ELECTROLYTES AND WATER TRANSPORT IN THE INTESTINE OF SHEEP

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ABSTRACT

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by Somsak Borvonsin

Two experiments were conducted on mature ewes to investigate the effects of two dietary levels of sodium and potassium and three rates of perfusion on: i) the fluxes of Na, (using ^{22}Na), K, Cl and water from a temporary Thiry-Vella loop of the jejunum, and ii) the osmolality and electrolyte compositions of intestinal contents and blood plasma.

Lower dietary intake of sodium and potassium resulted in lower concentrations of K in plasma and intestinal contents. Significantly lower rates of absorption of K and water from the fluid perfusing the jejunal loop were found for the low electrolyte intake period in which the perfusion fluid simulated the electrolyte composition of intestinal contents and was lower in potassium but high in sodium and osmolal concentrations. These and other results suggest that K and water absorption were passive processes and occurred along concentration gradients.

Increasing perfusion rates from 7 ml to 13 and to 26 ml/min reduced the transit time for fluid passing through the loop, but in general increased the permeability of the loop to water and electrolytes. These latter effects were presumably due to the increased volumes of fluid in loop found for the higher perfusion rates. Sodium efflux, net flux and influx all increased markedly but only the last resulted in statistically significant ($P < 0.05$) differences. Potassium absorption did not change significantly but chloride movement went from absorption at the low rate of perfusion to increasing secretion into the loop at the higher perfusion

rates. The variability of chloride fluxes was large and treatment differences were not statistically significant. The fluxes of sodium and chloride appeared to be linked. Statistically significant increases in water absorption were found for increasing perfusion rates. It was concluded that the increased permeability of the loop with increasing perfusion rates were due to the larger volumes of fluid in the loop causing a larger fluid-mucosa interface and generally increased mechanical and metabolic activity of the gut wall.

In the second experiment two possible causes of the variability found in the first experiment were examined. It was found that use of 100 times the amount of ^{22}Na used in the first experiment, gave more consistent measures of transit time and transfer rate constant. Also the data obtained from half-hour collections of effluent fluid from the loop for net flux measurements, which depend on the differences between input and output, suggest that little of the variability in net fluxes found in the first experiment could have been due to the length of collection period, which was of one hour duration.

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INTRODUCTION

In natural circumstances, a living cell maintains internal concentrations of electrolytes which differ from those in the ambient medium. It was pointed out by Claude Bernard that the extracellular fluid is an 'internal environment' of the body, and that its constituents are regulated so that the cell can maintain its normal electrolyte composition. The cells of the body can continue to live and function properly only as long as the composition of the extracellular fluid is maintained within certain normal limits. When intake of electrolytes is insufficient an animal cannot maintain extracellular fluid volume because the kidneys cannot retain the water in the presence of insufficient electrolytes.

Extracellular fluid, both that of plasma and of interstitial fluid, contains large quantities of sodium and chloride, moderate amounts of bicarbonate, and small amounts of potassium, calcium, magnesium, phosphate, sulphate and organic acid anions. These electrolytes may be called 'the electrolyte framework' of the fluids.

In the dynamic steady state of the body, there is a constant exchange of ions between the cell and its environment. The rate of exchange into and out of cells must be equal in order to maintain a constant internal concentration and volume. Studies have shown the presence of a 'sodium pump' in cell membranes. The sodium pump removes sodium from the intracellular fluid and is dependent on a supply of energy. The movement of chloride into and out of the cell usually accompanies the movement of sodium. Water movement into and out of the cell follows osmotic gradients and thus occurs when concentrations are changed. An interruption of the activity of the sodium pump leads to a loss of cellular potassium ions

and increases in intracellular sodium, chloride and water.

The transport of sodium ions across the intestinal mucosa also occurs in a similar manner to that in other tissues. Potassium, chloride as well as other ions and water are found transported across the intestinal membrane. The duodenum and jejunum appear to have a process of secretion of sodium into the lumen since hypotonic solutions introduced into the gut tend to become isotonic with blood prior to absorption taking place. In other regions of the gut, on the other hand, the absorption of sodium and chloride takes place from hypotonic solutions.

The transport of sodium from intestinal lumen into blood appears to be against electrochemical potential gradients, and is thus an active process, requiring energy from metabolic processes in the cells. Chloride transport generally seems to occur passively, following the active transport of sodium. Water movement across the intestinal membrane is believed to be passive secondarily to the inward movement of solutes such as sodium and chloride. The transport of potassium is also apparently a passive mechanism. However, there is evidence that the potassium and sodium transport mechanisms in the intestinal mucosa may be linked.

There are only few reports on the transport of electrolytes and water across the ruminant small intestine. The present report is on an investigation of the movements of monovalent electrolytes and water into and out of the sheep small intestine. Studies on the unidirectional fluxes of sodium, net fluxes of sodium, potassium, chloride and water in 'temporary' Thiry-Vella loops of sheep jejunum were the objectives of the present experiment. Related parameters studied were the intestinal volumes, osmolalities of intestinal contents and blood plasma and plasma electrolyte concentrations.

LITERATURE REVIEW

I.

BODY FLUIDS AND DISTRIBUTION OF ELECTROLYTES THEREIN

The water of the body and its dissolved (inorganic and organic) solutes is called body fluid (75). An adult animal consists of about 60 per cent of weight of water (57,93). Forty-three per cent of the body weight is represented by intracellular fluid (ICF) while extracellular fluid (ECF) accounts for about 17 per cent. The ECF is composed of 4% of blood plasma, 13% of interstitial fluid and small amounts of transcellular fluid, e.g. digestive secretions, sweat, cerebrospinal fluid (146).

The total body water (TBW) of a sheep is about 43 to 68% by weight of the body weight (22,51,140). The ECF volume accounts for 15%; of which 4 to 5% is plasma (52,81,140). In sheep, unlike monogastric animals, the alimentary fluids account for about 25% of TBW, or 8% of the body weight (22).

The major cations of ICF are potassium* (K^+) and magnesium (Mg^{++}); the major anions are proteins and organic phosphates. The major cation and anions of ECF are Na^+, Cl^- and HCO_3^-. Potassium, Ca^{++}, Mg^{++}, $PO_4^{\equiv}$ $SO_4^{=}$ and organic acid ions are present in small amount in the ECF (110).

Approximately similar concentrations of Na^+, K^+, and Cl^- have been found in the plasma of ruminants, swine and equine (29,77,87). Sodium and K concentrations in sheep plasma are about 144 and 5 mEq/l respectively (25,78). The plasma concentration of Cl is approximately 108 mEq/l (103).

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*For convenience, in this context:

K refers to potassium,
K^+ refers to potassium ion,
$[K^+]$ refers to potassium ion concentration,
Na refers to sodium, and so forth.

Functions of Electrolytes and Water

It was pointed out by Claude Bernard that the ECF is an "internal environment" of the body, and that its constituents are accurately regulated so that the cell can maintain its properties and functions with minimal changes in spite of general environmental fluctuations. The "internal environment" is maintained in a dynamic state of equilibrium by the constant expenditure of energy derived from cellular metabolism (146). Cannon invented the term "homeostasis" to describe the same process (75).

Water is the solvent of electrolytes (and other solutes), and is distributed passively along with electrolytes (146). Thus their functions are closely related.

The main functions of body water and electrolytes are to provide the medium for the "vital processes" by maintaining relatively constant acid-base equilibrium, osmotic concentration and body temperature.

The role of electrolytes in biological systems was discussed by Black (12). These functions may be summarized as following:

1. Maintenance of electroneutrality. Electrolytes carry positive or negative charges which attract each other. The total positive and negative charges must be equal in order to maintain the electroneutrality.

2. Maintenance of osmotic equilibrium in the body. Sodium is the major ion in accounting for any change of the osmotic concentration of the ECF. Despite the 20 times smaller mass relative to that of protein by weight, Na contributes about 140 milliosmoles per liter (mOsm/1) of the osmolality whereas protein osmolality is less than 2 mOsm/1 (24). Furthermore, the anions neutralizing sodium have a similar osmolality, so that electrolytes contribute almost all of the osmolality of plasma, which is about 280 to 310 milliosmoles per kilogram of water (mOsm/kgH_2O).

3. Relation to energy metabolism. Calcium, magnesium and potassium are required for enzyme activities. Potassium is also related to carbohydrate metabolism (143).

4. Special functions. Na and K are involved in the maintenance of membrane potential which is necessary for the propagation of nerve impulses, muscle contraction and other body function. Ca is involved in blood coagulation, irritability of contractile tissue and, with the appropriate proportion of phosphate, Ca plays a role in bone formation (75). Bicarbonate and phosphate ions are important in buffer systems of the ECF (114).

II

REQUIREMENTS, BALANCE AND CONTROL MECHANISMS FOR ELECTROLYTES AND WATER

A. Requirements

The Na, K, Cl and water needs of the animal depend on the requirements for growth, maintenance, lactation and pregnancy and the necessity to compensate for losses due to excretion via urine, faeces and sweating. In addition, Na requirement is dependent upon K content in the diet. Water intake depends on many factors. Among these factors are the daily intake and output of electrolytes.

Sodium requirement in sheep is approximately 0.5% of the diet fed in the form of NaCl (91). Potassium requirement is 0.3 to 0.5% of the air dry ration (23). Dietary levels of K and Na higher than 0.6% and 0.21% respectively do not affect the growth of lambs (46). Sodium intake as low as 1 gm/day concomitant with 1.2 gm of K in the diet meet the requirement for maintenance in wether lambs (41). Daily water consumption in sheep varies from 1 to 4 liters (88).

B. Balance

The external balances of water and electrolytes depend on the intake and output. Absorption of minerals and water occurs along the alimentary tract, while levels above requirement are excreted via the urine and faeces. In dairy cows, faecal K and Cl are the result of endogenous excretion (94).

C. Control mechanisms

1. Antidiuretic hormone (ADH). ADH from the posterior lobe of the pituitary gland controls water excretion. Tuttle and Schottelius (132) state that an increase in plasma osmotic pressure of only 2% stimulates

the secretion of ADH, and conversely a decrease in osmotic pressure inhibits the secretion. ADH exerts its water conserving effect primarily by increasing the permeability to water of the distal convoluted tubules and collecting duct cells of the kidney. A similar effect of ADH on water conservation has been shown in sheep (17).

2. Renal mechanism. The kidney adjust the urinary losses of Na, K and Cl according to variations in intake relative to body requirements. Pitts (110) states that when the Na intake is severely limited, reabsorption by the renal tubules can reduce the Na concentration of urine virtually to zero. Sodium is actively reabsorbed in the renal tubules. The reabsorption of chloride is passive in the proximal tubule and probably active in the distal nephron. Pickering (109) cited the results of other workers indicating that all the K filtered is reabsorbed in the proximal tubules. Thus, any K that appears in the urine is the result of secretion by the tubular cells of the distal segment; this K secretion is believed to be by an ion-exchange mechanism involving concomitant reabsorption of Na from the tubular urine.

3. Role of the adrenal glands. The mineralocorticoid hormones, aldosterone and deoxycorticosterone, from the adrenal glands, cause an increase in the reabsorption of Na and the excretion of K from the distal and collecting tubules of the kidney. In addition, mineralocorticoids regulate Na secretion by the salivary glands, the intestinal glands and the skin (56).

4. Role of the Intestine. Many studies have shown the role of the intestine in the regulation of water and electrolyte losses from the body. Phillips (101) stated that the absorption of water and osmotically active substances occurs mainly along the large intestine in steers. During

water restriction there is an increase in water absorption from the terminal gut. The work performed by English (51) indicated that during water restriction, the faecal water content of sheep decreased by 7%. Faecal losses of Na, K and Cl were also reduced during this period of water restriction. Similar evidence of an intestinal role in water and electrolyte regulations has also been shown in both sheep and cows by Renkema and his associates (113), Van Weerden (133) and Devlin and Roberts (41). Besides these effects, an increase in the osmotic pressure value of faecal press-juice of heifers was found by Weeth et al.(142) as a result of water intake restriction.

The influence of deoxycorticosterone on the intestine in the conservation of Na and the excretion of K has been shown in the dog (10). Crocker and Munday (31,32), claimed that aldosterone and angiotensin can increase the mucosal water and sodium transfer in rat jejunum.

5. Neural mechanism. Forbes (56) states that neural mechanisms can influence Na metabolism and its effect on the kidney, sweat glands, circulation and corticotropin. Bradley (16) pointed out that denervation of the kidney leads to an increased urine flow and excretion of sodium and other osmotically active solutes.

III

WATER, Na, K AND Cl TURNOVER BETWEEN THE BODY AND THE GASTROINTESTINAL TRACT

Water and minerals such as Na, K and Cl passing through the alimentary tract of ruminant are not only obtained by ingestion but also from secretions of the glands along the tract. Kay (74) states that in sheep, about 10 to 20 liters (l) of fluid secreted by the glands along the tract pour into the alimentary canal. These fluids, containing electrolytes and other substances, assist in maceration, digestion and transport of food. Parts of the canal gain water and electrolytes by secretions whereas other parts lose these substances and fluid by absorption. Generally, the intestines function to absorb the ingested electrolytes and water, and to reabsorb these substances secreted by the previous parts of the gut and by the intestine per se. Concomitantly, the intestine has to adjust the composition of the digesta to the requirements of digestion and propulsion of the digesta (74).

In an average-sized sheep (feed intake 700 - 1000 gms/day), 2 l of daily water intake (107) and 5 to 12 l/day of saliva pour into the approximately 4 l of ruminal fluid (50). The net movement of water in the rumen is not known (50). About 7 l of ruminal fluid flow into the omasum and 40 - 60% is absorbed therein (107). Thus it can be estimated that daily omasal absorption of water in sheep amounts to approximately 3 l (15). In the abomasum, the output of gastric juice from the fundic area is estimated to be 4 to 6 l per day (106). It is assumed that there is little or no absorption taking place in the abomasum since the quantity of water passing into the duodenum is approximately equal to the sum of that passing into and that secreted by the abomasal mucosa.

Water secreted into the intestine in bile, pancreatic juice and intestinal secretions amount to about 1.0, 0.5 and 2.0 1 respectively, per day (74). Most of the approximately 10 1 of water passing into and being secreted into the intestine is absorbed along the intestinal tract. This is illustrated by the calculation of Phillipson and Ash (107) which shows that the amount of digesta fluid passing from the terminal ileum into the caecum amounts to about 3.5 1 and that leaving the body as faecal water amounts to about 0.5 per 24 h. Thus about two-thirds of the intestinal water absorption is achieved in the small intestine.

Similarly, Na, K and Cl from saliva are added into the rumen. The concentrations of Na, K and Cl in mixed saliva of sheep are reported to be 160 to 180, 8 to 20 and 17 mEq/l respectively (13, 44, 73). The rumenal concentrations of Na, K and Cl, on the other hand, vary from 60 to 97, 39 to 71, and 16 mEq/l respectively (120,92). This might suggest that there is net absorption of Na, net secretion of K and no net movement of Cl across the rumenal wall. The concentrations of these electrolytes in the abomasal juice of sheep has been found to be quite variable. Phillipson (106) compiled the data obtained by various groups of workers and reported the electrolyte concentrations in the gastric juice of sheep as 21 to 167 for Na^+, 2 to 19 for K^+ and 138 to 172 mEq/l for Cl^-.

In the small intestine, Na, K and Cl are added by the secretions of bile and pancreatic juice and by intestinal secretion. The concentrations of Na and K in the bile are higher than those in plasma whereas that of chloride is slightly lower (67). In pancreatic juice, the concentrations of Na and K appear to be similar to those of plasma, and that of Cl seems to be slightly higher (127). The electrolyte composition of the intestinal juice will be discussed in the section of "Intestinal Secretion". However,

there is evidence that net secretions of electrolytes occur in the first part of the small intestine and net absorption in the rest of the tract (134, 89,99).

IV

INTESTINAL SECRETION

A. Definition

Hendrix and Bayless (70) in their review on intestinal secretion defined "secretion" as the process by which material is separated, elaborated, and discharged by cells, particularly by the epithelial cells of glands. Secretion may come via a duct or from a ductless gland. Saliva and enzymes from stomach and intestine are the result of the secretory activity from the digestive tract. The components of the digestive tract secretions are proteins, mucopolysaccharides, and solutions of water and electrolytes.

B. Secretory Cells

Hendrix and Bayless (70) suggested that the intestinal fluids are secreted by Goblet cells, Paneth cells, argentaffin cells (entero-chromaffin cells) and columnar cells which are derived from undifferentiated crypt cells found in the crypts of Lieberkühn. Goblet cells produce a mucus secretion containing water, electrolytes and a mixture of several different mucopolysaccharides. Serous fluids are mainly the product of Paneth cells. The function of argentaffin cells is believed to be the regulation of gastrointestinal motility through the release of serotonin. Columnar cells secrete a number of enzymes, e.g. leucine amino-peptidase, ATPase, dissacharidases, and alkaline phosphatase.

C. Regulation of intestinal secretions

Factors that influence the intestinal secretion can be summarized as follows:

1. Local stimulation. The presence of digesta or other source of mechanical stimuli in any segment of the gastrointestinal (G.I.) tract causes the glands in that region and its adjacent area to secrete digestive

juice. The distension of the small intestine causes copious secretion from the crypts of Lieberkuhn (64). A study by Harrison and Hill (68) indicated that, in sheep fed once daily, the duodenal secretion is at an average rate of 13 ml/hr whereas the rate of 26 ml/hr obtains in sheep fed 3 times a day.

2. Neural Regulation. Hendrix and Bayless (70) quoted the results of earlier workers that parasympathomimetic drugs such as pilocarpine and physostigmine produce copious intestinal secretion which is blocked by atropine. Guyton (64) emphasized that parasympathetic stimulation can increase intestinal secretion by 2 or 3 fold, but this is a small increase in comparison to the effects of local reflexes resulting from distension or irritation of the mucosa. Splanchnic nerves may have some effects on secretion (40).

3. Hormonal control. It is generally accepted that "enterocrinin" is a hormone that increases the secretion from intestine (70). It is liberated from intestinal mucosa by the presence of chyme (40).

D. Composition of intestinal secretion

Brooks (17) classified the composition of the secretions of the digestive glands into three types: (1) proteins, particularly those that constitute the digestive enzymes; (2) mucopolysaccharides, as in mucus; and (3) solutions of water and electrolytes, as in hydrochloric acid and bicarbonate.

Numbers of workers have been successful in obtaining the composition of intestinal juice in different species by means of Thiry fistula or some other modified device. Dukes (47) recorded the composition as: water 97.59%, protein 0.80%, other organic substances 0.73% and inorganic

salt 0.88%.

The concentration of electrolytes in the intestinal secretions has been investigated in the dog, man and sheep. Davenport (40) and Hendrix and Bayless (70) quoted experiments which showed that the concentration of sodium in duodenum, jejunum, ileum and colon of the dog and man is approximately similar to that of blood plasma. The concentration of potassium is slightly higher than that of plasma. Chloride concentration of duodenal secretion varies from lower to higher concentration relative to plasma chloride, and remains at a higher concentration in jejunal and then declines to slightly lower than that in plasma in ileal and colonic secretions.

A study by Scott (119) as quoted by Phillipson (106) showed that the concentrations of Na, K and Cl in the intestinal secretions of sheep were similar to those obtained in the dog and man. In upper jejunal secretion, the concentrations of Na, K and Cl were found to be 136.0, 8.1 and 134 mEq/l respectively, whereas 135.0, 9.0 and 105.0 mEq/l were the concentrations of these electrolytes in the lower ileum. The concentration of HCO_3^- in the fluid secreted by the upper jejunum of sheep was 11.8 mEq/l and in the lower ileum it was 41.5 mEq/l. In the jejunal secretions of the dog and sheep, the concentration of calcium appears to be a half that of plasma but is similar in concentration to plasma in ileal and colonic secretions (40,106).

The concentrations of Na and K in the intestinal content in ruminants have been investigated by a number of workers. Horrocks and Phillips (71) Rogers and Van't Klooster (115) Mylrea (90) and Perry, et al. (99) found that, in cattle, there is an addition of sodium in the upper intestine. Presumably this sodium is contributed by bile, pancreatic and intestinal gland secretions (115). The concentration of Na in intestinal contents declines progressively along the rest of the intestinal tract (71,70,99).

Similarly, the concentration of K in the intestinal content increases in the first part of the small intestine compared with that in the abomasum and decreases along the rest of the tract (99). On the other hand, it has been shown that the concentration of sodium in digesta of the horse and pig tended to increase going from the jejunum to the ventral colon and then decreased appreciably in the rest of the gut (2).

The concentrations of Na, K and Cl in the duodenal contents of sheep have been reported as 71, 21 and 116 mEq/kg water respectively (21). In the ileum, the concentrations of Na, K and Cl were shown by Bruce et al. (21) and Goodall and Kay (60) to vary from 134 to 137, from 13 to 17 and from 60 to 69 mEq/kg water respectively. Bruce et al.(21) reported faecal concentrations of Na, K and Cl of 41, 116 and 16 mEq/kg water respectively. Scott (119) quoted by Phillipson (106), reported that the concentrations of Na, K and Cl in the supernatant fluid of jejunal contents in sheep were 101, 25 and 140 mEq/l respectively.

V

TRANSPORT ACROSS BIOLOGICAL MEMBRANES

A. Transmembrane concentration and electrical potential differences.

The differences in concentrations between internal and external fluids of a cell reflect the characteristics of its membrane. Cells maintain low internal concentrations of Na^+ and Cl^- differing from that of the environment which has higher concentrations of these electrolytes. In contrast, K^+ concentration is higher in the cell. All electrolytes and ionized substances carry their own electrical charges, i.e. positive or negative. Woodberry (147) stated that there is in existence an electrical potential difference (PD) across the cell membrane, being negative inside the membrane and positive outside. The movement of electrolytes and other ionized substances across the cell membrane is influenced by the electrical forces.

B. Transepithelial concentration and transmural potential difference

In the digestive tract, there is a difference in concentrations of electrolytes between the lumen and plasma analogous to that between a cell and its environment. There is also in existence electrical potential differences between the mucosa and serosa of the intestine, the serosa being electrically positive with respect to the mucosal surface (58,62,106,117).

It has been found in experiments on the rabbit ileum (117) and the human colon (62) that the transmural PD (and the short circuit current) declines with time during the experiment. The cause of this decline is obscure. Schultz and Zalusky (117) suggest that it is not related to the rate of circulation or aeration of the buffer solution or changes in tissue morphology. However, the decline of the transmural PD obviously occurs concomitantly with the absorption. Thus it can be assumed that the decrease in concentration due to the absorption from the mucosal side causes a decrease in the

transmural PD. In the rat colon, there is evidence that as the luminal NaCl concentration increases the PD between the lumen and plasma increases (34). In sheep, the rumen potential is negatively correlated with $[Na^+]$ and positively with $[K^+]$ in the rumen (120).

C. Terminology

"Flux" is a term used to describe the amount of substance transported across unit area in a given unit of time (19).

"Undirectional flux" refers to the one-way passage of electrolyte (or water) from one side of the intestinal mucosa to the other, which occurs concurrently with one-way passage of the same ion in the opposite direction (7).

With regard to the intestinal tract, Berger (7) states that "outflux" is the flow out of the intestinal lumen, i.e. from gut to blood (136), while "influx" refers to the flow into the intestinal lumen, i.e. from blood to gut (136).

"Net flux" is the resultant of influx and outflux (17). When outflux exceeds influx, "absorption" occurs; similarly "secretion" is a net increase in the amount of electrolyte in the lumen resulting from a greater influx than outflux (7).

Code (27) has suggested the terms " insorption" for outflux (efflux*) and "exsorption" for influx. When insorption exceeds exsorption, the net gain to the body is "absorption". "Enterosorption" is the net gain in the opposite direction, i.e. secretion. The entire process, insorption, exsorption, absorption or enterosorption is designated "sorption".

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* In this context, the term "efflux" refers to "outflux".

The mechanism of flux can be classified into two types: passive and active transport. The passive transport is the movement that does not require energy expenditure, e.g. diffusion and osmosis. Active or carrier-mediated transport appears to be dependent upon the expenditure of metabolic energy in the biological system (36), and can proceed against a chemical and/or electrical gradient (33).

D. Passive transport.

Water and small water-soluble particles can pass through the membrane either driven by concentration differences or the latter may be carried by the flow of water (solvent drag) (33). The mode of membrane transport of a substance depends on many factors. Guyton (64) states that the primary factor affecting diffusion of substances is the solubility in the lipoprotein matrix of the membrane. Also the size of the transported substance as it relates to the pore size of the membrane. Guyton further states that negatively charged ions can pass through the pores with the same ease as water molecules. The net diffusion is also influenced by the effect of concentration, electrical and pressure gradients.

"Osmosis" is the net flux of water in the direction of lower concentration through a semipermeable membrane (55). "Osmotic pressure" is the pressure required to stop "osmosis" (64). Florey (55) defined "osmotic concentration" as the total number of nonpermeating particles per unit volume. The difference in osmotic concentrations between the solutions on either side of a semipermeable membrane is referred to as the "osmotic gradient". Differences in concentration of any one chemical between the two compartments separated by a membrane are called "concentration gradients".

Brown (19) stated that the water activity is evaluated by the total

concentration of all particles dissolved in it, rather than by its concentration. The greater the concentration of dissolved particles, the more reduction in water activity.

E. Active transport.

Curran and Schultz (36) state that "active transport" includes all transport processes for which there is no acceptable physical explanation.

The absorption of sugar and amino acids appear to be sodium dependent transport (30,33,35). Csáky (33) states that the movement of all water-soluble substances, which are too large to pass through the pores, is by carrier-mediated transport. There is utilization of the high energy bond of adenosine triphosphate (ATP) in the transport. The particle is bound to the carrier on one side of the membrane, moves through the membrane, and is released from the carrier on the other side. The carrier then returns to its original site (19). Brown (19) states that there are many factors that may influence active transport. Chemicals (drugs) or hormone (aldosterone, etc.) control the rate of active transport. Passive permeability which may be involved at one side of the membrane also influences the rate of active transport. The circulation affects the transport by means of metabolic mechanisms and by maintaining concentrations of the transported particles.

Pinocytosis, as defined by Csáky (33), is the process by which particles are invaginated or engulfed by the membrane surface, similar to phagocytosis. This process is linked directly to energy supplying reactions (19).

F. The transport mechanisms for Na, K, Cl and water across the small intestine epithelium.

The mechanism of Na^+ transport is believed to be an active process. A concept of the "carrier" has been proposed to explain the active transport

mechanism of sodium ion. Crane (30) postulated that the 'carrier' possesses a specific binding site for substrates (sugar) and the second specific binding site for Na^+, and that the binding of Na^+ to this site is essential for the activity of the carrier to equilibrate the substrates across the brush border membrane.

There is a controversy on the mechanism of K^+ transport. Clarkson and Rothstein (26) suggested that K^+ moves across the small intestinal mucosa in the direction of electrochemical gradient. Gilman et al. (58), on the contrary, showed net movement of K^+ taking place against the concentration gradient.

Similarly, the mechanism of Cl^- transport has not been established. A study by Tidball (130) indicated that the chloride movement occurs against its electrochemical concentration gradient. However, many authors have found evidence of passive mechanism of Cl^- movement (34,37,95). Furthermore, Parsons (96) suggested that the movements of sodium and of chloride are independent of each other to a certain extent and cannot entirely be tightly coupled.

Two possible mechanisms of water absorption have been proposed, namely active and passive processes. The first postulation is that the mucosa contains anatomical structures capable of moving water. This theory emphasizes the possible role of pinocytosis (40). Studies conducted by Curran and his associates (34,37) indicate the passive mechanism of water transport. Moreover, the water movement in the small intestine appears to be closely coupled to solute (Na^+) absorption (95,38).

VI

METHODS TO STUDY THE INTESTINAL TRANSPORT OF ELECTROLYTES AND WATER

The method for the investigation of intestinal transport of substances was described in detail recently by Parsons (97). He classified the method into two major types, namely, in vitro and in vivo. Many techniques have been applied to these studies. Only the in vivo methods will be discussed here.

Techniques that have been employed for studying the intestinal transport or the absorption of electrolytes and water are, for example, intubation, chronic fistula, isolated intestinal segments, etc. (43,116,144)

The intubation technique has been used extensively in human experiments (43,144). The standard perfusion tube, as described by Whalen et al.(144), is made of polyvinyl material with inner diameter 1.6 to 1.8 mm. The tube has 3 lumina: one ending in the proximal (P) and another in the distal (D) aspiration tip, located 15 and 45 cm distal to the infusion tip (I), respectively. The segment of intestine between I and P is termed the "mixing segment", between P and D the "study segment". The test solution is delivered by a constant rate infusion pump. After an equilibration period, intestinal contents are sampled from P and D by aspiration. In this technique, the subject is usually fasted before study. The purpose of this technique seems to be to study the absorption of electrolytes and water from the infusing solutions rather than the absorption of such substances from the intestinal contents.

The transport of Na and K across the intestinal mucosa of the dog has been studied by means of chronic "Thiry"fistula (8,9). Berger et al. (8,9), claimed that the rates of sodium and potassium fluxes measured in experiments repeated over several years were not significantly different

from those conducted during a single day.

The "Thiry-Vella" loop which produces a segment of intestine with two fistulas has been used extensively in studies on intestinal absorption. Studies on absorption in dogs using this technique have been conducted both in anaesthetized (28,135) and in conscious animals (3,4,8,9,28,136). The possible effects of anaesthesia on absorption have been investigated. Code et al. (28) found that anaesthesia with pentobarbital sodium did not affect the rates of insorption or exsorption of either water or sodium by the ileum. In the duodenum, insorption of both water and sodium also was unaffected, but the exsorption of both substance was decreased. These authors did not have a satisfactory estimate of the physiological significance of the effect of anaesthesia on the reduction of the rates of exsorption of sodium and water into the duodenum. However, it may be supposed that during anaesthesia, there is decreased intestinal blood flow due to a reduction in cardiac output. The duodenum is the major site for Na (and water) secretion. Thus, there might have been a reduction in activity of intestinal glands which eventually brought about the decreases in Na and water exsorption.

It has been suggested that a major disadvantage of the Thiry-Vella loop is because it is permanently isolated from the main part of the digestive tract and consequently may atrophy from disuse (108). Because of this, some studies on absorption from the ruminant intestine have utilized a temporary Thiry-Vella loop formed between two pairs of re-entrant cannulae implanted in the intestine. The double re-entrant cannulae provide that the digesta flow through the loop of intestine except when isolated for an experiment. Ash (4) designed and described the

manufacture of intestinal re-entrant cannulae for use in sheep. Goodall and Kay (60) employed the re-entrant cannulae for the study of digestion and absorption in the large intestine of sheep. Also, Phillipson and Storry (108) used two pairs of re-entrant cannulae in a study of calcium and magnesium absorptions from the small intestine of sheep. However, there are disadvantages of the re-entrant cannula system. Goodall and Kay (60) observed that the main difficulty was the occasional blockage of digesta in the cannulae. When this happened the sheep soon stopped eating, but after the blockage was removed the sheep recovered their appetites within a few hours. Another difficulty was that the sheep lay on the side in which the cannulae had been established, and so had to be dissuaded from doing so during the digesta sampling. These disadvantages have also been observed in the present study. However, it was observed that the sheep resumed eating immediately after the removal of the blockage.

Two other techniques have been used to study absorption from the G.I. tract of ruminants. The first of these is the cannulation of different sites of the G.I. tract. The absorption of substances is calculated from differences in concentrations of substances in samples taken from two successive cannulae usually in reference to a non-absorbable marker. Rogers and Van't Klooster (115) used this technique to study the absorption of minerals from the alimentary tract of dairy cows.

The second method for studying the absorption from the ruminant alimentary tract has been to use the distribution of the concentration of minerals in digesta at various points along the tract. Horrocks and Phillips (71) and Perry et al. (99) studied the absorption of minerals from the alimentary tract of cattle using this technique.

In addition, absorption of solutes may be inferred from changes in the freezing point depression of the intestinal contents. Decreases in freezing point depression of digesta from along the digestive tract might indicate the absorption of osmotic solutes or secretion of water. Such studies have been undertaken in cattle (101,112,133). For studying the absorption of water, a non-absorbable indicator such as phenol red, haemoglobin or polyethelene glycol (PEG) can be used. These materials have been used to serve as a reference substance for the determination of intestinal water absorption in the human and the rat (34,144).

The application of radioisotope in studying the bidirectional fluxes of electrolytes across the intestinal mucosa.

In the intestine, there are simultaneous movements of ions and water, into and out of, the lumen and the blood. The absolute rate of movement of such substances across the intestinal mucosa in either direction cannot be studied unless the rate in the opposite direction becomes zero. The availability of isotopes makes it possible to study absolute rates of the movement in either direction by using an isotope of the element being studied. Such an isotope is generally mixed in the perfusion fluid and it is assumed that the return of isotope is zero during a short term experiment. Visscher and his colleagues (136,137) pioneered the use of radioactive sodium, chloride and deuterium in studies on bidirectional fluxes across the intestinal wall in dogs. Several other groups of workers in the following decades have used isotopes in the study of fluxes across the intestinal mucosa of the dog (8,9,27,63,135). Reports on the use of isotopes to study the fluxes across the intestinal wall in ruminants have not been found.

VII

ABSORPTION OF Na, K, Cl AND WATER FROM THE SMALL INTESTINE

A. Factors that influence absorption

1. The epithelial permeability. The epithelial permeability of intestinal mucosa depends on the presence of villi, number and sizes of pores and size of particles relative to the pore size. The villi decrease in density from jejunum to ileum with a corresponding decrease in surface area. The jejunal pores are fewer and longer in length than those in the ileum. A mean pore diameter of 8 A°ngstroms (A°) in jejunum suggests high permeability to water which has a diameter of 3 A° and to K^+ and Cl^- with diameters of about 3.5 A°. Sodium ion with a diameter of 5.5 A° will be moderately permeable. In contrast, the ileum has pore size of 3 to 4 A° which presumably limits the efficient absorption of all substances except water (64).

2. The pH of the intestinal contents. At the anterior part of the duodenum, the contents are of lower pH due to gastric acid secretion. In the rest of the intestine the acidity is neutralized by the bicarbonate in pancreatic, bile and intestinal secretions. McHardy and Parsons (83) found that the absorption rates of water and sodium in the jejunum and ileum of the rat were greater from alkaline than from acid solutions, over the pH range 4.4 to 7.2. The results abtained by Code et al. (28) agree with those of McHardy and Parsons (83).

3. Osmolality of the luminal contents. This effect will be discussed separately in the particular sections on electrolyte and water absorption.

4. Blood supply. The transported substances must be carried away from the intestinal mucosa into the blood otherwise there will be a reduction in absorption due to the back diffusion gradients. Therefore, an efficient absorption requires a high rate of intestinal blood flow (20).

5. Flow rate of the digesta. The flow rate of the digesta may affect absorption rate. It appears that an increase in flow rate increases both intestinal volume and absorptive capacity (79). Love et al. (79) suggest that the increase in absorptive capacity may be explained by: (1) an increase in absorptive area due to stretching of the bowel; (2) an increase in absorptive area due to some contribution of a rising hydrostatic pressure in the lumen; and (3) an increase in absorptive area due to a more near approximation to continuous flow. In addition, Devroede and Phillips (43) suggested that the greater the quantity of substances being passed through, the more the availability for absorption; and that the increase in volume provides more intimate contact between solutions and absorptive area.

B. Absorption of sodium and chloride

Sodium transport is interdependent with the transport of sugars and amino acids (30,34,35,39,54,59,65,69,95,116,118).

The previous results obtained by Visscher and his colleagues (138,139), by placing autogenous serum and isotonic solutions of NaCl and Na_2SO_4 in equiosmotic proportion in the ileal segements of the dogs, showed that there was a developing hypotonicity of original isotonic solutions. Such a phenonenon indicates the absorption of Na and Cl.

Evidence has been found that the absorptions of Na and Cl depend on the osmolality in the intestinal lumen. Vaughan (135) Grim (63) and

Annegers (3) found, in the dog, an increase in Na and Cl absorption rates as the luminal concentrations of Na and Cl are increased. In the dog, absorption can occur from a luminal Na concentration of 32 mEq/l. At the luminal Na concentration of 80 mEq/l the absorption rate is about 50 μEq/min, while at a concentration of 220 mEq/l the absorption rate increases to 200 μEq/min (135). An increase in chloride absorption rate, on the other hand, was found to take place as the NaCl solution in the lumen was increased to 1.8% (3).

A few studies with the perfusion technique have been made in human (43,79,144). Love et al. (79) and Devroede and Phillips (43) found increased sodium and chloride absorption with increasing rates of perfusion.

In ruminants the pattern of Na secretion into the duodenum and net absorption of Na (and K) in the rest of the small intestine appears to be qualitatively similar to that shown in non-ruminants (122). Quarterman, et al. (112) and Van Weerden (133) showed, in cattle, the decreasing osmolality of the contents along the intestinal tract indicating that there was an absorption of electrolytes along the tract. The progressive decrease in Na concentration of the contents along the intestinal tract, to levels lower than that in plasma, leads to the conclusion of active absorption of Na from the gut (71,99).

C. Absorption of potassium

Very little is known about the absorption of K from the small intestine. Smith (123) claimed that K is absorbed from the ruminant small intestine, except the duodenum. Perry et al. (99) estimated that an average of 36 gm of K was absorbed from the calf small intestine daily. This group of workers further showed progressively decreasing concentrations

of K in intestinal contents from the duodenum through to the ileum, which suggests the absorption of K in these regions. In addition, Mylrea (90) suggested that K is nearly completely absorbed (90 to 97%) from the small intestine.

D. Absorption of water.

Several lines of study have demonstrated the dependency of water absorption on the luminal Na and Cl concentrations (3,26,38,63,83,135). In the dog small intestine, the absorption of water was found to decrease in approximately linear fashion with increase in the luminal concentrations of Na and Cl (3,63,135). The absorption was found to be greatest with hypotonic fluid in the lumen. It is evident that the absorption of Na and Cl in the small intestine depend on the osmolality of the lumen (3,63,135,137). Therefore, the absorption of water appears to be related to the absorption of solutes (3,35,96,98). Furthermore, there is a linear relationship between water and solute movement (26).

In ruminants, the amount of water remaining with the digesta at the ileum is usually related to the amounts of Na and K remaining at this site (122). This observation also suggests a close relationship between water and solute absorptions. It is further suggested that most of the water absorption from the ruminant small intestine occurs secondarily to the absorption of osmotically active solutes (123).

VIII

UNIDIRECTIONAL FLUXES OF Na, K, Cl AND WATER ACROSS THE INTESTINAL MUCOSA.

Extensive investigations of unidirectional fluxes of monovalent electrolytes and water across the intestinal mucosa have been carried out in the rat (34,37,38,86) the dog (4,8,9,28,63,135,138,139) and in the human (6,48,62,79). There is very little information on unidirectional fluxes of these electrolytes and water in ruminants.

A. Unidirectional fluxes of Na^+

The studies of Na flux in rat ileum and colon by Curran and his colleagues (37,38) indicated that the Na efflux (lumen to plasma) is a linear function of NaCl concentration in the lumen. Vaughan (135) also found a similar effect of Na^+ concentration in the lumen on the rate of Na^+ efflux from the dog small intestine. The influx of Na^+ appears to depend on Na^+ concentration in the lumen and is not entirely the result of simple diffusion from a reservoir of constant Na concentration (the plasma) (37). However, the regression coefficient of the influx is significantly smaller than that of the efflux for both species.

Code et al. (28) found that the influx (exsorption) of Na^+ into the duodenum of the dog was significantly increased after feeding. They also showed the efflux of Na^+ (insorption) from the duodenal and ileal loops was depressed by acidification of the intestinal contents.

Studies on the dog jejunum by Visscher, et al. (138) indicated that the unidirectional fluxes of Na^+ from NaCl solution were lower in the presence of $MgSO_4$ and $MgCl_2$ than in the presence of Na_2SO_4. Comparing chloride and sulphate solutions, Visscher et al. (137) found that the rate of Na efflux was greater from an isotonic chloride than an isotonic

sulphate solution, although the Na concentration in the gut was higher in the latter case.

In the dog jejunum, Grim (63) and Visscher et al. (136) showed the similar result of the greater rate of efflux to influx. In contrast, Berger et al. (8) found that the rate of influx was greater than that of efflux. Visscher et al. (136) studied the Na^+ flux in a 30-40 cm Thiry-Vella loop whereas Berger et al. (8) used a 20 cm chronic Thiry fistula. Rates of efflux and influx of 580 and 538 μEq/min were obtained by Visscher et al. (136). Those obtained by Berger et al. (8) were 68 for the efflux and 80 μEq/min for the influx.

Love et al. (79) found that the bidirectional fluxes of Na^+ in the entire human intestinal tract varied with rates of perfusion. At perfusion rates of 33, 50 and 84 ml/min, the rates of Na^+ efflux obtained were about 9, 17 and 20 mEq/min respectively. Based on the same perfusion rates, the rates of Na^+ influx were about 8, 15 and 19 mEq/min respectively. Increases in perfusion rate would certainly bring about comparable increase in flow rate. Thus Love and his co-workers (79) showed that as flow rates increase both volume and absorptivecapacity of the intestine increase to reach a plateau at high flow rates.

B. Unidirectional fluxes of K^+

Two groups of investigators have studied the unidirectional fluxes of K^+ in the canine small intestine. The first group is Code and his colleagues (28) who showed the effect of feeding on the unidirectional fluxes of K^+. These workers found that the rate of insorption (efflux) of K^+ from the duodenum significantly increased in the second 15-minute period after feeding, i.e. increased from 1 to 5 mEq/min.

C. Unidirectional fluxes of Cl^-

For the rat ileum and colon, there is evidence that the chloride efflux is a linear function of concentration in the lumen. There is no evidence for the dependence of Cl influx on concentration in the lumen (34,38). Similar results on the dependence of Cl efflux and independence of Cl influx on concentration in the lumen had been shown earlier by Visscher et al. (137). Curran and Solomon (38) suggested that the Cl influx would not be expected to be dependent on concentration in the lumen, since the influx takes place from a reservoir of constant concentration (the plasma).

D. Unidirectional fluxes of water.

In a chronic Thiry fistula of the dog, Berger et al. (8) found that approximately 1 ml of water was transferred into and out of a 20 cm length of intestine per minute. Code et al. (28) showed that for each liter of water insorbed from contents of the dog small intestine to the blood, about 60 mEq of sodium and 2 mEq of potassium were insorbed simultaneously. This might indicate water movement is dependent on sodium transport at least at certain concentrations of the latter, since the study by Visscher et al. (137) showed that in the dog ileum the efflux of water was as high as 2 ml/min from one-third isotonic solution and this rate was more than twice that found with hypertonic solutions. Regarding water influx, Visscher et al. (136) found that the rate of movement was nearly independent of the osmotic pressure in the gut.

IX

EFFECTS OF DIETARY SODIUM AND POTASSIUM ON INTESTINAL ABSORPTION AND ON OSMOTIC COMPOSITION OF BLOOD PLASMA

A. Effects of dietary levels of Na and K on absorption

Severe restriction of dietary sodium in the dog causes a great decrease in sodium concentration in the contents of the terminal ileum (53). This indicates that there is an increase in sodium absorption in the upper G.I. tract during the period of dietary sodium restriction.

Devlin and Roberts (41) showed a reduction in faecal Na of lambs when fed low levels of Na in the diet, and an increase in faecal excretion of Na when dietary Na was increased. In calves, when 0.25 gm Na and 1.2 gm K per 100 gm D.M. were fed, the mean Na to K molar ratios in the proximal duodenum and distal ileum respectively were about 2.2 and 3.2. When the calves were put on to pasture containing about 0.09 gm Na and 2.3 gm K per 100 gm D.M. these rations changed over a period of 1 to 2 weeks to 0.5 and 0.4 respectively (123). The results obtained in lambs and calves suggest that during dietary sodium restriction there is an increase in the absorption of Na from the intestine. The Na:K ratios further suggest that concomitant with the increase in Na absorption, there is an increase in K secretion in order to maintain a constant osmolality of the intestinal contents. On the other hand, as the dietary Na increase, there is a decrease in faecal excretion of K whereas urinary K remains constant (41). The cumulative K balance of the low treatment sheep was significantly less than the sheep receiving the medium and high levels of Na. This might suggest an increase in K absorption from the intestine when high dietary level of Na is fed.

In sheep, it was found that the amount of faecal Na was significantly greater for the group fed with high dietary K than for the group fed medium or low K. It was suggested that poorer absorption of Na occurs with high dietary K (23).

There are no published results on the effects of dietary Na and K on the absorption of these electrolytes, Cl and water from Thiry-Vella loops of the sheep small intestine.

B. Effects of dietary levels of Na and K on blood plasma Na, K, Cl concentrations and haemotocrit.

It has been found, in sheep, that variation in potassium levels in the diet did not significantly affect plasma Na concentration (23,46), but that high dietary K significantly decreased plasma chloride in lambs and steers (41,46,125).

Thelevel of K in the diet has been found to affect serum K level. In animals fed with high K, the $[K^+]$ in plasma was high (128); and plasma K was low in animals receiving low level of K in the diet (111,125,128).

Driedger (46) found an increase in plasma Na concentration in lambs receiving low K and high Na. An increase in haematocrit value was found in sheep and lactating cows receiving low K (111,128).

A level of 1.2% NaCl in drinking water was found to increase serum K and Na in cattle (141). In sheep, on the other hand, Meyer and Weir (85) found that high NaCl intake (up to 13.1% in the feed) did not affect blood haemotocrits or serum Na, but an increase in serum chloride was observed.

MATERIALS AND METHODS

EXPERIMENT I

In this study the sheep were fed on a continuous feeding device at about 1300 gm of feed consumed per 24 hours. The study was conducted by perfusing solutions into the proximal end of a jejunal Thiry-Vella loop, and collection of the loop effluents at the distal end of the loop. Three different rates of perfusion were used. The concentrations of Na and K in the perfusate were such as to simulate the concentrations of Na and K in the intestinal contents collected from the individual ewe on the day previous to an experimental day. The main objectives of this experiment were to study the effects of levels of dietary Na and K, and of the rates of perfusion on the transport of Na, K, Cl and water in the jejunum.

The purpose of the use of the continuous feeder.

Circadian rhythms have been observed in ruminants as well as in many other species. Gordon and McAllister (61) postulated that the diurnal variation is directly caused by behaviour and indirectly by feeding time. Ibrahim (72) indicated that the continuous feeding system resulted in a constant rate fermentation system in the rumen, and a uniformity in composition of rumen contents. From this evidence, it was assumed that fluctuations in the rate of flow and composition of the digesta presented to the successive parts of the alimentary tract would also be reduced by the continuous feeding regime. Thus variation in fluxes of electrolytes and water across the intestine might also be reduced.

Animals.

Three mature cross breed ewes, weighing about 60 kg, were used in

the experiment. Two re-entrant fistulae were fitted per animal, so as to provide an isolated loop of jejunum when required. The first re-entrant fistula was established by placing a pair of cannulae at the beginning of jejunum and the second was placed about 250 cm aborally to the first. A similar preparation has been described by Phillipson and Storry (108). The cannulae used in the experiment were described by Ash (5). The sheep were operated on at least 2 months prior to the start of the experiments.

The animals were trained to a continuous feeding regime on an automatic feeder for at least three weeks. Chopped alfalfa- bromegrass hay, to which a mineral mixture was added, was fed during this period. During this time they were trained to sample collection procedures and a preliminary study was undertaken. The purpose of the preliminary study was to try to determine the duration of perfusion required to establish a steady state of flow of perfusion through the loop.

Water was given ad lib., and the amount consumed daily was recorded. Wood shavings were used for bedding.

Diets.

The experiments comprised two dietary periods. In dietary period A (period A), the basal diet was supplemented with Na and K at the nominal levels of 0.5% and 0.8% respectively of the air-dry ration. In dietary period B (period B), Na and K were added at 0.5 and 0.4% respectively to the basal diet. The level of Na in the diet and the variation in dietary level of K were established on the assumption that at these levels the physiological functions of the body could be maintained normally. The establishment of these dietary levels of Na and K were based on results obtained by Campbell (23) Driedger (46) and the N.R.C. requirements (91).

TABLE 1

FORMULATION FOR THE BASAL DIET

Ingredient	Percentage
Ground barley straw	59.7
Dried brewers grains	30.0
Corn starch	6.0
Sugar	2.0
Vegetable oil	2.0
Minerals*	0.3

* Mineral mixture (expressed as kg per 1000 kg of feed):
Calcium phosphate (commercial), 3.24 kg; Cobalt chloride, 0.28554 gm; and potassium iodide, 0.41290 gm.
Vitamin supplements per 1000 kg of feed: Vitamin A, 1,000,000 I.U.; Vitamin D, 250,000 I.U.

The diet was given in the form of pellets. The main components of the pellets were barley straw and brewers grains. The straw had been previously soaked and washed with water to leach out the minerals. After leaching, the straw contained 12.5% protein, 0.02% and 0.08% of Na and K respectively.

The levels of Na and K in the basal diet were 12 and 28 mEq/kg air-dry diet respectively.

Mineral supplements

Mineral supplements to give the desired levels of Na and K were supplied in the form of reagent grade chemicals. The mixtures of salts used (Table 2) were designed to supply the desired levels of Na and K and to supply a constant level of chloride (Table 3). It was realized that the necessary variations in amounts of metabolisable anions supplied in order to achieve these requirements would result in variations in the acid-base turnover by the sheep. However, in normal diets the variations in potassium levels encountered also occur in conjunction with similar variations in organic anions.

The mixture of supplemental sodium and potassium was spread evenly on the column of pellets on the conveyor of the automatic feeder. Daily feed intake and water consumption were recorded. The weight of each sheep before and after each experimental period was also recorded.

Adjustment periods.

The length of time required for the adjustment period was based on a study on steers by Thorlacius (129) who found that the majority of the physiological responses had occurred by 8 days after changes in dietary Na and K from one level to another. Thus the adjustment period in the present study was for 10 days prior to experimental period A and for

TABLE 2

SUPPLEMENTAL SALTS IN MILLIMOLES/DAY

BASED ON FEED INTAKE OF 1300 gm/DAY

Period	Potassium Chloride	Sodium Bicarbonate	Sodium Acetate	Sodium Citrate	Potassium Bicarbonate	Potassium Acetate	Potassium Citrate
A	110	97	97	97	49.7	49.7	49.7
B	110	97	97	97	1.7	1.7	1.7

TABLE 3

CALCULATED DAILY ANION AND CATION INTAKES (mEQ) BASED

ON FEED CONSUMPTION OF 1300 gm/DAY.

Period	Sodium	Potassium	Chloride	Bicarbonate, Acetate and Citrate
A	303	287	110	481
B	303	143	110	337

9 days prior to experimental period B. During these periods the sheep were fed with the particular experimental diets.

Experimental procedures.

On the day before a perfusion experiment, intestinal loop contents were sampled and analysed for Na and K concentration. The composition of the perfusate solution was designed to simulate the concentrations of Na and K in the sample of intestinal content. NaCl and $KHCO_3$ were used to supply the Na and K in the perfusate. Polyethelene glycol 4000 (PEG) at 1 mg/ml was added to the perfusate as a non-absorbable marker.

A. Rates of perfusion

The study was composed of 3 different nominal rates of perfusion, namely 7, 13 and 26 ml/min.

The studies by Singleton (121) Harris and Phillipson (66) and Phillips and Dyck (104) suggested that, in sheep, the flow rate of digesta from abomasum into the duodenum varied from about 300 to 1200 ml/hr. In the present experiments, the nominal perfusion rate of 13 ml/min was based on the average flow rate obtained by these workers, and on the fact that there is an increased volume added by the secretions of bile, pancreatic juice and succus entericus (intestinal glands). The lower and higher rates of perfusion were used in order to study the effects of variation in perfusion rate on electrolyte and water transport.

B. Experimental schedule

The rates of perfusion of 7, 13 and 26 ml/min were designated as "low", "medium" and "high" respectively. Each sheep received perfusions on 3 occasions at 4 day intervals. One hour was required for the establishment of the steady state of the loop just prior to commencement

of a one-hour perfusion study. The perfusion study was undertaken by the collection of the effluent from the loop at various time intervals. Since the perfusion studies on each day were of long duration, variations of the sequences of applying the different perfusion rates were used to minimize the possibility of systematic errors.

In addition, varying the sequence of application of different perfusion rates allowed investigation of possible carry-over effects from one perfusion rate to the subsequent ones within the daily experimental schedules used. The sequences of perfusion rates used were:

Day	Sequences of perfusion rates
1	Low, medium and high
2	Medium, high and low
3	High, low and medium

The collections of effluent from the loop were made into "Whirl-pak" bags and stored at 7° C until analysed. Similar schedules for effluent collection were used on every perfusion day. A typical protocol was:

Perfusion rate	Time (Min)	Treatment and effluent collection
"Low"	0 to 60	The establishment of the steady state of the loop.
		Collections of effluent were made at 15 minute intervals.
	60th	2 ml of ^{22}Na-T1824 solution was rapidly injected into the proximal end of the loop while perfusion continued.
	60 to 120	Collections of effluent were made. This period was designated "perfusion study".

Perfusion rate	Time (Min)	Treatment and effluent collection
	60 to 75	Effluent was collected at 2 to 3 minute intervals. In practice, the volume collected sometimes was insufficient therefore the time interval was occasionally extended.
	75 to 90	Collections were made at 5 minute intervals.
	90 to120	Collections were made at 10 minute intervals.
"Medium"	120 to 180	The establishment of steady state for the "medium" rate. Samples were collected at 15 minute intervals i.e. as for 0 to 60 min.
	180 to 240	Medium rate perfusion study. Collection schedule similar to that during 60 to 120 minutes.
"High"	240 to 300	Similar to that during 0 to 60 min.
	300 to 360	Similar to that during 60 to 120 min.

C. Technique of perfusion

Prior to the perfusion experiment, the two pairs of cannulae were disconnected. The first cannula was joined with the fourth so that the digesta flow was by-passed into the distal part of the intestinal tract. The second cannula was used for perfusion, and the effluent was collected from the third; i.e. the gut between the second and the third cannula was a Thiry-Vella loop.

A Y-tube arrangement was fitted to the proximal cannula of the loop, so that rapid and quantitative addition of the marker solutions could be achieved without any change in the continuous administration of perfusate.

Part of an intravenous infusion set-up was adapted for this purpose.

The perfusate was continuously pumped from a reservoir by one or two "Buchler" micropumps, model #2-6000 at the required rates. The perfusate reservoir was maintained at a constant temperature close to the body temperature.

D. Preparation of radioisotope solution.

A batch of 0.2 millicurie (mCi) of ^{22}NaCl in 1 ml of aqueous solution was obtained in May 1970. Working and standard solutions of 0.1 μCi/ml were made by step-wise dilutions with distilled water.

E. Preparation of ^{22}Na and T1824 solutions.

The dual purpose for the use of T1824 (Evans Blue) was to use the dye directly as a marker to measure mean transit time, and also to act as a readily visible indicator of the appearance of the marker dose of ^{22}Na in the effluent solution. Five boluses of marker doses of 2 ml of mixed solution of ^{22}Na and T1824 were prepared in 2.5 ml syringe at the same time on the day before a perfusion study. Two of these were used as counting standards and the others for injections for the perfusion studies. The mixed solution consisted of 1 ml of ^{22}Na (0.1 μCi), 0.1 ml of 1 mg/100 ml T1824 and 0.9 ml of the perfusate solution

F. Catheterization and blood collection

Jugular catheterization was made at the beginning of each perfusion experiment. The catheterization technique of jugular vein for blood collection described by Phillips (102) was applied in this experiment. About 10 ml of blood were collected about 5 minutes before each introduction of ^{22}Na-T1824 solution, and at the end of each perfusion study. Blood was withdrawn into a syringe containing 0.1 ml of 1000 units/ml

of heparin-ammonium in dead space. Part of the first blood sample of each perfusion experiment was analysed for PCV and haemoglobin within 15 minutes, and was then centrifuged for plasma. Plasma samples were kept at -18° C until analysis.

ANALYTICAL TECHNIQUES

Effluents. The various successive samples of effluent from the distal end of the loop collected after an introduction of ^{22}Na-T1824 solution were weighed and the volumes recovered were recorded. Analyses for the recoveries of ^{22}Na and T1824 were made from these samples. Fifty percent aliquots of each of these samples were taken to make 10 min pooled-samples. The 10 min pooled-samples were analysed for Na, K, and PEG concentrations, and osmolality.

Plasma. Plasma samples were analysed for ^{22}Na activity, Na, K and Cl concentrations, and osmolality.

Radioactive sodium analysis

An Analyzer/Scaler model 8725 and a Well Scintillation Detector model DS-202(V) were used for the ^{22}Na analysis. The Analyzer/Scaler was calibrated before using. The window width was set at 10 per cent of the full scale energy of 200 kev, the base at 1, the window at 2, and the wide differential was operated for analysis. The operation potential of 792 volts with 2 ml of sample was selected by its maximal count production. The efficiency of counting for these conditions was approximately 18%.

Prior to the analysis, the Analyzer/Scaler was run for at least 1 hour and the background counted for 30 minutes. The background, counts per minute (cpm) was set on the background channel for automatic subtraction during analyses of the standard and unknown. Standards or unknowns were analysed by placing the cuvette into the detector for 5 minutes. The average count of cpm/ml was taken.

The total counts of each sample of effluent was obtained by the product of cpm/ml and volume recovered. Decay was neglected as standards and

unknowns for each experiment were counted within a few hours.

References to amounts and concentration of ^{22}Na actually mean cpm or cpm/volume and not the radionuclide per se.

Analytical method for fluxes of electrolytes

The calculation of transit time, unidirectional fluxes and net flux of electrolytes as described by Love et al. (79) were adopted in the present experiment.

Transit time (tt) for a bolus of marker does of ^{22}Na and T1824 passing through the intestinal loop was determined from:

$$tt = \frac{\Sigma Qr\bar{t}_1}{\Sigma Qr}$$

whereas $\bar{t}_1$ is the time from introduction of a bolus of marker dose to the midpoint of sample collection and Qr is the total quantity of ^{22}Na recovered.

The average flow rate ($\bar{F}$) of the perfusate passing through the loop was calculated from the mean of inflow (F_i) and outflow (F_o) volumes.

The product of tt and $\bar{F}$ gives the intestinal volume or capacity of the loop (I.V.).

The mean concentration of Na in the loop contents, [$\bar{Na}$], was calculated as the mean of the sodium concentrations in the perfusate and in the respective effluent.

The efflux of ^{22}Na, λ , was calculated from the fraction of the dose of ^{22}Na recovered and the tt,

$$Qt = Q_a e^{-\lambda tt}$$

This formula is re-written as

$$\lambda = \frac{1}{tt} \ln \frac{Qa}{Qt}$$

where Qa is the dose of ^{22}Na introduced, and Qt is the quantity of ^{22}Na recovered in the effluent.

The values for λ can be expressed as per cent per minute of the total ^{22}Na activity introduced into the loop being transferred from lumen to plasma.

The efflux of normal sodium (uEq/min) was obtained from the product of the amount of sodium in the loop and λ .

Thus, efflux of sodium can be written in a formula as

$$\text{Sodium efflux} = \bar{F} \times tt \times [\bar{Na}] \times \lambda$$

Net flux of sodium was calculated from the differences between the quantity of sodium introduced into the loop and the amount in the respective effluent. The data for the whole 60 min of the perfusion study were used.

Sodium influx was calculated from the difference of efflux and net flux.

Analytical method for water absorption

Water absorption was calculated from the differences between F_i and F_o volumes (direct measurement) and by polyethylene glycol 4000 (PEG) technique.

The concentrations of PEG recovered in the effluent fluid were analysed on a "Coleman Nepho-Colorimeter" Model 9. A Blank filter was used in the determination. The procedures of solution preparation and PEG analysis described by Dyck (49), were adapted with some techniques modified. A calibration standard curve of percentrge of light transmittance was constructed using concentrations of PEG varying from zero to 0.2 mg/ml. The light transmittance readings for experimental samples were interpolated on the standard curve to give PEG concentration. Increase in PEG concentra-

tion in the effluent compared with the perfusate indicates the absorption of water.

Rate of water absorption was calculated as:

(1) Percent of water recovery in the effluent

$$= \frac{\text{concentration of PEG in the perfusate}}{\text{concentration of PEG in the effluent}} X\ 100$$

(2) Quantity of water recovered during a given time period (F_o)

$$= \frac{\text{\% of water recovery X quantity of water perfused } (F_i)}{100}$$

(3) Thus, water absorption rate (for given time period)

$$= F_i - F_o$$

The analysis for T1824

The recovery of dye was determined photometrically using a Coleman-Model 9, Nepho-Colorimeter, in which a #8-212 monochromatic filter transmitting light of 550 to 560 mu was used. The recovery of the dye in the effluent samples was determined from percentage of light transmittance using the effluent collected before introduction of dye as blank.

Sodium and potassium analyses

An "EEL" flame photometer was used for the analyses of Na and K. Corrections for mutual interference were made by using standard curves which had been determined previously.

A. Preparations of standard solutions and standard curves

The stock standard solutions at 200 mEq/1 of Na and of K were prepared in de-ionized water using reagent grade chloride salts.

The reagents were dried at 100° C in an oven overnight and in a dessicator for 24 hours before weighing. The stock solutions were kept in plastic bottles with tight fitting screw caps and stored at 7° C.

"Working solutions" of 200 µEq/l were prepared from the stock solutions. Mixtures of these working solutions with addition of distilled water were used to prepare intermediate standards from which the mutual interference standard curves were constructed. Na:K ratios used were 1:10, 1:4, 1:1, 4:1 and 10:1. Ten different concentrations varying from 20 to 200 µEq/l were used for each ratio. Full scale deflection on the photometer was set using 200 µEq/l solution for both Na and K.

B. Dilution of samples

Effluents. The dilutions for Na analysis mainly were 1:1000, but some of them were 1:1250 and 1:1500. The dilution for K were 1:150 and 1:200, depending on the concentration in the effluent.

Plasma. The dilutions were 1:1000 and 1:1050 for Na and 1:50 for K.

Chloride analysis

A "Buchler-Cotlove" chloridometer was used for chloride analysis.

Osmolality

The sample osmolality was determined on an "Advanced osmometer". Two-step calibration adjustment was made prior to the sample determination according to the method described by the manufacturer (1).

Haemoglobin

A Coleman '25' photohaemoglobinometer was used on oxyhaemoglobin method for haemoglobin analysis. Each blood sample was photoelectrically compared with a sealed standard of fixed and exactly known haemoglobin content. Therefore, each measurement was referred to a single 'reference standard'

and expressed in grams of haemoglobin per 100 grams of blood (gm %).

Packed cell volume

An 'International' micro-haematocrit centrifuge was employed for packed cell volume determination. The 79 mm. capillary tubes filled with blood and sealed at one end, were centrifuged at 12,000 rpm for 5 minutes. The tubes were then read in the 'International' capillary tube reader.

EXPERIMENT II

The objectives of this experiment were to investigate the variations on intestinal transport of electrolytes and water in consecutive 30 minute periodsand from day to day. In this experiment the sheep was maintained in a "steady state" for 4 days. Doses of injected ^{22}Na used were 100 times higher than those used in experiment I, and longer preliminary perfusion equilibration periods were used. Only one dose of ^{22}Na/day was used, but perfusion and effluent collections were conducted for two hours to study net Na, K, Cl and H_2O fluxes.

Only the experimental procedures which differed from experiment I are described below.

Animal

Sheep #2 of the first experiment was re-used, started at weight of 60 kg. The animal was trained to a continuous feeding regime for one month prior to the experiment. Chopped alfalfa-bromegrass hay to which a mineral mixture was added was fed during this period.

Diets

In this experiment, the animal was fed with chopped alfalfa-bromegrass hay, 1300 gm a day. The composition of the diet obtained by analysis is shown in table 4.

Adjustment period

The adjustment period was for 12 days. During this time the animal was trained to sample collection procedure and a preliminary study was undertaken to determine the time required for the establishment of a steady state for perfusion of the intestinal loop. This was found to be about 1½ hours.

In order to study the digesta composition for this experiment, intestinal samples were taken from the second and the third cannulae for Na and K analyses at 800, 1200 and 1600 hours on days 7 to 11. The average of Na and K concentrations in duplicate analysis of samples for each collection were used.

Experimental period

The study was of 4 days duration. The perfusate solution was formulated to contain Na and K concentrations the same as the average concentrations of these electrolytes in samples collected from the second cannula on the day prior to perfusion study. The rate of perfusion was 13 ml/min.

TABLE 4

COMPOSITION OF THE DIET

Ingredient	Percentage
Protein	18.06
Ca	1.38
P	0.22
Na	0.03
K	2.30
Cl	not significant.

Experimental procedure

On each experimental day, the study was performed for 3½ hours. One and a half hours were occupied in the establishment of a steady state of the loop and two hours for the perfusion study.

At 90th min, the ^{22}Na-T1824 solution was injected into the loop. The collection protocol during the perfusion study was similar to that in the first experiment.

Blood collections were made 5 min before the introduction of ^{22}Na-T1824 solution, and two successive samples were taken at the end of the first and the second hours of the perfusion study period.

The application of radioisotope

The activity of ^{22}Na used in this experiment (10 μCi/ml of ^{22}NaCl solution) was 100 times greater than that used in the first experiment. Only one introduction was performed on each experimental day. The increase in activity of ^{22}Na in this experiment was to eliminate the counting error that might have been caused by the low activity of ^{22}Na recovered in the effluent samples in experiment 1 and to investigate the activity of ^{22}Na in the blood plasma. The activity of the duplicate standard ^{22}Na solutions were 1 μCi/ml.

Analytical analysis

^{22}Na activity. The operating potential of 819 volts was used in this experiment. The length of time required for unknown and standard analyses was 2 min.

Analysis of T1824. A standard curve was constructed from various concentration of T1824 added into the pooled fluid of effluent samples collected before the introduction of ^{22}Na-T1824 solution. The different

concentration of T1824 (μg/ml) were read as optical density of the Nepho-Colorimeter. The recovery of dye in the effluent was calculated from interpolation of the standard curve.

The analyses for Na, K, Cl, water and osmolality of the effluent were done on samples which were pooled at 13, 20, 30, 40 min intervals and so forth by taking 25% aliquots from each fractional sample.

Dilution of sample for Na and K analyses

Two sets of "Fisher" diluter, models 240 and 250, were employed to dilute the samples. The effluent samples were diluted 1:1000 for Na and 1:400 for K. Plasma samples were diluted 1:50 and 1:1000 for K and Na respectively.

Statistical analysis

Statistical methods used were analysis of variance and Duncan's multiple range test as described by Steel and Torrie (126) Programma 101, codes 6.10 and 6.11 (145) were used for the calculation of analysis of variance. Orthogonal comparisons by partitioning the sum of squares and linear, quadratic and cubic of orthogonal components in regression described by Snedecor and Cochran (124) were also used. F values exceeding the tabulated value for the five percent level of probability were taken as significant.

RESULTS

EXPERIMENT I

A particular difficulty that arose in this experiment was the decrease in feed intake which led to the decreases in sodium and potassium intakes. Based on the record of the daily feed intake the average of daily sodium and potassium intakes were 150 and 142 mEq respectively in dietary period A. However, these levels are higher than the N.R.C. requirements of 83 mEq/day for Na (91) and 102 mEq/day suggested by Campbell for K (23). In dietary period B, the average of sodium and potassium intakes were 43 and 23 mEq per day respectively which are very much lower than the avove levels. Therefore, the results of this experiment were obtained from high sodium and high potassium in dietary period A and from low of both in period B. The factor that caused the decrease in feed intake is not known, but it might be due to the "unpalatability" of the diets in which the main ingredient was the leached straw. Besides the minerals, the "flavour" of the straw might have been leached out during soaking and washing.

The concentrations of Na^+ and K^+ in the jejunal digesta

The concentrations of Na^+ and K^+ in samples of the jejunal contents collected on the day prior to the perfusion experiments were quite variable among sheep and among days of collection, except those from sheep III which had quite constant concentrations (Appendix A - Table 2). The average concentrations of these electrolytes are shown in Table 5.

The osmolality of the perfusate solutions

The mean values of the osmolality of perfusate solutions which had been prepared according to the concentrations of Na^+ and K^+ in the jejunal contents were 219.7 and 250.0 mOsm/kg H_2O for period A and B respectively.

Flow rates ($\bar{F}$)

Flow rates were calculated as the arithmetic mean of the respective perfusion (F_i) and effluent volumes (F_o) per minute. Flow rates reflect mean perfusion rates (Table 6). The flow rates were slightly less than the perfusion rates indicating net absorption of water (see below).

Transit time (tt)

In analysis of effluent for ^{22}Na, the major concentrations of ^{22}Na recovered were found in samples collected during the first 20-25 minutes following injection of the bolus of marker dyes into the proximal end of the loop. However, low concentrations of radio-activity were found even in effluent collected during 50-60 min after injection into the loop. These low concentrations caused a considerable skew in the recovery curve of ^{22}Na but this was not reflected in the recovery curve of T1824. Therefore, all counts of such samples of effluent which were less than twice background were regarded as zero. The rationale for this was examined further in experiment II.

Analysis of samples of the effluent collected from the aboral end of the isolated jejunal loop for radioactivity due to ^{22}Na, and for changes in percentage of light transmittance due to the amounts of T1824, indicated in all cases that peak concentration of these two markers coincided. The excretion patterns of ^{22}Na (Figures 1 and 2) and the calculated transit times for ^{22}Na (Table 7) indicate that the markers passed through the loop more or less as a bolus. Thus, the time of peak concentration of the marker gave a reasonably accurate estimate of transit time.

The tt was not affected by diet. The mean values for the tt for the two dietary treatments and for the low, medium and high rates of perfusion

TABLE 5

MEAN VALUES OF $[Na^+]$ AND $[K^+]$ OF THE JEJUNAL CONTENTS COLLECTED PRIOR TO PERFUSION EXPERIMENTS (MEAN ± S.E.; N:9 FOR PERIOD A; N:8 FOR PERIOD B).

Dietary period	Electrolyte concentration, mEq/l	
	Na	K
A	108.7 ± 5.6 *a	12.7 ± 1.4 a
B	135.1 ± 5.8 b	6.5 ± 0.7 b

*a, b Means of each electrolyte concentration not showing the same superscript letter are significantly different.

TABLE 6

MEAN PERFUSION RATES AND FLOW RATES
FOR THE TWO DIETARY PERIODS.
(MEAN ± S.E.; PERIOD A, N=9, PERIOD B, N=8)

	Perfusion rate ml/min		Flow rate (ml/min)	
	Period A	Period B	Period A	Period B
Low	6.9 ± 0.05	6.8 ± 0.13	6.3 ± 0.14	6.5 ± 0.15
Medium	12.7 ± 0.14	12.5 ± 0.39	11.8 ± 0.21	12.1 ± 0.40
High	25.1 ± 0.20	23.8 ± 0.78	23.9 ± 0.35	23.1 ± 0.85

are shown in Table 7. Mean for low rate was significantly longer than for high rate of perfusion and the difference in mean tt between low and medium rate was almost significant at 5% level of prbability. There was no significant difference in mean tt between medium and high perfusion rate.

By taking the overall mean tt and the length of the intestinal loop estimated during surgical operation at approximately 250 cm, the rate of passage of a bolus through the loop was about 30 cm per minute.

The transit times for sheep II were usually longer than those for other sheep (Appendix A - Table 4 and 5).

Intestinal volume

Intestinal volume means the volume of perfusate solution in the loop at any given time and is the product of flow rate and transit time ($\bar{F}$ x tt = I.V.).

Diets did not affect the intestinal volume but the effect of perfusion rate on the I.V. was statistically significant (Table 7). The increase in intestinal volume with increasing perfusion rates appeared to be linear within the conditions of this experiment (Figure 3).

It was observed that the intestinal volumes of sheep II were higher than those of other sheep reflecting the longer transit times for sheep II.

Amount of sodium in the loop

The amount of sodium in the loop was calculated as the product of intestinal volume and the mean concentration of sodium in the loop, [$\bar{N}a$]. The mean concentrations of sodium (Table 8) were obtained from the sodium concentrations in the perfusate and that in the respective effluents.

There was no significant difference in the amount of sodium in the loop due to diet. Rates of perfusion significantly affected the amount of

Figure 1. The typical pattern of ^{22}Na recovery within 30 minutes after introduction into the loop (period A).

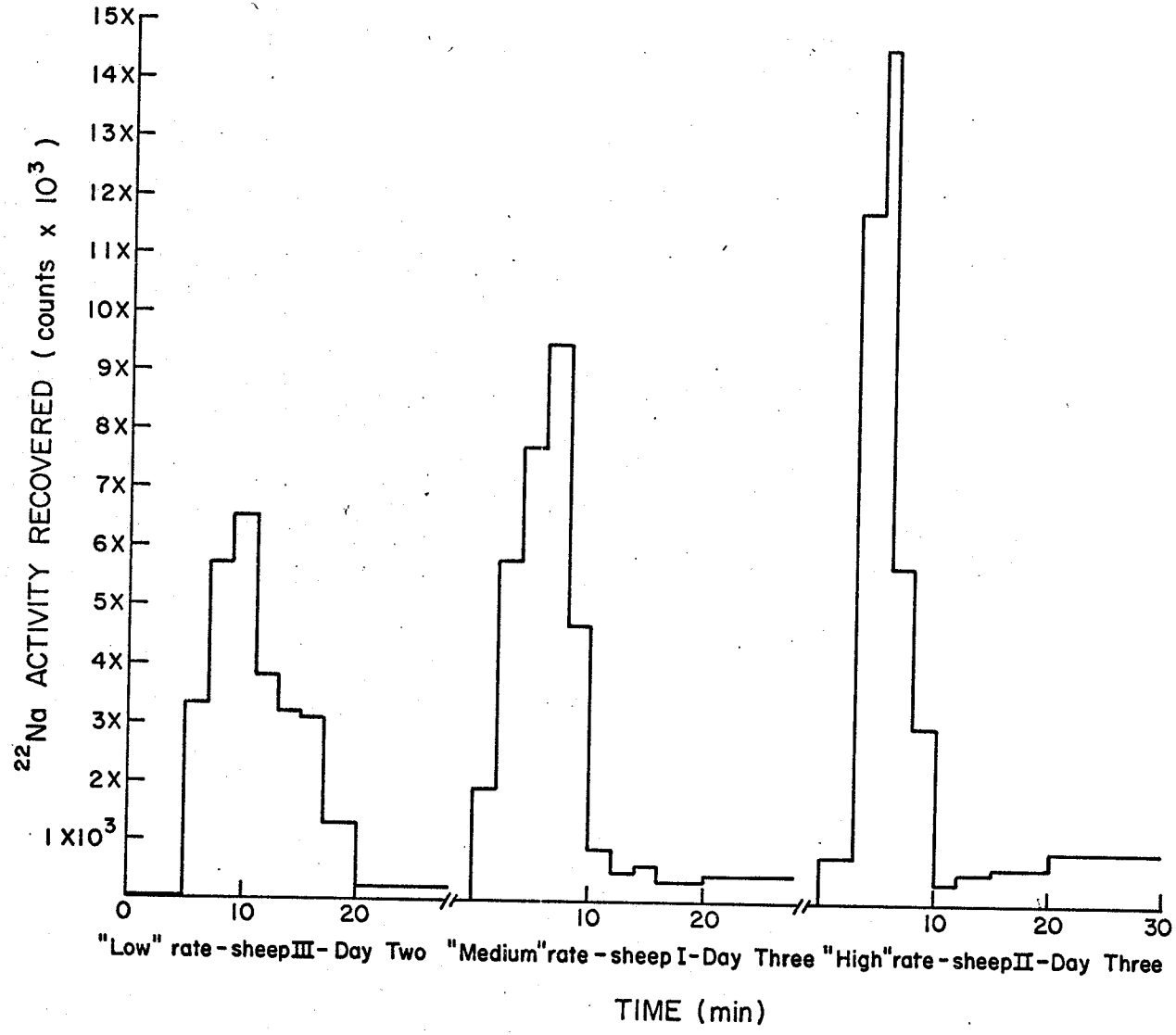


Figure 2. The typical pattern of ^{22}Na recovery within 30 minutes after introduction into the loop (period B).

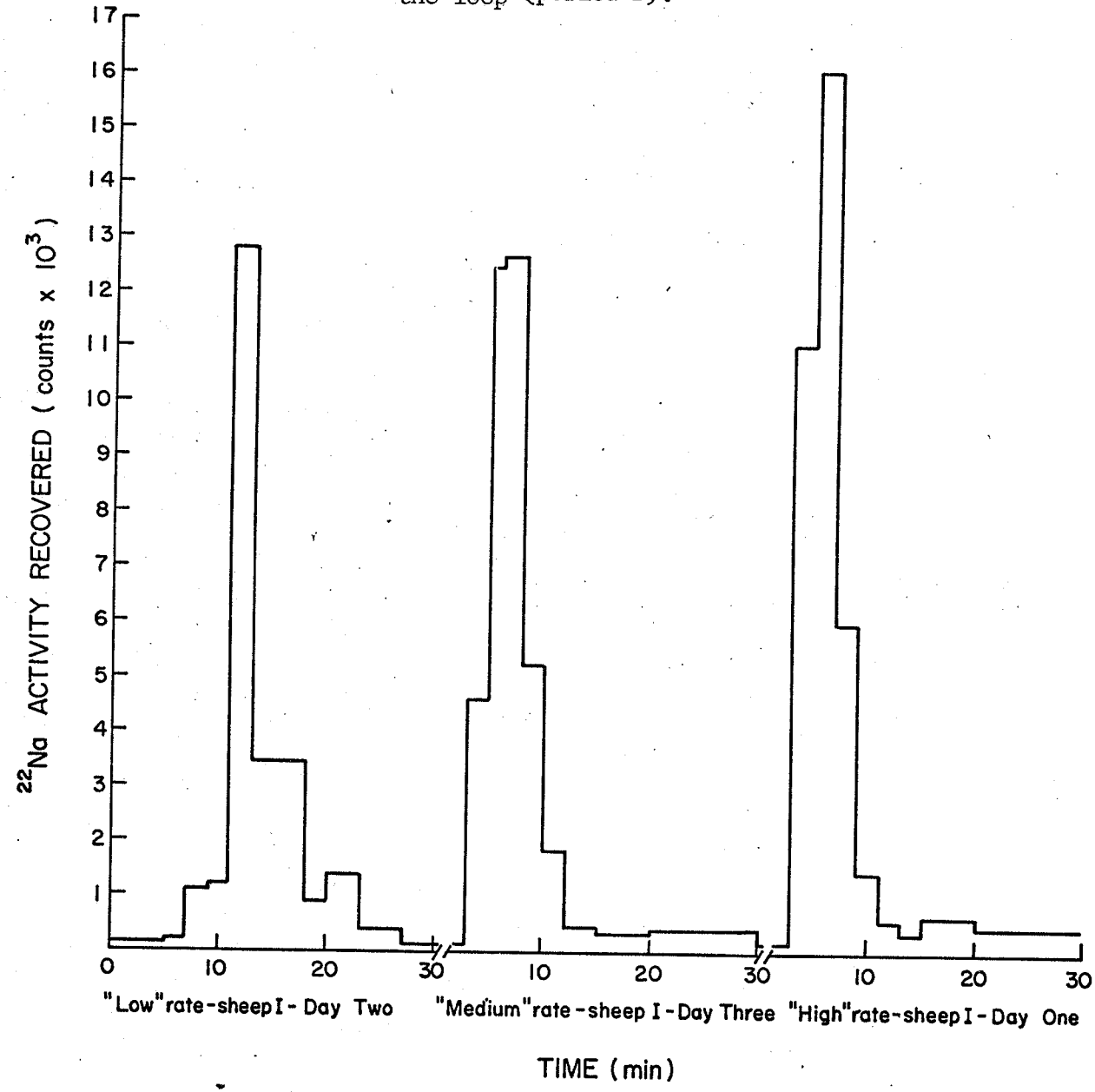


Figure 3. Intestinal volumes at three different rates of perfusion.

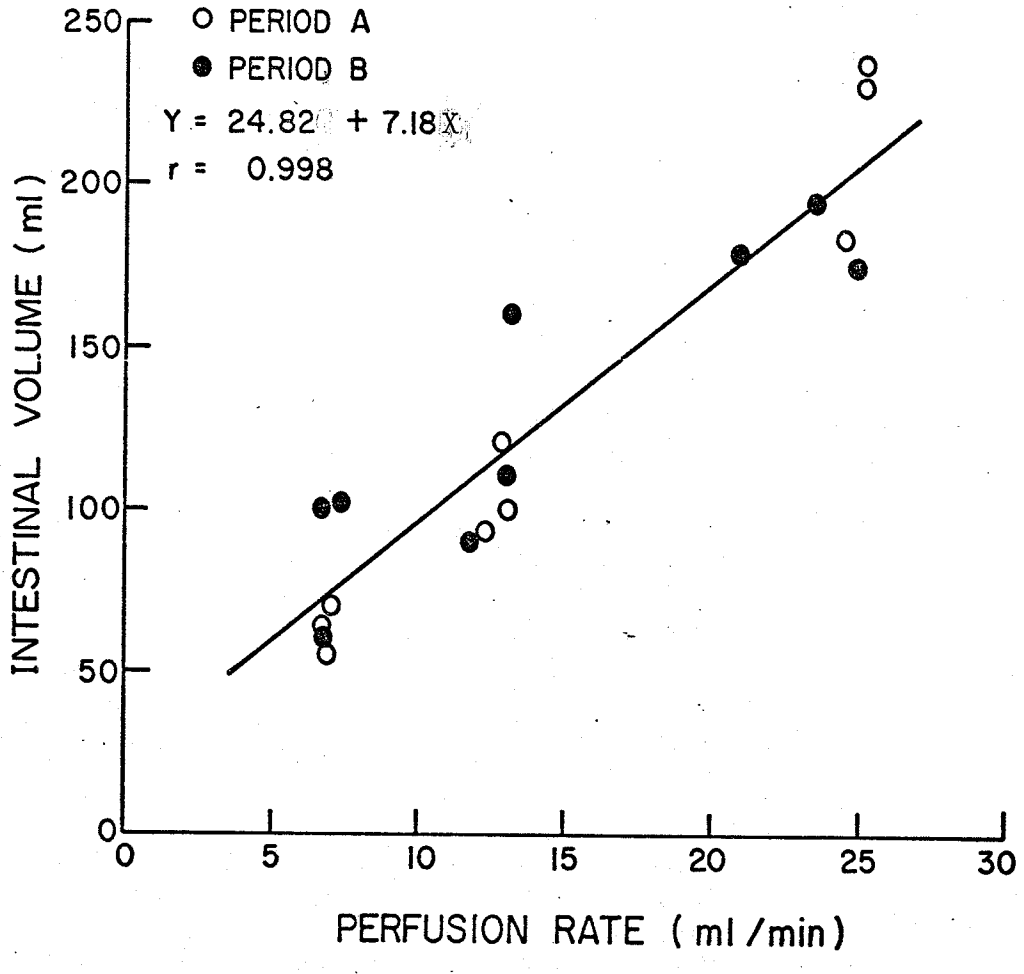


TABLE 7

MEAN TRANSIT TIMES AND INTESTINAL VOLUMES FOR
THE TWO DIETS AT THREE DIFFERENT RATES OF PERFUSION

Source of variation	Transit time (min)	Intestinal volume (ml)
Diets		
A	9.4	128.9
B	10.6	131.5
S.E.*	0.68	7.43
Rates		
Low	11.9^{a}	76.0^{a}
Medium	9.4^{ab}	113.2^{b}
High	8.6^{bc}	201.4^{c}
S.E.	0.83	9.16

abc means within class not showing the same superscript letter are significantly different.

S.E.* Standard error of the mean (N:9 for diet; N:6 for rate).

TABLE 8

MEANS OF SODIUM CONCENTRATION [$\overline{Na}$] IN THE INTESTINAL LOOP FOR THE TWO DIETS AT THREE DIFFERENT PERFUSION RATES (MEAN ± S.E.: PERIOD A, N=9, PERIOD B, N=8).

Perfusion rate	[$\overline{Na}$] (mEq/l)	
	Period A	Period B
Low	122.9 ± 4.55	141.0 ± 4.67
Medium	123.8 ± 4.10	145.3 ± 5.81
High	118.9 ± 7.12	146.6 ± 5.06

sodium in the loop (Table 9). The amount of sodium in the loop increased significantly in linear fashion with increasing perfusion rates (Figure 4). These results reflect the pattern for intestinal volume.

The constant rate of ^{22}sodium transfer (λ)

The transfer rate of ^{22}Na from lumen to plasma was not significantly affected by diet nor by rates of perfusion (Table 9). The mean values of ^{22}Na transfer rate were 0.0292, 0.0277, and 0.0216 per minute for low, medium and high perfusion rates respectively. These values can be expressed as 2.92, 2.77 and 2.16% per minute of the total ^{22}Na activity introduced into the loop being transferred from lumen to plasma.

Fluxes of sodium

Sodium efflux

The transport of normal sodium is assumed to take place proportionally to and in a similar manner to radiosodium. Thus the values of efflux of sodium were obtained from the products of the amounts of sodium in the loop and the rates of ^{22}Na transfer (λ) from lumen to plasma.

There was no significant difference in sodium efflux due to diet. The efflux of sodium increased with increasing rates of perfusion and the differences were almost statistically significant at 5% level of probability (Table 10 and Figure 5).

Sodium net flux

Sodium net fluxes were calculated from the differences between the quantities of sodium introduced into the loop in the perfusion solution and the amounts recovered in the respective effluent during the 1 hour perfusion studies.

Neither diet nor rates of perfusion affected the sodium net flux (Table 10). However, it appeared that the rate of sodium net flux increased

TABLE 9

AMOUNT OF SODIUM IN THE INTESTINAL LOOP
AND THE CONSTANT RATE OF ^{22}SODIUM TRANSFER (λ)
OF THE TWO DIETARY PERIODS AT THREE DIFFERENT RATES OF PERFUSION

	Amount of sodium (mEq)	(λ) (percent per min)
Diets		
A	15.68	2.93
B	18.81	2.30
S.E.	1.17	0.32
Rates		
Low	10.05[a]	2.92
Medium	15.18[b]	2.77
High	26.51[c]	2.16
S.E.	1.48	0.39

abc Means within class not showing the same superscript letter are significantly different.

Figure 4. Amount of sodium in the intestinal loop at three different perfusion rates.

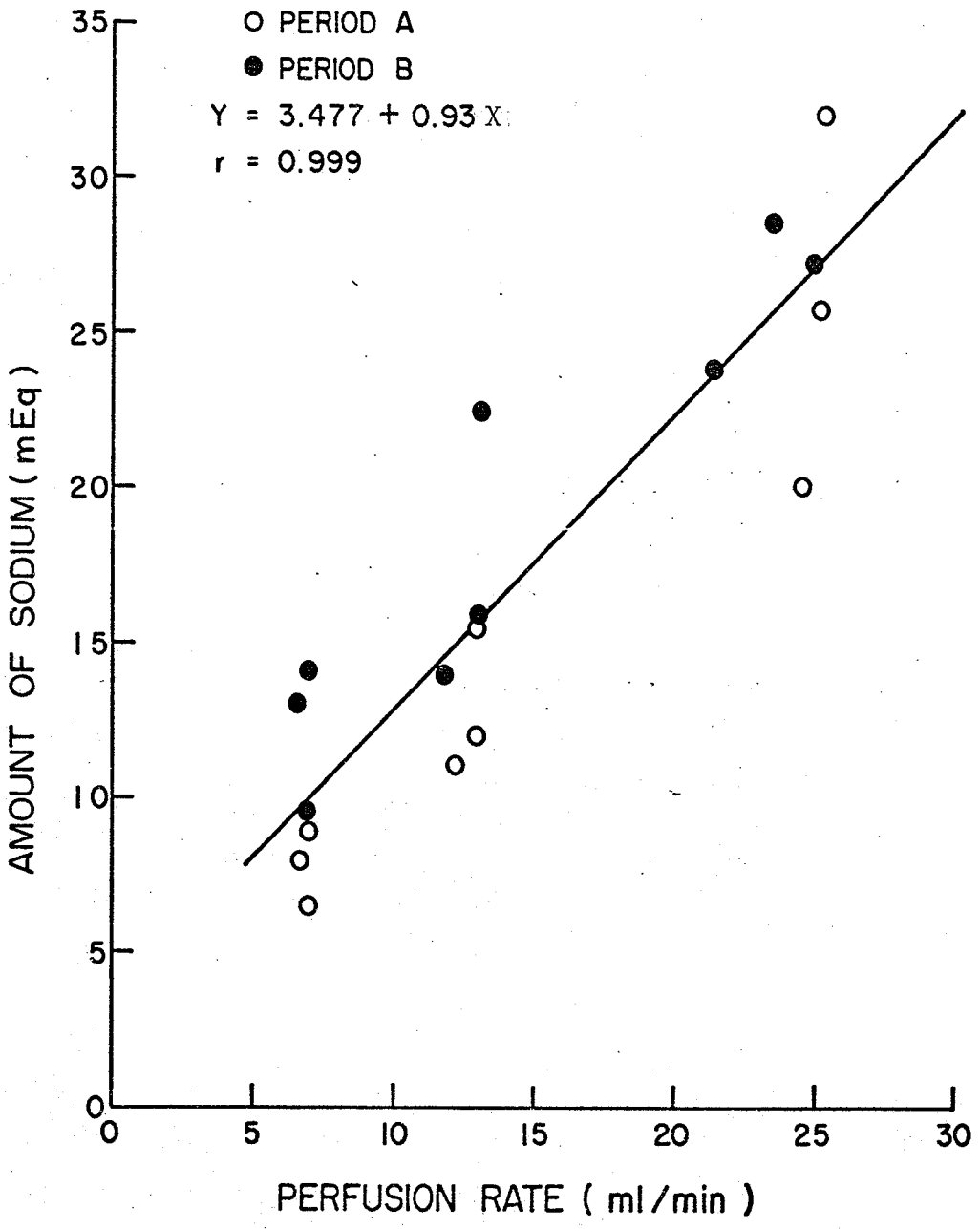


TABLE 10

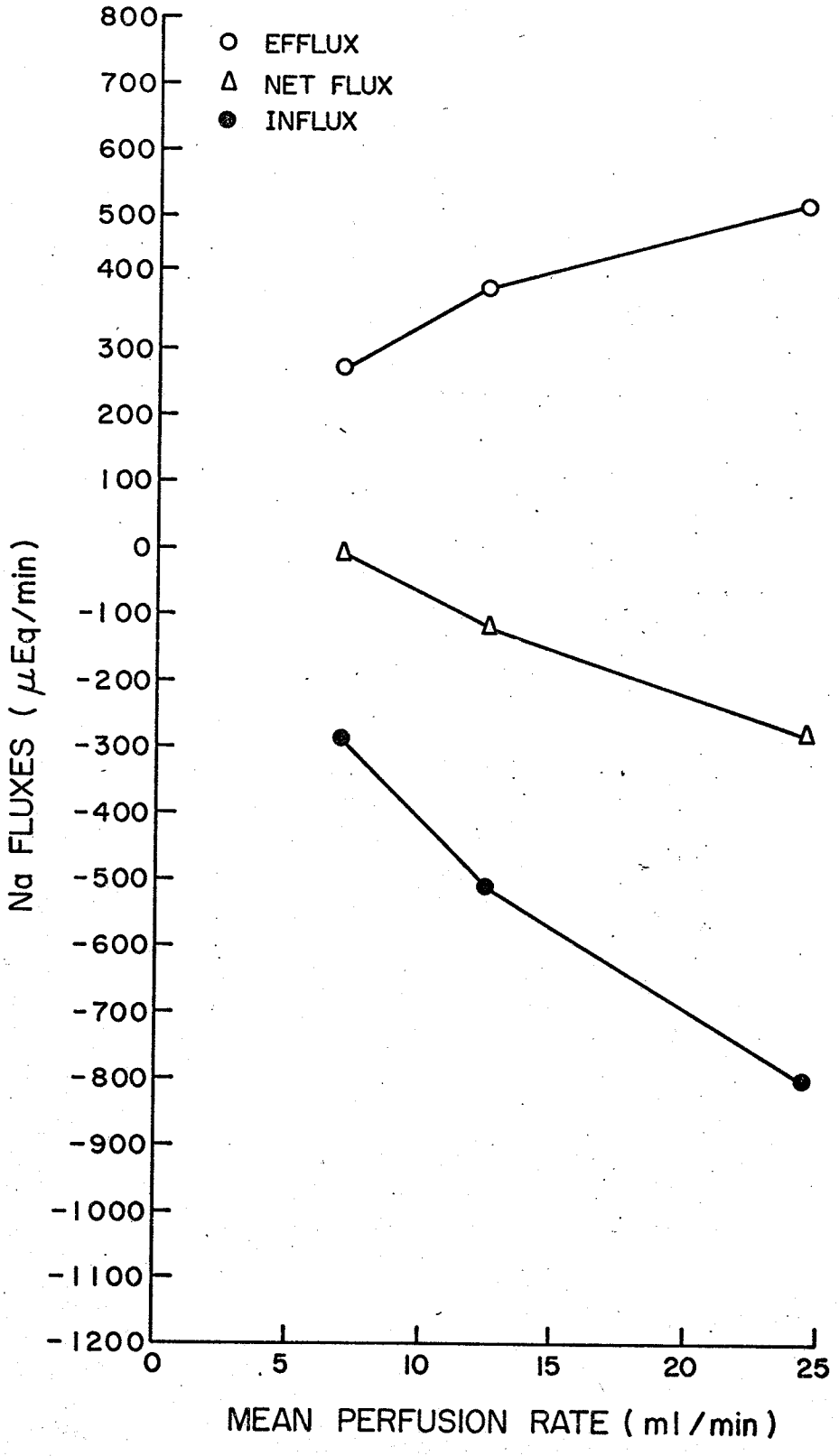
THE MEAN EFFLUX, NET FLUX AND INFLUX OF SODIUM
FOR THE TWO DIETS AND THREE DIFFERENT RATES OF PERFUSION

Source of variation	Efflux (μEq/min)	Net Flux* (μEq/min)	Influx (μEq/min)
Diets			
A	363.4	130.5	493.9
B	431.9	142.7	574.7
S.E.	53.82	76.07	92.56
Rates			
Low	277.7	9.4	287.1^{a}
Medium	395.5	121.2	516.7^{ab}
High	519.9	279.2	799.1^{b}
S.E.	65.92	93.17	113.36

* indicates net secretion of sodium.

ab means within class not showing the same superscript letter are significantly different.

Figure 5. Mean sodium efflux, net flux and influx at three different perfusion rates.



with increasing perfusion rates. The results suggest net secretion of sodium into the loop (Figure 5).

Sodium influx

The influx of sodium was calculated from the difference between efflux and net flux.

There was no significant difference on sodium influx due to diet. The influx was found to increase significantly with increasing rates of perfusion (Table 10 and Figure 5). The difference in sodium influx between the low and medium rates of perfusion, and that between medium and high rates were not significantly different, but the difference in sodium influx between the low and high rates of perfusion was statistically significant.

Net flux of potassium

Potassium net flux was calculated from the difference between the quantity of potassium introduced into the loop with perfusate and the amount recovered in the effluent during the 1 hour perfusion study.

It was found that there was net absorption of potassium. The difference between means for period A and B was significant and presumably this was due to differences in dietary levels of Na and K (Table 11). There were no significant differences due to rates of perfusion (Table 11).

Net flux of chloride

The net flux of chloride was obtained on the same basis that was used for sodium and potassium net fluxes

The net flux of chloride was quite variable. In period A there was mean net absorption of chloride. In contrast, there was mean net secretion in period B. There was net absorption of chloride at the low rate of perfusion whereas at the medium and high rates there was net secretion

TABLE 11

THE MEAN NET FLUXES OF POTASSIUM AND CHLORIDE FOR THE TWO DIETS AND THREE DIFFERENT RATES OF PERFUSION

Source of variation	Potassium Net Flux µEq/min	Chloride Net Flux* (µEq/min)
Diets		
A	52.8[a]	2.6
B	4.1[b]	-23.3
S.E.	5.70	66.60
Rates		
Low	26.8	31.8
Medium	30.1	-4.6
High	28.4	-58.3
S.E.	6.98	81.57

* Negative values denote net secretion; positive values denote net absorption.

ab Means within class not showing the same superscript letter are significantly different.

(Table 11). However, because of the large variation in chloride fluxes, there were no significant differences in net flux of chloride due to diet or rates of perfusion.

Comparisons of net fluxes of Na, K and Cl are shown in Figure 6.

Net flux of water

The results suggest net absorption of water. The absorption of water was significantly greater in period A than that in period B. The rate of water absorption increased significantly with increasing perfusion rate (Table 12). As determined by direct measurement there were no significant differences in water absorption between low and medium nor between medium and high rates of perfusion, but the rate of water absorption at high rate of perfusion was significantly greater than that at the low rate obtained for the 3 perfusion rates. By PEG technique, the mean absorption rates of water were significantly different from one another.

A comparison of net water absorption rate obtained by direct measurement and PEG technique is shown in Figure 7. It is apparent that the rate of water absorption was greater on PEG technique than on direct measurement. This may be due to the interference of protein during analysis for PEG.

Osmolality of the effluent

There was a significant difference on osmolality of the effluent due to diet. The osmolality in period B was significantly greater than that in period A (Table 12), presumably reflecting the differences in osmolality of the perfusate solution. The mean increase in osmolality of the effluent compared to the respective perfusion fluid was 43.2 mOsm/kg for period A and 38.7 mOsm/kg for period B. There were no significant differences on effluent osmolality due to rates of perfusion. The average means of the

Figure 6. The mean net fluxes of K^+, Cl^- and Na^+

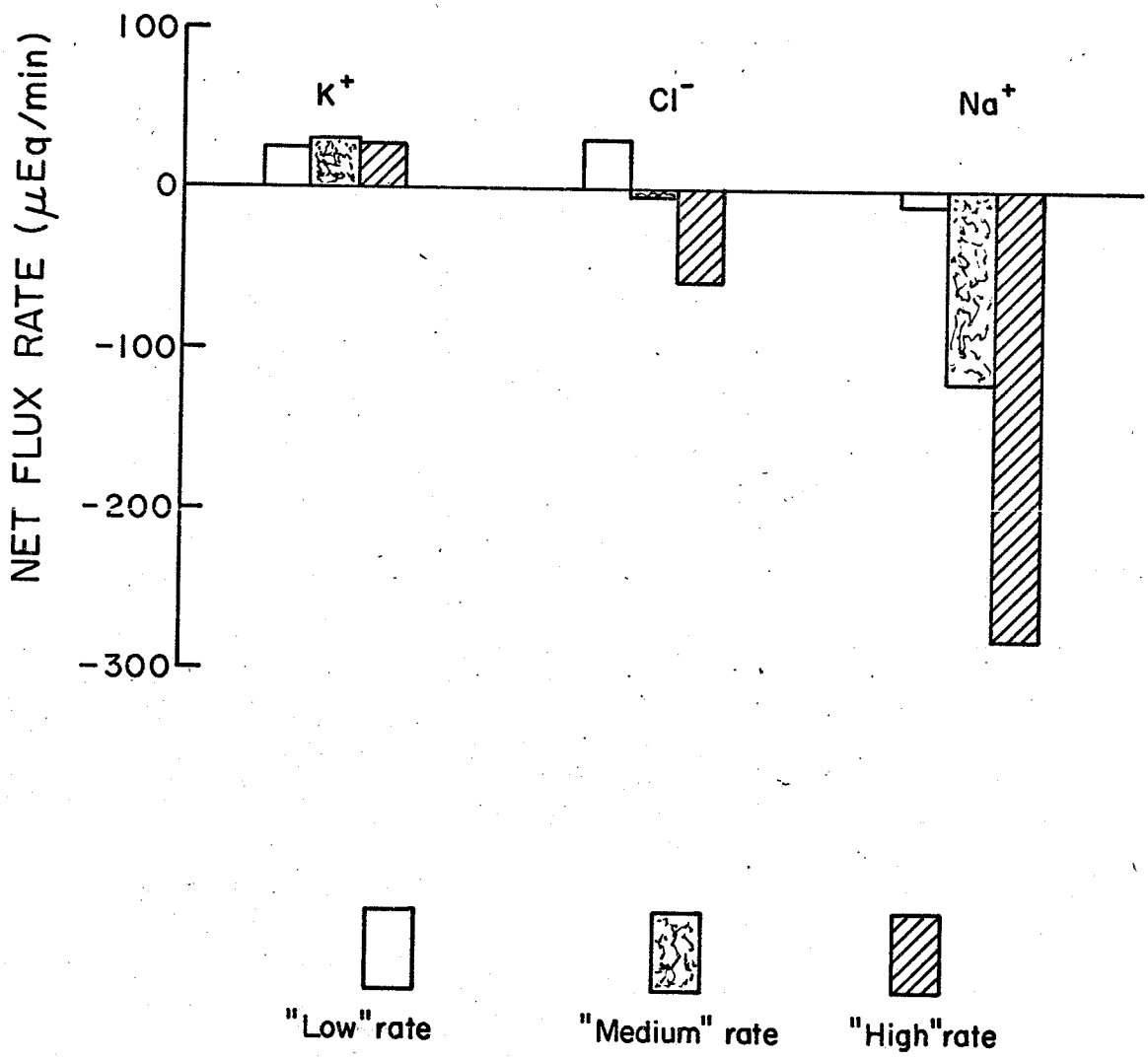


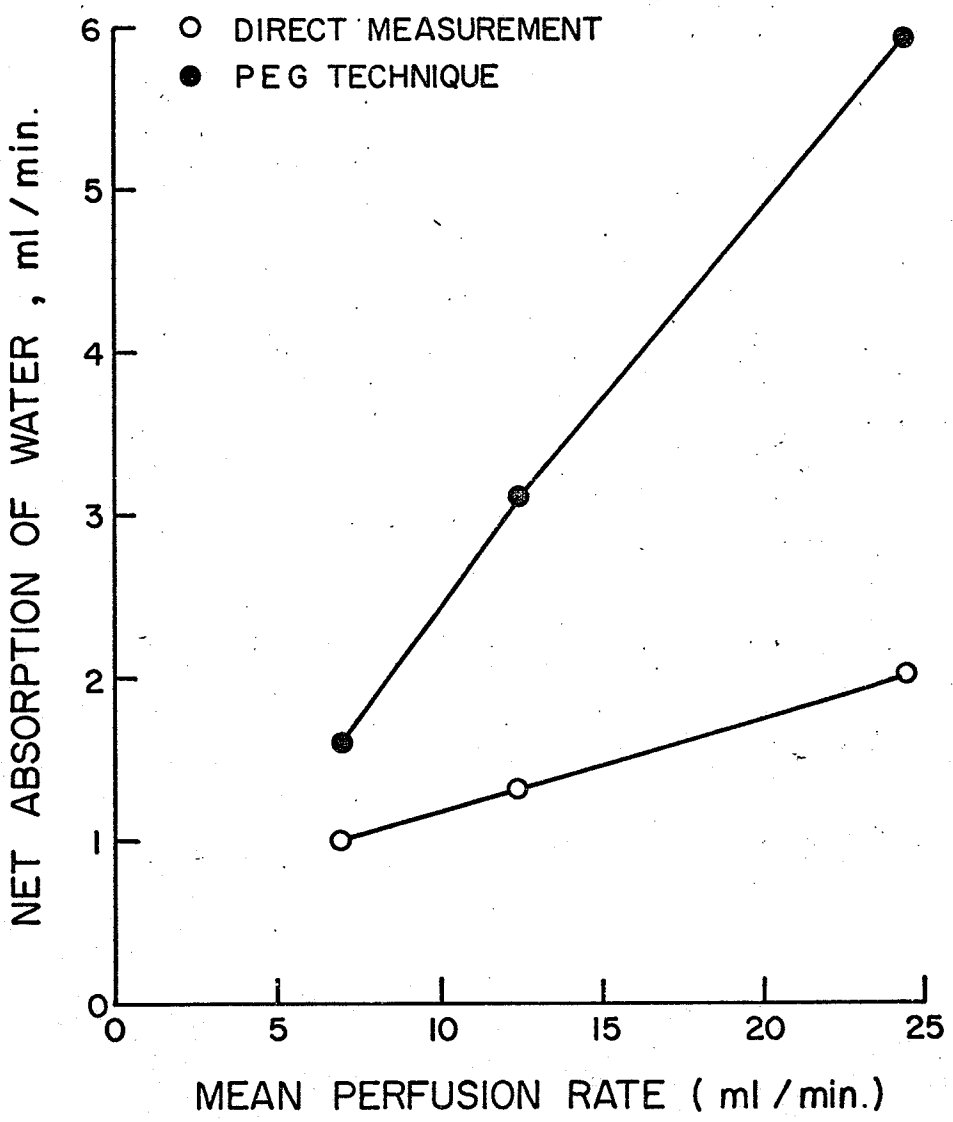
TABLE 12

MEAN VALUES FOR WATER ABSORPTION RATES OBTAINED BY DIRECT MEASUREMENT AND BY PEG TECHNIQUE AND FOR OSMOLALITY OF THE EFFLUENT FOR THE TWO DIETS AND THREE DIFFERENT RATES OF PERFUSION

Source of variation	Direct measurement ml/min	PEG technique ml/min	Osmolality mOsm/kg H_2O
Diets			
A	1.9^{a}	4.2^{a}	262.9^{a}
B	1.0^{b}	2.9^{b}	292.7^{b}
S.E.	0.21	0.31	6.66
Rates			
Low	1.0^{a}	1.6^{a}	278.8
Medium	1.3^{ab}	3.1^{b}	282.0
High	2.0^{b}	5.9^{c}	272.5
S.E.	0.26	0.38	8.16

abc Means within class not showing the same superscript letter are significantly different.

Figure 7. Net water absorption rate obtained by direct measurement and by PEG technique.



osmolality [Na^+], [Cl^-] and [K^+] are shown in Figure 8.

Blood plasma osmolality

There were no significant differences in blood plasma osmolality due to diet nor rate of perfusion (Table 13).

Blood plasma electrolytes

Plasma sodium and chloride were not affected by dietary levels of Na and K nor by rates of perfusion. Plasma potassium was affected by the levels of Na and K in the diet. The greater plasma potassium concentration for period A compared with period B was presumably directly due to the level of K in the diet. Rates of perfusion did not affect the concentration of potassium in plasma (Table 13).

Figure 8. Osmolality, $[Na^+]$, $[K^+]$ and $[Cl^-]$ of the effluent for the two diets.

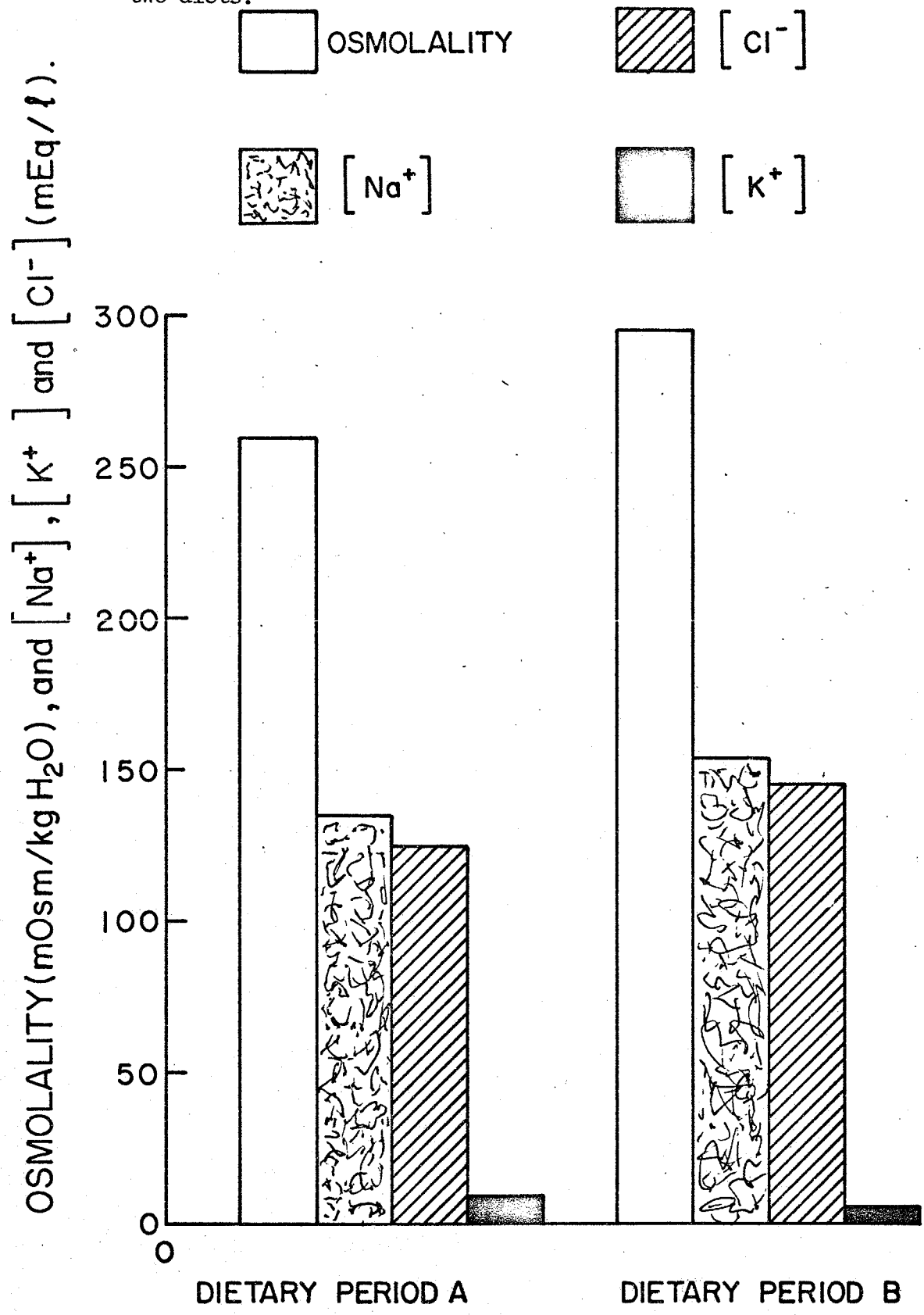


TABLE 13

OSMOLALITY AND CONCENTRATION OF SODIUM, POTASSIUM AND CHLORIDE IN BLOOD PLASMA

Source of variation	Osmolality mOsm/kg H_2O	$[Na^+]$ mEq/l	$[K^+]$ mEq/l	$[Cl^-]$ mEq/l
Diets				
A	287.2	148.9	3.3[a]	96.9
B	292.7	148.9	2.7[b]	106.1
S.E.	3.05	5.89	0.15	3.44
Rates				
Low	288.7	148.9	2.9	101.0
Medium	290.8	150.8	3.1	102.2
High	290.2	146.9	2.9	101.3
S.E.	3.73	7.22	0.18	4.21

ab Means within class not showing the same superscript letter are significantly different.

EXPERIMENT II

Sodium and potassium concentrations in the perfusate solution

The perfusate solution was formulated to contain the same concentrations of Na and K as were found in the intestinal contents collected from the first cannula (before entering the loop). The average concentrations of the three collections collected at 800, 1200 and 1600 hours were taken. The concentrations of these electrolytes and the osmolality of the perfusate are shown in Table 14.

The recovery of ^{22}sodium and its activity in blood plasma

The pattern of ^{22}Na recovery was similar to that found in Experiment I, i.e. the maximum recovery occurred from 5 to 10 min and declined appreciably until 20th min after administration. The accumulative recovery shown in Figure 9 was very steep during the first 20 min. The total recovery on day 1 was considerably lower than that for the three successive days.

The radioactivity in plasma found on the day after an experiment but before the next introduction of ^{22}Na indicate that there was a small amount of ^{22}Na remaining in the body fluids. On days 3 and 4 there was an increase in activity of ^{22}Na in plasma of about 70 to 90 cpm/ml one hour after the infusion of the bolus of radiosodium but by the second hour the count had declined by 25 to 45 cpm/ml (Figure 10). The pattern differed for day 2 in that the value at the end of the first hour was lower than that for hour 2.

Flow rates

The flow rate mainly depends on perfusion rate. The mean flow rates were slightly less than the mean perfusion rates (Table 15) indicating the absorption of water.

TABLE 14

CONCENTRATIONS OF Na AND K IN THE INTESTINAL CONTENTS AND OSMOLALITY OF PERFUSATE SOLUTIONS

Day	$[Na^+]$ (mEq/l)	$[K^+]$ (mEq/l)	Osmolality (mOsm/ kg H_2O)
1	45.0	46.1	170.0
2	68.6	42.8	205.4
3	45.5	46.9	170.8
4	46.9	50.1	180.2

Figure 9. The accumulative recovery of radioactive sodium.

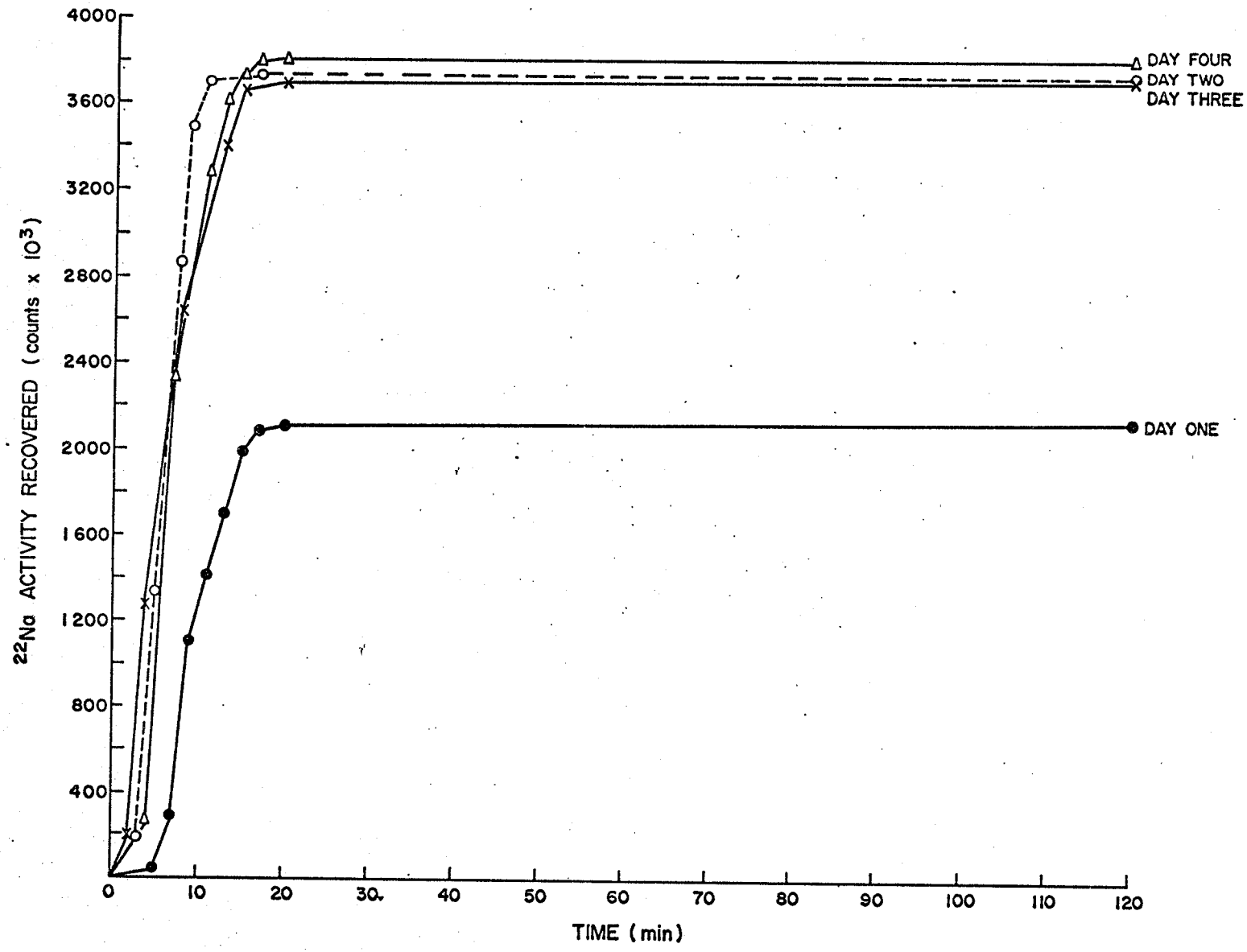


Figure 10. The activity of ^{22}Na in blood plasma over two hour period after introduction of ^{22}Na solution.

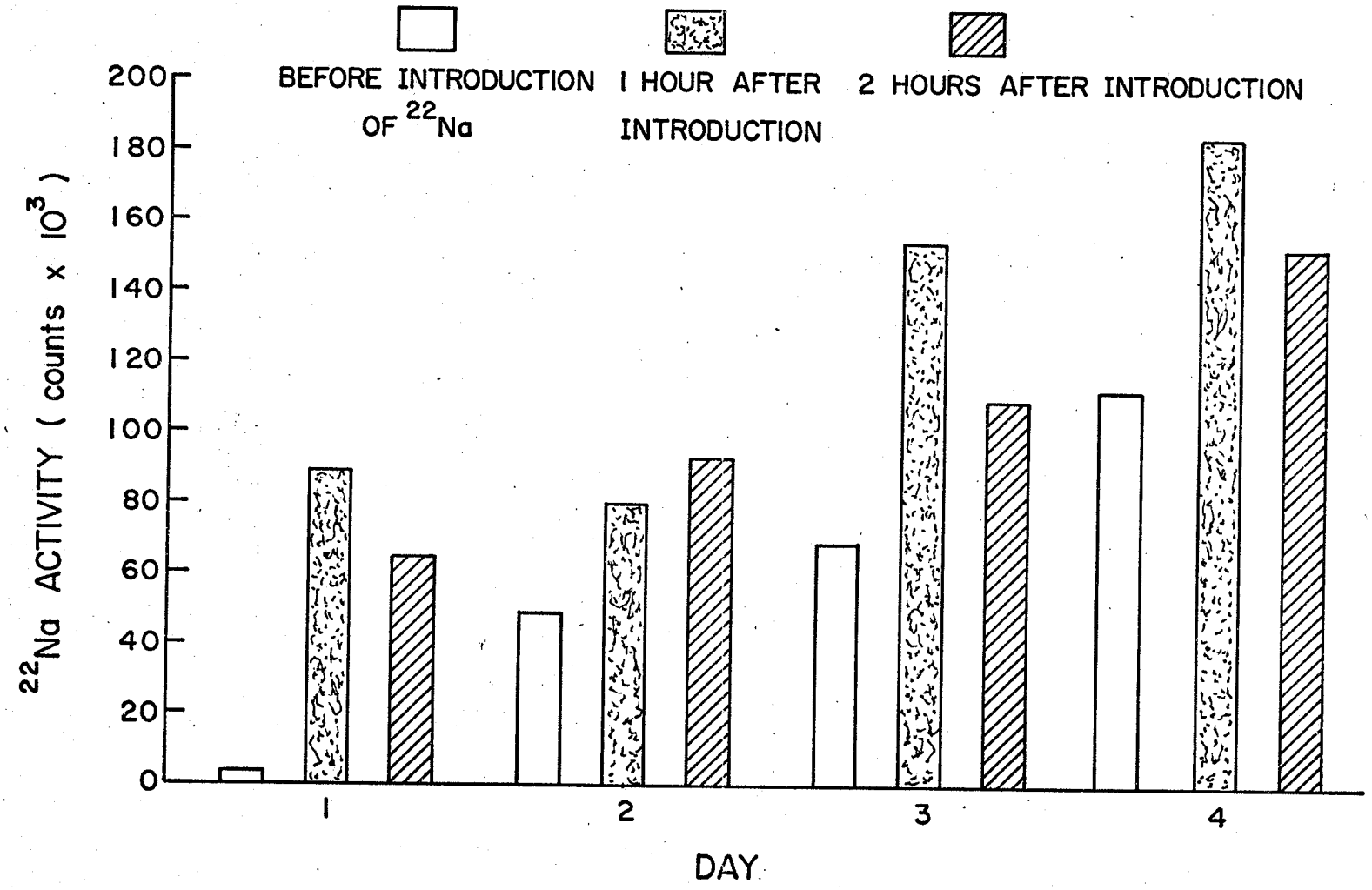


TABLE 15

MEAN PERFUSION RATES AND FLOW RATES AT DIFFERENT TIME INTERVALS AND ON DIFFERENT DAYS (MEAN ± S.E.; N=12)

Day	Perfusion rate ml/min	Flow rate ml/min	Time intervals (min)	Perfusion rate ml/min	Flow rate ml/min
1	13.0 ± 0.17	12.5 ± 0.55	0 - 30	12.6 ± 0.15	12.1 ± 0.26
2	13.0 ± 0.20	11.9 ± 0.19	30- 60	12.8 ± 0.18	11.5 ± 0.36
3	13.0 ± 0.15	11.5 ± 0.26	60- 90	13.2 ± 0.10	12.0 ± 0.25
4	12.9 ± 0.13	11.5 ± 0.28	90-120	13.3 ± 0.13	11.9 ± 0.51

The mean transit time

The mean transit times of ^{22}Na-T1824 solution passing through the loop are shown in Table 16. The mean tt for the 1st day was longer than those for the three successive days. There was little difference between the times obtained by the two methods except for the first day. The longer tt obtained by T1824 analysis compared with that determined from the recovery of ^{22}Na on the 1st day may be due to the interference of protein during photocolorimetric analysis or due to technical error.

Intestinal volume

There were significant differences in intestinal volumes among days. The intestinal volume on day 1 was significantly greater than those on days 2, 3 and 4 (Table 18). This is due to the differences in tt. There were no significant differences in intestinal volumes among days 2, 3 and 4. However, the lowest value for intestinal volume was found for day 2 and this reflects the shortest tt. The intestinal volumes presumably affected the net flux of sodium; and their relationship is shown in Figure 11.

The intestinal volumes among time intervals were not significantly different. The average means for time intervals of 0-30, 30-60, 60-90 and 90-120 minutes were 89.9, 85.8, 89.0 and 88.3 ml respectively.

Amount of sodium in the loop

The amount of sodium in the loop among time intervals were not significantly different but there were significant differences among days. The amount of sodium in the loop on day 1 was significantly greater than the amounts found for the three succeeding days; that of day 2 was significantly different from those of days 3 and 4 and that of day 3 significantly different from that of 4 (Table 18). The significant differences between

TABLE 16

TRANSIT TIMES FOR ^{22}Na AND T1824 PASSING THROUGH THE LOOP.

Day	By ^{22}Na Analysis (min)	By T1824 Analysis (min)
1	9.7	10.7
2	6.0	6.1
3	6.6	6.3
4	7.3	7.5

TABLE 17

MEAN SODIUM CONCENTRATIONS [Na] IN THE INTESTINAL LOOP AT DIFFERENT TIME INTERVALS AND ON DIFFERENT DAYS (MEAN ± S.E.: N=12)

Day	$[\bar{Na}]$	Time interval (min)	$[\bar{Na}]$
1	66.6 ± 0.61	0 - 30	65.5 ± 2.31
2	79.7 ± 0.49	30- 60	66.7 ± 2.46
3	59.5 ± 0.38	60- 90	68.6 ± 2.29
4	62.2 ± 0.65	90-120	67.2 ± 2.43

TABLE 18

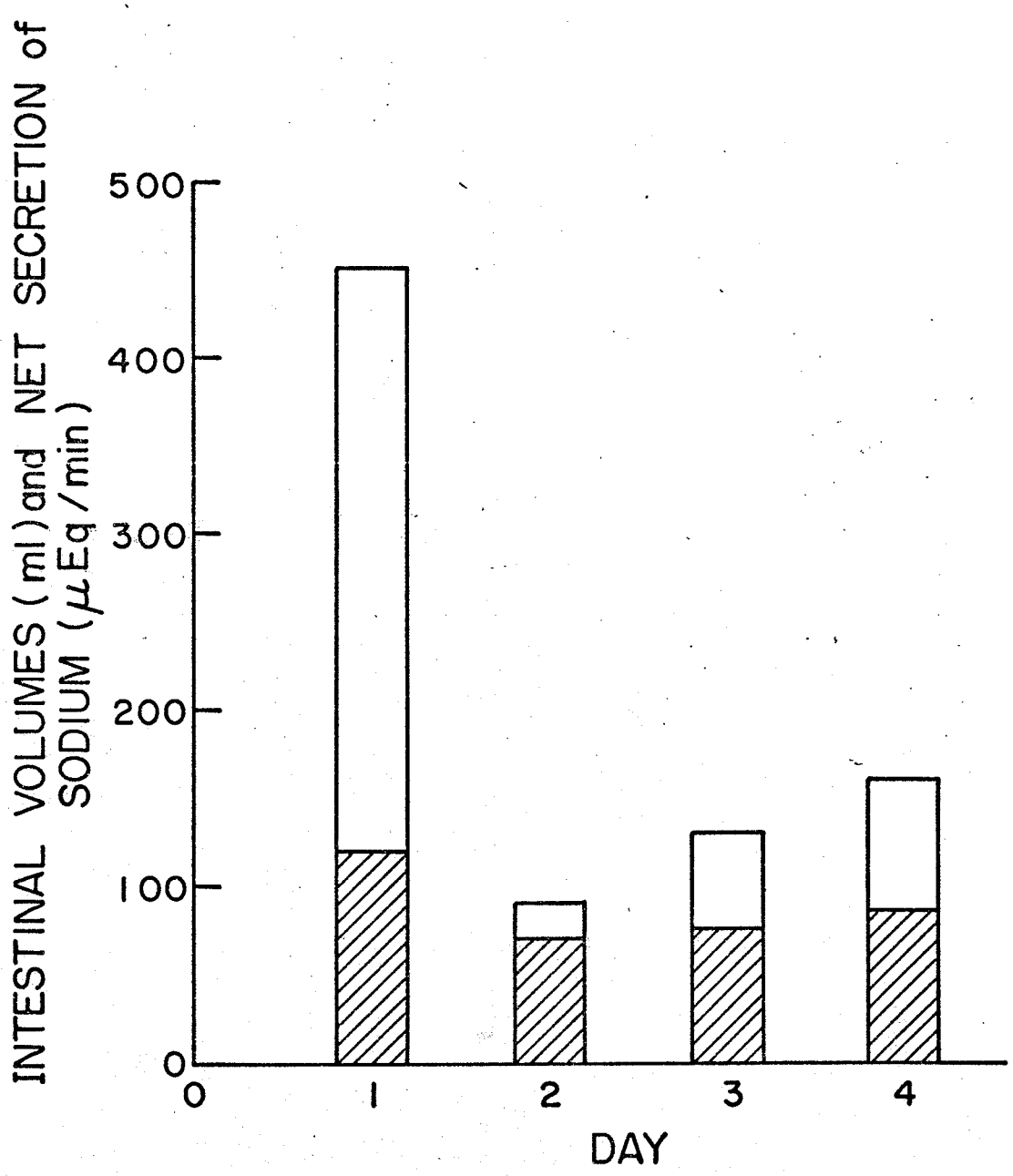
MEANS OF INTESTINAL VOLUMES AND AMOUNTS OF SODIUM IN THE LOOP FOR THE FOUR EXPERIMENTAL DAYS

Day	Intestinal Volume (ml)	Amount of sodium (mEq)
1	121.3^{a}	8.06^{a}
2	71.5^{b}	5.69^{b}
3	75.9^{b}	4.52^{c}
4	84.4^{b}	5.24^{d}
S.E.	3.63	0.21

abcd mean values within class not showing the same superscript letter are significantly different.

Figure 11. The relationship between intestinal volume and net (secretion) flux of sodium during 4-day study.

The total bar area represents the secretion rate of sodium; the shaded area represents the intestinal volume.



the value for day 2 and those for days 3 and 4 are presumably due to the considerably higher sodium concentration for day 2 (Table 17).

The constant rate of ^{22}Na transfer

The transfer rates of ^{22}Na, from lumen to plasma, were 8, 4, 4 and 3 per cent of the total activity introduced into the loop for days 1, 2, 3 and 4 respectively. The high efflux value for day 1 was associated with the low total recovery of ^{22}Na on that day.

Fluxes of sodium

Sodium efflux

There were no significant differences in sodium efflux due to time intervals but the means for days of experiment were significantly different (Table 19). The efflux of sodium on day 1 was significantly greater than the effluxes on the other three successive days. The efflux on day 2 was also significantly greater than those on days 3 and 4 but there was no significant difference between the effluxes on days 3 and 4. It may be noted that the efflux of sodium decreased progressively from day to day.

Sodium net flux

The net flux of sodium suggests net secretion into the loop.

Time intervals did not significantly affect sodium net flux. However, there appeared to be an increase in sodium net flux during the second 30 minute interval of each hour (Figure 12).

There were significant differences in sodium net fluxes due to days of study. The mean net flux for day 1 was significantly greater than those for days 2, 3 and 4 (Table 19). This might have been due to the greater intestinal volume for day 1 compared with the other three days. Although the net flux of sodium on day 2 was considerably lower than fluxes on days 3 and 4 there were no significant differences between the net flux

Figure 12. The unidirectional and net fluxes of sodium over two-hour period.

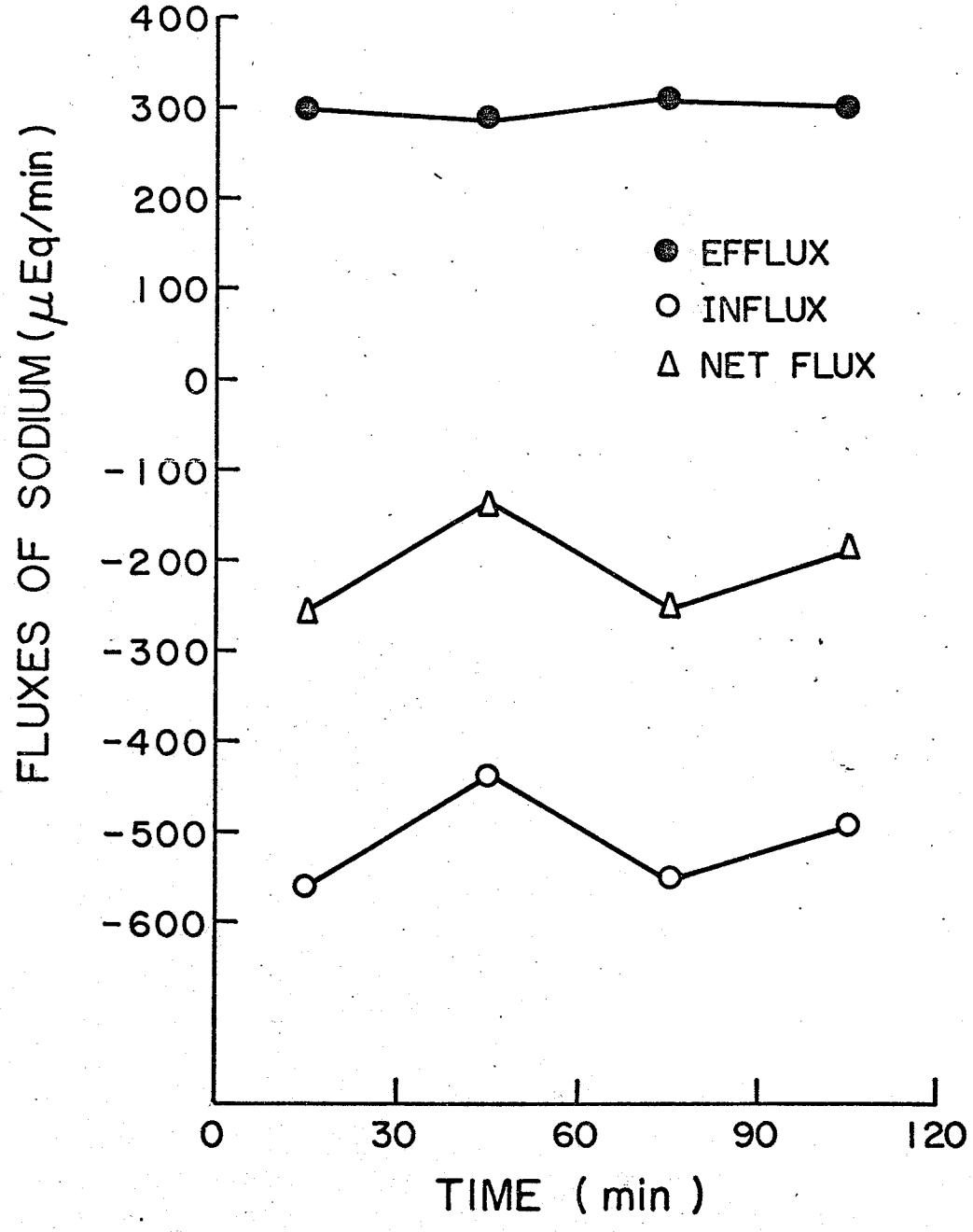


TABLE 19

MEAN VALUES FOR FLUXES OF SODIUM FOR THE FOUR EXPERIMENTAL DAYS

Day	Efflux μEq/min	Net flux μEq/min	Influx μEq/min
1	644.9^{a}	452.9^{a}	1092.8^{a}
2	227.9^{b}	92.3^{b}	320.3^{b}
3	180.8^{c}	132.3^{b}	313.1^{b}
4	157.5^{c}	159.1^{b}	316.5^{b}
S.E.	16.38	61.64	84.86

a b c Mean values within class not showing the same superscript letter are significantly different.

on day 2 and those of days 3 and 4. The relationship between intestinal volume and net flux of sodium is illustrated in Figure 11.

Sodium influx

Time intervals did not affect the influx of sodium. The means at different time intervals are shown in Figure 12. There were significant differences in sodium influx among days. The influx on day 1 was significantly greater than the influxes on days 2, 3 and 4 whereas there were no significant differences among days 2, 3 and 4 (Table 19).

Net influx of potassium

There were no significant difference in K net flux among 30 minute intervals and among days of perfusion study. The average net flux indicating absorption of K among days and time intervals are shown in Table 20 and Figure 13 respectively. It appears that as the secretion of Na increases, there is a decrease in K absorption. Similarly, when the secretion of Na decreased, there was an increase in K absorption (Figure 13).

Net flux of chloride

The net flux of chloride was quite variable. The mean for the first 30 minute interval suggests net secretion (Figure 13) whereas the others indicate net absorption. There were no significant differences among time intervals.

There were significant differences among days of perfusion study (Table 20). On day 1, the data indicate net secretion and this mean was significantly different from those for the other three days. The means for days 2, 3 and 4 were not significantly different from one another and all indicate absorption of chloride.

The absorption of water

The net flux of water indicates net absorption. There was no

TABLE 20

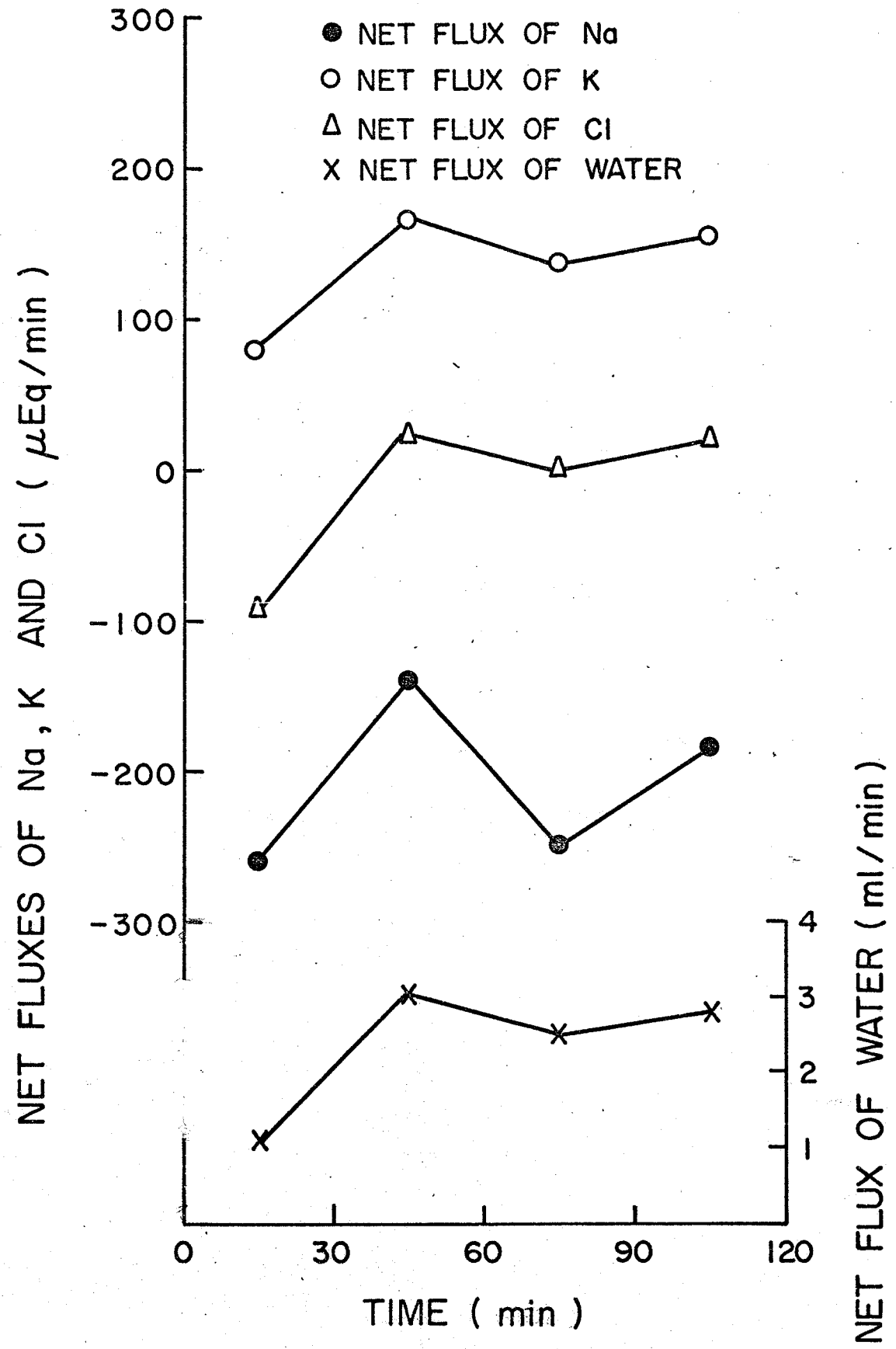
MEAN NET FLUXES OF POTASSIUM AND CHLORIDE
FOR THE FOUR EXPERIMENTAL DAYS

Day	K Net Flux µEq/min	Cl Net Flux* µEq/min
1	131.2	-236.2[a]
2	136.4	91.5[b]
3	123.0	39.5[b]
4	151.7	61.7[b]
S.E.	33.48	56.78

* Negative value denotes net secretion; positive ones denote net absorption.

a b Mean values within class not showing the same superscript letter are significantly different.

Figure 13. Net fluxes of Na, K, Cl and water (by direct measurement).



significant difference in mean water absorption among days and among time intervals within days obtained by direct measurement. By PEG technique, the mean water absorption rate on day 1 was significantly lower than on days 2, 3 and 4. The rate on day 2 was significantly lower than on days 3 and 4. The rates of absorption were not significantly different between days 3 and 4 (Table 21). Time intervals did not affect the rate of water absorption obtained by PEG technique.

Osmolality and electrolyte compositions of the effluent

The osmolality of the effluent was significantly different due to time intervals and due to perfusion study days (Table 22). The interaction between time intervals and days was also significant.

The osmolality significantly increased from the beginning of perfusion study until the third 30 minute interval and significantly decreased during the last 30 minutes of the 2-hour period. Among days of perfusion study, the results suggest that the mean osmolalities of the effluent for days were significantly different from one another.

The osmolality and electrolyte composition of the effluent for time intervals and perfusion study days are shown in Figures 14 and 15 respectively. The osmolality is contributed approximately, one-third by $[Na^+]$, one-fourth by $[Cl^-]$ and one-fifth by $[K^+]$. In this experiment the osmolality of the effluent not accounted for by these three electrolytes i.e. about one-fifth or 40-50 mOsm/kg was presumably due to bicarbonate with smaller contributions of organic substances etc.

The concentrations of Na, K and Cl in the effluent are shown in Table 23.

Blood plasma osmolality and electrolyte concentrations

The means for plasma osmolality and plasma electrolyte concentrations for the 4 perfusion study days are shown in Table 24. The osmolality,

TABLE 21

MEAN ABSORPTION RATES OF WATER OBTAINED BY DIRECT MEASUREMENT AND BY PEG TECHNIQUE OVER TWO HOUR PERIOD AND FOR THE FOUR PERFUSION STUDY DAYS

Day	By direct measurement (ml/min)	By PEG technique (ml/min)	Time interval (min)	By direct measurement (ml/min)	By PEG technique (ml/min)
1	1.3	1.1[a]	0 - 30	1.1	2.4
2	2.1	1.9[b]	30- 60	3.0	2.3
3	2.9	3.3[c]	60- 90	2.5	1.9
4	3.0	2.7[c]	90-120	2.8	2.5
S.E.	0.86	0.20	S.E.	0.86	0.20

TABLE 22

MEAN OSMOLALITY OF THE EFFLUENT FOR 30 MINUTE INTERVALS AND FOR THE 4 PERFUSION STUDY DAYS

Day	Osmolality mOsm/kg H_2O	Time interval (min)	Osmolality mOsm/kg H_2O
1	240.8^{a}	0 - 30	227.6^{a}
2	243.3^{b}	30- 60	235.8^{b}
3	222.6^{c}	60- 90	241.9^{c}
4	236.3^{d}	90-120	237.6^{d}
S. E.	0.37	S.E.	0.37

a b c d Mean values within class not showing the same superscript letter are significantly different.

Figure 14. Osmolality and electrolyte composition of the effluent over two-hour period.

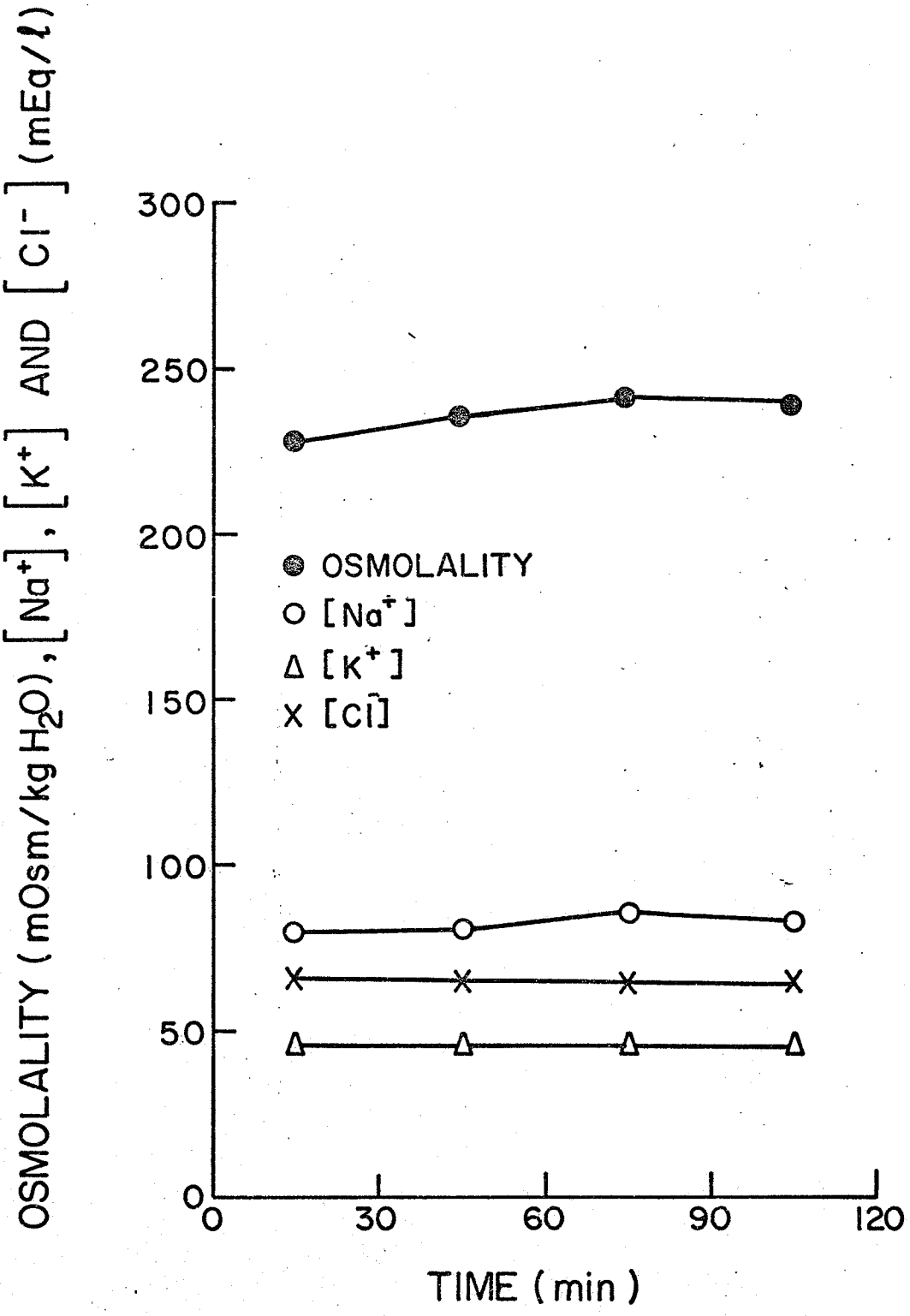


Figure 15. Osmolality and electrolyte composition of the effluent for the four perfusion study days.

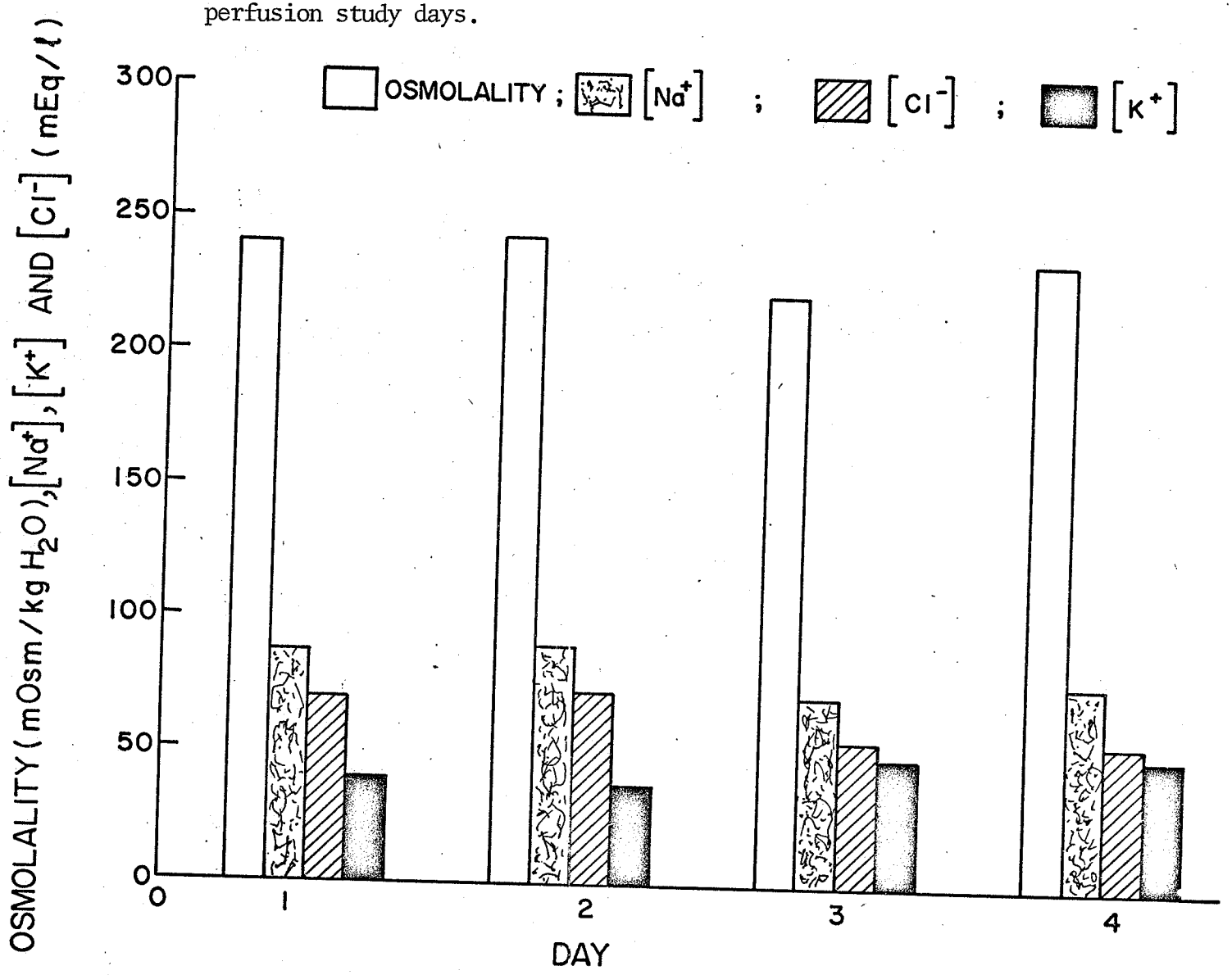


TABLE 23

CONCENTRATIONS OF Na, K AND Cl IN THE EFFLUENT
FOR TIME INTERVALS AND DAYS OF STUDY

Day	Concentrations (mEq/l)			Time	Concentrations (mEq/l)		
	Na	K	Cl				
1	88.2 ± 1.24	39.9 ± 0.22	69.4 ± 1.65	0 - 30	79.1 ± 2.29	44.3 ± 1.56	64.3 ± 3.09
2	90.8 ± 0.99	38.6 ± 0.24	73.6 ± 1.14	30- 60	81.3 ± 2.65	44.3 ± 1.57	63.7 ± 2.87
3	72.7 ± 0.98	48.5 ± 0.16	55.2 ± 0.72	60 -90	85.7 ± 2.37	44.3 ± 1.60	62.6 ± 2.39
4	77.4 ± 1.32	50.1 ± 0.23	55.1 ± 0.71	90-120	82.9 ± 2.38	44.3 ± 1.46	62.7 ± 2.52

TABLE 24

MEANS FOR BLOOD PLASMA OSMOLALITY, $[Na^+]$, $[K^+]$ AND $[Cl^-]$ DURING ADJUSTMENT AND PERFUSION STUDY PERIODS

Item		Adjustment Period	Time of Collection (Perfusion study period)		
			0	1 hour	2 hours
Osmolality	(mOsm/kg H_2O)	-	304.3	310.0	317.2
$[Na^+]$	(mEq/l)	154.0	154.5	155.5	157.0
$[K^+]$	(mEq/l)	5.1	4.3	4.4	4.2
$[Cl^-]$	(mEq/l)	118.2	122.1	128.0	130.5

$[Na^+]$ and $[Cl^-]$ tended to increase during the 2 hour period of the perfusion study. It is observed that $[Cl^-]$ during the experiment period was greater than that of the adjustment period.

DISCUSSION

EXPERIMENT I

For fluid flowing through a tube of fixed volume changes in flow volume can only be accommodated by proportionate changes in linear flow rate or in other words transit time through a given length of the tube. Alternatively, if the transit time is held constant then changes in flow rate will be accompanied by compensatory change in the volume of fluid in the tube.

It is apparent that within the limits of flow rate studied in the present experiment, increasing the volume of fluid flowing through the jejunal loop resulted in decreased transit time and increased intestinal volume. Thus, both the parameters varied with flow rate. The relationship between perfusion rate, which was practically the same as flow rate, and intestinal volume was linear between about 7 ml and 26 ml per minute. Love, et al (79) found in human that increasedintestinal volume for the whole alimentary tract tended to plateau with high flow rates. However, they used rates of up to 100 ml/min in subjects which were only about 50% higher in body weight than the sheep used in the present experiment. Although there were compensatory decreases in transit time with higher flow rates, these were relatively modest. Also the differences observed between input (perfusion) and output (effluent) volume, representing the absorption of water, were quite small and although water absorption rates increased significantly with increased perfusion rates the actual changes in volume of water absorbed were small compared with the concurrent changes in perfusion volume.

Therefore, the rate of perfusion was the major determinant of intestinal volume, i.e. volume of fluid in the loop, which was calculated as the product

of average flow rate and transit time.

The amount of sodium in the loop of jejunum and therefore available for exchange was calculated from the product of intestinal volume and the concentration of sodium in the fluid, the latter being taken as the arthmetic mean of the sodium concentrations in the perfusion and effluent solutions. The dominant parameter appeared to be intestinal volume for although higher concentrations of sodium were used in the perfusate solution for period B, the greater amounts of sodium in the loop found for Period B were not significantly different than those for Period A. However, the large differences in intestinal volume due to perfusion rate were reflected in parallel significant differences in amounts of sodium in the loop.

The constant rate of ^{22}Na transfer was calculated from the proportion of the administered dose of radioisotope which was recovered in the effluent and the transit time. In spite of significant differences in the latter related to changes in perfusion rate, there were no significant differences in rates of ^{22}Na transfers. As was mentioned earlier there were in some cases low counts of radioactivity in samples of effluent taken 1 hour after introduction of the ^{22}Na into the loop. This apparent radioactivity could not be accounted for by possible return of ^{22}Na from the plasma to the loop solutions. Therefore, counts of less than twice background were ignored in calculating both transit time and total recovery of radioactivity. Whether for this reason or some other, the recovery of ^{22}Na for the loop was quite variable among sheep, among days and among perfusion rates as can be seen in Table 3, appendix A, the same dose of ^{22}Na having been administered in each case. Thus the transfer rates were variable within diets and within perfusion rates, resulting in large standard errors.

Sodium efflux from the loop was calculated as the product

of the constant transfer rate of ^{22}Na from gut to blood and the amount of sodium in the loop. Thus, the values for sodium efflux are subject to all the assumptionsand experimental errors inherent in determining all the foregoing parameters. The mean sodium effluxes were remarkably increased with higher rates of perfusion but the differences in means did not quite reach significance at the 5% level probability.

Net fluxes for sodium, potassium, chloride and water (by direct measurement) were determined from the differences in quantities entering the loop in the perfusion solution and leaving the loop in the effluent fluid over a 1 hour period. The possible sources of error in such determination include, in addition to the physical and chemical measurements of fluid volumes and concentrations of electrolytes, a biological variant related to the peristaltic mode of flow of fluids through the intestine. The input of fluid into the loop was constant, within the limits of the pumps used, but the effluent was produced as spurts of fluid. Individual spurts were estimated to vary rather randomly from about 5 ml up to about 20-30 ml. Thus significant beginning and end time errors could have occurred depending on whether the fixed time period for collection of effluent began (and or ended) just before or just after a large spurt. The possible magnitude of such sources of error an the net fluxes were not determined. However, they could account at least in part for the wide variations found for instance in chloride fluxes. But such end time errors should have resulted in comparable variations in all the fluxes and this was not the case. Therefore, it can be deduced that the relatively poor repeatability of the flux values from day to day and from time to time was due to variations in the activity of the intestinal loop <u>per se</u> and that the

attempt to limit these by prior use of and training to the continuous feeder by the sheep was not too successful.

Somewhat similar problems have been encountered by other workers attempting to obtain similar data in other species as will be apparent in the ensuing discussion.

Effects of diet

The experimental design was to reduce only the potassium and related organic anion intakes in Period B compared with Period A, but owing to the capricious appetites of the sheep this was not achieved. Instead there was a reduction in total food intake in Period B so that both Na and K intakes were reduced. However, there was a difference in the Na:K intake ratios from approximately 1:1 for Period A to 2:1 for Period B.

Statistically significant effects of this change were found in lower concentrations of K in plasma and jejunal contents and higher concentration of Na in the latter for Period B. Both potassium and water fluxes (absorption) were also lower for Period B. There were no significant differences in sodium fluxes or in the parameters contributing to the estimation and calculation of those fluxes. Mean chloride fluxes indicated a small net absorption for Period A and a net secretion for Period B but owing to the wide variability these means were not significantly different. The osmolality of the effluent fluid was significantly higher for Period B, but this was mainly due to the higher osmolality of the solution used for perfusion, but which reflected the composition of the intestinal contents.

There is little published information on the effects of dietary change on these parameters. However, Bergen et al (8) showed that moderate salt loading by saline intravenous infection or per se did not alter the rate of sodium fluxes across the small intestine of the dog.

Water absorption

The absorption of water obtained by both techniques, direct measurement and PEG application, was significantly greater in period A than that in period B. A major factor that directly caused the greater absorption rate in period A is believed to be the osmolality in the lumen. By direct measurement, the mean absorption rate for period A was 1.9 ml/min with an introduced osmolality in the perfusate of 219.7 mOsm/kg H_2O. In period B the osmolality was 250.0 mOsm/kg H_2O and the rate of water absorption was 1.0 ml/min. The data suggest that water can be absorbed effectively from a lower hypotonic solution and the rate of absorption decreases with increasing tonicity. Grim (63) suggests that the greatest absorption rate of water occurs from hypotonic fluid in the lumen. As the osmolality increases the absorption rate becomes smaller; when the luminal fluid is hypertonic, the absorption rate becomes negative, i.e. there is a net increase in volume of the luminal fluid. Annegers (3) also found that the net fluid absorption decreased as the concentration of chloride or heoxose increased, and that the progressively more hypertonic solutions evoked increasing loop secretion. In the present experiment, there was greater net secretion rate of sodium and secretion of chloride in period B thus tending to increase the osmolality in the lumen. A lower rate of water absorption was found in this period relative to that in period A. These results are similar to those obtained by Annegers (3) and Grim (63).

Parsons (96) suggested that water transport is closely coupled to the absorption of sodium chloride and other solutes. A linear relation between the absorption of water and that of solutes has been shown by MacHardy and Parsons (83) and by Curran and his associates (34,37,38). The present

experiment suggests that the absorption of water is not necessarily solely dependent upon the absorption of sodium and chloride. To illustrate this a comparison of the net flux of Na, Cl and water in both periods can be made. In period A, sodium was secreted at the rate of 130.5 μEq/min, whereas chloride and water were absorbed at the rates of 2.6 μEq/min and 1.9 ml/min respectively. In period B, the secretion of sodium was greater that that in period A, i.e. 142.7 μEq/min, chloride was secreted at the rate of 23.3 μEq/min and the absorption of water decreased to 1.0 ml/min. This comparison suggests that the absorption of water does not depend on the absorption of Na and Cl. It is possible that water is absorbed along with other solutes such as K. In period A, the absorption of K was significantly greater than that in period B, i.e. 52.8 versus 4.1 μEq/min for periods A and B respectively.

The greater concentration and absorption rate of K in period A might have caused the higher rate of water absorption. From the data obtained by Goodall and Kay (60) it can be inferred that 12% of water, 4% of Na, 2% of Cl and 47% of K are absorbed in the small intestine of sheep. These values lead to the conclusion that, in the small intestine of sheep, water is more closely related to the absorption of K rather than to the absorption of Na and Cl. Therefore, it is suggested that the absorption of water, in the present experiment, was partly dependant upon, or was coupled with the absorption of K eventhough there is lack of proportionality between the rates of water and K absorption in the two periods.

Another factor that might have caused the higher rate of absorption of water in period A could have been the daily water consumption. Average daily water consumption in period B was smaller than that in period A (Appendix A

Table 1). The lower water consumption could then have resulted in less water presented for absorption. Phillips (101) showed that there was a tendency for an increase in freezing point depression of the contents in the small intestine in steers which received half water supply relative to the full water supply group. In the present study there was lower water consumption in period B than in period A possibly resulting in the increased osmolality of the luminal contents which eventually brought about the decrease in water absorption.

Effects of Perfusion Rates

Increasing perfusion rates (and therefore flow rates) resulted in significant decreases in transit time and significant increases in intestinal volume, total sodium in the loop, sodium influx and water absorption rate. There were also large but not statistically significant increases in sodium efflux and net flux and in fluxes of chloride which went from a slight absorption at the low rate to increasing secretion from the medium to the high rate of perfusion. There were no consistent effects of perfusion rate on sodium concentration in the loop, on flux of potassium or on osmolality of the effluent.

The general effect of increasing flow rates therefore appears to be increased permeability (i.e. absorption and/or secretion) of the jejunal mucosa to sodium, chloride and water in the absence of changes in osmolality and in the concentration of these electrolytes in the luminal fluid. This is similar to the results found for the entire human intestine by Love, et al (79). The most likely explanation for the change in permeability can be sought in the parallel changes in volume of intestinal fluid and the presumably matching changes in functional surface area of intestinal mucosa.

Thus the combination of more fluid in the loop and larger area of active mucosal surface for the higher perfusion rates resulted in greater net and unidirectional flux rates. However potassium fluxes appear anomalous in this regard.

In addition increase in perfusion rate also caused decreased transit time, or alternately, increased rate of passage through the loop. Thus the loop was not only mechanically transporting more fluid per unit time but also at greater speed, so that there must have been marked increase in motor activity of the gut wall and also in mechanical stimulation of the mucosa. Increased metabolic activity could be implied in these circumstances resulting in increase fluxes of water and electrolytes.

A study of human colon suggested that increased volumes of perfusate by the more rapid perfusion provide more intimate contact between perfusate and colonic absorptive surface (43). Inasmuch as the functional morphology of and intestinal transport in the colon are not much different from those in the jejunum (131), changes in perfusion rate could result in a similar effect in the latter.

Increased motor activity tends to increase blood flow if the contractions are not excessively vigorous (84). In addition, it was found that a mechanical stimulation of the cat jejunal mocosa with PVC tube induced an increase in jejunal blood flow amounting to 30-100% above control. After rapidly flushing the jejunal segment with saline, jejunal blood flow increased 15-50% (11). Moreover, the gut itself appears to originate some of the stimulus for an increased blood flow during absorption of nutrients (84). The increase in perfusion rate may cause or be accompanied somehow by an increase in intestinal blood flow of the loop region. Thus, the rates of

perfusion facilitate an increase in the rate of transport of sodium across the jejunal mucosa.

The variation in rates of sodium flux was large. A big variation as such has been found in the previous studies (8,135,136,144). Visscher et al. (136) found that there were greater variations in rates of sodium flux in jejunum and ileum than in the colon. The variations in the jejunum and ileum resulted from changes in the state of the animal from day to day for which the causes were not ascertained. According to Vaughan's view (135) the great variation of unidirectional flux rates possibly implies that the mixing of radioactivity in the lumen or in the plasma is not complete, and/or the tracer is sequestered in an intermediate location.

The large variation found in the present study may have been due to some of those aforementioned factors. Since there is a simultaneous flux of sodium into and out of the intestinal mucosa, in this situation, therefore, the mixing of radioactivity in the lumen and in plasma may not have been complete. The slightly fluctuation in each perfusion rate from time to time may have caused variations in intestinal motility and in intestinal blood flow which could produce variations in flux rate.

Secretion or absorption in the jejunal region.

There is controversy on the evidence of sodium status in the jejunum. For example, a study in the dog by Grim (63), suggests that the efflux exceeds influx from an isotonic NaCl solution in the jejunal loop. On the contrary, Berger et al. (8) found that the rate of influx was greater than that of efflux in a Thiry fistula. The first study indicated absorption whereas the second showed secretion in the region. However, Visscher et al. (136) suggested that there was a great variation in sodium

flux in the dog jejunum, and they found both negative and positive net flux, i.e. secretion and absorption respectively. In the present experiment a similar fluctuation in sodium fluxes were found and an identical conclusion may be drawn.

In ruminants, Smith (123) and Rogers and Van't Klooster (115) claimed that sodium is secreted into the duodenum and is absorbed in the rest of the small intestine. The secretion of sodium into the duodenum is derived from bile, pancreatic juice and the secretion of Brunners glands. Perry et al. (99) fed calves with 3 rations, semipurified, concentrate and concentrate + hay, and studied the distribution of Na concentration in the gut content. They divided the small intestine into 3 sections, and found that there was an addition of Na in the proximal section compared to that in abomasum and decreases in Na concentration of the intestinal contents in the distal two sections. The proximal section represented duodenum, and the middle and distal sections represented the jejunum and ileum respectively, thus confirming the result by Rogers and Van't Klooster (115).

Horrocks and Phillips (71) divided the small intestine of slaughtered steers into 2 parts. These workers showed an increase in Na in the first part of the small intestine, relative to that in the abomasum, and a decrease in the second part. Mylrea (90) also suggested that in calves, secretion of Na occurs in the proximal half and absorption in the distal half of the small intestine.

Studies by Bruce et al. (21) and Pfeffer et al. (100) in sheep suggest that sodium concentration of the ileal content is greater than that in the duodenal content. Thus it may be inferred that there is a secretion of sodium in the region between duodenum and ileum i.e. in the jejunum. On

the other hand, an increase in sodium concentration in this region could also infer the absorption of water. The data from the present experiment suggest both occurrences, i.e. secretion of sodium and water absorption.

Goodall and Kay (60), Bruce et al. (21) and Pfeffer et al (100) agree on the absorption of sodium in the large intestine of sheep. Goodall and Kay (60) estimated that 96% of sodium in the alimentary tract is absorbed from the large intestine. The present study shows that sodium influx exceeded efflux for every rate of perfusion examined and it appears that in the sheep jejunum there is net secretion of sodium (Figure 5). However, absorption is not excluded. It is also suggested that sodium can be transported across jejunal mucosa against the concentration gradient. This suggestion is based on the fact that sodium transport from lumen to plasma was found to take place from the lower concentration in the loop to the higher concentration in blood plasma.

Potassium net flux

The mean net flux of K indicates absorption in the jejunum. The present experiment is in agreement with that shown in the previous studies on the absorption of K from the small intestine of sheep (21,60,100) and from the small intestine of calves and cows (90,99,115,123). The result obtained by Goodall and Kay (60) suggests that about 50% of K is absorbed in the small intestine; and part of this region is certainly the jejunum in which the absorption of K takes place. In addition, the present experiment suggests that the absorption of K in jejunum occurs along the concentration gradients.

A significant difference between diets for the mean net flux of K was found (Table 11). This effect is presumably due to the levels of Na and K

in the diets. It is apparent that the higher levels of K (and Na) in the diet bring about the greater absorption rate of K. The increase in net absorption of K from the small intestine for the diet containing high Na agrees with the data obtained by Devlin and Roberts (41). However, it might be expected that the low dietary level of K would increase net absorption of K but it was not evident in the present experiment.

In calves, it has been shown that during feeding with 0.25 gm Na and 1.2 gm K per 100 gm. D.M. the Na:K mole ratios in the proximal duodenum and distal ileum were 2.2 and 3.2 respectively. When the calves were on pasture containing 0.09 gm Na and 2.3 K per 100 gm. D.M. these ratios were0.5 for duodenal and 0.4 for ileal contents (123). These data suggest that the quantity in the intestinal content presented for absorption, at least varies with the level of K (and Na) in the diet. Thus it is feasible to suggest that, in the present experiment, the lower rate of absorption in dietary period B is due to the low levels of K (and Na) in the diet.

Net flux of chloride

There was considerable variation in the mean fluxes of chloride. It is apparent that at the low perfusion rate Cl is absorbed approximately in equal rate to that of K. In contrast, at the medium and high rates Cl is obviously secreted along with the secretion of Na but at a lower rate than that of Na.

In the jejunum of the dog and rat, it is suggested that there is absorption of Cl (95,3). In contrast, in the ruminant, many authors suggest that Cl is effectively absorbed in the large intestine (90,60,133). The absorption of Cl in sheep large intestine is estimated to be 98% of the Cl passing through the intestinal tract (60). The absorption of Cl is

believed to take place following the absorption of Na. The absorption of Cl following Na absorption has been shown in rats (95,96). The data obtained by many investigators (21,60,90,100,133) on the absorption of Na and Cl in the large intestine of sheep can be applied to the absorption of Cl following Na absorption. Furthermore, in the rat large intestine, Cl is absorbed in an exchange process with the secretion of HCO_3 (105,96). A similar Cl^- -HCO_3^- exchange mechanism is believed to occur in the large intestine of sheep.

Based on the information that, in ruminants, the majority of Cl passing through the G.I. tract is absorbed in the large intestine, it is assumed that Cl is secreted in the small intestine, particularly in the region in which there is secretion of sodium. The secretion of Cl probably follows the secretion of Na. The present experiment suggests the secretion of Na in the jejunum, thus Cl is secreted along with Na secretion but in lower molar proportion moles. Parson (95) states that in the rat jejunum Na is absorbed faster than Cl. In this experiment the data suggest the secretion of Na and Cl. Therefore, there seems to be no doubt that the rate of secretion of Cl is lower than that of Na which occurs in a similar manner in the rat.

Data obtained by a number of investigators can be adduced to support the evidence of Cl secretion into the sheep jejunum as described in the present experiment. The concentration of Cl in the upper jejunal content increases up to 140 mEq/l (119) in spite of the concentration of Cl in the secretions of bile and pancreatic juice which are 93 and 118 mEq/l respectively (67,127). The increase in Cl concentration of the jejunal content is believed to be contributed by the secretion from the intestinal glands which secrete the jejunal juice containing [Cl^-] of about 134 mEq/l (119). In

view of Na secretion into the loop and increase in Cl concentration found in the effluent, therefore, it is suggested that there is Cl secretion in the jejunum and the rate of secretion depends on the rate of sodium secretion.

A possible mechanism of Cl^- secretion in exchange with HCO_3^- absorption is suggested in the jejunal region. The higher pH in the ileum has been suggested to be due to the secretion of HCO_3^- and absorption of Cl^-. Bicarbonate in the jejunal secretion is about 12 mEq/l whereas that of ileal secretion is 41 mEq/l. The pH in the jejunum is about 7 whereas that in the ileum is 8 (106). The lower pH in the jejunum than in ileum may be due to the absorption of HCO_3^- since it has been shown that HCO_3^- disappears rapidly from the jejunum (90). The disappearance of HCO_3^- with the increase in $[Cl^-]$ in the jejunum, therefore, suggests the similar mechanism of Cl^- and HCO_3^- exchange as that in the ileum and colon, but in reciprocal manner.

Plasma electrolytes

A significant difference in plasma K was found due to diet. Plasma K concentration for period A was greater than for period B. This was probably due to the level of K in the diet. In finishing beef steers, serum K increased with an increase in K level in the diet (42). A similar observation has been made in lambs (23,41).

Plasma chloride concentration was found to be significantly different due to diet. An increase in plasma $[Cl^-]$ was found in spite of the intake of Na and K which were low and presumably the intake of Cl was also low in period B. An increase in plasma Cl during feeding low Na and K has been demonstrated in the previous studies. Devlin et al. (42) fed steers with different levels of dietary K varying from 0.27 to 0.85%, they found that

the serum [Cl^-] increased significantly as the levels of dietary K decreased. Campbell (23) also observed the increase in serum chloride in lambs fed low K. A study by Devlin and Roberts (41) indicated that lambs which were fed with Na at 44 mEq/d had higher serum chloride concentrations than the group fed 129 mEq/d.

The increase in plasma [Cl^-] in period B may have been due to greater efficiency, compared with that of Na, in Cl conservation by kidneys and intestine. This assumption is based on the fact that Cl is more permeable than Na in the aspects of reabsorption by the kidneys and absorption from the intestine. Another explanation for the increased [Cl^-] in plasma when the intake was low, was the lower water consumption in period B compared with period A. MacFarlane et al. (80) showed that the plasma [Cl^-] increased up to 28% during water deprivation. The increase of plasma Cl in this situation accompanies increases of plasma osmolality and Na. In the present experiment there was no increase in plasma [Na] nor a significant increase in plasma osmolality accompanying the increased plasma [Cl^-]. However, there was a tendency for an increase in plasma osmolality in period B.

EXPERIMENT II

Considerable variation was found for several of the major parameters measured in experiment I. Variation between replicate observations within the same animal were of particular interest and experiment II was conducted to see whether better repeatability could be found by simply making the same set of observations under the same conditions on one sheep on each of 4 successive days. The only variation was the composition of the perfusate which was based for each day, on the composition of the intestinal contents on the previous day. In addition there was the problem of skewing of the ^{22}Na recovery curve due to low counts of radioactivity found in effluent samples as long as 50-60 minutes after administration of the marker bolus into the loop. In experiment II the marker boluses contained 100 times the ^{22}Na activity used in experiment I.

Except for day 1 recoveries of radioisotope in the effluent were complete by about 20 minutes and the cumulative recovery curves were virtually identical for the three days. These results, together with similar results for recovery of T1824 administered at the same time, indicate that the markers passed through the loop as a bolus with only limited dispersion. The higher dosage rate of ^{22}Na gave more definitive recovery curves so that more confidence can be put in the transit times which where calculated therefrom. The good repeatability in calculated transit times for days 2, 3 and 4 suggest that, inspite of gradually increasing radioactivity in the blood plasma, influx of ^{22}Na into the loop solution had little or no effect on the isotope recovery curve.

The results for day 1 cannot be explained. Both total recovery of isotope in the effluent and the time course of recovery were markedly at

variance with the values for the succeeding 3 days. Furthermore a similar pattern was found for the T1824 marker, except that transit times calculated for the two markers differed from one another more on the first day. All the other parameters estimated for day 1, which depend directly or indirectly on ^{22}Na measurements, also were markedly at variance with the values for the other days. It could be suggested that some systematic error in the measurement of volumes or analysis of effluent samples was responsible for the aberrant results. However other measurements made did not result in values consistent with such a result. For instance net flux of sodium, (secretion) was much greater for day 1 than for the other days and chloride secretion was found for day 1 but absorption for days 2, 3, and 4. If the low total recovery of ^{22}Na was due to loss of effluent by spillage or undermeasurement of effluent solution then the sodium and chloride fluxes would have been similarly underestimated. Effluent samples and standard solution of ^{22}Na were counted on several occasions and so an error in radioisotope measurement seems unlikely. Finally it could be argued that for some reason the absorption of ^{22}Na from the loop was greatly enhanced on day 1. But the pattern of changes in ^{22}Na counts were similar for all four days and furthermore estimation of total radioactivity absorbed into the body, based on plasma counts and estimations of body fluid volumes, coult not account for the missing radioactivity. The results for day 1 must be discarded for the purposes of further interpretation and discussion which will be limited to the results from day 2, 3 and 4.

Comparison of the results for the 3 days indicate a good degree of repeatability in nearly all the parameter that were measured and calculated.

A significant difference was found in Na efflux, however this can be related to the difference in sodium concentrations in the loop fluid due to differences in composition of the perfusion. The latter also resulted in differences in osmolality in effluent fluids which were noted. The differences in water absorption were probably due to the osmolality differences as was discussed for experiment I. Therefore, it can be said that the simplified experimental procedure used in experiment II resulted in good repeatability of the observed parameters. The half hour collection periods for estimation of net fluxes also resulted in fairly repeatable results except for chloride and osmolality of the effluent. The variability in the latter was statistically significant but the standard error was in fact quite small. It would seem then that beginning and end collection errors as were discussed for experiment I did not seem to be an important source of variability in experiment II. Thus such collection errors may be judged not to have contributed very much to the variability found in experiment I.

SUMMARY AND CONCLUSIONS

1) Two experiments were conducted to study the unidirectional fluxes of sodium and the net fluxes of potassium, chloride and water from temporary, approximately 250 cm long, Thiry-Vella loops of the jejunum in mature female sheep.

2) In the first experiment three sheeps were used and the experimental variables studied were a) two dietary treatments resulting in intakes of 150 and 142 mEq/day of sodium and potassium respectively in dietary period A and 42 and 23 mEq/day of sodium and potassium respectively in period B; b) for each dietary treatment perfusion fluids were pumped into the proximal end of the Thiry-Vella loops at approximately 7, 13 and 26 ml/min on each of 3 days. There was thus a total of 9 perfusion studies on each of the sheep for each dietary treatment.
Radioactive sodium (^{22}Na) and T-1824 were used to study transit times of fluid through the loops and the ^{22}Na was also used to determine movements of sodium from out of the loop fluid. Net fluxes of electrolytes and water were measured as the net differences between input to the loop in the perfusion fluid and output collected as effluent fluid at the distal end of the loop. Water fluxes were also measured using PEG 4000 as a non-absorbable marker in the perfusion fluids.
During prior equilibration periods and during the experimental periods the sheep were fed from an automatic feeder in an attempt to minimize variations in metabolic status within the sheep. Various samples of intestinal contents and plasma were analysed for electrolyte and osmolar composition and for ^{22}Na.

3) The second experiment was conducted to examine some of the possible sources of variation found among replications in the first experiment.

One sheep was maintained on the automatic feeder as before and identical single perfusion studies on each of 4 successive days. The dose of ^{22}Na used was 100 times that used in experiment I and net fluxes were determined for 4 successive one-half hour collections periods. The perfusion rate was 13 ml/min.

4) The only statistically significant ($P < 0.05$) differences found between dietary treatments were in the Na and K concentrations in intestinal contents and plasma and in the absorption rates for potassium and water.

   The lower intake of potassium (and also sodium) in dietary period B resulted in intestinal contents containing only about half the concentration of potassium compared with period A, but sodium concentration was increased by about one-third so that total Na + K concentration was higher for period B. The dietary restriction of electrolytes also resulted in lower plasma potassium concentrations. The perfusion fluids were formulated to simulate the electrolyte composition of intestinal contents for each perfusion study. Thus the lower potassium and water absorption rates for period B were consonant with the lower potassium concentrations and higher osmolality of the perfusion fluids on the basis of passive movements of these substances.

5) The imposed variations in flow rate of fluid through the loop resulted in a number of significant differences in measured parameters. Higher flow rates resulted in shorter transit times of fluid through the loop and increased volume of fluid in the loop. Neither the concentration of sodium in the loop fluid, nor the transfer rate of ^{22}Na out of the loop were affected by flow rate. Efflux of sodium from the loop is

calculated from volume of loop fluid, concentration of sodium in the fluid and the sodium transfer rate. Although higher rates of flow resulted in markedly higher mean sodium efflux the within group variations were so large as to preclude statistical significance. Higher rates of perfusion were also accompanied by higher net fluxes of sodium, chloride and water but statistical significance was found only for the last mentioned. There was net secretion of sodium into the loop at all rates of perfusion, but there was absorption of chloride at the low rate and secretion at the two higher rates. Rates of water absorption by direct measurement were consistently lower then by the PEG technique but significantly increased absorption rates with higher perfusion rates were found by both methods. Influx of sodium into the loop was calculated as the difference between efflux and net flux. The different rates in perfusion caused significantly different sodium influxes.

No differences in plasma electrolytes and osmolality were found in relation to the different perfusion rates.

6) In experiment II the results for the first day were considerably different in several respects than those for the 3 succeeding days. A careful evaluation of possible technological errors and biological variations failed to indicate the reason for these aberrant results which were then disregarded from further consideration. The 100 times higher dosage of ^{22}Na resulted in more clearly defined and fairly highly reproducible measures of transit time and transfer rate constant. Comparisons of half hour collection periods for estimation of net fluxes indicated relatively modest variability. Beginning and

end collection errors are inherent in time collection systems where flow is intermittent as was observed in effluent collection in period A. From the data for experiment II it is concluded that such errors probably contributed relatively little to the variability found in experiment I using 1 hour collections.

7) Consideration of the results both for different diets and different rates of perfusion and including experiment II lead to the conclusion that net sodium and potassium movements occurred along concentration gradients. In some cases chloride movement was against the concentration gradient and appeared instead to be related to the rate of sodium movement but not on a mole for mole basis. Water moved from solution of lower osmolality and the magnitude of the osmolality gradient positively affected rate of water absorption.

8) Increasing the rate of perfusion of fluid into the loop, and thus the flow rate had a principle effect of increasing the volume of fluid in the loop. It is concluded that the latter was primarily responsible for the fairly generally observed increase of permeability of the jejunum to water and electrolytes presumably by increasing the interface between fluid and mucosa and possibly also by generally increasing the mechanical and metabolic activity of the gut wall.

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APPENDIX A

EXPERIMENT I

TABLE 1

DAILY FEED INTAKE, WATER CONSUMPTION,
AND SODIUM AND POTASSIUM INTAKE

Daily Intake	Sheep No.		
	I(9074A)	II(D.S.28)	III(D.S.29)
Feed (gm)			
Period A	600	330	1007
Period B	143	300	186
Water (l)			
Period A	1.7	1.4	1.2
Period B	1.4	1.1	1.2
Sodium (mEq)*			
Period A	140	77	235
Period B	34	70	44
Potassium (mEq)*			
Period A	132	73	222
Period B	16	33	21

* Based on daily feed intake.

TABLE 2

SODIUM AND POTASSIUM CONCENTRATIONS AND OSMOLALITY OF THE PERFUSATE SOLUTIONS

Day	Sheep No.	$[Na^+]$ & $[Cl^-]$ mEq/l	$[K^+]$ mEq/l	Osmol.* mOsm/KgH_2O
Period A				
One	I	84.0	14.5	182
	II	134.0	8.8	260
	III	100.8	16.7	212
Two	I	135.4	6.0	260
	II	112.0	9.6	214
	III	106.0	13.5	214
THREE	I	95.5	20.2	210
	II	110.0	11.6	218
	III	100.8	13.5	205
Period B				
One	I	105.0	8.4	206
	II	145.0	4.0	269
	III	136.0	10.0	265
Two	I	141.0	5.2	266
	II	122.0	5.6	230
	III	156.0	5.6	291
Three	I	127.0	5.4	240
	II	-	-	-
	III	149.0	8.0	282

* By analysis.

TABLE 3

RECOVERY OF TOTAL RADIO-ACTIVITY (cpm) DURING COLLECTION PERIODS.

Day	Sheep No.	Perfusion Rate	Period A	Period B
		"LOW"		
One	I		32,103	25,974
	II		31,018	33,237
	III		20,583	36,211
Two	I		30,499	22,771
	II		43,131	35,135
	III		28,659	36,247
Three	I		41,524	36,176
	II		28,121	-
	III		29,238	37,052
		"MEDIUM"		
One	I		35,988	40,864
	II		38,630	38,317
	III		37,684	38,305
Two	I		31,878	38,986
	II		38,740	40,561
	III		31,817	37,286
Three	I		34,920	40,459
	II		38,282	-
	III		35,453	40,721
		"HIGH"		
One	I		29,079	40,681
	II		39,634	40,331
	III		57,311	42,169
Two	I		43,284	42,456
	II		48,636	43,446
	III		40,176	44,127
Three	I		44,942	48,827
	II		40,794	-
	III		27,329	44,662

TABLE 4

PERIOD A

PERFUSION RATE (P.R.), MEAN FLOW RATE ($\bar{F}$), TRANSIT TIME (tt), AND RATE CONSTANT OF ^{22}Na TRANSFER (λ)

Day	Sheep No.	P.R. (ml/min)	$\bar{F}$ (ml/min)	tt (min)	λ (per min)
		"LOW"			
One	I	7.00	6.84	7.96	0.0183
	II	7.20	6.63	10.67	0.0192
	III	6.70	5.94	8.11	0.0726
Two	I	7.10	6.58	7.77	0.0565
	II	7.05	6.45	10.89	0.0231
	III	7.10	6.53	12.30	0.0226
Three	I	6.90	6.11	8.79	0.0011
	II	6.75	5.35	13.42	0.0361
	III	6.80	6.28	11.18	0.0588
		"MEDIUM"			
One	I	13.00	11.74	7.80	0.0233
	II	13.30	12.86	8.15	0.0190
	III	12.60	11.70	8.20	0.0429
Two	I	12.60	12.13	7.90	0.0603
	II	12.25	11.71	12.20	0.0212
	III	12.00	10.65	6.46	0.0336
Three	I	13.30	12.76	9.57	0.0118
	II	12.75	12.68	8.83	0.0434
	III	12.50	11.38	10.12	0.0588
		"HIGH"			
One	I	26.00	22.35	12.36	0.0341
	II	25.30	24.58	12.76	0.0091
	III	25.30	23.51	7.56	0.0045
Two	I	25.50	25.03	8.79	0.0273
	II	25.25	24.24	7.41	0.0063
	III	24.60	23.57	8.35	0.0078
Three	I	24.50	23.67	8.50	0.0082
	II	25.75	25.53	8.54	0.0187
	III	24.10	22.44	8.27	0.0647

TABLE 5

PERIOD B

PERFUSION RATE (P.R.), MEAN FLOW RATE ($\bar{F}$), TRANSIT TIME (tt), AND RATE CONSTANT OF ^{22}Na TRANSFER (λ).

Day	Sheep No.	P.R. (ml/min)	$\bar{F}$ (ml/min)	tt (min)	λ (per min)
		"LOW"			
One	I	6.10	5.69	16.59	0.0234
	II	6.70	6.51	12.75	0.0133
	III	6.60	6.25	12.49	0.0034
Two	I	7.20	7.19	14.63	0.0484
	II	7.25	6.52	18.73	0.0222
	III	7.15	6.97	9.15	0.0469
Three	I	6.75	6.27	16.36	0.0099
	II	-	-	-	-
	III	6.85	6.68	6.86	0.0330
		"MEDIUM"			
One	I	12.10	11.05	10.46	0.0203
	II	13.10	12.75	13.68	0.0123
	III	10.35	10.18	7.85	0.0031
Two	I	13.50	13.08	8.14	0.0344
	II	13.30	12.72	11.79	0.0107
	III	11.65	11.13	8.31	0.0569
Three	I	13.40	13.17	8.98	0.0126
	II	-	-	-	-
	III	13.30	12.78	7.69	0.0024
		"HIGH"			
One	I	22.50	20.71	8.69	0.0183
	II	18.90	18.09	8.44	0.0170
	III	24.50	24.38	7.44	0.0353
Two	I	23.97	23.81	7.62	0.0232
	II	24.75	23.81	8.73	0.0282
	III	26.05	24.91	7.84	0.0418
Three	I	24.40	23.70	9.55	0.0075
	II	-	-	-	-
	III	25.10	25.00	6.05	0.0259

TABLE 6

PERIOD A

SODIUM, POTASSIUM, AND CHLORIDE CONCENTRATIONS OF THE EFFLUENT, AND THE QUANTITY OF WATER RECOVERED DURING THE COLLECTION PERIODS

Day	Sheep No.	Perfusion Rate	Sodium (mEq/l)	Potassium (mEq/l)	Chloride (mEq/l)	Water (ml/hr.)
		"LOW"				
One	I		117	11.3	119.2	400.5
	II		141	6.2	138.3	363.8
	III		144	10.4	126.4	311.2
Two	I		155	5.6	156.7	363.4
	II		144	5.4	132.2	352.0
	III		151	3.8	135.1	357.0
Three	I		134	11.8	112.1	318.6
	II		114	6.2	118.0	237.0
	III		133	9.9	116.1	345.0
		"MEDIUM"				
One	I		148	16.1	108.2	629.3
	II		152	8.1	149.3	745.2
	III		137	14.0	110.2	648.4
Two	I		149	5.0	141.4	699.5
	II		152	6.5	141.7	670.2
	III		138	10.1	124.1	557.7
Three	I		124	16.1	112.5	624.7
	II		119	9.0	112.3	757.5
	III		131	10.8	114.5	615.2
		"HIGH"				
One	I		101	14.1	88.9	1122.0
	II		180	10.5	175.2	1431.7
	III		111	13.9	118.4	1303.2
Two	I		154	6.0	152.8	1473.4
	II		150	7.3	143.0	1394.3
	III		131	11.9	109.8	1352.5
Three	I		103	18.0	96.0	1370.3
	II		113	10.2	107.2	1518.7
	III		119	12.3	98.1	1246.9

TABLE 7

PERIOD B

SODIUM, POTASSIUM, AND CHLORIDE CONCENTRATIONS OF THE EFFLUENT, AND THE QUANTITY OF WATER RECOVERED DURING THE COLLECTION PERIODS

Day	Sheep No.	Perfusion Rate	Sodium (mEq/l)	Potassium (mEq/l)	Chloride (mEq/l)	Water (ml/hr.)
		"LOW"				
One	I		133	6.6	129.6	316.8
	II		158	3.6	149.2	379.6
	III		151	7.5	140.9	353.9
Two	I		143	4.7	142.5	430.9
	II		138	5.0	139.2	347.0
	III		163	4.9	160.1	407.5
Three	I		138	5.2	131.6	347.2
	II		-	-	-	-
	III		151	6.1	140.9	390.3
		"MEDIUM"				
One	I		133	8.4	132.1	600.1
	II		154	3.9	141.2	744.5
	III		167	9.6	159.9	600.3
Two	I		180	6.2	180.9	760.0
	II		132	5.4	129.8	788.2
	III		178	5.4	176.3	636.1
Three	I		147	6.0	125.2	775.9
	II		-	-	-	-
	III		152	6.7	140.2	735.2
		"HIGH"				
One	I		149	10.4	141.2	1135.4
	II		144	4.0	140.4	1036.6
	III		169	10.7	159.1	1455.0
Two	I		186	6.4	176.5	1419.4
	II		129	5.7	120.6	1372.5
	III		169	5.9	169.0	1425.6
Three	I		166	7.8	121.8	1380.3
	II		-	-	-	-
	III		153	7.1	141.6	1494.5

TABLET 8

INTESTINAL VOLUMES (I.V.), AMOUNT OF SODIUM IN THE LOOP AND ITS MEAN CONCENTRATION THEREIN [$\bar{Na}$]

Day	Sheep No.	Perfusion rate	Period A I.V. (ml)	Period A Amt.Na (mEq)	Period A [$\bar{Na}$] (mEq/l)	Period B I.V. (ml)	Period B Amt. Na (mEq)	Period B [$\bar{Na}$] (mEq/l)
		"LOW"						
One	I		54.45	5.47	100.5	94.40	11.23	119.0
	II		70.74	9.73	137.5	83.00	12.58	151.5
	III		48.17	5.90	122.4	78.06	11.20	143.5
Two	I		51.13	7.43	145.3	105.19	14.94	142.0
	II		70.24	8.99	128.0	122.12	15.88	130.0
	III		80.32	10.32	128.5	63.78	10.17	159.5
Three	I		53.71	6.17	114.8	102.58	13.59	132.5
	II		71.80	8.04	112.0	-	-	-
	III		70.21	8.21	116.9	45.83	6.88	150.0
		"MEDIUM"						
One	I		91.57	10.62	116.0	115.58	13.75	119.0
	II		104.81	14.99	143.0	174.42	26.08	149.5
	III		95.94	11.41	118.9	79.91	12.11	151.5
Two	I		95.83	13.64	142.3	106.47	12.09	160.5
	II		142.86	18.86	132.0	149.97	19.05	127.0
	III		68.80	8.39	122.0	92.49	15.45	167.0
Three	I		112.54	12.36	109.8	118.27	16.20	137.0
	II		111.96	12.82	114.5	-	-	-
	III		115.17	13.35	115.9	98.28	14.79	150.5
		"HIGH"						
One	I		276.25	25.55	92.5	179.97	22.86	127.0
	II		313.64	49.24	157.0	152.68	22.06	144.5
	III		177.74	18.82	105.9	181.39	27.66	152.5
Two	I		220.01	31.86	144.8	181.43	29.66	163.5
	II		179.62	23.53	131.0	207.86	26.09	125.5
	III		196.81	23.32	118.5	195.29	31.74	162.5
Three	I		201.20	19.98	99.3	226.34	33.16	146.5
	II		218.03	24.31	111.5	-	-	-
	III		185.58	20.40	109.9	151.25	22.84	151.0

TABLE 9

PERIOD A

RATE OF UNIDIRECTIONAL FLUX OF SODIUM, AND NET FLUXES OF SODIUM, POTASSIUM AND CHLORIDE (μEq/min)

Day	Sheep No.	Perfusion rate	Sod. Efflux	Sod. Net Flux*	Sod. Influx**	Pot. Net Flux*	Chlor. Net Flux*
		"LOW"					
One	I		100.14	-192.98	293.12	25.93	-208.00
	II		186.76	- 9.87	196.63	25.46	126.60
	III		428.08	- 71.52	499.60	58.23	19.77
Two	I		419.72	22.92	396.80	8.26	12.62
	II		208.50	- 55.20	263.70	35.53	13.73
	III		233.25	-145.85	379.10	72.76	-51.55
Three	I		6.78	- 52.23	59.02	76.91	64.05
	II		290.29	292.20	- 1.91	53.61	277.58
	III		482.61	- 79.32	561.92	34.88	17.85
		"MEDIUM"					
One	I		247.50	-460.17	707.67	18.90	- 42.83
	II		284.91	-105.63	390.54	16.44	- 72.48
	III		489.37	-210.43	699.81	59.33	79.18
Two	I		822.26	- 30.43	852.70	76.63	57.37
	II		399.79	-325.83	725.62	45.00	-211.35
	III		288.02	- 10.72	292.92	67.47	118.40
Three	I		145.82	- 20.22	166.03	100.97	99.10
	II		556.39	- 99.88	656.27	33.64	- 16.30
	III		784.84	- 83.18	868.03	57.30	85.88
		"HIGH"					
One	I		871.35	295.30	576.05	113.33	521.16
	II		448.10	-904.90	133.00	- 28.62	-790.13
	III		84.70	139.32	- 54.65	120.56	22.50
Two	I		869.72	-327.75	1197.47	4.92	-298.28
	II		148.24	-657.75	805.99	72.06	-496.93
	III		181.91	-345.37	527.28	62.28	132.75
Three	I		163.83	- 11.37	175.19	84.35	146.45
	II		454.60	- 27.72	482.31	40.02	116.82
	III		220.56	- 43.73	264.30	69.11	389.98

* Positive values denote net absorption whereas the negative ones denote net secretion.

** The negative value denotes that net flux exceeds efflux.

TABLE 10

PERIOD B

RATE OF UNIDIRECTIONAL FLUX OF SODIUM, AND NET FLUXES OF SODIUM, POTASSIUM, AND CHLORIDE (μEq/min)

Day	Sheep No.	Perfusion rate	Sod. Efflux	Sod. Net Flux*	Sod. Influx	Pot. Net Flux*	Chlor. Net Flux*
		"LOW"					
One	I		262.86	- 64.73	327.59	15.97	-144.05
	II		167.25	- 28.12	195.37	3.83	27.00
	III		38.09	6.95	31.14	21.47	66.05
Two	I		722.95	- 11.78	734.73	3.11	- 8.68
	II		352.44	86.40	267.04	11.68	78.93
	III		477.08	8.35	468.73	6.22	27.85
Three	I		134.56	58.68	75.88	5.90	95.32
	II		-	-	-	-	-
	II		226.83	38.40	188.43	14.79	103.97
		"MEDIUM"					
One	I		279.21	- 59.72	338.93	17.63	- 51.42
	II		320.73	- 11.38	332.11	3.64	146.33
	III		37.53	-263.23	300.76	7.45	-193.10
Two	I		587.85	-376.50	964.35	- 8.71	-388.15
	II		203.79	20.57	183.22	8.09	46.28
	III		878.87	- 69.70	948.57	7.04	52.00
Three	I		204.15	-199.15	403.30	- 5.23	81.58
	II		-	-	-	-	-
	III		331.32	119.20	212.12	23.32	262.68
		"HIGH"					
One	I		418.27	-454.08	872.35	- 7.80	-310.80
	II		375.06	252.67	122.39	5.63	314.52
	III		976.45	-766.25	1742.70	-14.48	-526.18
Two	I		688.21	-1020.37	1708.58	-27.94	-796.35
	II		735.64	68.62	667.02	8.21	260.08
	III		1326.54	48.37	1278.17	5.70	47.42
Three	I		248.69	-720.03	968.72	-47.68	296.10
	II		-	-	-	-	-
	III		591.52	-710.83	1302.35	23.95	212.13

* Positive values denote net absorption whereas the negative ones denote net secretion.

TABLE 11

PERIOD A

NET ABSORPTION OF WATER, BY DIRECT MEASUREMENT AND BY PEG TECHNIQUE AND OSMOLALITY OF THE EFFLUENT.

Day	Sheep No.	Perfusion rate	Water (Dir. Measure) ml/min	Water (PEG technique) ml/min	Osmolality mOsm/kg H_2O
		"LOW"			
One	I		0.33	2.78	247.27
	II		1.14	1.49	278.77
	II		1.51	1.98	277.18
Two	I		1.04	2.38	294.12
	II		1.18	1.71	255.95
	III		1.15	2.82	278.74
Three	I		1.59	2.64	276.50
	II		2.80	1.03	258.87
	III		1.05	2.79	264.18
		"MEDIUM"			
One	I		2.50	4.56	223.60
	II		0.88	3.22	325.35
	III		1.79	2.71	255.40
Two	I		0.94	3.97	272.68
	II		1.08	5.00	292.05
	III		2.81	4.17	266.32
Three	I		2.89	4.19	263.95
	II		0.13	0.49	240.22
	III		2.25	4.71	266.03
		"HIGH"			
One	I		7.30	4.56	187.17
	II		1.44	8.87	329.98
	III		3.58	5.53	233.40
Two	I		0.94	9.76	284.90
	II		2.01	10.74	285.92
	III		2.06	6.27	239.57
Three	I		1.66	6.46	231.55
	II		0.44	0.95	233.05
	III		3.32	7.47	234.35

TABLE 12

PERIOD B

NET ABSORPTION OF WATER, BY DIRECT MEASUREMENT AND BY PEG TECHNIQUE AND OSMOLALITY OF THE EFFLUENT.

Day	Sheep No.	Perfusion rate	Water (Dir. Measure.) ml/min	Water (PEG technique) ml/min	Osmolality mOsm/kgH_2O
		"LOW"			
One	I		0.82	1.74	272.97
	II		0.37	0.93	290.10
	III		0.70	1.22	300.27
Two	I		0.03	0.99	282.85
	II		1.58	1.40	274.33
	III		0.36	0.88	318.15
Three	I		0.96	0.38	267.52
	II		-	-	-
	III		0.35	1.03	297.62
		"MEDIUM"			
One	I		2.10	4.72	267.92
	II		0.69	1.61	286.87
	III		0.35	2.63	330.28
Two	I		0.83	4.63	357.30
	II		1.16	2.22	260.85
	III		1.05	2.40	343.82
Three	I		0.47	1.44	260.53
	II		-	-	-
	III		1.05	1.86	289.88
		"HIGH"			
One	I		3.58	9.42	291.90
	II		1.62	2.88	287.58
	III		0.25	6.17	321.75
Two	I		0.31	9.13	349.85
	II		1.88	3.42	251.50
	III		2.29	4.34	326.70
Three	I		1.40	1.81	253.05
	II		-	-	-
	III		0.19	6.40	293.25

TABLE 13

PERIOD A

CONCENTRATIONS OF SODIUM, POTASSIUM AND CHLORIDE IN BLOOD PLASMA AND PLASMA OSMOLALITY AT THE END OF EACH PERFUSION RATE

Day	Sheep No.	Standard Sol. (cpm/2ml)[1]		Background (cpm)[2]		Plasma[1*]	
		Period A	Period B	Period A	Period B	Period A	Period B
				"LOW"			
One	I	37133	38218	45	49	7000	13500
	II	38045	39369	46	52	21200	12000
	III	37093	37775	44	48	25440	9300
Two	I	35571	38058	48	48	13500	10800
	II	39553	36381	43	49	15900	12000
	III	36287	38633	48	43	74120	18550
Three	I	37674	38938	46	43	13500	13500
	II	38586	-	49	-	13250	-
	III	37066	38942	44	49	12000	13250
				"MEDIUM"			
One	I	37133	38218	45	49	4200	8100
	II	38045	39369	46	52	13250	9600
	III	37093	37775	44	48	43248	15000
Two	I	37807	38058	42	48	8100	8100
	II	39553	36381	43	49	21200	12000
	III	36287	38633	48	43	10900	23850
Three	I	37674	38938	46	43	8100	13500
	II	38586	-	49	-	13250	-
	III	37066	38942	44	49	12000	15900
				"HIGH"			
One	I	37133	37986	45	46	5600	10800
	II	38045	39369	46	52	13900	12000
	III	37093	37775	44	48	35616	18600
Two	I	35571	38058	48	48	10800	16200
	II	39553	36381	43	49	15900	12000
	III	36287	38633	48	43	7540	18550
Three	I	37674	38938	46	43	13500	18900
	II	38586	-	49	-	13250	-
	III	37066	38942	44	49	4800	15900

1 Average of the 5 minute counting.
2 Average of the 30 minute counting.
1* Based on plasma volume being 5% of total body weight.

TABLE 14

PERIOD A

CONCENTRATIONS OF SODIUM, POTASSIUM, AND CHLORIDE IN BLOOD PLASMA, AND PLASMA OSMOLALITY AT THE END OF EACH PERFUSION RATE.

Day	Sheep No.	Perfusion Rate	Sodium (mEq/1)	Potassium (mEq/1)	Chloride (mEq/1)	Osmolality mOsm/kgH_2O
		"LOW"				
One	I		125.8	4.3	80.4	269
	II		137.0	2.3	115.7	291
	III		147.0	3.2	87.6	291
Two	I		165.9	3.8	62.4	275
	II		120.0	2.2	113.4	288
	III		150.2	3.6	87.2	294
Three	I		152.3	3.4	104.3	288
	II		153.3	2.6	111.2	283
	III		151.2	3.4	101.7	294
		"MEDIUM"				
One	I		132.3	3.4	79.9	269
	II		141.0	2.8	114.4	296
	III		143.9	3.3	82.4	293
Two	I		139.7	3.6	79.3	278
	II		166.0	3.1	110.8	288
	III		156.5	3.8	102.2	293
Three	I		152.3	4.0	111.9	293
	II		168.0	2.5	109.1	286
	III		153.3	3.6	94.2	294
		"HIGH"				
One	I		126.0	3.4	78.5	267
	II		146.0	2.6	118.1	299
	III		147.1	3.5	86.8	290
Two	I		143.9	3.8	67.8	294
	II		154.0	2.4	114.0	284
	III		156.5	3.8	89.7	295
Three	I		152.3	3.4	107.7	292
	II		189.0	2.6	109.0	281
	III		150.2	3.3	96.5	289

TABLE 15

PERIOD B

CONCENTRATIONS OF SODIUM, POTASSIUM, AND CHLORIDE IN BLOOD PLASMA, AND PLASMA OSMOLALITY AT THE END OF EACH PERFUSION RATE.

Day	Sheep No.	Perfusion Rate	Sodium (mEq/l)	Potassium (mEq/l)	Chloride (mEq/l)	Osmolality mOsm/kgH_2O
		"LOW"				
One	I		141.0	3.0	103.2	284
	II		184.8	2.6	101.7	280
	III		141.0	2.5	103.1	304
Two	I		144.0	2.5	106.0	278
	II		172.2	2.6	109.3	309
	III		130.0	2.3	112.6	296
Three	I		133.4	2.8	103.2	276.
	II		-	-	-	-
	III		152.3	3.0	108.2	303
		"MEDIUM"				
One	I		146.0	3.0	99.7	288
	II		184.8	2.7	104.1	290
	III		159.0	2.8	104.2	297
Two	I		108.0	2.6	107.9	272
	II		143.9	3.3	108.6	308
	III		142.0	2.5	108.7	294
Three	I		144.9	3.2	106.1	287
	II		-	-	-	-
	III		169.1	2.9	109.8	310
		"HIGH"				
One	I		144.0	2.4	102.6	287
	II		165.9	2.7	103.8	290
	III		125.0	2.2	105.3	294
Two	I		133.0	2.9	102.5	272
	II		186.9	2.6	110.0	313
	III		120.0	2.3	111.2	293
Three	I		98.7	3.2	100.6	276
	II		-	-	-	-
	III		130.2	2.7	112.2	306

TABLE 16

HAEMOGLOBIN (Hb) AND PACKED CELL VOLUME

Day	Sheep No.	Hb (gm %)		P.C.V. (%)	
		Period A	Period B	Period A	Period B
One	I	9.50	9.75	29.00	26.75
	II	11.50	10.50	31.50	33.50
	III	9.75	8.25	30.75	26.00
Two	I	9.50	9.00	27.00	26.50
	II	11.25	12.00	31.50	31.50
	III	6.00	8.25	25.50	25.00
Three	I	6.50	9.25	26.50	27.70
	II	10.75	-	31.50	-
	III	8.00	9.00	21.00	23.00

TABLE 17

MEANS SQUARES FOR SODIUM AND POTASSIUM CONCENTRATIONS IN THE INTESTINAL CONTENT, TRANSIT TIME (tt), INTESTINAL VOLUME (I.V.) AMOUNT OF SODIUM IN THE LOOP AND EFFLUX OF ^{22}Na (λ)

Item Source of Variation	Diets	Perfusion rate	Interaction	Error
Degrees of Freddom	1	2	2	12
$[Na^+]^1$	2,947.48*	-	-	279.43
$[K^+]^2$	163.24*	-	-	11.92
tt	6.75	17.64*	8.08	4.14
I.V.	32.40	24,889.98*	1,595.10	503.65
Amt. Na in Loop	44.05	425.43*	8.70	12.12
λ	18,049.99	9,585.72	16,335.50	9,556.44

* ($P < 0.05$)

1, 2 Error mean squares were 15.

TABLE 18

MEAN SQUARES FOR SODIUM, POTASSIUM AND CHLORIDE FLUXES

Item Source of Variation	Diets	Perfusion rate	Interaction	Error
Degrees of Freedom	1	2	2	12
Sodium Efflux	21,156.24	88,052.12	54,412.73	26,080.81
Sodium Net Flux	676.14	110,262.54	18,313.39	52,087.49
Sodium Influx	29,433.84	394.593.14*	132,895.45	77,110.63
Potassium Net Flux	10,682.35*	16.60	411.69	293.43
Chloride Net Flux	3,010.88	12,323.18	1,074.19	39,924.81

* ($P < 0.05$)

TABLE 19

MEAN SQUARES FOR WATER ABSORPTION (DIRECT MEASUREMENT AND PEG TECHNIQUE), AND EFFLUENT OSMOLALITY

Item Source of Variation	Diets	Perfusion rate	Interaction	Error
Degrees of Freedom	1	2	2	12
Water Absorption (Direct Measurement)	2.93*	1.57*	0.071	0.39
Water Absorption (PEG Technique)	6.83	29.02*	0.10	0.87
Effluent Osmolality	4,004.83*	141.21	246.70	399.44

* ($P<0.05$)

TABLE 20

MEAN SQUARES FOR PLASMA ELECTROLYTE CONCENTRATIONS AND OSMOLALITY

Item Source of Variation	Diets	Perfusion rate	Interaction	Error
Degrees of Freedom	1	2	2	12
Sodium	0.03	22.62	117.49	313.18
Potassium	1.25*	0.06	-	0.21
Chloride	375.38	2.47	1.66	106.71
Osmolality	135.57	6.84	0.87	83.83

* ($P < 0.05$)

APPENDIX B

EXPERIMENT II

TABLE 21

SODIUM AND POTASSIUM CONCENTRATIONS AND OSMOLALITY OF THE PERFUSATE SOLUTIONS, HAEMOGLOBIN (Hb) AND PACKED CELL VOLUME (PCV)

Day	Na (mEq/l)	K (mEq/l)	Osmolality* (mOsm/kg H_2O)	Hb (gm %)	PCB (%)
1	45.03	46.13	170.00	12.50	29.00
2	68.60	42.80	205.40	11.50	28.25
3	45.50	46.93	170.80	11.25	30.75
4	46.90	50.13	180.20	12.00	30.00

* Measurement

TABLE 22

MEAN TRANSIT TIME (tt) OBTAINED BY
RADIOACTIVE AND T1824 CALCULATIONS, AND
RATE CONSTANT OF ^{22}Na TRANSFER (λ)

Day	tt (min)		λ (per min)
	Radioactive	T1824	
1	9.73	10.70	0.08
2	5.96	6.05	0.04
3	6.59	6.30	0.04
4	7.26	7.53	0.03

TABLE 23

TOTAL RADIOACTIVITY IN THE EFFLUENT AND BLOOD PLASMA FOR THE FIRST AND SECOND HOUR FOLLOWING INTRAJEJUNAL INTRODUCTION, AND TIME AT WHICH TOTAL T1824 RECOVERED

Day	Effluent[1]		PLASMA[2]		Time for T1824 (min)
	1st Hour	2nd Hour*	1st Hour	2nd Hour	
1	2,135,905	2,127,259	89,244	67,536	30 - 40
2	3,762,313	3,785,181	81,926	93,974	11 - 13
3	3,725,695	3,703,873	156,624	110,841	13 - 15
4	3,840,140	3,836,051	183,129	154,214	13 - 15

1, 2 Half life was corrected.

* Small correction of tracer returned to the lumen was made.

TABLE 24

INFLOW (Fi) OUT-FLOW (Fo), AVERAGE FLOW ($\bar{F}$) OF WATER, INTESTINAL VOLUME, AMOUNT OF SODIUM IN THE LOOP, AND MEAN SODIUM CONCENTRATION AT DIFFERENT INTERVAL COLLECTIONS (Min) DURING THE PERFUSION STUDY.

Item	Day	0 -13	13-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100	100-110	110-120
Fi	1	156	84	130	130	130	140	130	130	140	140	130	130
(ml)	2	156	84	140	130	125	125	130	130	140	140	130	130
	3	164.5	88.5	127	120	135	135	135	135	125	125	135	135
	4	164.5	88.5	137	120	130	130	130	130	135	135	130	130
Fo	1	149.8	100.8	132.0	144.9	95.4	115.6	118.4	76.4	138.4	31.7	195.9	111.1
(ml)	2	156.4	73.1	101.0	108.9	119.2	84.5	125.9	119.0	91.7	86.4	125.5	115.5
	3	109.6	104.1	97.8	102.7	80.1	96.6	103.4	111.4	99.2	97.6	76.6	103.6
	4	136.6	82.3	110.5	82.5	112.2	43.9	107.5	98.3	107.6	111.1	98.2	102.1
$\bar{F}$	1	11.8	13.2	13.1	13.8	11.3	12.8	12.4	10.3	13.9	8.6	16.3	12.6
(ml/min)	2	12.0	11.2	12.1	12.0	12.2	10.5	12.8	12.5	11.6	11.3	12.8	12.1
	3	10.5	13.8	11.2	11.1	10.8	11.6	11.9	12.3	11.2	11.1	10.6	11.6
	4	11.6	12.2	12.4	11.1	12.1	8.7	11.9	11.4	12.1	12.3	11.4	11.6
I.V.	1	114.46	128.04	127.07	133.86	109.61	124.16	120.28	99.91	134.83	83.42	158.11	122.22
(ml)	2	72.00	67.20	72.60	72.00	73.20	63.00	76.80	75.00	69.60	67.80	76.80	72.60
	3	69.30	91.08	73.92	73.20	71.28	76.56	78.54	81.18	73.92	73.26	69.96	78.54
	4	84.68	89.06	90.52	81.03	88.33	63.51	86.87	83.22	88.33	89.79	83.22	84.68
Amt. Na in	1	7.55	8.19	8.26	8.70	7.34	8.19	8.30	7.09	9.10	5.67	10.59	7.76
Loop	2	6.50	5.33	5.61	5.75	5.77	5.12	6.32	5.91	5.59	5.61	6.09	5.68
(mEq)	3	4.28	5.36	4.35	4.31	4.12	4.50	4.82	4.86	4.46	4.27	4.29	4.62
	4	4.95	5.30	5.41	5.02	5.34	4.10	5.69	5.29	5.65	5.79	5.16	5.29
$[\bar{Na}]$	1	66.0	64.0	65.0	65.0	67.0	66.0	69.0	71.0	67.5	68.0	67.0	63.5
(mEq/1)	2	77.8	79.3	77.3	79.8	78.8	81.3	82.3	78.8	80.3	82.8	79.8	78.3
	3	61.8	58.8	58.8	58.8	57.8	58.8	61.3	59.8	60.3	58.3	61.3	58.8
	4	58.5	59.5	59.8	62.0	60.5	64.5	65.5	63.5	64.0	64.5	62.0	62.5

TABLE 25

SODIUM, POTASSIUM AND CHLORIDE CONCENTRATIONS, AND OSMOLALITY OF THE EFFLUENT AT DIFFERENT INTERVAL COLLECTIONS (min) DURING THE PERFUSION STUDY.

Item	Day	0 -13	13-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100	100-110	110-120
$[Na^+]$ (mEq/l)	1	87	83	85	85	89	87	93	97	90	91	89	82
	2	87	90	86	91	89	94	96	89	92	97	91	88
	3	78	70	70	70	68	70	77	74	75	71	77	72
	4	70	72	72	77	74	82	84	80	81	82	77	78
$[K^+]$ (mEq/l)	1	38.4	39.2	39.2	40.0	40.0	40.4	40.4	40.2	41.2	39.2	40.4	40.4
	2	40.0	39.2	39.2	38.8	38.8	37.2	37.6	37.6	38.0	38.8	38.8	39.2
	3	47.2	48.4	48.4	48.4	48.4	49.2	48.4	48.8	48.8	49.2	48.0	48.8
	4	50.4	50.8	50.8	50.8	50.8	48.8	49.6	51.2	50.0	50.0	49.2	49.2
$[Cl^-]$ (mEq/l)	1	74.00	74.00	78.00	77.00	73.00	65.00	71.00	66.50	62.00	63.50	65.00	63.50
	2	72.00	77.00	71.00	71.00	73.00	77.00	68.50	79.50	69.00	70.00	77.50	78.25
	3	58.50	53.00	56.00	54.00	53.50	57.00	58.50	52.00	55.50	51.00	57.50	56.50
	4	52.50	53.00	53.00	52.00	53.50	59.00	58.00	57.00	54.00	56.50	55.50	58.00
Osmol. (mOsm/kg H_2O)	1	245.1	231.2	239.2	239.0	243.0	240.0	245.0	242.0	244.0	243.5	241.3	236.0
	2	233.3	239.0	239.0	241.0	243.7	250.0	249.6	247.7	245.4	245.0	243.4	243.1
	3	215.4	206.8	213.0	219.2	217.0	224.0	233.2	229.0	230.1	224.4	229.0	230.0
	4	220.4	222.0	227.0	233.0	232.0	248.0	250.0	244.0	243.0	237.0	239.0	240.00

TABLE 26

FLUXES OF SODIUM (μEq/min) AT DIFFERENT INTERVALS (min) DURING THE PERFUSION STUDY

Item	Day	0-13	13-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100	100-110	110-120
Efflux	1	604.0	655.2	660.8	696.0	587.0	655.2	664.0	567.2	728.0	453.6	847.2	620.8
	2	224.0	213.2	224.4	230.0	230.8	204.8	252.8	236.4	223.6	224.4	243.6	227.2
	3	171.2	214.4	174.0	172.4	164.8	180.0	192.8	194.4	178.4	170.8	171.6	184.8
	4	148.5	159.0	162.3	150.6	160.2	123.0	170.7	158.7	169.5	173.7	154.8	158.7
Net Flux**	1	-463	-655	-537	-647	-264	-376	-516	-157	-616	341	-1159	-326
	2	-224	-117	91	-99	-203	64	-317	-167	116	122	-250	-124
	3	-82	-466	-107	-141	69	62	-182	-210	-175	-124	24	-132
	4	-142	-254	-153	-72	-220	250	-293	-176	-239	-278	-146	-186
Influx*	1	1067.0	1310.2	1197.8	1343.0	851.0	1031.2	1180.0	724.2	1344.0	112.0	2006.2	946.8
	2	448.0	330.2	133.4	329.0	433.8	140.8	569.8	403.4	107.6	102.4	493.6	351.2
	3	253.2	680.4	281.0	313.4	95.8	242.0	374.8	404.4	353.4	294.8	147.6	316.8
	4	290.5	413.0	315.3	222.6	380.2	-127.0	463.7	334.7	408.5	451.7	300.8	344.7

* The negative value denotes that net flux exceeds efflux.

** Positive values denote net absorption whereas the negative ones denote net secretion.

TABLE 27

NET FLUXES OF POTASSIUM AND CHLORIDE AND NET ABSORPTION OF WATER OBTAINED BY DIRECT MEASUREMENT AND PEG TECHNIQUE AT DIFFERENT INTERVALS (min)

Item	Day	0 -13	13-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100	100-110	110-120
Potassium* (μEq/min)	1	111	-11	83	20	218	179	122	294	76	522	-191	151
	2	34	105	194	133	72	221	83	109	250	264	69	103
	3	196	-127	123	66	246	159	133	76	103	107	266	128
	4	104	37	126	183	82	438	122	149	139	121	169	150
Chloride* (μEq/min)	1	-313	-526	-445	-531	-111	-121	-256	76	-228	429	-688	-121
	2	- 43	19	243	119	-12	207	30	-54	327	355	-81	--12
	3	83	-213	30	- -9	185	63	9	35	18	71	173	29
	4	42	-30	57	134	10	351	-14	50	53	5	65	18
Water Direct Measurement (ml/min)	1	0.5	-2.4	-0.2	-1.5	3.5	2.4	1.5	5.4	0.2	10.8	-6.6	1.9
	2	-0.03	1.6	3.9	2.1	0.6	4.1	0.4	1.1	4.8	5.4	0.5	1.5
	3	4.2	-2.2	2.9	1.7	5.5	3.8	3.2	2.4	2.6	2.7	5.8	3.1
	4	2.1	0.9	2.7	3.8	1.8	8.6	2.3	3.2	2.7	2.4	3.2	2.8
Water (PEG) (ml/min)	1	0.8	0.7	1.1	1.6	1.6	1.2	-	1.1	0.9	1.2	2.0	1.6
	2	1.4	1.8	2.4	2.2	2.1	0.8	2.2	2.2	1.7	1.7	2.2	2.0
	3	4.3	3.9	4.3	2.3	2.6	3.6	2.7	2.7	3.0	3.0	4.2	3.6
	4	2.7	2.6	2.9	3.0	1.5	5.8	1.5	1.5	3.3	3.3	2.7	2.4

* Positive values denote net absorption whereas the negative ones denote net secretion.

TABLE 28

MEAN SQUARES FOR FLUXES OF SODIUM, POTASSIUM, CHLORIDE AND WATER, AND INTESTINAL VOLUME, AMOUNT OF SODIUM IN THE LOOP AND OSMOLALITY OF THE EFFLUENT

Item Source of Variation	Time	Day	Interaction	Error
Degrees of Freedom	3	3	9	32
Sodium Efflux	480.94	634,608.74*	95.73	3,219.95
Sodium Net Flux	40,860.39	1,807,160.12*	12,432.04	86,423.58
Sodium Influx	19,391.38	314,417.05*	6,863.88	45,596.41
Potassium Net Flux	17,551.91	1,748.85	2,733.94	13,458.31
Chloride Net Flux	35,430.14	276,346.25*	16,171.34	38,699.31
Absorption of Water (Direct measurement)	8.33	8.08	1.67	8.93
Absorption of Water (PEG technique)	0.84	11.24*	0.67	0.53
Intestinal Volume	431.04*	6,161.63*	23.07*	146.59
Amt. Na in Loop	0.32	28.15*	0.07	0.57
Osmolality of the Effluent	431.04*	1,025.74*	43.01*	16.42

* ($P < 0.05$)

TABLE 29

MEAN SQUARES FOR THE ORTHOGONAL COMPARISON OF FLUXES OF SODIUM, CHLORIDE AND WATER (PEG TECHNIQUE) INTESTINAL VOLUME, AMOUNT OF SODIUM IN THE LOOP, AND OSMOLALITY OF THE EFFLUENT

Item							
Source of Variations	Day[A]			Time[B]			Error
	C_1	C_2	C_3	C_1	C_2	C_3	
Degrees of Freedom	1	1	1	1	1	1	32
Sodium Efflux	1,872,906.29*	27,655.60*	3,264.33	-	-	-	5,386.48
Sodium Net Flux	871,111.11*	44,055.01	28,085.04	-	-	-	45,596.41
Sodium Influx	5,421,174.91*	235.08	70.38	-	-	-	95,486.29
Chloride Net Flux	812,702.25*	13,366.13	2,970.38	-	-	-	38,699.31
Water Absorption	20.78*	10.73*	2.04	-	-	-	0.53
Intestinal Volume	17,453.27*	593.86	437.76	-	-	-	146.59
Amt. Na in Loop	75.99*	5.30*	3.19*	-	-	-	0.57
Osmolality	404.01*	1,548.46*	1,124.77*	784.82*	467.50*	40.84	16.42

A C_1 Day 1 vs 2, 3 and 4;
C_2 Day 2 vs 3 and 4;
C_3 Day 3 vs 4.

B C_1 Linear comparison;
C_2 Quadratic comparison;
C_3 Cubic comparison.

* ($P < 0.05$)

TABLE 30

PLASMA CONCENTRATIONS* OF Na, K AND Cl, AND OSMOLALITY **
BEFORE, 1 AND 2 HOURS AFTER INTRODUCTION OF
A BOLUS MARKER DOSE

Item		Days 1	2	3	4
Na	Before	156	151	150	161
	1 H after	151	159	153	159
	2 H after	151	155	152	160
K	Before	4.20	3.90	4.45	4.55
	1 H after	4.85	3.80	4.45	4.65
	2 H after	3.85	3.75	4.55	4.65
Cl	Before	117.0	118.0	137.5	119.0
	1 H after	113.5	115.0	165.0	119.0
	2 H after	131.5	172.5	97.0	121.0
Osmolality	Before	299	305	303.2	310.0
	1 H after	298.5	308	306.5	327.0
	2 H after	306	305	323.5	334.2

* mEq/l

** mOsm/kg H_2O