as reference

basic during all three periods. There are no significant differences present.

The potential difference across the intestine varies from animal to animal. This fact is evident when one examines the data in Table 6. The low mean potentials, sometimes negative and other times positive, are a result of the variability among animals. There does not appear to be a definite trend which the potentials follow; however, the average potentials are positive throughout hemorrhage, while they are negative during control and recovery. No significant change is present among control, experimental and recovery.

Osmotic pressure (Table 6) within the lumen was significantly greater during hemorrhage than control. Similarly, the effect was long lasting in that the osmotic pressure was significantly greater during recovery as when compared with control and experimental. Osmotic pressure reaches a constant value during the last 30 minutes of the control and post-hemorrhage periods.

Calculated and observed Ussing ratios for sodium appear on Table 7. It can be seen that the observed ratios are greater than calculated values during all time intervals of all three periods.

USSING RATIOS

TABLE 7

TIME (min)

PERIOD	NO. OF DOGS	0 - 15	15 - 30	30 - 45	45 - 60
			Ussing Ratio (calculated)		
I	5	0.59 (0.06)	0.58 (0.08)	0.53 (0.08)	0.49 (0.08)
II	5	0.59 (0.07)	0.56 (0.07)	0.52 (0.08)	0.48 (0.08)
III	5	0.57 (0.06)	0.53 (0.06)	0.53 (0.08)	0.51 (0.09)
			Ussing Ratio (observed)		
I	5	0.78 (0.18)	2.82 (0.11)	1.15 (0.08)	2.09 (0.33)
II	5	1.18 (0.14)	1.38 (0.36)	1.11 (0.08)	1.38 (0.46)
III	5	1.17 (0.15)	1.15 (0.35)	1.25 (0.83)	1.54 (0.18)

Values are Mean ± (S.E.M.)

V.

DISCUSSION

DISCUSSION

The results indicate that water absorption remains constant while there is a significant increase in the amount of sodium absorbed from the ileum following hemorrhage. Thus, one may consider the maintenance of a constant rate of water absorption and increased sodium absorption as a compensatory mechanism tending to restore or maintain the volume of the plasma.

The decreased blood flow after hemorrhage is due to hemorrhage itself, plus splanchnic vasoconstriction associated with hemorrhage. Following reinfusion, flow rate is at a maximum during the first 15 minutes and then declines. The initial elevation of blood flow is probably due to local vasodilation due to accumulated metabolites and CO_2. As soon as they are removed, blood vessel diameter decreases, and flow declines.

It is interesting to note that after reinfusion of all blood previously withdrawn mean blood pressure (Table 1) never reaches control values. This is probably due to sympathetic venodilation, and pooling of blood in various organs following reinfusion (c.f. Mountcastle, Vol. I, p 270, 1968).

Regression and correlation analysis showed no significant relationship between salts or water absorbed and blood flow or blood pressure. Absorption of sodium, chloride, 3-O-methyl glucose and water are independent of blood flow and mean blood pressure within the ranges encountered in these

experiments. These results are not in agreement with those of Nelson and Beargie (1963) who induced intestinal ischemia by occlusion of the superior mesenteric vein. They found a decrease in absorption of sodium and glucose following occlusion. However, occlusion causes an increase in capillary pressure which may account for the different findings.

The mechanism by which increased absorption of sodium occurs is fairly clear cut. There is a significant reduction in sodium influx to the lumen while efflux remains unchanged. Thus, the system is absorbing more sodium, not by increasing uptake, but rather by decreasing its loss into the intestine. Reinfusion of the blood restores influx to control values. ANOV showed no significance between Period III versus I and II. This implies the mechanism decreasing influx following hemorrhage is not long lasting.

A possible cause for reduction of sodium influx is that decreased blood flow reduces available absorptive and secretive areas in the lumen. If the influx of sodium into the gut is passive it should obey the equation:

$$J_{in} = -DA \frac{dC}{dX} e^{EF/RT}$$

Where J_{in} is the flux of water into the lumen, D is the diffusion coefficient which is a characteristic of the membrane, A is the luminal surface area available for diffusion, C is the concentration of sodium and X the width of the gut. The term e is equal to 2.313 raised to the power of EF/RT. E is

the transmural potential times the valence of sodium, F the faradays, R the gas constant and T the temperature in degrees kelvin. The term EF/RT is therefore a relationship between the electrical and thermal energies of the system.

Since $\frac{dC}{dX}$ and E do not change greatly after hemorrhage, and it is unlikely that D change, a decreased area available for diffusion seems the most likely explanation for decreased influx of sodium. Some villi are probably not perfused at all, which renders them inactive. Since there is less blood flow per gram tissue than normal, there are diminished amounts of sodium perfusing the tissue, and less available for influx. Areas that are being supplied with oxygen maintain their normal influx. These same areas may increase the rate at which the sodium pump works to maintain a constant efflux out of the lumen.

Regression analysis shows significant correlation ($P < 0.01$) between sodium influx and mean blood pressure during hemorrhage but not control. The correlation is a positive one indicating as mean blood pressure decreases, influxes diminish. This seems to indicate further that cardiovascular changes causing decreased blood pressure concomitantly cause decreased influx of sodium into the lumen.

It is impossible to identify positively the process by which sodium is absorbed. However, application of the Ussing criteria can narrow the

possibilities. It can be clearly seen from Table 7 the observed Ussing ratios do not lie on the same line as the calculated values, but rather are greater. Since the ratio of observed to calculated is not unity, sodium is leaving the gut by a process other than diffusion. Although a difference between observed and calculated ratios only eliminates passive diffusion, one can be relatively sure the process involved is active sodium transport. Further evidence for active transport is obtained from the fluxes. There is no correlation between sodium influx and efflux in any period, implying an absence of exchange diffusion.

Though transmural potentials were not significantly changed from Period I to II, they did reverse sign, and this in itself is significant. Increasing positivity within the lumen implies sodium is moving out actively and diffusing back in passively.

Constant sodium efflux throughout all three periods implies integrity of the sodium pump despite relative ischemia. It appears as though the pump works optimally as long as an adequate amount of metabolic energy is available, and it is not dependent upon blood supply within the experimental limits. As has been previously mentioned, perhaps maintenance of constant efflux results from perfused areas increasing their rate of transport to counter balance loss of pump activity in hypoxic regions. Another possibility is that oxygen extraction increases, and that the reduced blood flow is counteracted

by increased removal of oxygen from the blood.

There is significant correlation between control, experimental and recovery between sodium absorbed and water absorbed. The regression equations are in Table 8. The equations are in the form of $Y = aX + b$, where Y is the average amount of sodium absorbed over the entire period, a is the slope which gives the concentration at which sodium leaves with water, X is the average water absorbed, and b the Y intercept, which indicates how much sodium leaves independent of water. Values in parenthesis are standard errors. It becomes apparent upon examination of the regression equations that the concentration at which sodium leaves the gut when water is absorbed concomitantly is constant for all three periods.

The fact that water enters the lumen can be explained by the presence of $MgSO_4$. The solution within the gut contains $MgSO_4$, which is an osmotically active particle. It tends to keep net water fluxes close to zero by drawing water into the lumen.

Water movements from mucosal to serosal surfaces can be explained according to Diamond and/or Curran's theories. Sodium is actively transported across a membrane with a high reflection coefficient into a channel. Sodium creates a local osmotic gradients pulling water in down an activity gradient. Increasing volumes of water in a compartment increases hydrostatic pressure, which forces water to the serosal surface (interstitial space

REGRESSION EQUATIONS

TABLE 8

PERIOD	NO. OF DOGS	Avg. Y (uEq)	Slope a (uEq/ml)	x	Avg.X (ml)	Intercept b (uEq)
		Sodium Absorbed (Y) versus Water Absorbed (X)				
I	5	0.78 (1.80)	99.75 (11.90)		-0.02 (0.02)	3.15
II	5	3.84 (1.00)	100.01 (16.32)		0.005 (0.01)	3.35
III	5	1.36 (1.02)	95.08 (17.19)		-0.01 (0.01)	2.28
		Chloride Absorbed (Y) versus Water Absorbed (X)				
I	5	3.90 (2.19)	117.86 (16.10		-0.01 (0.02)	6.71
II	5	4.38 (1.00)	84.28 (20.63)		0.005 (0.01)	3.97
III	5	3.36 (0.99)	66.16 (22.47)		-0.01 (0.01)	4.00

Values are Mean ± (S.E.M.)

or lumen of capillaries) across a freely permeable membrane or an open pore. The presence of $MgSO_4$ creates a counteracting osmotic pressure which almost exactly counter balances the osmotic pressure of sodium. No conclusive evidence is available for localization of either membranes or compartments. Movement of water in the opposite direction is due to magnesium osmotically attracting water into the lumen. The direction which water moves is dependent upon the relative strengths of the two forces. If the osmotic force is greater than the activity difference created by sodium, water will be secreted. If local osmotic gradients created by sodium are greater than luminal osmotic pressure, water is absorbed.

It is difficult to determine the cause of reduction in water influx. The most likely explanation is that reduced blood pressure reduces the driving force on water exerted at the large pore surface of the epithelial cells. If hydraulic conductivity through the large pores remained constant while the pressure dropped, a decreased influx of water would result. The significant correlation between mean blood pressure and water influx only during the experimental period supports this explanation.

Chloride appears to move out of the gut via an active process. Chlorides maximum concentration within the gut is approximately 78 mEq/l. It is moving into extracellular fluid whose concentration is at least 30 mEq/l higher. Therefore, chloride is leaving against a concentration gradient.

Similarly, it is moving against an electrical gradient. During hemorrhage it leaves an electrically positive lumen and passes to a negative serosal surface. This transport during control and recovery is also against an electrochemical gradient. The equilibrium potential for chloride calculated by the Nernst equation is -18 mV. This means a potential of -18 mV (mucosa negative) would have to be present for chloride to be diffusing out. Any movement of chloride from a less negative region would be against an electrical gradient.

Chloride absorbed correlates significantly with water absorbed. The regression equations showing these relationships are in Table 8. When comparing the intercepts with those in the equation comparing sodium and water, it is clear that more chloride moves out independent of water than does sodium. Further analysis of the equations reveals chloride leaves the gut in a less concentrated solution during Period II and III as compared to sodium. Active chloride transport is probably drawing some water out with it; however, its effect is not as profound as that of sodium.

Although bicarbonate secreted does not change significantly following hemorrhage, the role of this ion is important. When one calculates bicarbonate concentration from the equation:

$$pH = pKa + \frac{[HCO_3^-]}{apCO_2}$$

(where 'a' is the solubility coefficient for CO_2), and compares this value to the chloride sodium and potassium concentration, it can be shown that HCO_3^- accounts for the difference in the concentration of positive and negative charges. Thus, bicarbonate maintains electrical neutrality of charges in the lumen.

The significant increase in osmotic pressure during the experimental period is obviously due to an increase in the number of particles within the lumen. Investigation of intestinal mucosa following hemorrhage (Bounous, 1965) showed increased trypsin like activity with a concomitant rise in lumenal protein. It would appear a similar process may be occurring in the present study. Certainly, increased proteins or mucopolysaccharides (sloughed off glycocolyx) within the lumen would account for increased osmotic pressure.

The mechanism by which potassium is absorbed or secreted by the gut has been obscure for many years. In these experiments potassium is probably diffusing down its electrochemical gradient, and comes into electrochemical equilibriun. It has previously been reported (Phillips and Code, 1966) that at luminal potassium concentrations which are less than plasma, potassium is secreted.

VI.

SUMMARY

SUMMARY

This study has shown that following 20% hemorrhage there is a significant decrease in mucosal blood flow and mean blood pressure. Concomitant with reduced blood flow are decreased influxes of water and sodium which probably result from diminished blood perfusion of intestinal mucosa.

An increased absorption of sodium subsequent to hemorrhage compensates for other lost electrolytes. The increased absorption is a result of decreased influx to the lumen rather than increased efflux out of the gut.

The ability of the intestine to absorb water following hemorrhage has been studied. Absence of increased water absorption is due to the presence of an osmotically active substance in the lumen. Even with magnesium present, no net water is lost after hemorrhage. This conservation of water is due to decreased water influx.

Chloride absorbed, potassium and bicarbonate secreted and net 3-O-methyl glucose flux were unchanged during intestinal ischemia.

VII.

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