Some Characteristics of the Rabbit Vermiform Appendix as a Secreting Organ

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ABSTRACT It has been confirmed that the rabbit vermiform appendix secretes spontaneously at a relatively rapid rate (1–12 $ml \cdot h^{-1}$; $1.4 \pm 0.24\ \mu l \cdot min^{-1} \cdot cm^{-2}$). The electrolyte composition is similar to that of ileal fluids and independent of the secretory rate. The transmural potential difference is about 12 mV, mucosa negative. Of the major electrolytes, only HCO_3^- is secreted grossly against its electrochemical potential difference. This finding plus the low hydraulic (or osmotic) permeability (L_p) and high secretory pressures of the organ strongly suggest that the secretion is an active one. The passive permeability to Na^+ and Cl^- appears to be, at most, somewhat less than for small bowel. Permeability to mannitol was estimated at $2.5 \times 10^{-7}\ cm \cdot s^{-1}$. On the basis of reasonable assumptions and results with luminal test solutions of differing osmolarities, it was concluded that (*a*) the L_p of the appendiceal epithelium is in the lower range of values reported for small bowel and colon; (*b*) the L_p is higher for osmotic absorption than for osmotic secretion; and (*c*) the rate of spontaneous secretion is insensitive to luminal anisotonicity over a wide range of values. But sufficiently hypotonic solutions can reverse net secretion to net absorption, more by inhibiting spontaneous secretion than increasing osmotic absorption. The rabbit vermiform appendix appears to be a useful model for the elucidation of intestinal secretory processes.

INTRODUCTION

Although numerous studies have increased our knowledge regarding the mechanisms of intestinal absorption, comparatively little is known about intestinal secretion. Most of the studies of net fluid and solute entry into the lumen have employed some kind of physical, chemical, or nervous stimulation which reverses the direction of the usual absorptive state of the epithelium (1–3). The "pathological" intestinal secretion associated with cholera has been intensively studied in recent years (4, 5). Additional pathological conditions characterized by diarrhea presumably due to intestinal secretion include bacterial infections, sprue, certain pancreatic tumors, medullary thyroid carcinoma, neural crest tumors, and villous adenomas.

A model of spontaneously secreting intestinal epithelium would be desirable. Florey, Wright, and Jennings (1) found the Brunner's gland areas to be spontaneously secretory in the horse, dog, cat, goat, sheep, rabbit, and pig. More recently the chicken duodenum (which has no Brunner's glands) has also been reported to be secretory (6). These duodenal preparations have the disadvantage of relatively low secretory rates per unit area. Powell, Malower, and Plotkin recently reported a consistent net secretion by the luminally perfused guinea pig small intestine in vivo (7); whether the unperfused preparation secretes is not stated. Lindsay, Ralston, and Lifson suggested the rabbit vermiform appendix as a model for the study of intestinal secretion (8).

The wall of the rabbit appendix is mostly composed of a layer of tall conoid lymph nodules, each associated with a flask-shaped gland that Crabbe and Kelsall describe as resembling an Erlenmeyer flask with its base inverted nearly to the neck (9). The glands are formed in the embryo by the confluence of intervillus crypts surrounding an abortive villus with fusion of adjacent villi to form the roof. The epithelium of the abortive villus becomes the lympho-epithelium bottom of the gland while the ends of the intervillus crypts form the glandular rim. Contributions to secretion could be made by these glands, the surface epithelial cells, and the crypts of Lieberkühn.

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Except for the abstract of Lindsay et al. (8), practically all published information on the physiology of appendiceal secretion has been provided by Wangensteen and co-workers (10–19). In a study of a large variety of mammalian species, a true appendix (in the sense of a long tubular organ distinguishable from the cecum) was found only in man, the chimpanzee, gibbon, gorilla, orangutan, rabbit, and wombat. The rabbit appendix secreted 20–80 ml of fluid per day; the human organ, 1–3 ml per day. Both the human and rabbit organs could generate a secretory pressure of some 100 cm H_2O. The composition of the secretion was much like that of ileal fluids. Data were presented by Wangensteen et al. from which it was concluded that the secretion rate was decreased by cooling, local radiation, systemic cortisone treatment, and the mucosal application of 5% silver nitrate; and that the rate of secretion did not correlate with the animal's age or the presence of lymphoid tissue in the wall. Due to the animal-to-animal variability or the small number of cases, or both, it would appear that these latter conclusions in a number of instances must be taken as only suggestive.

The purpose of the present paper is to report further observations on the characteristics of this spontaneously secreting organ which bear chiefly on its suitability as a model for the study of intestinal secretion in general.

METHODS

Male white New Zealand rabbits weighing approximately 2 kg, without evidence of diarrhea, were injected intravenously with pentobarbital, 15–20 mg/kg, plus 10 mg of diazepam. The animals were observed for a satisfactory response to the anesthetic agents, i.e., maintenance of spontaneous respiration, normal color, and slight response to external stimuli. After the appendix was delivered through a midline incision, a soft rubber catheter was inserted into it via a small opening in the cecum and its contents washed with bicarbonate Ringer solution warmed to 38°C. With avoidance of the blood supply, a cannula was inserted into the base of the organ and tied with umbilical tape. The appendix was then placed in its natural position in the abdomen and the cannula and tubing brough to the outside through a stab wound in the right flank. The abdomen was closed with towel clips and covered with moistened sponges. A temperature probe was placed just under the anterior abdominal wall adjacent to the appendix, and another probe was inserted into the rectum. Both temperatures were maintained at 38°C, by means of external heat lamps and heating pads.

In some experiments, accumulated secretion was collected from an initially emptied organ by simple drainage under mineral oil, the drainage tubing being placed so as to maintain a hydrostatic pressure not exceeding 5 cm of water. In others, the appendiceal lumen was filled with either 2 or 5 ml of test fluid. Two types of fluid were used: (*a*) simulated appendiceal secretion, which contained the following electrolyte concentrations (meq/liter): Na^+, 146; K^+, 7.0, Cl^-, 93; and HCO_3^-, 60 (osmotic pressure 306 mosM; (*b*) sodium-free Ringer's solution, which contained the following electrolyte concentrations (meq/liter): Na^+, O; K^+, 6.1; Cl^-, 7.4; Ca^{++}, 1.3; Mg^{++}, 2.5; $SO_4^=$, 2.5; HPO_4^-, 1.3. Without mannitol, the osmotic concentration of this solution was close to 25 mosM. Various amounts of mannitol were added to make solutions ranging from 150 to 1500 mosM.

Mannitol permeability. [^{14}C]Mannitol was added to non-sodium Ringer's solution made isosmotic with ordinary mannitol to give a final activity of approximately 580,000 dpm/ml. After cannulation and emptying of the appendix, 1 ml of the solution was placed in the lumen for 1 h. Hydration of the animal and a high urine output was maintained by infusing 5% dextrose in water intravenously at 1 ml/min. Urine, secretion, and blood samples were taken at the end of the time period. The appendiceal lumen was washed with 200 ml isotonic salt solution. The appendix was then weighed and divided into 500-mg portions for oxidation in a Packard Tri-Carb sample oxidizer, model 305 (Packard Instrument Co., Inc., Downers Grove, Ill.). The radioactivity in the various fluids and tissue was counted and estimates made of the mannitol recovery. For purposes of calculating the mannitol in the body from the serum concentration, mannitol was assumed to be distributed in 25% of the total body weight.

To test the possibility of mannitol metabolism with the production of tagged small fragments, [^{14}C]mannitol was purified by column chromatography (Bio-Gel P-2,) Bio-Rad Laboratories, Richmond, Calif. and thin-layer chromatography (TLC).[1] Two peaks of radioactivity were found. The smaller one, containing 1.8% of the counts and showing a TLC mobility different from that of mannitol, was removed. [^{14}C]Mannitol thus purified was then incubated for 1 h at 38°C as follows: (*a*) in the appendiceal lumen, (*b*) with 500-mg appendiceal contents, and (*c*) with 500 mg homogenized appendix. The supernate from these solutions was reapplied to the same column and revealed a single peak identical with that of the original purified peak. No evidence was thus obtained that mannitol is significantly metabolized by bacteria or mucosal enzymes of the appendix.

Mucosal electrical potential difference (PD). The PD was measured by a previously described technique (20) similar to that used by Andersson and Grossman (21) with substitution of isosmolar sodium chloride for potassium chloride in the agar bridge. The venous bridge was placed in the femoral vein. Measurements in this laboratory of the PD of human stomach and duodenum have yielded results like those of Andersson and Grossman.

Analytic methods. Sodium and potassium were determined by flame photometry (IL Flame Photometer model 143 with automatic diluter, model 144, Instrumentation Laboratory, Lexington, Mass.). Chloride was determined amperometrically (Aminco Clinical chloride titrator and dilution pump model 4-44-13, American Instrument Co., Inc., Silver Springs, Md.). Osmolalities were determined by a freezing point-depression method (Osmette Precision osmometer model 2007, Precision Systems, Inc., Natick, Mass.). Bicarbonate was determined by the standard manometric Van Slyke method. [^{14}C]-Mannitol was determined by scintillation counting (Packard Tri-Carb liquid scintillation spectrometer, model 3375, Packard Instrument Co., Inc.).

Mucosal area of appendix. In 39 rabbits, the appendix was removed leaving as little mesentery, fat and connective tissue adherent to the organ as possible. The appendix was then immediately weighed to the nearest 0.1 g, opened longitudinally, and lightly stretched over waxed paper. The mucosal area employed for normalizing transport rates was measured by planimeter with the dimensions determined by the inherent elasticity of the organ wall.

[1] *Abbreviations used in this paper:* L_p, osmotic permeability; PD, potential difference; $\bar{\pi}_L$, average luminal osmolarity.

RESULTS

Relationship between mucosal area and weight of appendix. The mucosal area was found to average 4.43 $cm^2 \pm 0.07$ (SEM) per g of tissue. The correlation coefficient between serosal area and weight was 0.95. This value for mucosal area assumes that the mucosa is perfectly smooth and continuous, i.e., mucosal area is equal to the serosal area. Although there are no significant evaginations of the mucosal surface, two types of invaginations are present, as noted above: the flask-like glands and crypts of Lieberkühn.

An estimate of the flask-like gland area was made in five formalin-fixed appendices, by counting five 1-cm^2 areas in each, under a dissecting microscope. An average of 230 ± 43 [SD) gland openings per cm^2 were found (range = 134–295). The number of openings in the tip of the organ ($260 \cdot cm^{-2}$) differed significantly ($P < 0.005$) from that at the base ($191 \cdot cm^{-2}$). The diameters of the glands and gland openings (necks) were measured in hematoxylin and eosin sections of 10 appendices by use of a Reichert "Visopan" microprojector and a micrometer (American Optical Corp., Scientific Instrument Div., Buffalo, N. Y.). The diameter of the flask-like glands averaged 0.61 ± 0.09 mm (Mean±SD); the openings, 0.13 ± 0.01 mm in width. The gland necks leading to the surface openings were 0.16 ± 0.09 mm long and the openings occupied about 3% of the measured surface area.

On the assumption that the flask-like gland was a sphere of the above diameter (0.61 mm), the average surface area/gland was calculated to be 1.29 mm^2. Assuming the necks to be right angle cylinders, one calculates the surface area of the necks to average 0.064 mm^2, giving a total area of 1.33 mm^2. The total flask-like gland mucosal area/cm^2 serosal area was estimated to be 3.06 $cm.^2$ On this basis, the mucosal area/serosal area ratio would be increased to about 4.0 by the glands.

The area of the epithelium of the crypts of Lieberkühn was not similarly measured; but, depending on their state of dilitation, their contribution to a further increase in the ratio could be important, since there are many crypts per gland.

It is emphasized that these measurements were made on histological sections of the appendix. When the mucosa of the opened appendix is observed in vivo through a dissecting microscope during rapid secretion, the openings of the flask-like glands may be much greater and form a much larger percentage of the smooth surface area. The architecture of the mucosa in vivo is undoubtedly much less static than indicated by the measurements on fixed tissue.

In the present paper all values will be expressed per square centimeters of smooth mucosal area.

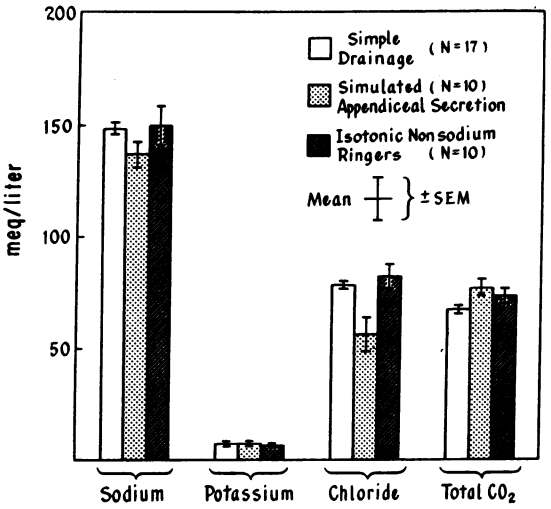

FIGURE 1 Graph of electrolyte concentrations in the secretion. Concentrations were determined directly in the secretion collected by simple drainage and calculated for the instillation experiments. Serum values in seven of the animals were (mean±SEM) sodium 143 ± 6; potassium 3.6 ± 0.7; chloride 102 ± 3; and CO_2 content 26 ± 2 meq/liter.

Spontaneous secretory rate. The spontaneous secretory rate determined by simple drainage of the appendix was found to be 1.4 ± 0.24 (SEM) $\mu l \cdot cm^{-2} \cdot min^{-1}$. For the average rabbit weighing 2.5 kg this amounts to some 3 $ml \cdot h^{-1}$ or about 50% of the plasma volume per day. Wangensteen actually collected daily volumes of this magnitude (12). Similar rates of secretion occurred when the organ was initially filled with nonsodium Ringer's solution made isosmolar with mannitol (1.5 ± 0.24 $\mu l \cdot cm^{-2} \cdot min^{-1}$), or with simulated appendiceal secretion (1.2 ± 0.28 $\mu l \cdot cm^{-2} \cdot min^{-1}$). These values are comparable with rates of choleragenic secretion per square centimeters of smooth mucosal area by rabbit small intestine. One can estimate that Leitch, Iwert, Burrows, and Stolle (22, 23) found the cholera-induced ileal secretion to range from 1.1 to 3.0 $\mu l \cdot cm^{-2} \cdot min^{-1}$ of smooth mucosal area. Serebro, Iber, and Yardley (24) found the rate to be 3.4 $\mu l \cdot cm^{-2} \cdot min^{-1}$. In the human ileum the data of Banwell, Pierce, and Mitra (25) indicated a cholera toxin-induced output of some 2.7 $\mu l \cdot cm^{-2} \cdot min^{-1}$.

Composition of the spontaneous secretion, and passive permeability to Na^+ and Cl^-. The composition of the directly collected secretion is shown in Fig. 1. Serum values are given in the legend of the figure for comparison. The secretion is isosmolar with serum. The largest differences in individual electrolyte concentrations are in the relatively lower concentrations of chloride (78 meq/liter) and higher concentrations of bicarbonate (67 meq/liter) in the secretion. The sodium concentration was 148 meq/liter and the potassium 7.4 meq/

TABLE I

Composition of Previously Reported Ileal Fluids

Model	Na^+	K^+	Cl^-	HCO_3^-	References
Dog—mechanical stimulation	151	4.7	78	84	de Beer et al. (27)
Human—aspiration	140	6	60	60	Fordtran and Locklear (28)
Cholera					
Human	146	5.7	121	42	Banwell et al. (26)
Dog	143	9.6	87	66	Carpenter et al. (29)
Rabbit	153	4.9	56	68	Norris et al. (30)

liter. The alkaline pH of 7.9 of the secretion is consistent with its bicarbonate concentration, a P_{CO_2} in the secretion equal to the normal serum P_{CO_2} and a pK′ of 6.1.

The electrolyte concentrations of the appendiceal secretion are similar to those of ileal fluids collected during mechanical stimulation, and application of cholera toxin, in that $[HCO_3^-]$ is higher than plasma, and the $[Cl^-]$ lower, (except for that in Banwell's study [26]) (Table I).

For the experiments in which a test fluid was placed in the lumen, the apparent electrolyte concentrations in the secretion were calculated from the ratio of (*a*) the net secretion of the electrolyte to (*b*) the net secretion of water, a ratio denoted in the figures by (J_s/J_v), where the subscript "*s*" is the ion in question.

When isotonic nonsodium Ringer was the test fluid, the apparent composition of the secretion was practically identical with that of the directly collected secretion (Fig. 1). The results thus indicate that despite a concentration difference for diffusion for Na^+ and Cl^- into the lumen, net entry of these ions over and above that expected from the secretion alone was not definitely detected. The direction of the PD (see below) adds to the diffusive driving force for Na^+. Even ignoring this effect, one can calculate that if the permeability to Na^+ (P_{Na^+}) had been 10^{-5} cm·s^{-1}, the additional Na^+ secretory flux would have been detected.[2] Values for P_{Na^+} per unit serosal area are typically 10^{-4} cm·s^{-1} for small intestine or colon (31, 32). In the small intestine, correction for the increase in mucosal area due to the villi (31) would lower the values to some 10^{-5} cm·s^{-1} (33). The rabbit appendiceal epithelium thus appears to be less permeable to Na^+ compared with that of the small intestine or colon, though how much less is uncertain. Corresponding calculations yield the same conclusion for P_{Cl^-}.

When simulated appendiceal secretion was the test fluid, the only difference of significance in composition as compared with directly collected secretion was a moderately lower $[Cl^-]$.

Relationship between rate of secretion and its composition. The average concentrations of the individual electrolytes in the secretion, whether determined directly or calculated when test fluids were used, were essentially independent of the rate of secretion, as illustrated for Na^+ in Fig. 2, and for HCO_3^- in Fig. 3. The simplest explanations for this result are (*a*) either that the collected secretion consists mainly of a single fluid component of constant composition from a single cell type; or (*b*) that if several cell types contribute, the electrolyte pattern from these cell types is similar. The results also imply that if the secretion is formed in the flask-like glands of the epithelium or crypts of Lieberkühn, its composition is not greatly modified in passing into the collected luminal fluid over the usual

[2] The diffusive secretion of Na^+ was estimated from:

$$(\Delta Na^+)_{diff} = P_{Na}A([Na^+]_p - [Na^+]_L)\Delta t)$$

in which $(\Delta Na^+)_{diff}$ is the amount of Na^+ entering the lumen diffusively during an experimental period, A is the mucosal area of the appendix, $[Na^+]_p$ is the sodium concentration in the plasma, $[Na^+]_L$ is the mean sodium concentration in the lumen during the period, and Δt is the duration of the period. The increase in the apparent $[Na^+]$ in the spontaneous secretion, J_{Na}/J_V due to this diffusional component would be equal to $[\Delta Na^+]_{diff}/\Delta V$, where ΔV is the observed volume increase during the period. When typical values of $A = 40$ cm^2, $[Na^+]_p - [Na^+]_L = 100$ μeq/cm^3, $\Delta t = 3600$ s, and a relatively high value of $\Delta V = 5$ cm^3 were employed, the increment in J_{Na}/J_V for $P_{Na} = 10^{-6}$ cm·s^{-1} amounts to some 2.5 meq/liter; for $P_{Na} = 5 \times 10^{-6}$ cm·s^{-1}, $J_{Na}/J_V = 12.5$ meq/liter; for $P = 10^{-5}$ cm·s^{-1}, $J_{Na}/J_V = 25$ meq/liter. The most appropriate feature of the results to be examined is whether the mean J_{Na}/J_V for sodium diffusion would make isotonic nonsodium Ringer fluid (observed $J_{Na}/J_V = 150 \pm 10$ meq/liter) significantly greater than the mean for simulated appendiceal secretion (observed $J_{Na}/J_V = 137 \pm 10$ meq/liter). The observed difference of 13 meq/liter between these two observed means is not significant ($P \sim 0.25$). If the difference had been 25 meq/liter, the difference would have been significant at better than the 5% level ($P < 0.05$). On this basis a value for P_{Na} of 10^{-5} cm·s^{-1} would very probably have been detected.

Another approach was to measure the diffusive component for the above two test solutions as the extrapolated intercepts at $J_V = 0$ of a plot of J_{Na} vs. J_V. These intercepts were not significantly different from zero, but would have become so for the isotonic non-Na Ringer solution if $P_{Na}+$ had been 10^{-5} cm·s^{-1}.

range of rates of secretion. Further, if net secretion is a resultant of simultaneous secretion and absorption, either the absorbate has practically the same composition as the secretion or the rate of absorption is a constant fraction of the rate of secretion, neither of which arrangements appears to us very likely. Moreover, according to the findings, the effect of the absorbate on the rate and composition of the secretion would be similar whether the secretion was being drained from the organ or added to nonsodium Ringer fluid.

Permeability of the appendiceal epithelium to mannitol. After instillation of [^{14}C]mannitol into the lumen less than 1% was recovered in the extracellular fluid or urine. Using the same calculation as for Na^+ permeability the permeability to mannitol was no greater than 2.5×10^{-7} cm·s^{-1}. From the point of view of transepithelial transport, mannitol can at least tentatively be considered as an impermeant with a reflection coefficient of essentially 1.0.

Effects of anisosmotic luminal solutions on rate of spontaneous secretion and on osmotic permeability. The relationship between the rate of net or total fluid transfer $(J_v)^t$ and the mean osmolarity of the luminal fluid during the test period (1 h) is shown in Fig. 4. The most dilute test fluid contained the nonsodium salts of Krebs-Ringer bicarbonate (about 25 mosM or 1/12 isosmotic). The other more concentrated fluids were prepared by adding mannitol to this solution to raise the osmolarity to 150, 300, 600, 900, 1200, and 1500 mosM. The group average values for these nonsodium luminal solutions during the test period were close to 65, 190, 300, 700, 850, and 1030 mosM, respectively. Luminal hyperosmolarity increases, and luminal hypoosmolarity decreases the rate of net secretion as compared with the isosmolar fluid in accordance with the expected direction of the osmotic flow. In the case of

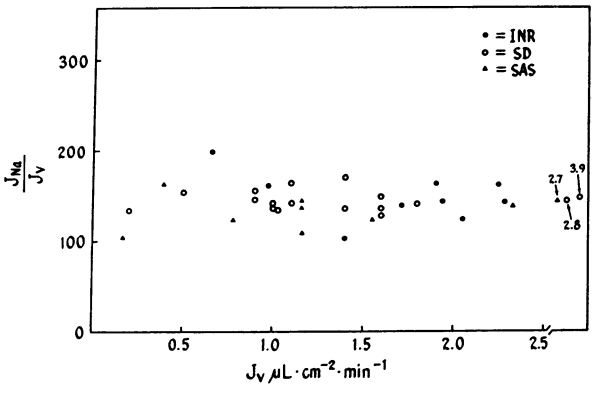

FIGURE 2 Graph of sodium concentrations as a function of the spontaneous secretion rate. INR, isotonic nonsodium Ringer's instilled, SD, simple drainage without instillate; SAS, simulated appendiceal secretion instilled.

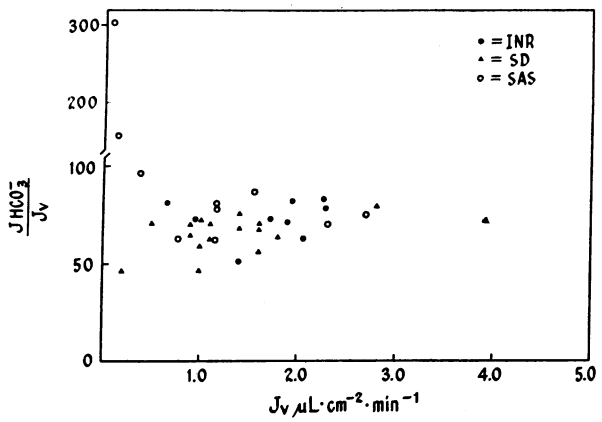

FIGURE 3 Graph of the bicarbonate concentrations plotted against the spontaneous secretion rates. INR, isotonic nonsodium Ringer's instilled; SD, simple drainage without instillate; SAS, simulated appendiceal secretion instilled.

the 25 mosM test solution, net secretion was reversed to net absorption.

When an anisotonic solution is placed in the lumen, we are provided with an example of a situation in which driving forces for two transepithelial fluid-transport processes are simultaneously present: spontaneous isotonic secretion, probably active, and passive osmosis. With hypertonic luminal fluids the driving force for osmosis is in the same direction as for spontaneous secretion; with hypotonic luminal fluids the driving forces are opposite in direction. Recent advances in our understanding of the mechanisms and routes of fluid

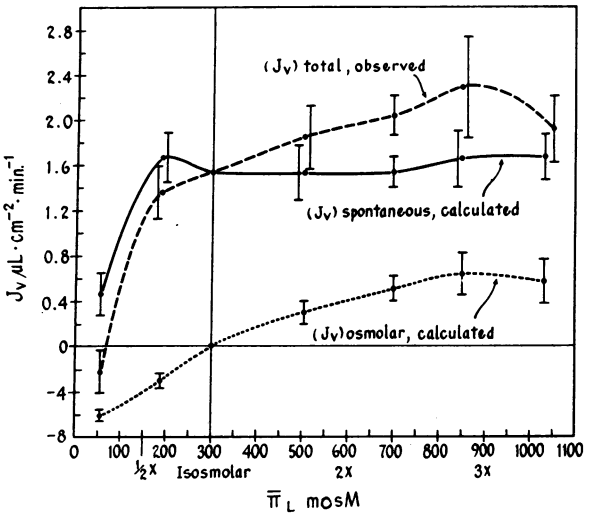

FIGURE 4 Graph of osmotic experiments. The volume output is plotted against the average luminal osmolarity ($\bar{\pi}$). Each point represents approximately 10 studies except at $\bar{\pi}$ = 190 where the results of two experiments were omitted because of obvious technical errors.

movement across epithelia (34) enhance interest in the kinds of interaction taking place between volume transfer processes of different types.

For the purposes of analysis of the present experimental results, $(J_v)^t$ will be considered to be the sum of spontaneous secretion $(J_v)^{sp}$ and osmotic volume flow $(J_v)^{osm}$:

$$(J_v)^t = (J_v)^{sp} + (J_v)^{osm} \quad (1)$$

In view of the implications of the independence of the composition of the secretion from rate of secretion, an active absorptive flow has been omitted, though, to be sure, it cannot be ruled out.

In order to quantitate the separate contributions of spontaneous secretion and osmotic flow to the total flow, assumptions will be necessary.

As noted above, the calculated composition of the secretion (J_s/J_v) when isosmotic test fluids were employed was similar to the directly collected secretion; and thus for these luminal fluids Na^+ may be taken to enter the lumen practically entirely with the spontaneous secretion at a concentration of some 150 meq/liter. It will be assumed that the same holds for fluid transfer when the anisosmotic test solutions were used. Accordingly, $(J_v)^{sp}$ was taken as directly proportional to the rate of total Na^+ secretion, and was calculated by dividing J_{Na} by the $[Na^+]$ in the spontaneous secretion (150 meq/liter, or 0.15 $\mu eq \cdot \mu l^{-1}$); i.e.,

$$(J_v)^{sp}(\mu l \cdot cm^{-2} \cdot min^{-1}) = \frac{J_{Na}(\mu eq \cdot cm^{-2} \cdot min^{-1})}{0.150(\mu eq \cdot \mu l^{-1})}. \quad (2)$$

The rate of osmotic flow $(J_v)^{osm}$ is then the difference between $(J_v)^t$ and $(J_v)^{sp}$ thus estimated.

The above assumptions on which equation 2 is based will be discussed later. This approach yields the relationship shown in Fig. 4 between $(J_v)^{osm}$ and the average luminal osmolarity, $\bar{\pi}_L$. The rate of spontaneous secretion calculated from J_{Na} by equation 2 remains essentially constant between $\bar{\pi}_L$ = 190 mosM and $\bar{\pi}_L$ = 1030 mosM (i.e., 0.63 × to 3.4 × isosmotic) at a rate of 1.5–1.7 $\mu l \cdot cm^{-2} \cdot min^{-1}$. Thus deviations of osmolarity from about 100 mosM hypo-osmolar to more than 600 mosM hyperosmolar have surprisingly little effect on the rate of spontaneous secretion by the appendix.

However, in the case of the most hypo-osmotic solution ($\bar{\pi}_L$ = 65 mosM) J_v^{sp} fell by 70% to 0.47 $\mu l \cdot cm^{-2} \cdot min^{-1}$. This reduction in the rate of spontaneous secretion by extreme hypo-osmolarity could be due to a relatively direct effect of this environmental factor on the secretory process (its driving force, conductance, or both), or some interaction, perhaps in the epithelium, between osmotic flow and spontaneous secretion. It is noteworthy that an effect of hypo-osmolarity on the rate of spontaneous secretion does not become manifest until the luminal solution is sufficiently hypo-osmotic to cause actual reversal of net flow from secretion to absorption, i.e., to dominate the direction of net flow.

To calculate the hydraulic or osmotic permeability (L_p) of a "membrane" from osmotically induced volume flow, it is necessary to have values for the Staverman reflection coefficients (σ) of the solutes. If σ of the quantitatively important ions, chiefly Na^+ and Cl^-, and of mannitol is 1.0, L_p can be estimated from:

$$L_p = \frac{(J_v)^{osm}}{\bar{\pi}_L - \bar{\pi}_{iso}} = \frac{(J_v)^t - (J_v)^{sp}}{\bar{\pi}_L - \bar{\pi}_{iso}} = \frac{(J_v)^t - \frac{J_{Na}}{0.150}}{\bar{\pi}_L - 300} \quad (3)$$

in which $\bar{\pi}_{iso}$ is the osmotic pressure of the plasma or interstitial fluid.

The values of $(J_v)^{osm}$ as a function of $\bar{\pi}_L$ are shown in Fig. 4, and the values of L_p as a function of $\bar{\pi}_L$ are shown in Fig. 5. When the luminal fluids are made progressively more hypertonic (hypertonic = hyperosmotic since all solutes are assumed to have σ — 1.0), L_p declines relatively uniformly but not significantly ($P > 0.1$) from a value of 1.5 × 10^{-3} $\mu l \cdot cm^{-2} \cdot min^{-1} \cdot mosM^{-1}$ at $\bar{\pi}_L$ = 1.67 × isotonic to 0.74 × 10^{-3} $\mu l \cdot cm^{-2} \cdot min^{-1} \cdot mosM^{-1}$ at $\bar{\pi}_L$ = 3.4 × isotonic.

The values of L_p at $\bar{\pi}_L$ = 65 mosM and $\bar{\pi}_L$ = 190 mosM are not significantly different from each other. However, in the case of these two hypotonic fluids, L_p was about 80% greater than for even the least hypertonic fluid ($\bar{\pi}_L$ = 1.67 × isotonic), a difference which was highly significant. This kind of asymmetry in the magnitude of the osmotic permeability depending on the direction of the osmotic driving force has frequently been found (35–38), most recently for the bullfrog jejunum (39). According to Bentzel, Parsa, and Hare

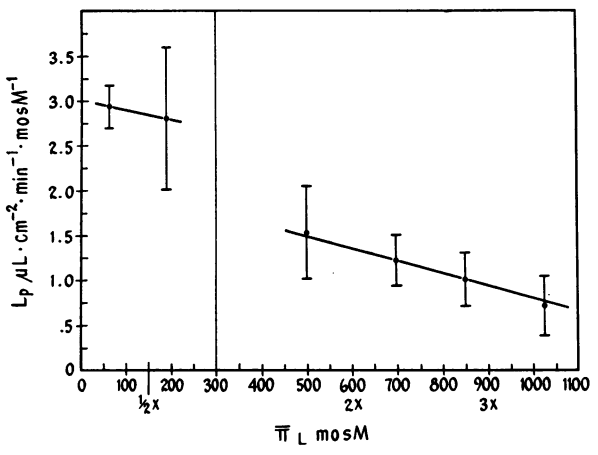

FIGURE 5 Osmotic permeability plotted as a function of the average luminal osmotic pressure.

(38) and Loeschke, Bentzel, and Csáky (39), the pathway of osmotic absorption through the epithelial cell layer is different from that for osmotic secretion; and they suggest that the asymmetry is due to different osmotic permeabilities in the two pathways. Diamond has concluded that in the case of the rabbit gallbladder in vitro there is a decrease in water permeability with increasing osmolarity of the bathing solutions (37).

According to this analysis, the change from net secretion at $\bar{\pi}_L$ = 190 mosM to net absorption at $\bar{\pi}_L$ = 65 mosM is due more to a decrease in the rate of spontaneous secretion than an increase in osmotic absorption.

It will be recalled that the rate of spontaneous secretion as calculated from equation 2 was based on two assumptions, namely, that the only way in which Na^+ was added to the luminal fluid was in spontaneous secretion and that in essence the secretion was isotonic, containing Na^+ at a concentration of 150 meq/liter. The assumption that the only source of secreted Na^+ is the spontaneous secretion maximizes the calculated value for $(J_v)^{sp}$; for if Na^+ had in fact entered the lumen by some other way, presumably diffusion, the value used for the numerator in equation 2 overestimated the amount of Na^+ in the spontaneous secretion. The actual reduction in $(J_v)^{sp}$ by extreme hypotonicity ($\bar{\pi}_L$ = 65 mosM) would then have been even greater than indicated.

On the other hand, the assumption that the $[Na^+]$ in the spontaneous secretion is 150 meq/liter for all test fluids would minimize the calculated value for $(J_v)^{sp}$, at least if the spontaneous secretion is not hypertonic with respect to plasma. However, it can be calculated that the denominator in equation 2 (the $[Na^+]$ in the spontaneous secretion), would have to fall to about 50 meq/liter in the case of the most hypotonic fluid in order for $(J_v)^{sp}$ not to have been reduced by the hypotonicity.

Instead of employing equation 2 to calculate $(J_v)^{sp}$, a second more arbitrary approach to the analysis of the effects of the anisotonic solutions on fluid transfer can be made by assuming that the rate of spontaneous secretion in equation 1 remains constant, and hence equal to $(J_v)^t$ when the isotonic luminal fluids were used ($[J_v]^{osm}$ = zero). However, by the first approach used above, $(J_v)^{sp}$ was in fact found to remain practically constant for all but the most dilute fluid. Hence the conclusions from this second approach would be similar to those already described except with respect to the mechanism of the reversal of direction of net fluid transfer from secretion to absorption in the most hypotonic test solution. The reversal would then be attributed solely to a large increase in osmotic fluid absorption conditioned by a severalfold increase in L_p. In view of the reasons given above for believing that the calculated estimate of $(J_v)^{sp}$ by equation 2 is a reasonable one and probably a maximal one for this fluid, the first approach appears to us by far the more reliable.

The calculation of L_p for both approaches involves the assumption that reflection coefficients are 1.0. For Na^+ this assumption is supported by the facts that (*a*) the reflection coefficient for Na^+ in the human ileum has been found to be approximately 1.0 (32, 33) and (*b*) the appendix has a very low apparent passive permeability to Na^+ and Cl^-. It may be noted that this assumption is more important for the hypotonic fluids, in which larger portions of the luminal osmotic pressure are made up of the nonmannitol solutes. σ for mannitol for intestinal mucosa would be expected to be at least as high as that for Na^+.[3] Consistent with this expectation is the low diffusive permeability of this solute, and the fact that the osmotic fluid transfer appears to be in the direction expected on the assumption that mannitol and NaCl have similar reflection coefficients. It seems very unlikely that the above calculations of L_p are seriously in error due to the assigned value of the reflection coefficients.

It is also possible that the results are due in part to changes in the rate and composition of an active absorptive stream; however, for the present we have no reason to assign an important role to such a process except that the epithelium is intestinal.

Transmucosal PD; active transport of HCO_3^-. The time course of the values for the transmucosal PD is shown in Fig. 6 for a 4 h period. During the 1st h, it averaged 11.9±1.1 mV (mucosa negative). It was relatively stable, decreasing only by 13% in the succeeding 3 h. This is a value well within the range found for other intestinal segments (41–49). The magnitude of the PD was in the indicated range even

[3] A value of 1.0 for the reflection coefficient of mannitol is also supported by the following considerations:

The expression for σ for a homogenous membrane is given by Kedem and Katchalsky (40) as:

$$\sigma = 1 - \frac{\omega \bar{V}_s}{L_p} - \frac{K f_{sw}}{\phi w (f_{sw} + f_{sm})}$$

where ω is the diffusive permeability of the solute at constant volume, $\bar{V}_s$ is the partial molar volume of the solute, K is the distribution coefficient of the solute between membrane and solution, ϕ_w is the volume fraction of water in the membrane, f_{sw} is the frictional coefficient between water and solute, and f_{sm} is the frictional coefficient between membrane and solute.

From the values for L_p and the mannitol permeability, $\omega \bar{V}_s / L_p$ is of the order of 10^{-13}, and would be negligible even if many orders of magnitude too large. The remaining term to be subtracted from 1 is also likely to be small since f_{sm} is probably very large relative to f_{sw}.

If permeation is through water-filled pores, the last term on the right is equal to A_s/A_w (40), the ratio of the diffusive permeability of the solute to that of water in the membrane. The ratio is of the order of only 0.01 for mannitol.

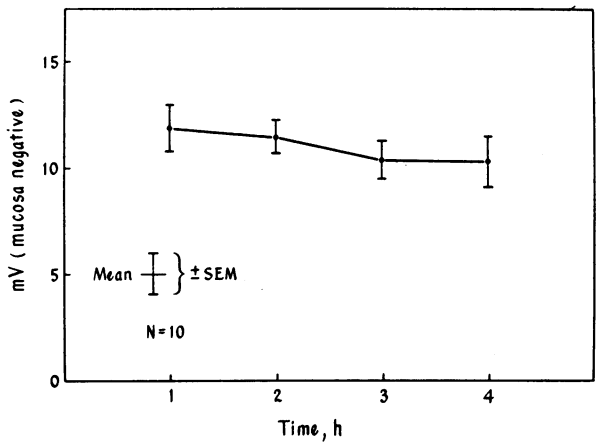

FIGURE 6 Graph of the mucosal PD of the rabbit vermiform appendix.

when the luminal solutions were simulated appendiceal secretion, isotonic nonsodium Ringer, nonsodium Ringer, or 2–5 × isotonic nonsodium Ringer.

The figure for the PD together with the values for concentrations in serum and secretion may be used to assess whether a given ion has moved grossly against its electrochemical potential difference. Of the quantitatively major ions (Na^+, Cl^-, HCO_3^-), it is only HCO_3^- that does so. This ion moves into the lumen against both the concentration and electrical driving forces. Moreover, since the $[HCO_3^-]$ in the secretion is greater than in plasma (or interstitial fluid), solvent drag cannot account for the higher $[HCO_3^-]$ in the secretion, a result also characteristic of lower intestinal secretions in general.

It is recognized that other processes such as active secretion of OH^- or absorption of H^+ could yield the same results. Also even though the net transport of Na^+, K^+, or Cl^- may not be uphill, the electrochemical activity profile across the epithelial cell may well be such as to require uphill transport for these ions at one or another of the cell membranes traversed by them.

DISCUSSION

Table II lists the main results. The observations confirm the previous reports concerning the rate and composition of this spontaneous secretion. Evidence is, in addition, presented that the average electrolyte concentrations of the secretion are independent of the rate of secretion, a relationship which suggests that the secretion as collected consists essentially of a single fluid component of constant composition. The rate of spontaneous secretion, calculated on the basis of reasonable assumptions, remains remarkably unaffected over a range of luminal osmolarity from 0.63 × to 3.4 × isosmolar. It is only with extreme luminal hypo-osmolarity (0.22 × isotonic) that this rate is significantly reduced, to about one-third the normal value; and net fluid transfer becomes absorptive.

Certain aspects of the passive permeability properties of the appendiceal mucosa have been investigated. The hydraulic or osmotic permeability (L_p) is in the lower portion of the range reported for other intestinal epithelia (31–33) and the measurements offer another example of higher values for L_p when the direction of the presumed osmotic fluid transfer is absorptive than when it is secretory. Maximum passive permeability to Na^+ and Cl^- per unit serosal area was estimated to be somewhat smaller than the corresponding values for other intestinal epithelia; and the permeability to mannitol is likewise very low.

Bicarbonate was secreted from a lower to a higher concentration against the electrical PD, which was found to be in the range previously reported for gut. Thus, this ion appears to be actively secreted by the conventional criterion of net transport from low to

TABLE II

Some Transport Characteristics of Rabbit Appendiceal Epithelium

Smooth mucosal area (serosal area)	4.43 ± 0.07 (SEM) $cm^2 \cdot g^{-1}$
Area of glands/serosal area*	3
Spontaneous rate of secretion	1.4 ± 0.24 $\mu l \cdot cm^{-2} \cdot min^{-1}$
Composition	Like lower ileal fluid
Composition of secretion as a function of rate	Independent
Potential difference (PD)	11.9 ± 1.1 mV (mucosa negative)
Hydraulic or osmotic permeability, (L_p)	
Hyperosmolar luminal fluid	1.5×10^{-3} $\mu l \cdot cm^{-2} \cdot min^{-1} \cdot mosM^{-1}$
Hypo-osmolar luminal fluid	2.8×10^{-3} $\mu l \cdot cm^{-2} \cdot min^{-1} \cdot mosM^{-1}$
Diffusive permeability to Na^+ (P_{Na}) or Cl^- (P_{Cl})	$<10^{-5} \cdot cm \cdot s^{-1}$
Permeability to mannitol ($P_{mannitol}$)	2.5×10^{-7} $cm \cdot s^{-1}$
Secretory pressure (8, 13)	80–100 cm H_2O

* Formalin-fixed sections.

high electrochemical activity, not explainable by solvent drag. From the relationship between its pH and bicarbonate content there is no reason to assume a significant P_{CO_2} gradient between mucosal blood and secretion.

If the secretion is passive, the most obvious possibility is that it is a filtrate driven by mucosal tissue fluid pressure. However, this mechanism is made extremely unlikely by the low hydraulic permeability (L_p) measured osmotically during secretion. As in the case of the remainder of the intestine, the value of L_p is such that tissue fluid pressure can be ignored as a driving force for fluid transfer. (When tissue fluid pressure increases L_p, secretory filtration can occur [50].) Another argument against filtration in the appendix is the high secretion pressure, some 80–100 cm H_2O (8, 13). If tissue fluid pressure during secretion is in the usual range, the pressure difference between lumen and tissue fluid would produce absorption rather than secretion. The participation of secretory pressure is central to the view of Wangensteen of the pathogenesis of appendicitis (51). Appendiceal secretion is thus most probably an active process in the sense that its driving force is created within the epithelium at the expense of metabolic energy.

With the exception of the apparently low permeability to net diffusive transport of Na^+ or Cl^-, the above features of appendiceal secretion are similar to those found elsewhere in the intestinal tract, especially the ileum. Moreover, the composition of the secretion is practically identical with that of fluid collected in other secretory states of the ileum. It is interesting that the electrolyte composition is also practically identical to that reported for fluid removed by micropuncture from interlobular ducts of the rabbit pancreas (52) probably representing the primary secretion of the gland.

These considerations, together with its high rate of spontaneous secretion, availability, and ease of study, favor the rabbit appendix as a useful model for the elucidation of intestinal secretory processes. The low permeability to Na^+ and Cl^-, and probably other solutes, should enhance this usefulness by reducing "contamination" of the spontaneous secretion by other transport processes. It may be that the appendiceal epithelium approaches an example of secreting intestinal epithelium without significant absorptive functions.

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