Response of the Small Intestine to the Application of a Hypertonic Solution

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The morphologic and functional alterations caused by a commonly used hypertonic radiographic dye (Hypaque®-50%) were compared with changes observed during the absorption of 150 mM saline in closed segments of the ileum of 2- to 3-kg rabbits. Hypertonic dye caused a rapid decrease in height and width of the villi, a decrease in height of the epithelial cells and closure of the intercellular space. Concomitantly, the tissue fluid content of the bowel wall and the volume of venous outflow from the segment of ileum decreased, presumably in response to the osmotic gradient between ileal lumen and blood. The fluid added to the luminal contents was hypotonic and contained sodium, potassium, chloride and bicarbonate. In contrast, the ileum exposed to 150 mM saline had prominent intercellular spaces between adjacent epithelial cells and absorbed the solution at isotonic conditions. These studies indicate that production of diarrheal fluid by this hypertonic solution is different from that reported for enteric pathogens (Am J Pathol 73:747–764, 1973).

Malabsorption of nutrients, overactivity of the intestinal musculature or intestinal infection were thought, in the past, to be the major causes of diarrheal syndromes. Recently, it has been shown that a great many different mechanisms all result in the production of diarrhea.[1] Intestinal infections caused by bacteria have received the most attention. Application of hypertonic solutions directly onto the mucosa of the small bowel also causes diarrhea. While many of the pathophysiologic consequences of application of a hypertonic solution to the mucosa of the gastrointestinal tract are known,[2–9] the morphologic injuries resulting from this interaction are much less well appreciated.[8,9] The purpose of this study is to describe the morphologic and some of the functional alterations caused by one hypertonic solution (Hypaque®-50%, Winthrop Laboratories), a radiographic dye used frequently

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in clinical medicine, when applied to the mucosa of the small bowel and to compare these alterations with the changes observed during absorption of 150 mM saline.

Materials and Methods

The *in vivo* ileal loop preparation in 2- to 3-kg rabbits was used for this study. This model has been used extensively in the study of diarrheal diseases of infectious origins. Preparation of these loops has been described in detail elsewhere.[10] In summary, a series of closed segments of ileum 6 cm in length were made. Between each ileal loop a 4-cm segment of ileum was interposed. The responses of two to eleven *in vivo* ileal loops were studied in each animal. In each rabbit the response of the ileum to both hypertonic and control solutions was studied. Closed segments that served as controls received either 2.0 ml of 150 mM sodium chloride or were left empty (referred to hereafter as saline or empty loops). Sodium diatrizoate (Hypaque-50%, w/v; 3,5-diacetamido-2,4,6-triiodobenzoate) has an osmolality of 1560 mOsm/kg, a sodium concentration of 768 mEq/liter and a molecular weight of 636 daltons. Closed segments used to study the response to the hypertonic solution received either 2.0 ml of Hypaque-50% or 2.0 ml of Hypaque-25% prepared by diluting the Hypaque-50% solution with sterile distilled water (referred to hereafter as 50% and 25% Hypaque loops). The ileal loops were resected at 0, 1, 2½, 5, 10, 20, 40, 60, 90 and 120 minutes after instillation of the solutions into the lumen of the loops with needle and syringe. At resection the drained Hypaque loops weighed 1.4 $\pm$ 0.1 g, and the emptied control loops weighed 1.5 $\pm$ 0.1 g (mean $\pm$ SE). A total of 272 Hypaque loops and 143 control loops was studied in 46 rabbits.

Morphologic Studies

The majority of control and test loops were surveyed histologically after fixing in chilled buffered formalin, embedding in paraffin, and staining with hematoxylin and eosin. Tissue from each animal was always processed together in an identical manner.

The mucosa and submucosa of 34 Hypaque, 8 empty and 16 saline loops from 8 animals were studied by electron microscopy. For these studies, 2.0 ml of 1200 mOsm/kg Karnovsky fixative [11] was injected into the closed loop immediately after it had been resected. Two to 5 minutes later the loops were opened and a portion of the small bowel minced. Tissue was processed as previously described [10] and studied with an RCA 3-G electron microscope. Two other methods of morphologic preservation were also used.

Freeze Substitution

Small pieces of one control and one test loop from the same animal were quenched in liquid propane chilled in liquid nitrogen. The test loop had been in contact with 50% Hypaque for 5 minutes. The control loop had been in contact with 150 mM NaCl for a similar period of time. Tissue was then transferred to liquid nitrogen and trimmed with chilled scissors before being placed in small bottles at −70 C. Two and one-half ml of a solution of osmium tetroxide chilled to −70 C (340 mg% solution diluted with acetone dried in molecular seive, Linde) was poured over the tissue. Tissue was kept at −70 C for the next 9 days and was transferred to new vials containing fresh solution every 3 days. On the ninth and tenth days the procedure was repeated but at 0 C. On the eleventh and twelfth days, xylene at 0 C

was substituted for the osmium-acetone mixture. Tissue was subsequently brought to room temperature, embedded in paraffin, sectioned and stained with hematoxylin and eosin.

Freeze Drying

Tissue was also preserved by the freeze drying procedure of Benditt *et al.*[12] The tissue, after being brought to room temperature, was postfixed in paraformaldehyde vapor, vacuum-embedded in paraffin, cut and stained with hematoxylin and eosin.

Physiologic Studies

Immediately after resection, the luminal fluid was drained into volumetric cylinders.

Electrolyte Determinations

Sodium and potassium concentrations of the luminal contents and serum were determined by flame photometry, chloride concentrations with a Buchler-Cotlove chloridometer and bicarbonate by the microgasometric procedure. All samples were collected under oil to minimize the loss of bicarbonate. Osmolality was determined by freezing-point depression method with a Fiske osmometer.

Determination of Net Volume and Net Ion Flows

Net volume changes of the luminal contents were determined from direct measurement of the initial and final volumes. Net ion fluxes were calculated from the difference in the product of the ionic concentration and volume at the time of injection and resection.

Dry Weights

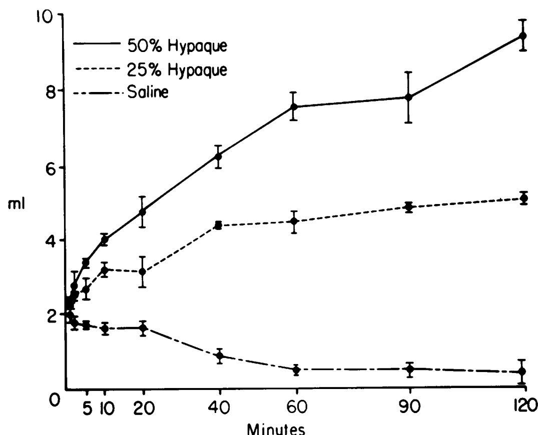
The dry weights of portions of 33 control and 46 50% Hypaque loops from 9 rabbits were determined. Wet weights were measured after opening the bowel, trimming away mesenteric fat and carefully blotting the tissue. These portions were dried in an oven at 100 C until constant weights were obtained (4 to 5 days). The amount of tissue fluid lost was then calculated.

Total Venous Outflow

Blood flow from an ileal loop was determined from timed collections of blood obtained after cannulation of the branch of the superior mesenteric vein draining the loop. At least six 30-second collections were made. The loop was empty during the initial collection. Two ml of Hypaque-50% were then injected, and another series of timed collections made over the next 5- to 10-minute interval. Calculations of venous outflow were based on the specific gravity of blood collected during each study period and the wet weight of each loop. A total of fourteen loops was studied in 7 animals.

Results

The amount of luminal fluid increased immediately after instillation of the hypertonic solution (Text-figure 1). In animals with several test loops, no significant difference was found when the responses of the proximal and distal test segments were compared. The intestinal loops in contact with the hypertonic solutions gradually increased in diameter



TEXT-FIG 1—Changes in intraluminal volume in 6-cm closed segments of rabbit ileum (mean ± SE).

and length (Figure 1), but during the time period studied they easily accommodated the increase in intraluminal volume. The closed segments that received 150 mM NaCl gradually absorbed fluid. Many were dry by 2 hours. The response of the empty loops was variable. Two closed segments resected at 2 hours contained 1.4 and 1.2 ml of luminal contents; all the others were dry.

By light microscopy the ileal mucosa that was in contact with saline had many tall villi (Figure 2A). Numerous intercellular spaces were present between absorptive cells (Figure 3A). These spaces could be seen at irregular intervals from the crypt-villous junction to the tip of the villus. The lamina propria and submucosa were easily identified and contained the usual complement of mononuclear cells. With one exception, the histology of the ileal mucosa of the empty loops was identical to the saline loops. No intercellular spaces could be identified in the empty loops.

The ileal mucosa in contact with the hypertonic solution, when compared with the saline or empty loops, demonstrated a significant decrease in the width and height of the intestinal villi (Figure 2B). The mucosal epithelium in contact with the hypertonic solution appeared much shorter and stained more densely (Figure 3B). (The figures used to compare the morphologic response to 150 mM saline and to the hypertonic solution are of adjacent loops from the same experimental animal.) Measurement of the epithelium in contact with the hypertonic solution revealed a reduction in height as great as 55% after 1 minute of exposure. At later times the reduction was less marked. An apparent decrease in the cellular population of the lamina propria occurred. The vasculature in the lamina propria and submucosa was extremely hyperemic. Ileal segments preserved by freeze substitution

or freeze drying showed similar morphology when studied by light microscopy.

With electron microscopy the intercellular space between adjacent epithelial cells in contact with saline was extremely prominent (Figure 4A). At the apex of the epithelial cell, the dilation of the intercellular space began just beneath the most distally located desmosome (Figure 5). At the nuclear area, the space was much more prominent (Figure 6A). Interdigitations between lateral projections of adjacent absorptive cells were occasionally present. The amount of surface contact between transmigrating lymphocytes in the space and the epithelial cells was small. Only rarely were interdigitations present between the absorptive cells and the lymphocytes. At the base of epithelial cells absorbing saline, the basement membrane was usually in direct contact with the epithelial cell cytoplasm. An area was occasionally present where the intercellular space was in direct continuity with the basement lamina (Figure 7A). No alterations were seen in the cytoplasmic organelles of the saline loops. The subepithelial capillaries (Figure 4A) were invariably dilated and contained an occasional erythrocyte.

The only significant difference between the saline and the empty loops was in the morphology of the intercellular space. Lateral cell membranes of adjacent epithelial cells from empty loops were usually in close proximity. Occasionally the space was dilated to a width of 600 to 800 Å. No differences were seen in the height or cytoplasmic density of the epithelial cells.

The height of the epithelium in contact with Hypaque-50% was markedly decreased (Figure 4B). The space between adjacent epithelial cells had narrowed to 200 Å. At the nuclear area of the epithelial cells (Figure 6B), the cell membrane of transmigrating lymphocytes was in almost complete contact with the adjacent epithelial cells and was frequently indented by the lateral plications of the epithelial cells. From the apex to the base of the intercellular space there were interdigitations between the lateral projection of adjacent epithelial cells. These interdigitations were particularly numerous at the base of the cell (Figure 7B).

The epithelial cells and the transmigrating lymphocytes in the intercellular spaces of Hypaque loops had a cytoplasm that stained more densely. Except for an occasional area of dilated endoplasmic reticulum, the organelles in the cytoplasm of the absorptive cells appeared unaltered. The subepithelial capillaries (Figure 4B) were filled with erythrocytes. Very little plasma was present in the vascular space.

Comparison of the rate of volume production with the osmotic

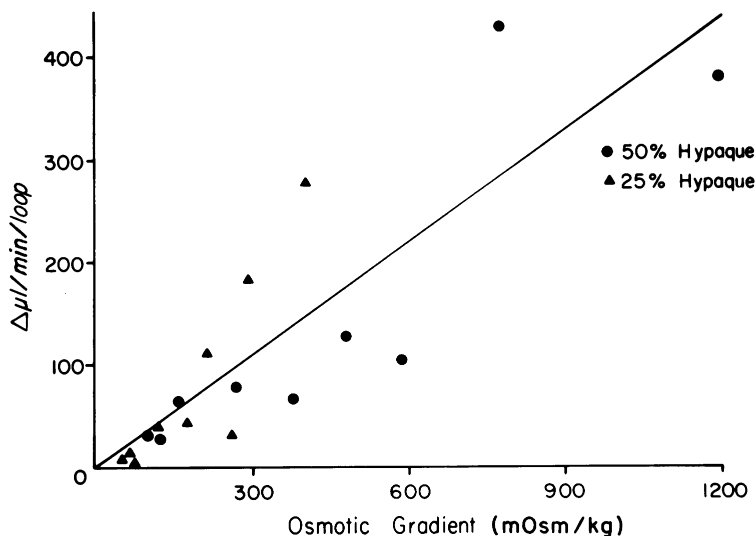
gradient between the luminal effluent and the serum demonstrated that the immediate increase in the volume of the luminal contents was related to the osmotic gradient between the effluent and the serum (Text-Figure 2). The rapid decrease in the osmolality of the luminal contents is depicted in Text-figure 3. Calculations based on a comparison of net volume flow and net sodium flow [20] indicated that the fluid added to the luminal contents of the 50% Hypaque loop has an osmolality of 108 and that added to the 25% Hypaque loops has an osmolality of 134 (Text-figure 4). Absorption of saline took place at close to isotonic conditions. Table 1 summarizes the measured amount of volume and of the ionic constituents that were added to the luminal contents between minutes 1 and 120. They indicate that a hypotonic solution containing sodium, potassium, bicarbonate and chloride accounts for almost all the ionic constituents added to the luminal contents.

Dry Weight Determinations

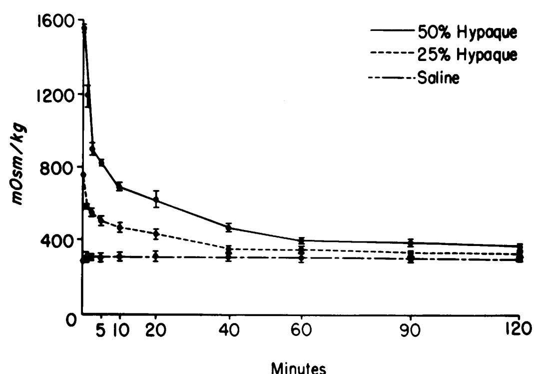
The bowel wall in contact with Hypaque was significantly drier ($P < .005$) than loops left empty or exposed to saline (Table 2). This difference was most marked immediately after injection of the compound but was still evident at 120 minutes.

Blood Flow Determinations

A significant decrease ($P < .001$) in measured venous outflow from the loop occurred immediately after instillation of the hypertonic solu-



TEXT-FIG 2—Comparison of the rate of volume flow in the Hypaque loops and the difference in osmotic gradient between the luminal contents and the serum.

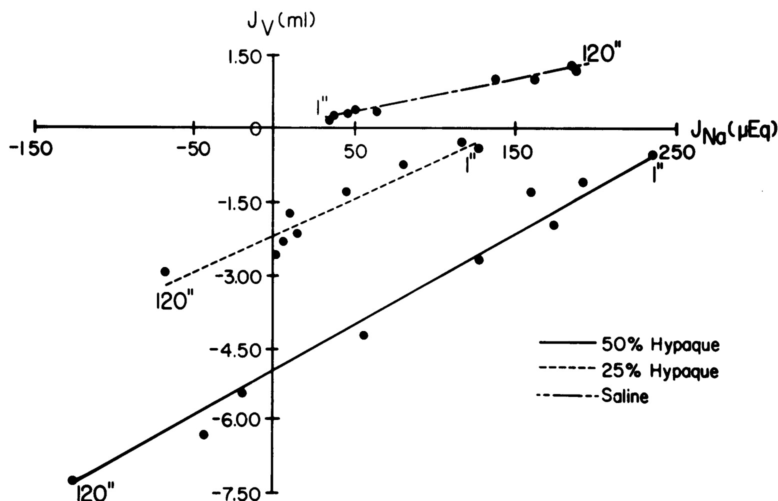


TEXT-FIG 3—Changes in osmolality of the intraluminal contents following instillation of solutions (mean ± SE).

tion onto the mucosa (Table 3). The decrease continued during the period of observation (5 to 10 minutes).

Discussion

These studies were undertaken to ascertain the effect of the application of a hypertonic solution on the light and electron microscopic appearance of the small bowel and to gain further insight into some of the pathophysiologic events that occur as the result of this application. Immediately following the application of the hypertonic solution there were marked alterations in the morphology of the small bowel mucosa. The osmotic gradient between the luminal contents and the



TEXT-FIG 4—The relationships between net volume flow and net sodium flow. Positive values denote absorption from the lumen and negative numbers denote secretion into the lumen. The slope of the least squares line corresponds to a fluid osmolality of 275 mOsm/kg for the saline loops, 134 mOsm/kg for the 25% Hypaque loops and 108 mOsm/kg for the 50% Hypaque loops.

Table 1—Net Volume and Ion Flows Between Minutes 1 and 120

Loop	Volume (ml)	μEq added to luminal contents Na^{+}	K^{+}	Cl^{-}	HCO_3^{-}	Total
50% Hypaque	6.9	357	16	165	208	746 μEq/6.9 ml
as mEq/liter		52	2.3	24	30	108 mEqX/liter
25% Hypaque	2.7	187	12	69	130	398 μEq/2.7 ml
as mEq/liter		70	4.5	26	48	149 mEq/liter
Rabbit serum						
as mEq/liter		141	4.8	93	31	270 mEq/liter

blood resulted in an instantaneous increase in luminal contents, with a concomitant decrease in tissue fluid content of the bowel wall per se, and in venous outflow from the segment of the ileum under study. The osmolality of the luminal fluid was decreased by the secretion into the luminal fluid of a hypotonic solution containing sodium, potassium, bicarbonate and chloride.

Previous investigations using hypertonic solutions of lower tonicity (550 mOsm/kg) in the small bowel indicated that only slight histologic damage occurred.[8,9] In the present experiments significant histologic alterations developed immediately after contact with Hypaque. The changes in heights and widths of the villus and the staining density of the mucosa could be directly attributed to the dehydrating effect of the hypertonic solution on the epithelium. This effect was present throughout the period of observation and correlated with the decreased amount of water in the bowel wall, per se. The absence of a cellular inflammatory response, electron microscopic evidence of damage to the cytoplasmic organelles, and the gradual return in the height of the mucosal epithelial and the gradual increase in the water content of the

Table 2—Tissue Fluid Content of the Ileum (%)

Time (min)	Type of Loop: Empty	Saline	50% Hypaque	P value*: Empty vs Saline	50% Hypaque vs saline
1	82.3	82.4	70.5	NS	<.005
2½	82.3	82.4	69.2	NS	<.005
5	82.3	82.4	70.6	NS	<.005
10	82.3	82.4	73.7	NS	<.005
20	80.9	80.6	72.1	NS	<.005
60		81.6	77.9	—	<.005
120		82.1	77.4	—	<.005

* Student paired **t** test

Table 3—Total Venous Outflow from Ileal Loops

	Blood flow rate* (Mean ± SE)
Contents of loop	
Empty	54.95 ± 4.2
Hypaque-50%	30.32 ± 2.5
P value†	<.001

* in ml/min/100 g of ileum (wet weight)
† Student paired t test

ileum in contact with the hypertonic solution indicate that the injury is reversible. Ultrastructural examination of the bowel demonstrated a marked difference in the configuration of the epithelial cells absorbing saline and the epithelial cells responding to the hypertonic solution. These differences in conformation were seen by light microscopy with three different means of morphologic preservation, indicating that they probably do not represent an artifact.

Marked dilation of the intercellular space of the mucosa has been associated with the isotonic transfer of water from gall bladder [13,14] or small bowel [15,16] lumen to the vasculature. A similar configuration occurred in the toad bladder [17] and in the distal convoluted tubule of the kidney, where net transfer was from the tubule lumen into the vasculature in response to antidiuretic hormone.[18] Diamond has recently proposed the standing-gradient model of fluid transport, which offers an explanation for this geometrical configuration.[14] Solute pumps located at the lateral border of epithelial cells transfer solute (mainly sodium) from the cell to the intercellular space. This results in local hypertonicity in the intercellular space. The solute diffuses down the concentration gradient toward the open mouth of the channel located at the junction of the epithelial cell with the underlying lamina propria. Water continually flows into the channel due to the osmotic gradient, resulting in the absorption of fluid at close to isotonic conditions. In a steady state, a standing osmotic gradient will be maintained in the intercellular space by active solute transport. The mathematical model for this type of transport predicts that the fluid transported will be isotonic when the shape of the intercellular space is long and narrow and hyperosmotic when the space becomes shorter.[14] Arrest of fluid transport from lumen to blood results in complete closure of this intercellular space. Heretofore, the closure of an intercellular space between adjacent absorptive cells of the small bowel has been associated with either an unknown functional state of the ileal absorptive epithelium or with the absence of

water and ion transfer from lumen to blood. These studies indicate that this configuration also occurred when net fluid movement was from the vasculature to the lumen of the bowel.

The closed intercellular space not only occurred with another hypertonic compound when studied in the gall bladder [13,14] but was also seen in diarrheal diseases due to the enterotoxins of *Vibrio cholerae*,[10] or *Escherichia coli*,[19] where water accompanies net solute movement at approximately isotonic proportions from blood to mucosal lumen.[20] In contrast to enterotoxin-produced diarrhea where sodium and water are thought to follow an extracellular route through the intercellular space from blood to lumen,[21] the mechanism for addition of a hypotonic solution to the luminal contents must of necessity include some process which causes the retention of solute so that a hypotonic fluid results. Diamond [14] also speculated that the addition of a hypotonic solution into the lumen of the bowel could occur through the intercellular space (the "backward" operation of the standing gradient flow system). In this speculation, solute is actively transported out of the intercellular space into the adjacent epithelial cell, making the fluid in the intercellular space hypotonic. The similar appearance of all regions of the epithelial cell may indicate that fluid is not only lost from the intercellular space directly into the lumen but also from the apical portions of the epithelial cells. Recently Oschman and Berridge have suggested that the microvillous border may be the site of standing osmotic gradients during fluid secretion.[22]

Does the alteration in morphology cause a concomitant change in mucosal function? Other studies have shown that some mucosal functions were severely altered in response to hypertonic solutions. Specifically, decreased absorptive capacity for chloride [6] or glucose [8] occurred in the small bowel exposed to hypertonic solutions.

This hypertonic solution elicited instantaneous effluent production with concomitant diminution in the osmolality of the luminal fluid. The events occurring with Hypaque were the result of its hypertonicity rather than a direct toxic effect, since 300 mOsm/kg solutions of Hypaque failed to cause effluent production.[23] The pattern of change in osmolality was similar to those which occurred with other poorly absorbed hypertonic solutions when applied to the small bowel mucosa.[3]

In the Hypaque loops, the changes in osmolality could be accounted for by the addition of a hypotonic solution. The calculated osmolality of the ions and water that was added to the luminal contents of the 50% Hypaque loops is in excellent agreement with measured volume flow and net ion fluxes, indicating that sodium, potassium, chloride

and bicarbonate account for all the ions added to the solution. In the 25% Hypaque loops the calculated osmolarity of 149 mOsm/liter was not significantly higher than the 134 mOsm/kg predicted from the net volume and net sodium change. The forces necessary for the ion fluxes to occur were probably passive in nature. In the saline loops, sodium and chloride were absorbed, and potassium and bicarbonate secreted into the lumen. Fluxes of ions in the saline loops occurred at close to isotonic conditions and were similar to those observed in previous experiments.[20]

The diminution in venous outflow probably resulted from a direct effect of the hypertonic solution on the microcirculation rather than being due solely to loss of fluid into the effluent. Each minute, 0.82 ml of blood flowed through a typical empty loop. During the first 10 minutes following exposure to 50% Hypaque, venous outflow decreased to 0.43 ml/min/loop, while effluent production accounted for only 0.18 ml/min/loop. Landis and Sage[24] have recently described the changes that occurred in small vessels when exposed to extravascular hypertonic solutions. The progressive loss of intravascular fluid resulted in hemoconcentration and finally stasis. In the Hypaque model, marked hyperemia and congestion of the subepithelial capillaries also occurred. Direct visualization of *in vivo* subepithelial capillaries along the outer portion of the villus revealed that a progressive diminution in blood flow and partial stasis occurred within minutes after exposure to Hypaque.[23]

Hypaque-50% was chosen as the test solution, not only because of its wide application in radiology, but also because it caused a diarrheal syndrome in infant rabbits that was indistinguishable from experimental cholera.[25] The mechanism of effluent production by the hypertonic solution is different from that resulting from stimulation of the ileal epithelium to hypersecrete as seen with the bacterial diarrheas due to enterotoxin production (*V cholerae* and *E coli*) or from the exudative inflammatory response elicited by *Shigella* or other enteropathic *E coli*.[1,10,19,26]

Hypaque-50% or related compounds containing diatrizoate have been used in a wide variety of clinical situations. Volumes ranging from 50 ml to 150 ml are commonly administered. These compounds are frequently used to localize the position of small bowel biopsy devices or the area of gastrointestinal perforation. Occasionally, they are instilled into the lumen of the gastrointestinal tract in an attempt to relieve an area of obstruction prior to laparotomy. This therapeutic procedure should be abandoned, as the cellular and vascular alterations caused by the hypertonic solution can only cause further damage to a compromised gastrointestinal tract. Hypaque-50% also has a variety of intravascular

applications including angiography, translumbar aortography, excretory urography and intraosseous venography. Deaths from hypovolemia following administration of this compound in children have been reported.[27] In adult patients, vascular spasm also has occurred during these procedures. Perhaps a similar series of events are occurring in other locations. Further studies along these lines are in progress.

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Legend for Figures

Fig 1—Gross photographs of loops of rabbit ileum 40 minutes (left) and 120 minutes (right) after the instillation of the test compounds. The top row received 2 ml of 50% Hypaque, the middle row, 2 ml of 25% Hypaque and the bottom row, 2 ml fo 150 mM saline. Progressive fluid accumulation was seen in Hypaque loops, and saline loops gradually became dry.

Fig 2—Low power photomicrographs of the villi of two loops from the same animal. Tissue from both loops was fixed and processed at the same time in an identical manner. **A**—Ileum in contact with 150 mM saline had numerous slits between adjacent absorptive cells. The lamina propria was easily identified. **B**—Ileum in contact with Hypaque-50% for 1 minute showed a marked decrease in the height and width of the villi. The lamina propria was substantially narrowed (H & E, × 120).

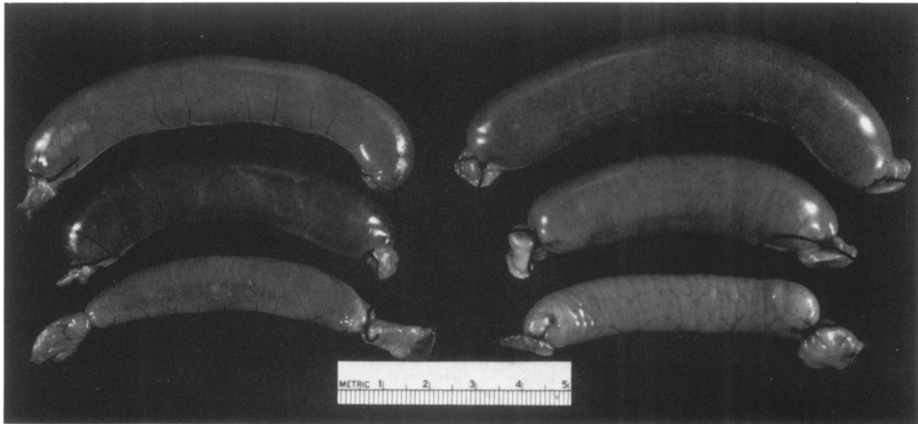
Fig 3—Higher power photomicrographs of a portion of the villus. Both villi were from ileal loops of the same animal and processed identically. **A**—Ileum in contact with saline exhibited numerous intercellular spaces (*arrows*) between adjacent absorptive cells. The goblet cells contained mucus. No alterations were noted in the vasculature of the lamina propria. **B**—Villus in contact with Hypaque-50% for 1 minute had mucosal epithelium reduced in height. Increased staining density of the cytoplasm was present. Many goblet cells had discharged their mucus and the subepithelial capillaries were severely congested (H & E, × 300).

Fig 4—Electron micrographs of mucosa absorbing saline for 10 minutes (**A**) or in contact with Hypaque-50% for a similar period of time (**B**). Both loops were from the same animal and the tissue was processed identically. The muocsa absorbing saline (**A**) showed numerous intercellular spaces containing an occasional transmigrating lymphocyte (*L*). The subepithelial vasculature was dilated (*C*). The mucosa in contact with Hypaque-50% (**B**) was significantly shorter and stained more densely. The intercellular spaces could not be identified at this magnification. Severe congestion of the subepithelial capillaries (*C*) occurred (× 4200).

Fig 5—Electron micrograph of the apex of an absorptive cell from a loop absorbing saline. The dilation of the intercellular space was limited by an apical desmosome (*arrows*). No alterations in cellular organelles are seen (× 19,600).

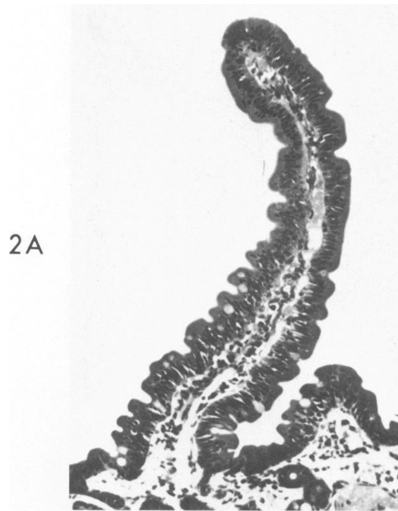
Fig 6—Electron micrograph of the nuclear area (middle portion) of epithelial cells absorbing saline (**A**) or in contact with Hypaque (**B**). In the ileum absorbing saline (**A**) the intercellular space was prominent. Large spaces were present between the transmigrating lymphocytes (*L*) and adjacent epithelial cells. The intercellular space between adjacent absorptive cells in contact with Hypaque (**B**) was obliterated. The lateral projections (*arrows*) of the absorptive cell interdigitated with the transmigrating lymphocytes (× 12,800).

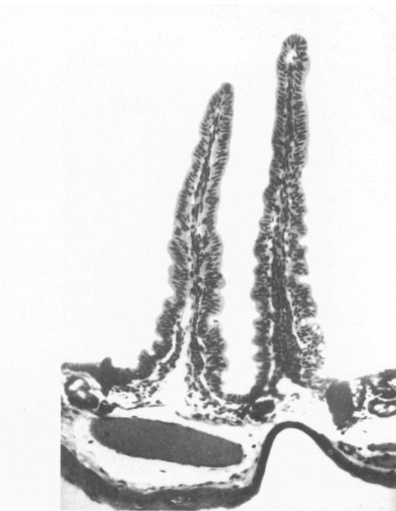
Fig 7—Basal portion of the epithelial cell absorbing saline (**A**) or in contact with Hypaque (**B**). The basal portion of the absorptive cells in contact with saline (**A**) had a prominent intercellular space which occasionally was in direct contact with the basal lamina. There were only a few interdigitations of the lateral plications of adjacent epithelial cells (*arrow*). The intercellular space of the loops in contact with Hypaque (**B**) was just visible and was filled with the interdigitations of the lateral projections of adjacent absorptive cells (*arrows*) (× 15,000).



1

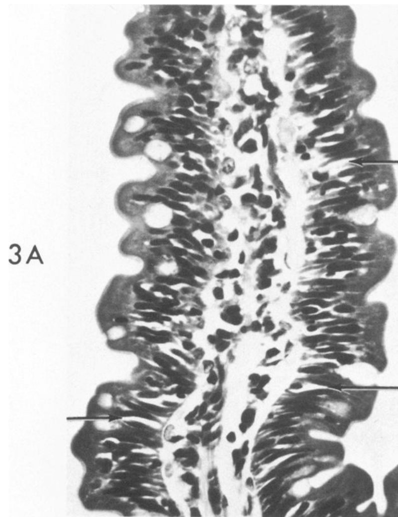
2A





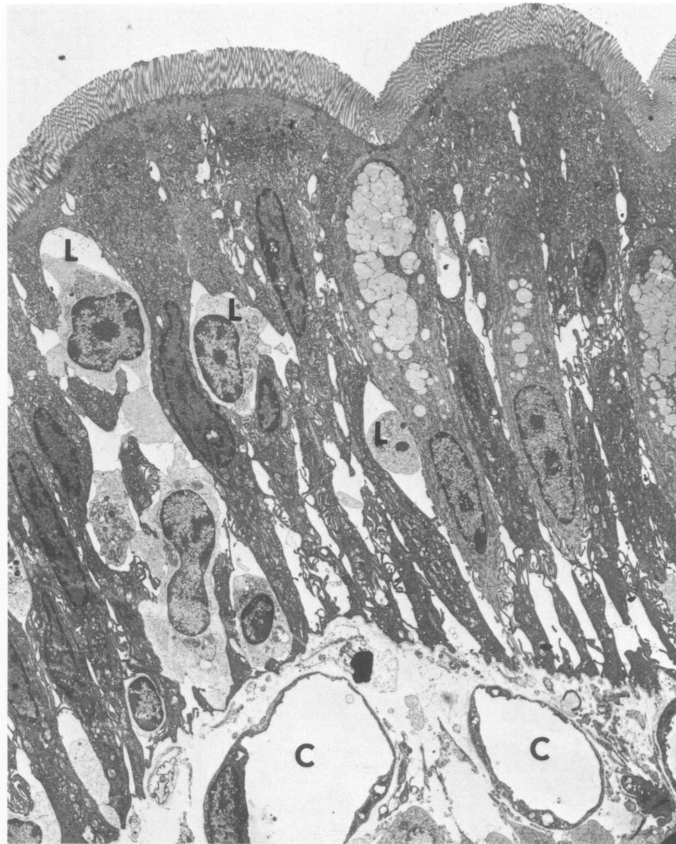
2B

3A



3B

4A



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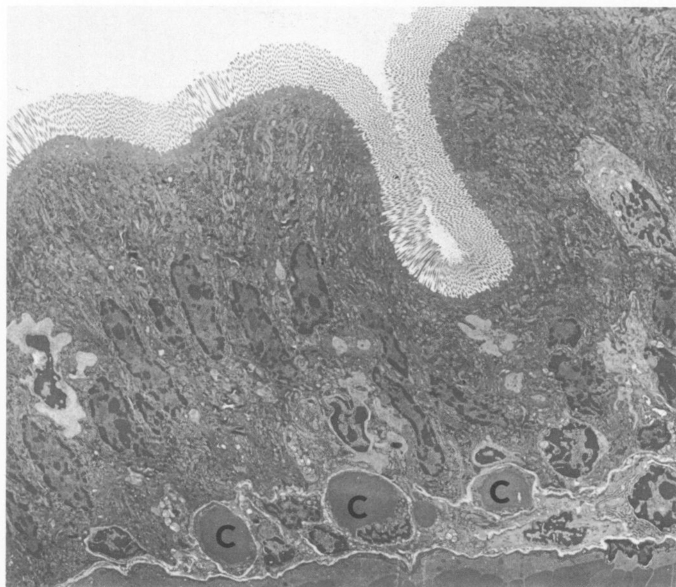

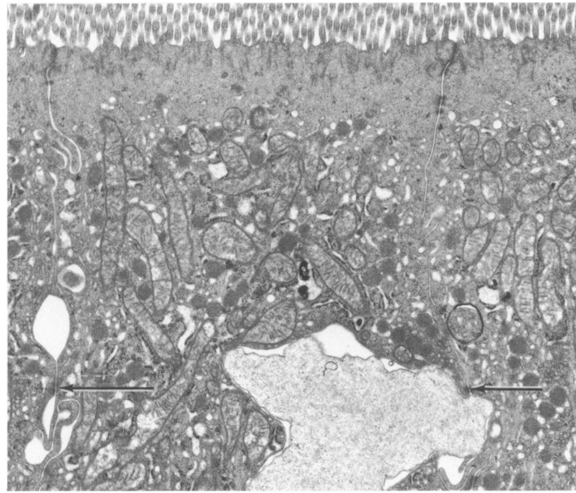
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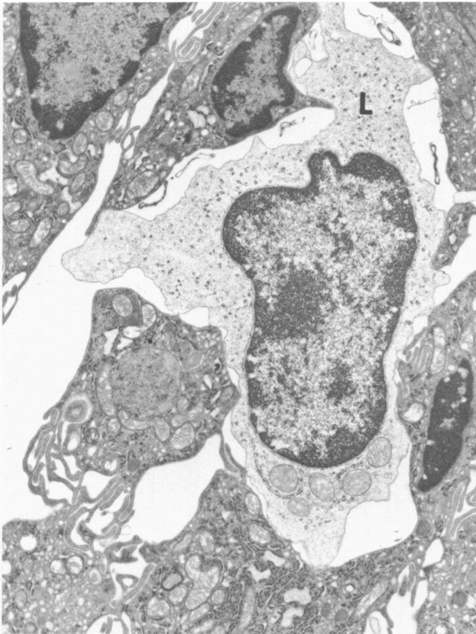
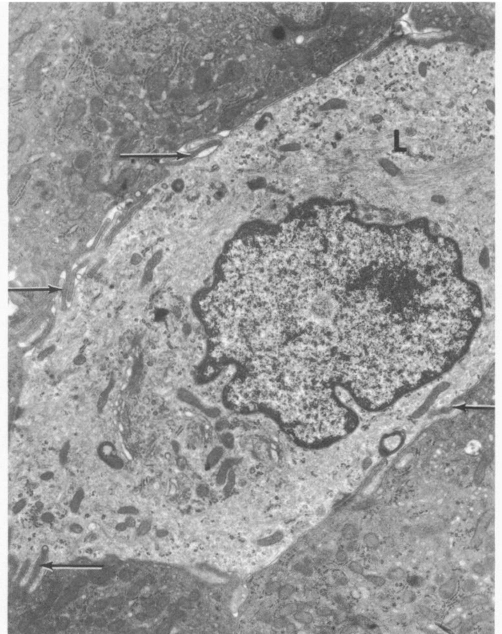
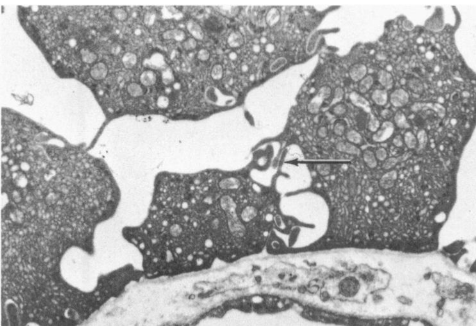
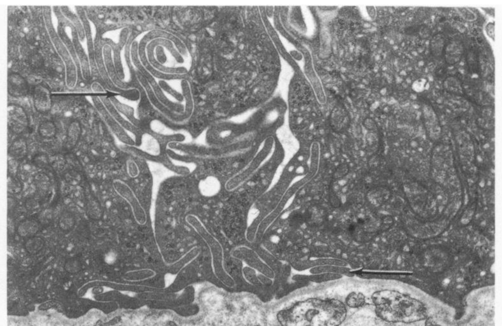


6A



6B


7A


7B

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