WATER, GLUCOSE AND ELECTROLYTE MOVEMENT IN THE JEJUNUM OF THE TRANSMISSIBLE GASTROENTERITIS-INFECTED PIG

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ABSTRACT

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By

James E. Rogers

The influence of transmissible gastroenteritis on jejunal fluid movement as it relates to glucose and electrolyte absorption was studied. Jejunal loops of 16 pigs (2 litters) 16 to 21 days of age were continuously perfused for 4 hours, using a solution of d-glucose (26mM) in Ringer's lactate solution. The pigs were perfused in pairs, one being infected and the other serving as a control. After the perfusion, absorption rates of water, glucose and electrolytes were determined and their relationships in intestinal loops of infected and control pigs compared.

The mean glucose absorption in the "infected loops" was 29 μM/cm/4 hr as compared with a mean glucose absorption of 72 μM/cm/4 hr in the "control loops." Not only did the TGE-infected loops not absorb glucose properly, but they actually secreted sodium chloride and water into the lumen.

The relationship between total net solute movement and net fluid movement was linear. Fluid movement in and out of the perfused solution was closely associated with total net solute movement and approached zero at zero fluid movement. Relationships of sodium and chloride to net fluid movement were also linear.

In view of the mucosal cellular changes, close association between solute and fluid movement, and demonstrated malabsorption of actively absorbed solute in pigs affected with the disease, interference with active solute absorption was suggested as the primary phenomenon in TGE diarrhea. The small intestine is prevented from absorbing the normal glandular secretions and ingesta added at the duodenal end of the tract, and the capacity of the colon to absorb water is exceeded. The secretory process is thought to contribute only secondarily to the diarrheal state.

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By

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Dedicated to

Dorothy Heyward Rogers

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INTRODUCTION

Transmissible gastroenteritis (TGE) is a specific, virally-induced diarrheal disease of young swine. Several features of TGE, including its reproducibility and the readily demonstrable lesions associated with it, make it useful for the study of enteric disease (Maronpot and Whitehair, 1967; Haelterman and Hooper, 1967). The pathogenesis of diarrhea in TGE and other malabsorption syndromes remains essentially unsettled. In view of the marked loss of total surface area due to the extensive atrophy of the villi and microvilli in the disease, interference with active solute absorption was expected.

To assess the importance of altered intestinal absorption and fluid and electrolyte movements, absorption of glucose and net movements of water and electrolytes in the pig jejunum were measured both in the presence and absence of TGE.

REVIEW OF LITERATURE

Transmissible Gastroenteritis (TGE)

Transmissible gastroenteritis (TGE) is a disease of swine caused by a virus and characterized by growth of the virus within the epithelial cells of the small intestine. The disease causes rapid and extensive atrophy of the villi, resulting in acute malabsorption, diarrhea, dehydration, and death in a high percentage of affected pigs under one week of age. Doyle and Hutchings (1946) were the first to describe the disease.

Pigs are infected with TGE most readily by the oral route (Haelterman and Hooper, 1967). After an incubation period of 12 to 48 hr, there is a sudden onset of vomiting and diarrhea, but some affected pigs continue to nurse to within a few hours of death (Blood and Henderson, 1963). Depression and dehydration are pronounced, and weakness and emaciation progress, with death occurring on the third to sixth day. Death is usually attributed to loss of body fluids and electrolytes (Maronpot and Whitehair, 1967).

In the surviving pigs, diarrhea usually stops between the fourth and sixth day (Haelterman and Hooper, 1967). Most pigs gain weight normally after recovery

but some remain severely emaciated and have slow weight gains (Maronpot and Whitehair, 1967).

Haelterman (1965) found that growth of the TGE virus was limited to the small intestine in baby pigs. The lesions of TGE resemble, superficially at least, those of human sprue (Haelterman and Hooper, 1967). A massive destruction and shortening of the villi extends throughout the small intestine except for the anterior duodenum, where the villi usually remain normal in appearance (Haelterman and Hooper, 1967). The mean villus-crypt ratio (length of the villi and depth of the crypts) in the jejunum of normal pigs is about 7:1 as compared with a ratio of less than 1:1 in TGE-infected pigs (Haelterman and Hooper, 1967).

Membrane Structure and Absorption

Since the luminal membrane of the intestine is lipoidal, non-lipid soluble substances such as water, electrolytes and glucose can penetrate either by passing through aqueous channels in the membrane or by movement via a membrane carrier. Since carriers are not available for water, movement occurs exclusively through the aqueous channels. The "equivalent pore radius" is a measure of the average pore size expressed as a statistical concept (Soloman, 1960). Lindeman and Soloman (1962) have measured the equivalent pore radius of the rat intestine

and found it to be 4Å. Fordtran et al. (1965) reported the equivalent pore radius in the human proximal jejunum to be 6.5Å. This is large compared with the radius of the sodium molecule, which is approximately 2.5Å (Schultz and Soloman, 1961). This means that sodium can freely penetrate the membrane through the aqueous channels. These relatively large pore radii of the mucosa of the anterior small intestine gradually change to those of the ileum and colon where the effective pore radius is only 3Å (Fordtran et al., 1965), and the sodium molecule is, for all practical purposes, restricted from passing through the luminal membrane via aqueous channels. However, active sodium transport is highly efficient in this area. Fordtran, Rector and Carter (1968) found that a mucosal solution bathing the ileum had to be made 110 milliosmols/liter hypertonic to a serosal solution in order to stop fluid movement from mucosa to serosa. Sodium absorption in the ileum is, therefore, principally a result of active sodium transport (Curran, 1965).

Characteristics of the Carbohydrate Transport Process

Crane (1960) suggested that hexoses containing a pyranose ring with another carbon atom attached at the C-5 position and a hydroxyl group attached at the C-2 position, in the same stereochemical position as d-glucose, can readily pass through the lipid membrane, whereas those

with a slightly different configuration do not. Fisher and Parsons (1953) found that the glucose absorptive process exhibited saturation kinetics analogous to Michaelis-Menten kinetics. Michaelis-Menten kinetics in membrane transfer studies refer to the situation in which the rate of transport of a substance through a membrane is analogous to the reaction rates between enzymes and their respective substrates. This means that the rate of glucose absorption does not increase proportionally with an increase in concentration, but approaches a maximum rate at high concentrations. Competitive interference between glucose and galactose absorption was shown *in vivo* by Cori (1926) and *in vitro* by Fisher and Parsons (1953). Jorgensen, Landau and Wilson (1961) concluded that all transported sugars compete for a common pathway. In all cases the competing sugar is thought to attach itself to one of the membrane carrier sites and hence reduce the number of sites available for transfer of the other sugars. The extent of competitive inhibition depends on the particular sugars used and on their concentrations. Riklis and Quastel (1958) showed the sodium ion to be essential to glucose absorption. This association between sugar and sodium transport has been confirmed *in vitro* (Bihler and Crane, 1962) and *in vivo* (Csáky and Zollicoffer, 1960). Since the glucose molecule has a radius of 4Å (Fordtran, *et al.*, 1965), it does not pass readily through pores in

the luminal membrane. However, it is well known that there are active transport mechanisms within the lipid membrane proper which can transport glucose across the intestinal mucosa against concentration gradients (Fisher and Parsons, 1953; Wilson, 1962).

Carrier Hypothesis

Modern concepts of carbohydrate transport rest heavily on the carrier hypothesis. A carrier is assumed to be a mobile component of the luminal membrane possessing specific chemical groups or sites to which the monosaccharide molecules can attach (Newey, 1967). Diffusion of the monosaccharide-carrier complex would move the sugar across the lipid barrier membrane, with subsequent release of the sugar into the cell. Such a carrier process would account for the characteristics of specificity, saturation and competition, but no reports have been made of the isolation and characterization of such a carrier (Christensen, 1960; Wilbrandt and Rosenberg, 1961).

Crane (1962) has postulated that sugar movement is dependent on the sodium gradient across the luminal membrane. He suggested that a sodium-sugar-carrier complex moves across the lipoidal luminal membrane in response to the difference in sodium concentration between the lumen and cell, this difference being maintained by an energy-dependent sodium pump which returns sodium to the lumen.

Water and Solute Movement

Most authors consider the movement of water to be completely passive and the result of a gradient of osmotically active solute particles (Curran, 1965; Fordtran, 1967; and Parsons, 1967). Water accompanies active solute movement across the intestinal mucosa in either direction, or it may move as a result of a difference in osmotic activity between the tissue extracellular fluid and lumen contents. In addition, movement of water secondary to active solute transport or osmotic pressure gradients can carry small solutes through aqueous channels in the intestinal membrane. This process is called "solvent drag" (Fisher, 1955).

Once a carbohydrate or amino acid molecule has moved across the luminal membrane, it tends to become trapped since it is too large to readily diffuse back, and water flows in the direction of solute movement. As this water flow occurs through aqueous channels, sodium is swept along with it by the "solvent drag" effect (Fordtran, 1967). Thus, a major driving force for sodium absorption in the upper small intestine is active transport of non-electrolytes such as glucose and amino acids. Therefore, in the jejunum and duodenum, water absorption is largely secondary to the active absorption of large organic molecules (Fordtran, 1967). These properties of the proximal mucosa gradually change to those of the ileum

and colon where the luminal membrane is less permeable and where sodium transport is highly efficient (Fordtran, Rector, and Carter, 1968). At this site, water absorption is largely dependent on active electrolyte absorption (Curran, 1965).

Electrolyte Absorption

That sodium can be absorbed against an electrochemical gradient is evidence that the mucosa contains a sodium pump (Vaughan, 1960), and it is known that metabolic energy is used in pumping sodium (Skou, 1965). Visscher (1944) reported that isotopically labeled sodium and water rapidly exchange in both directions between the blood and the lumen contents of the proximal jejunum.

The law of electroneutrality requires that each solution contain an equal number of cations and anions; thus absorption of sodium cannot occur without an equivalent transfer of anions in either direction. At least a portion of chloride absorption is merely a consequence of sodium absorption (Parsons, 1967). The ileum absorbs chloride more rapidly than sodium (Parsons, 1956). The law of electroneutrality is maintained by the secretion of bicarbonate into the lumen.

Active absorption of potassium has not been described; net absorption is probably a passive consequence of water absorption (Davenport, 1966).

The Study of Intestinal Absorption

The various methods for the study of intestinal absorption have been reviewed by Crane (1960), Smythe (1961), Quastel (1961), Wilson (1962), Wiseman (1961) and Spencer (1962).

Absorption may be measured *in vivo* in intact conscious animals by tolerance tests, e.g., glucose tolerance tests (Smythe, 1961). Such studies do not lend themselves, however, to investigation of absorption mechanisms at the cellular level, as absorption is affected by factors unrelated to mucosal transfer mechanisms. Such factors include motility of the alimentary tract, blood flow, and the metabolism of the liver, kidney, peripheral tissues and the gut flora (Levin, 1967). The classic *in vivo* technique is that of Cori (1925), in which intact animals are given the substance to be studied by stomach tube and killed a short time later. The amount remaining in the alimentary tract is subtracted from that fed, the difference being the amount absorbed. Here, the results may be complicated by gastric emptying, which controls the rate at which substances are delivered to the intestine (Fenton, 1945). With anesthetized animals, variations in gastric emptying and intestinal motility are avoided, but variations in blood flow and anesthetic effects are present. Techniques in anesthetized animals include the use of cannulated loops through which fluid is circulated by

a peristaltic pump (Serebro, 1968). In this method, a low concentration of solutes can be used, making it possible to measure the active transfer against the blood concentration. Here, the net loss of solute or fluid from the lumen is regarded as "absorption."

The choice of in vitro methods used in the study of intestinal absorption include: the everted sac (Wiseman, 1961), whole thickness strips (Kimberg, Schachter and Schenker, 1961), cut rings (Hakin, Lester and Lifson, 1963), and mucosal cells (Perris, 1966). The everted sac has been the most widely used of in vitro techniques.

MATERIALS AND METHODS

General Plan

The influence of TGE on jejunal fluid movement as it relates to glucose and electrolyte absorption was studied. Jejunal loops of 16 pigs (2 litters) 16-21 days of age were continuously perfused for 4 hours using a solution of glucose (26 mM) in Ringer's lactate solution. The perfusion method used was a modification of the method described by Serebro et al. (1968). The pigs were perfused in pairs, one member being infected and the other acting as a control. After the perfusion, absorption rates of water, glucose and electrolytes were determined, and results in infected and non-infected loops were compared.

Animal Procedures

Litter I was farrowed October 30, 1968, the offspring of a Yorkshire sow and a Hampshire boar. Litter II was purebred Yorkshire, farrowed January 9, 1969. Litter sizes were 10 (Litter I) and 6 (Litter II). Litter mates were paired at weaning according to weight and sex, placed in individual stainless steel cages and fed an artificial diet* (180 ml 3 x daily). One member of each pair

*SPF-LAC. Borden Co., New York, N. Y.

was fed 10^5 pig-infective doses of TGE virus* in the diet, and each pair was studied 24 hours after exposure of the one animal and following an 18 hour fast. The pigs were anesthetized with pentobarbital sodium given intravenously to effect.

Each abdomen was opened, and a loop of jejunum, approximately 24 cm long and 48 cm (Litter I) or 84 cm (Litter II) rostral to the cecum, was isolated. The ends of this loop were transected between vascular arcades to preserve the blood supply. Both cut ends were ligated to prevent spillage. The proximal loop was cannulated with 1/4 in (inside diameter) plastic tubing,** while latex rubber tubing (3/8 in inside diamater) was used distally. The isolated loops were returned to the abdominal cavity, and the abdomen was closed, leaving only the tubing exposed.

Perfusion

Loops of both pigs were simultaneously perfused with a solution containing 26 mM d-glucose and .3 mM phenol red (PSP) in Ringer's lactate. The PSP was used as a non-absorbable marker (Serebro, et al., 1968).

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*Courtesy of Dr. E. O. Haelterman, School of Veterinary Science and Medicine, Purdue University, Lafayette, Ind.

**Tygon. U. S. Stoneware, Akron, Ohio.

Using a single pump,* a sample of the test solution (100 ml) was continuously perfused, and the efflux from the loop was recirculated from individual reservoirs at a rate of 2 ml/min for 4 hours. Hydration was maintained with intravenous Ringer's lactate infused at a rate of approximately 60 ml/hr, using hourly determinations of packed cell volume (PCV) to monitor hydration. Rectal temperatures were monitored every 15 min, and the body temperature was maintained between 100° and 103° F with the aid of heat lamps. After 4 hours of perfusion, each animal was killed with a lethal dose of pentobarbital sodium and reopened. The fluid in each reservoir and that drained from the loops was measured. After inspection, the loops were excised and their lengths measured.

Initially, 3-ml samples were obtained from each reservoir for determination of initial glucose and PSP concentrations. Three-milliliter samples were collected from each reservoir at hourly intervals. All samples were frozen soon after collection and stored in a frozen state until determinations could be accomplished at a more convenient time. Prior to freezing and storage, protein-free filtrates of samples to be used in glucose determinations were prepared by diluting 1 ml of the original 3-ml samples 1:10 with sodium tungstate followed by centrifugation.

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*Sigmamotor Model T-8. Middleport, N. Y.

The glucose concentration was determined on each sample by the O-toluidine method described by Feteris (1965), using the Coleman Junior Spectrophotometer.*

Samples for sodium and potassium were diluted 1:200 and 1:50, respectively, with Sterox** and their concentrations read on the Coleman Flame Photometer.*** Chloride was determined by the method of Schales and Schales (1941). Phenol red (PSP) concentration was determined by the method of Schedl (1961) with two minor modifications. The sample was diluted 1:5 with 10% sodium tungstate and the pH of the described buffer was adjusted by the addition of 1N sodium hydroxide for maximum color intensity.

The final volume in each reservoir and loop was calculated by dividing the amount of PSP placed in the system by the final PSP concentration (Serebro et al., 1968). Glucose, sodium, chloride and potassium were calculated by subtracting from the amount placed in the system the amount in each sample and the amount remaining at the end (Serebro et al., 1968). Their respective rates

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*Coleman model 6D Junior Spectrophotometer. Coleman Instruments, Inc., 42 Madison Street, Maywood, Ill.

**Sterox. Hartman and Leddon Co., Philadelphia, Penn.

***Coleman Model 21 Flame Photometer. Coleman Instruments, Inc., 42 Madison Street, Maywood, Ill.

of absorption were calculated by dividing the total amount absorbed by the length of each intestinal loop in centimeters.

Tissue for Histopathological Examination

Sections were taken from five different levels of the intestinal tract, preserved in Zenker's fluid, and were stained with hematoxylin and eosin. These levels included: the perfused loop, 2 centimeters rostral to the perfused loop, 2 centimeters distal to the perfused loop, the ileum, and the duodenum. The histological procedures were according to the United States Armed Forces Institute of Pathology Manual of Histologic and Special Staining Techniques (1957).

Analysis of Data

The difference between the means of the data from the infected and control groups were analyzed, using the t test (Goulden, 1952). Lines in Figures 4-6 were determined by the method of least squares (Goulden, 1952).

RESULTS

Animal Data

At the time of perfusion, body weights of the pigs in Litter I averaged 4.3 kg, as compared with 3.2 kg in Litter II (Table 1). The average hemoglobin value in Litter II was 8.0 gm/100 ml as compared to 11.6 gm/100 ml in Litter I (Table 1).

Clinical Signs

The first sign of infection was usually vomition (16 to 24 hr after infection), soom followed by diarrhea. Stools of infected pigs were watery and often contained undigested curds of milk. Infected animals continued to eat until the fast was initiated 18 hr before perfusion. All infected pigs had a marked leukopenia with white blood cell counts averaging 3181/mm^3 24 hr after infection, as compared to normal white blood cell counts averaging 5837/mm^3 in the control pigs (Table 1). Despite a severe diarrhea, dehydration, as indicated by packed cell colume (PCV), did not become marked before perfusion (Table 1). The diarrhea subsided 8 to 10 hr after pigs were denied access to food and water.

All control pigs remained clinically normal throughout the experimental period.

Table 1. Hematologic and body weight values of infected (I) and control (C) pigs.

Date (1968)	Pig Number and Sex	Hb (Gm/100ml)	HCT (%)	WBC/mm^3	Body Weight (Kg)
10-11*	1I-M	11.3	34	9,050	3.9
10-15	"	14.2	43	6,050	4.0
10-18**	"	12.3	36	4,200	4.1
10-11*	1C-M	10.1	31	6,850	4.4
10-15	"	12.8	41	10,850	4.5
10-18**	"	10.8	31	7,500	4.7
10-11*	2I-M	11.0	32	8,600	3.7
10-15	"	12.3	39	10,100	4.0
10-19**	"	12.6	37	1,800	4.5
10-11*	2C-M	11.1	34	5,900	3.9
10-15	"	12.6	39	8,550	4.2
10-19**	"	13.0	41	6,050	4.6
10-11*	3I-M	12.8	35	8,600	2.1
10-15	"	12.6	39	11,150	2.5
10-19**	"	11.5	33	3,250	2.9
10-11*	3C-F	10.3	32	6,910	3.0
10-15	"	13.3	40	6,300	3.4
10-19**	"	12.2	39	5,100	3.6
10-11*	4I-F	9.8	30	7,700	3.6
10-15	"	12.0	39	5,200	4.4
10-20**	"	10.3	32	1,200	4.9
10-11*	4C-F	11.8	32	6,850	3.4
10-15	"	13.5	40	4,550	4.0
10-20**	"	12.0	36	3,850	4.3
10-11*	5I-F	12.0	35	6,600	4.0
10-15	"	13.3	41	5,300	4.5
10-20**	"	12.3	34	2,700	4.8
10-11*	5C-F	13.2	37	8,100	3.6
10-15	"	13.3	42	4,750	4.0
10-20**	"	12.3	38	5,300	4.6

*Date weaned

**Date perfused

Table 1 (Continued)

Date (1968)	Pig Number and Sex	Hb (Gm/100ml)	HCT (%)	WBC/mm^3	Body Weight (Kg)
1-21*	6I-M	8.6	26	6,600	2.5
1-24	"	-	-	-	2.7
1-28**	"	7.2	25	3,700	3.2
1-21*	6C-M	7.0	23	8,400	2.7
1-24	"	7.7	29	9,150	2.9
1-28**	"	7.3	30	5,200	3.5
1-21*	7I-M	8.2	26	11,100	2.3
1-24	"	9.8	35	11,850	2.5
1-29**	"	10.0	30	3,950	2.7
1-21*	7C-M	7.3	22	10,450	2.1
1-24	"	8.2	31	10,200	2.4
1-29**	"	7.3	23	8,200	3.2
1-21*	8I-M	8.7	26	13,900	2.4
1-24	"	8.0	31	10,200	2.8
1-30**	"	9.2	28	4,650	3.1
1-21*	8C-M	9.8	29	6,400	2.4
1-24	"	-	-	-	2.6
1-30**	"	7.0	22	5,500	3.4

*Date weaned

**Date perfused

Gross Lesions

Before prefusion, the infected small intestine was transparent and distended with a clear fluid containing milk curds. Intestinal vessels were slightly engorged, but neither inflammation nor necrosis was evident. Control loops appeared normal.

After perfusion, each loop was examined to insure that the blood supply was intact, that drainage was unobstructed and that over-distention of the loops had not occurred.

Histopathologic Lesions

The control small intestine and perfused loops (Figure 1) contained no histologic abnormalities.

Atrophy of the villi was present in all infected loops. Some villi were absent, many others were shortened, and fusion of two or more villi was prominent (Figure 2). Epithelial cells of the villi were cuboidal, and a decreased height of the brush border was present in all infected loops. The villus-crypt ratio was markedly decreased. A slight hyperemia and mild infiltration of the lamina propria with lymphocytes and granulocytes was observed.

Pathophysiology

Absorption data for the eight normal and eight infected loops are listed in Table 2. The relationships

Figure 1. Jejunal mucosa of control pig. Note the length of the villi and the depth of the crypts as compared with those in Figure 2. H & E Stain; x 190.

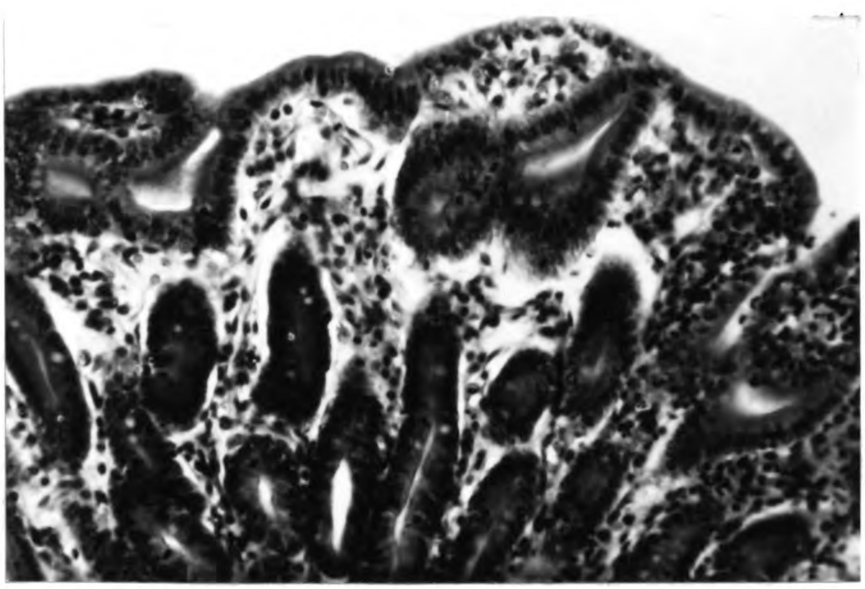

Figure 2. Jejunal mucosa of infected pig. The remaining villi are short, blunt, and fused. H & E stain; x 190.

Table 2. Absorption rates of infected and control loops.

Pair Number	Water Absorption (Measured)		Glucose Absorption		Sodium Absorption		Chloride Absorption	
	(ml/cm/4hr)		(μM/cm/4hr)		(μEq/cm/4hr)		(μEq/cm/4hr)	
	I*	C*	I	C	I	C	I	C
1	-9.09	0.44	29	61	-15	24	0	41
2	-0.31	0.65	28	41	-78	44	-44	63
3	-0.27	0.59	29	67	-56	27	-26	64
4	-0.21	0.25	13	46	-48	4	-26	38
5	-0.33	0.73	25	79	-75	41	-50	59
6	-0.16	0.17	47	81	-64	19	-48	41
7	-0.17	0.48	19	100	-47	16	- 3	64
8	-0.24	0.15	29	70	-44	17	-44	5
Mean	-0.22	0.43	29	72	-55	18	-31	45
Mean Difference	0.65 ± .098		40.8 ± 8.20		76.6 ± 10.0		77.0 ± 9.10	

*I = Infected; C = Control

Table 2 (Continued)

Pair Number	Potassium Absorption		Total Solute Absorption		Water Absorption (PSP)		Perfusate Specific Gravity	
	(μEq/cm/4hr)		(μosM/cm/4hr)		(ml/cm/4hr)			
	I*	C*	I	C	I	C	I	C
1	-1.5	-2.6	12	123	-0.18	0.44	1.010	1.009
2	-3.0	-5.6	-97	142	-0.37	0.74	1.007	1.009
3	-2.9	-4.1	-56	154	-0.22	0.68	1.008	1.008
4	-1.5	-2.5	-63	85	-0.11	0.10	1.009	1.008
5	-2.5	-1.8	-103	204	-0.46	0.96	1.008	1.008
6	-5.3	-5.2	-70	136	--	--	1.009	1.009
7	-1.9	-4.8	-47	175	-0.23	0.64	1.008	1.009
8	-2.8	-5.3	-62	77	--	0.22	1.010	1.008
Mean	-2.8	-4.2	-64	134	-0.25	0.54	1.009	1.009
Mean Difference	1.14 ± .41		197.8 ± 22.7					

*I - Infected; C = Control

between net solute movement and net fluid movement are summarized in Figures 3-6.

The mean glucose absorption in the infected loops was 29 μM/cm/4hr as compared with 72 μM/cm/4hr in the control loops (Figure 3). The difference between the means of the infected and control groups was significant at the 0.01 level. In the control loops, absorption of glucose was accompanied by net fluid absorption. In the infected loops, glucose absorption continued at a reduced rate despite the secretion of small quantities of isotonic fluid.

These data indicate that glucose absorption is seriously impaired in TGE infection.

The net mean sodium secretion in the infected loops was 55 μEq/cm/4hr as compared with a net mean absorption of 35 μEq/cm/4hr in the control loops (Figure 4). The difference between the means of the infected and control groups was significant at the 0.01 level. In the control loops, sodium absorption was associated with water absorption. On the other hand, net sodium secretion was associated with net fluid secretion in the infected loops. The relationship between net sodium movement and net fluid movement in infected and control loops was linear.

These data indicate that both sodium and water are secreted at this level of the TGE-infected gut and suggest a linear relationship between sodium movement and water movement.

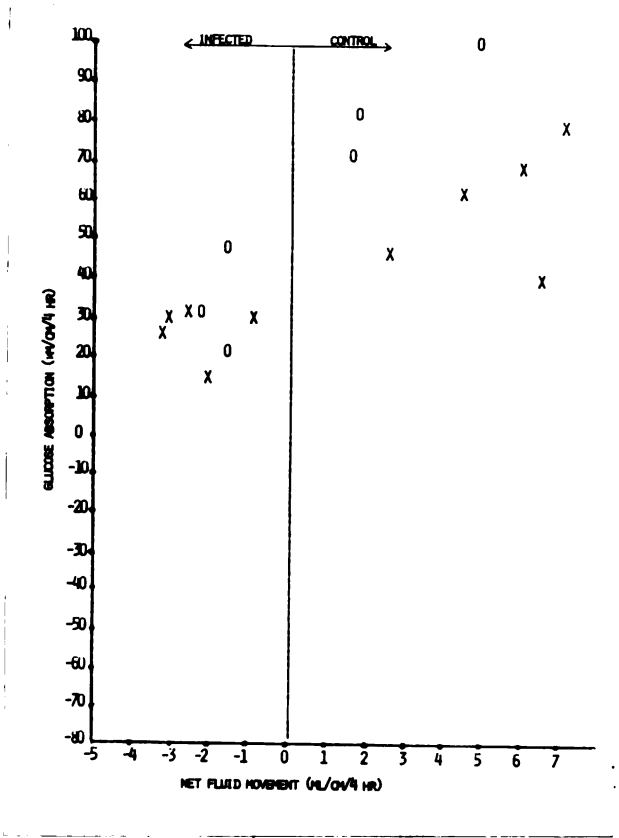

Figure 3. Relationship between glucose absorption and net fluid movement in control and TGE-infected jejunal loops. Glucose absorption is plotted along the vertical axis and net fluid movement along the horizontal axis. X = litter I (posterior jejunum); O = litter II (middle jejunum).

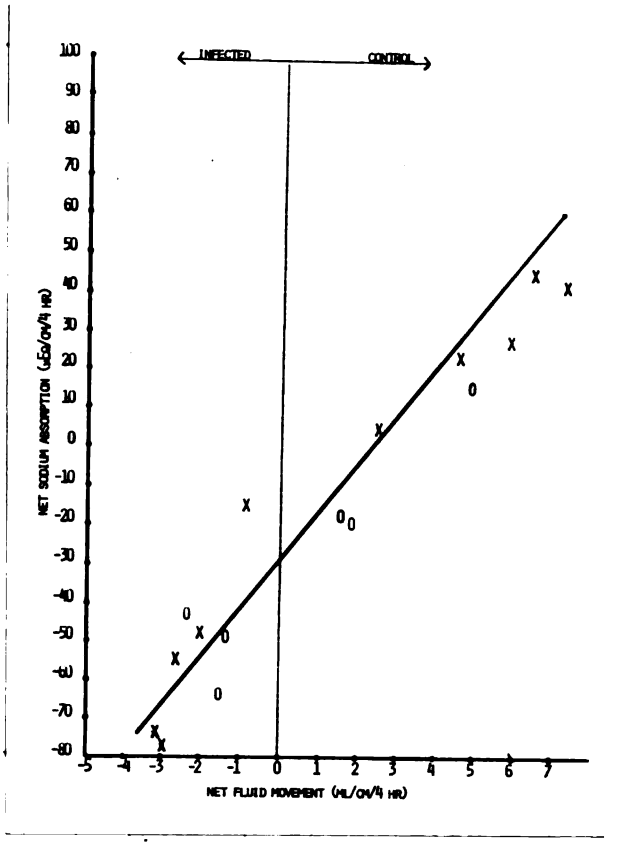

Figure 4. Relationship between net sodium movement and net fluid movement in control and TGE-infected jejunal loops. Net sodium absorption is plotted along the vertical axis and net fluid movement along the horizontal axis. X = litter I (posterior jejunum); O = litter II (middle jejunum).

The net mean chloride secretion in the infected loops was 31 μEq/cm/4hr as compared with a net mean absorption of 45 μEq/cm/4hr in the control loops (Figure 5). The difference between the means of the infected and control groups was significant at the 0.01 level. In the control loops, chloride absorption was associated with fluid absorption. On the other hand, net chloride secretion was associated with net fluid secretion in the infected loops.

A direct linear relationship between net chloride movement and net fluid movement existed in both infected and control loops.

The total net mean solute secretion in the infected loops was 64 μOsm/cm/4hr as compared with a total net mean solute absorption of 134 μOsm/cm/4hr in the control loops (Figure 6). The difference between the means of the infected and control groups was significant at the 0.01 level. In the control loops solute absorption was associated with fluid absorption. Conversely, total net solute secretion was associated with net fluid secretion in the infected loops.

The relationship between total net solute movement and net fluid movement was linear. Fluid movement in and out of the perfused solution was closely associated with total net solute movement and approached zero at zero solute movement.

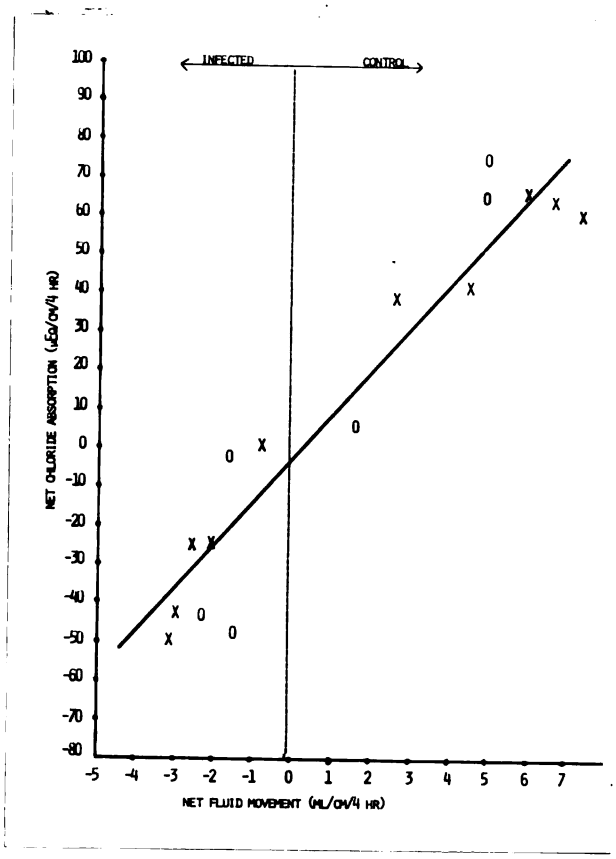

Figure 5. Relationship between net chloride movement and net fluid movement in control and TGE-infected jejunal loops. Net chloride absorption is plotted along the vertical axis and net fluid movement along the horizontal axis. X = litter I (posterior jejunum); O = litter II (middle jejunum).

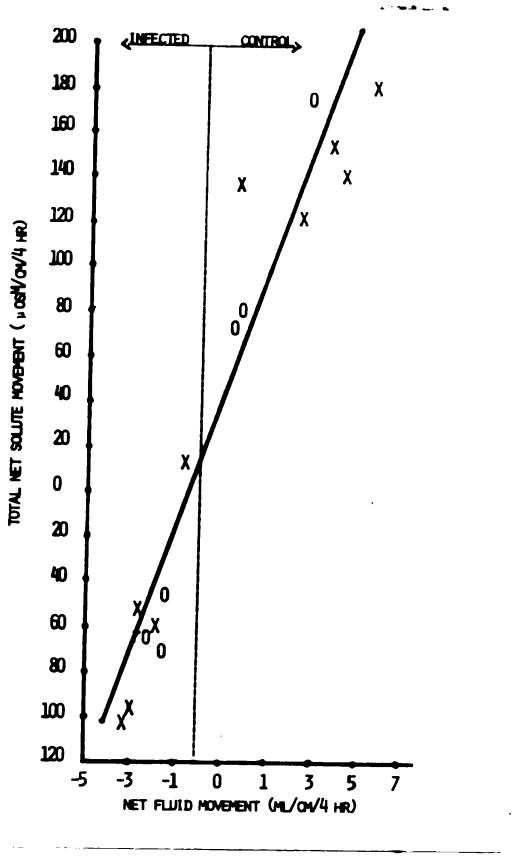

Figure 6. Relationship of total net solute (glucose, sodium, chloride and potassium) movement and net fluid movement in control and TGE-infected jejunal loops. Net solute absorption is plotted along the vertical axis and net fluid movement along the horizontal axis. X = litter I (posterior jejunum); O = litter II (middle jejunum).

DISCUSSION

Relationships of Sodium, Chloride and Glucose Absorption with Fluid Movement

The relationship between total net solute movement and net fluid movement was linear. Fluid movement in and out of the perfused solution was closely associated with total net solute movement and approached zero at zero fluid movement. These relationships suggest that water movement in the pig's jejunum is a passive process; it is the result of the movement of osmotically active solute particles.

Glucose was absorbed at a higher rate in Litter II than in Litter I (Table 2). This suggests that glucose absorption may occur at a faster rate in the middle jejunum than in the posterior jejunum. Chloride absorption closely paralleled sodium absorption (Figures 4 and 5). Since the larger part of chloride absorption is thought to be merely a passive consequence of sodium absorption, this observation is consistent with modern concepts. As the intestine does not distinguish between chloride and other anions, sodium and chloride are absorbed at different rates (Davenport, 1966).

Excellent reviews of the general concepts of solute and fluid movement are available by Curran (1965), Fordtran (1967) and Fordtran, Rector and Carter (1968).

Speculations on the Pathogenesis of TGE Diarrhea

In TGE, as in other malabsorption syndromes, there is a marked loss of total surface area due to the extensive atrophy of the villi and microvilli (Thake, 1968). Thus, a drop in glucose absorption with a proportionate fall in water and electrolyte absorption by the jejunum is to be expected. A relative impermeability to water and electrolytes should also logically be present. Here it is assumed that the remaining mucosa retains the permeability characteristics of the normal jejunal cells.

The results of these experiments indicate that the intestine infected with TGE is in a secretory state. Not only did the TGE-infected loops fail to absorb glucose properly, but they actually secreted sodium chloride and water into the lumen. The exact cause of this secretion is unknown, but decreased membrane permeability (effective pore radius) is a plausible explanation. It can be seen (Figure 4) that when the normal net sodium flow was extrapolated to zero fluid movement, there would be net sodium secretion. In decreased membrane permeability, the normal volume of intestinal sodium secretion would continue in the presence of impaired sodium absorption. Thus,

the active absorption of glucose and other large organic solutes would lose its effect on the movement of sodium chloride and other small solute particles, thereby retarding water absorption secondary to active solute transport. This would lead to fluid accumulation as a result of the osmotic effect of the secreted solute. Fordtran (1967) described a marked reduction in jejunal effective pore radius (below levels normally found in the ileum) in a case of human celiac sprue. A similar defect was described in a case of tropical sprue (Fordtran, 1967). Thus, such situations have the condition of an osmotic load being exposed to a relatively impermeable jejunal mucosa. Such an explanation would be consistent with the observation that, although glucose absorption was substantially reduced in the infected loops, it was associated with concomitant net sodium and fluid secretion.

Increased active sodium secretion is also another possible explanation for fluid and sodium chloride secretion in TGE-enteric disease. Such secretion could possibly originate from crypt cells which are abundant in this disease. Thake (1968) stated that the cells of the crypts and villi are similar ultrastructurally and histochemically in TGE-infected pigs. This indicated incomplete differentiation of the cells of the villi. As it has been hypothesized that the cells of the crypts of Lieberkühn respond to injury of the mucosa by secretion

(Nielson, Moon and Roe, 1968), it is possible that these "undifferentiated" cells could lead to excessive sodium secretion and fluid accumulation.

One current hypothesis to explain the severe diarrhea of cholera involves alteration of the mucosal and vascular permeability by cholera toxin (Gordon et al., 1966; Philips, 1966). Although the extent to which the virus affects endothelial and mucosal cell permeability is unknown, it cannot be ruled out as a possible factor in solute and fluid accumulation in TGE. However, the absence of signs of significant edema or inflammation do not support this as a primary phenomenon in the secretory process.

Whatever the cause, this secretory process probably contributes significantly to the diarrhea in TGE infection and is a probable explanation of the fluid-filled intestinal loops consistently observed during laparotomy and post-mortem examination.

If active sodium secretion with subsequent fluid accumulation were the sole cause of the diarrhea in TGE, one would expect the diarrheal state to continue during fasting, which is not the case. Hooper (1965) observed a cessation of diarrhea when infected pigs were fasted or fed only water or isotonic diets. Further, it seems unreasonable to assume that the absorptive capacity of the colon could be exceeded by the small quantity of fluid

secreted. Thus, the above observations and demonstrated malabsorption of actively absorbed solute suggests that the bulk of stool fluid lost in TGE diarrhea results from interference with active solute absorption. Therefore, the small intestine is prevented from absorbing the normal glandular secretions and ingesta added at the duodenal end of the tract and the capacity of the colon to absorb water is exceeded.

SUMMARY AND CONCLUSIONS

The influence of transmissible gastroenteritis on jejunal fluid movement as it relates to glucose and electrolyte absorption was studied. Jejunal loops of 16 pigs (2 litters) 16 to 21 days of age were continuously perfused for 4 hours, using a solution of d-glucose (26 mM) in Ringer's lactate solution. The pigs were perfused in pairs, one being infected and the other serving as a control. After the perfusion, absorption rates of water, glucose and electrolytes were determined and their relationships in intestinal loops of infected and control pigs compared.

The mean glucose absorption in the "infected loops" was 29 μM/cm/4hr as compared with a mean glucose absorption of 72 μM/cm/4hr in the "control loops." Not only did the TGE-infected loops not absorb glucose properly, but they actually secreted sodium chloride and water into the lumen.

The relationship between total net solute movement and net fluid movement was linear. Fluid movement in and out of the perfused solution was closely associated with total net solute movement and approached zero at zero fluid movement. Relationships of sodium and chloride to net fluid movement were also linear.

In view of the mucosal cellular changes, close association between solute and fluid movement, and demonstrated malabsorption of actively absorbed solute in pigs affected with the disease, interference with active solute absorption was suggested as the primary phenomenon in TGE diarrhea. The small intestine is prevented from absorbing the normal glandular secretions and ingesta added at the duodenal end of the tract, and the capacity of the colon to absorb water is exceeded. The secretory process is thought to contribute only secondarily to the diarrheal state.

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VITA

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The author is married to the former Dorothy Heyward of Atlanta and they have two daughters, Allison, five, and Beryl, two.

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