The Influence of Structural Abnormality on Ion Transport in Rabbit Ileum[1] (35103)

QAIS AL-AWQATI,[2] RAFAEL GARCIA-BUNUEL, MICHAEL FIELD, AND WILLIAM B. GREENOUGH III
(Introduced by Philip S. Norman)

Departments of Medicine and Pathology, The Johns Hopkins University School of Medicine, and Hospital and Baltimore City Hospitals, Baltimore, Maryland 21205; and Department of Medicine, Harvard Medical School, Beth Israel Hospital, Boston, Massachusetts 02115

Diarrhea may result when the normal processes of intestinal absorption of salt and water are deranged. In clinical syndromes characterized by mucosal pathology there is generally mild to moderate fluid loss from the bowel (1). In certain acute diarrheal illnesses such as cholera there is massive intestinal fluid loss without any detectable epithelial damage (2, 3). The short-circuited rabbit ileum, an *in vitro* model which has been used extensively to study the normal transport of water and electrolytes (4), was recently shown to respond to the exotoxin of *V. cholerae* with reduction of the normal net sodium absorption and the production of chloride secretion (5). During the course of our studies on cholera we found that some of our control rabbits had diarrhea. When ion transport was studied in such rabbits, no net absorption of either sodium or chloride was found. Furthermore, histological studies in these animals showed various degrees of abnormality of the intestine. This report compares electrolyte movement in the normal and abnormal short-circuited ileal mucosa of the rabbit.

Materials and Methods. White New Zealand rabbits weighing approximately 2 kg were anesthetized with pentobarbital and the terminal 15 cm of ileum was excised, opened along its mesenteric border, and cleaned of its contents by rinsing it with buffer. The excised ileum was then placed in buffer at room temperature and vigorously oxygenated. Small pieces were cut and placed mucosa downwards on a Lucite half-chamber. With the aid of a magnifying glass and fine pointed forceps, the serosa and the two layers of muscularis were stripped away leaving the mucosa intact which was then clamped as a flat sheet between two Lucite half-chambers. The exposed area of the mucosa measured 1.13 cm^2. Both half-chambers were connected to water jacketed reservoirs through which water at 37° was pumped from a constant temperature bath. The reservoirs contained 10 ml of buffer which was continuously circulated and aerated by a gas lift device with 95% O_2–5% CO_2. The tips of salt agar bridges were placed 1 mm from both sides of the membrane and connected to a high impedance potentiometer. For short-circuiting, external current from a battery and variable resistance was applied using immersed Ag–$AgCl_2$ electrodes connected by salt–agar bridges to the extreme ends of the chamber. The composition of the buffer used was (mmoles/liter) Na, 141; K, 10; Ca, 1.25; Mg, 1.1; Cl, 127; HCO_3, 25; HPO_4, 1.6; H_2PO_4, 0.3. Glucose 15 m*M* was added to the serosal solution and an equimolar amount of mannitol was added to the mucosal solution.

In the cholera experiments, the rabbit anterior abdominal wall was anesthetized with Xylocaine and a 10–15-cm loop of distal ileum was constructed. The loop was evacuated

[1] This work was supported by The United States-Japan Cooperative Medical Science Program administered by The National Institute of Allergy and Infectious Diseases of the NIH, Department of Health, Education and Welfare Grant 5R22 AI 08209-03.

[2] Present address: Renal Unit, Department of Medicine, Massachusetts General Hospital, Boston, Massachusetts 02114.

by washing with saline and 3 ml of an isotonic solution containing crude *V. cholerae* exotoxin (NIH Lot No. 001, 1 g/30 ml) was instilled. The loop was then returned to the abdominal cavity. Three to 5 hr later the rabbit was anesthetized, the loop was excised and stripped mucosal segments were mounted in the chamber.

^{22}Na and ^{36}Cl were added to the mucosal or serosal solution 30–40 min after mounting, and the tissue was short-circuited by application of external current taking into consideration the resistance of the film of fluid between the tips of the potential-probing bridges and the tissue. Twenty min after the addition of the isotopes, the first 1-ml samples of fluid were taken from both reservoirs. Thirty to 50 min later additional samples were taken. The short-circuit current (the current necessary to nullify the spontaneous potential difference) remained stable during this period and was read every 10 min. At the conclusion of the experiment 15 m*M* glucose was added to both the mucosal and the serosal solutions and the change in short-circuit current was recorded. Theophylline (10 m*M*) was then added and the additional change in short-circuit current was recorded. Samples were counted for ^{22}Na in an automatic well counter (Picker Model Autowell II). Bray's solution (6) 15 ml was added to the ^{36}Cl samples which were then counted in a liquid scintillation counter (Packard Tricarb 3375). Correction was made for the beta emissions of ^{22}Na in each sample from a standard curve constructed by measuring the activity of varying amounts of this isotope in both the gamma well and liquid scintillation spectrometers.

The unidirectional fluxes were calculated according to the following formula:

$$J_i = \frac{(C_2 - C_1)\, V}{\Delta T\, p^*\, A},$$

where J_i = flux (Eq cm^{-2} hr^{-1}); C = cpm of the samples taken at the beginning (C_1) and end (C_2); V = volume of reservoir (ml); ΔT = time (hr); p^* = specific activity (cpm $mole^{-1}$); A = membrane area (cm^2); and

$$J_{net} = J_{ms} - J_{sm},$$

where J_{net} is the net flux, J_{ms} the unidirectional flux from mucosal-to-serosal reservoirs, and J_{sm} from serosal-to-mucosal. The unidentified ion flux was calculated as

$$SCC - J_{net^{Na}} + J_{net^{Cl}}.$$

Segments of ileum from the same loop were fixed in 5% formaldehyde and the paraffin sections were then stained with hematoxylin and eosin. All pathologic examinations were made by an independent observer without prior knowledge of the results of the ion fluxes. The data are expressed as mean ± 1 standard error.

Results. In histologically normal tissues there was significant ($p < .05$) net mucosal-to-serosal (m→s) movement of both sodium and chloride in the absence of electrical or chemical gradients. No net transport of either sodium or chloride was seen in tissues with abnormal histology (Table I). In addition there was a significant ($p < 0.05$) decrease in both m→s and s→m sodium fluxes. There was no significant change in chloride movement.

The short-circuit current (SCC) was significantly lower in damaged as compared to normal tissues. The net fluxes of sodium and chloride did not entirely explain the short-circuit current in either instance. The unidentified ion flux was 2.3 μEq/cm^2/hr in normal tissue while in abnormal tissue it was 1.6 μEq/cm^2/hr. Electrical resistance of the normal rabbit ileum was 39 ± 2 ohms/cm^2 (range 32–47) while pathologic tissues had a mean resistance of 55 ± 4 ohms/cm^2 ($p < .001$) (range 28–86). All normal tissues had resistances below 50 while two-thirds of the pathological tissues had resistances of 50 or above.

Addition to the mucosal solution of glucose produced a rise in short-circuit current in normal tissues. This rise is well correlated with the concomitant increase in net (m→s) sodium flux and an excellent measure of the absorption of glucose itself (7, 13). Tissues with abnormal histology exhibited no response to glucose in six instances and a smaller response in the remaining four tissues. The mean rise in short-circuit current after mucosal application of glucose was 45 μA ±

TABLE I. Sodium and Chloride Fluxes, Resistance, and Current in Rabbit Ileum.

	Sodium			Chloride				
	ms	sm	net	ms	sm	net	R	SCC
Normal control ($n = 19$)	14.3 ± 0.6	12.6 ± 0.7	+1.6 ± 0.3[c]	8.6 ± 0.7	7.8 ± 0.5	+1.3 ± 0.6[c]	38 ± 1	3.0 ± 0.2[c]
Pathologic ($n = 12$)	10.2 ± 1.0[b]	10.6 ± 0.7[b]	−0.3 ± 0.9[b]	8.0 ± 0.9	8.1 ± 0.5	−0.1 ± 0.9	57 ± 6[b]	2.3 ± 0.2[b,c]
Cholera ($n = 16$)	9.4 ± 0.6[b]	9.8 ± 0.7[b]	−0.4 ± 0.7[b,c]	5.8 ± 0.4[b]	8.3 ± 0.5	−2.6 ± 0.5[b,c]	52 ± 2[b]	3.6 ± 0.2[b,c]

[a] Unidirectional and net sodium and chloride fluxes (μEq/cm² hr). m→s = mucosal-to-serosal; s→m = serosal-to-mucosal; net = m→s—s→m; R = resistance (ohms/cm²); SCC = short-circuit current (μEq/cm²/hr). All values are mean ± 1 SE.

[b] Significantly different from normal control value ($p < 0.05$).

[c] Net ion flux significantly different from zero ($p < 0.05$).

8 in normals and 5 μA ± 2 in abnormals ($p < .001$) (Fig. 2).

Both normal and abnormal mucosa responded equally to the serosal addition of theophylline (a substance that leads to an increase in the intracellular level of adenosine 3′,5′-monophosphate (cyclic AMP) by inhibiting the enzyme phosphodiesterase). The rise in short-circuit current observed in normal tissue is derived from the difference between an inhibition of the normal sodium and chloride absorption and the net secretion of chloride ion which is evoked by this agent (15). In damaged tissue, since there is no net absorption of sodium or chloride, the change in SCC reflected only the degree to which net chloride secretion was stimulated. The rise in SCC in normals was 39 μA ± 4 while in damaged tissue it was 45 μA ± 7 ($p > 0.1$)

Similar studies to those performed above were done 3 hr after instilling 100 mg of a lyophilized crude exotoxin of *V. cholerae* into the terminal ileum of a rabbit. It is evident from Table I and Fig. 1 that in cholera net sodium absorption is inhibited and net chloride secretion is evoked. There was a rise in short-circuit current and in resistance. The glucose mediated rise in SCC was not affected by cholera (Fig. 2): however, there was

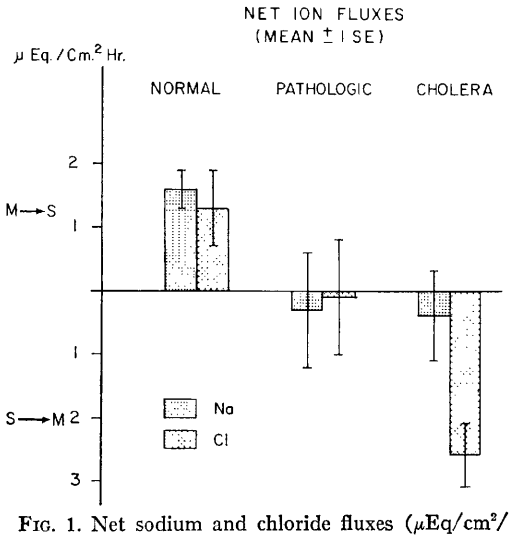

FIG. 1. Net sodium and chloride fluxes (μEq/cm²/hr) is isolated rabbit ileal mucosa, with normal and pathologic histology compared to mucosa exposed to exotoxin of *V. cholerae*. M and S refer to mucosa and serosa, respectively (mean ± 1 SE).

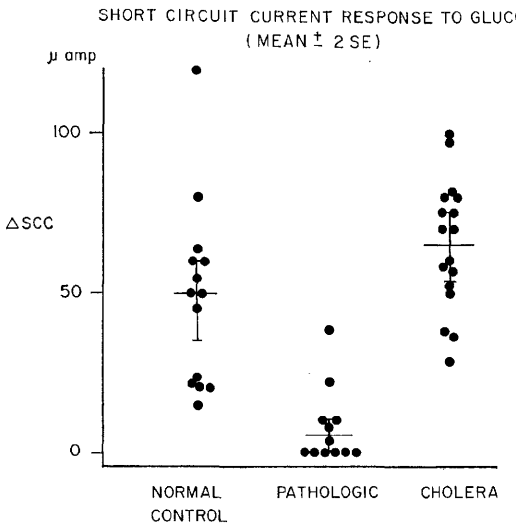

FIG. 2. Change in short-circuit current (μA) following the addition of 15 mM glucose to the mucosal solutions in stripped rabbit ileal mucosa with normal and pathologic histology as compared to mucosal exposed to the exotoxin of *V. cholerae* (mean ± 2 SE).

virtually no increase in the SCC after theophylline (Fig. 3).

The nature of pathologic alterations. Normal intestine was characterized by uniform, slender, tapering intestinal villi, clothed by intact epithelium and showing only small numbers of lymphoid cells in the lamina propria (Fig. 4a). Deviations from this picture covered a wide spectrum of morphological alterations and probably several etiologies. One of the diseases recognized in the abnormal tissue was coccidiosis, a common parasitic infection of the intestinal tract of rabbits (10). In our series, coccidia (Eimeria) in various stages of its life cycle, were demonstrated in the intestinal epithelium in 6 out of 12 animals with abnormalities of the ileal mucosa (Fig. 5).

The structural abnormalities ranged from mild to severe. The former were characterized by blunting of intestinal villi, with or without intervillous adhesions, and a moderate increase in lymphoid cells in the lamina propria with occasional small foci of eosinophilic leukocytes, usually in the depths of the crypts (Fig. 4b, c).

The more severely affected tissues showed an ulcerative-granulomatous enteritis. This was characterized by focal ulceration and regenerative hyperplasia of the intestinal epithelium with marked disorganization of the villous pattern of the intestinal mucosa, and intense inflammatory cellular reaction in the lamina propria, composed of lymphocytes, plasma cells, histocytes, and eosinophilic leukocytes (Fig. 4d). In many of the abnormal tissues there was greater disruption of villi as compared to the crypts.

Discussion. The rabbit ileum has been used extensively in the recent past for investigation of electrolyte transport. The small intestine of mammals, especially rodents, harbors various bacteria (8). Many of these bacteria, *e.g., E. coli,* have been associated with diarrhea similar to cholera (9), and functionally the exotoxins of *E. coli* and *V. cholerae* are similar (10). Furthermore, inflammatory and granulomatous disease of the small intestine is extremely frequent (11) and very difficult to eradicate from rabbit colonies (12). Hence, data from experiments performed on rabbits must be interpreted with caution unless suitable precautions have been taken to insure that they are at least free of structural damage. Because of the

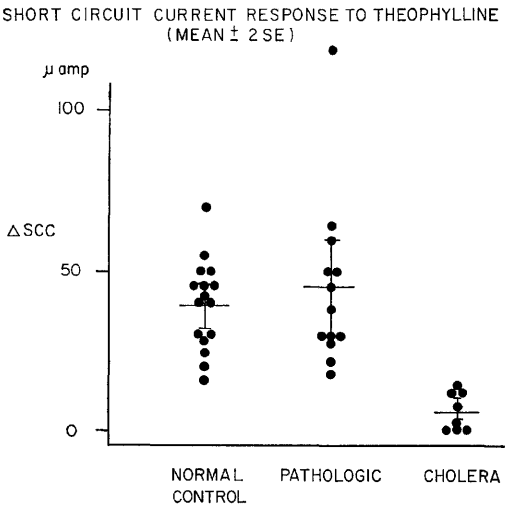

FIG. 3. Change in short-circuit current (μA) following the addition of 10 mM theophylline to the serosal solution in stripped rabbit ileal mucosa with normal and pathologic histology as compared to mucosal exposed to the exotoxin of *V. cholerae* (mean ± 2 SE).

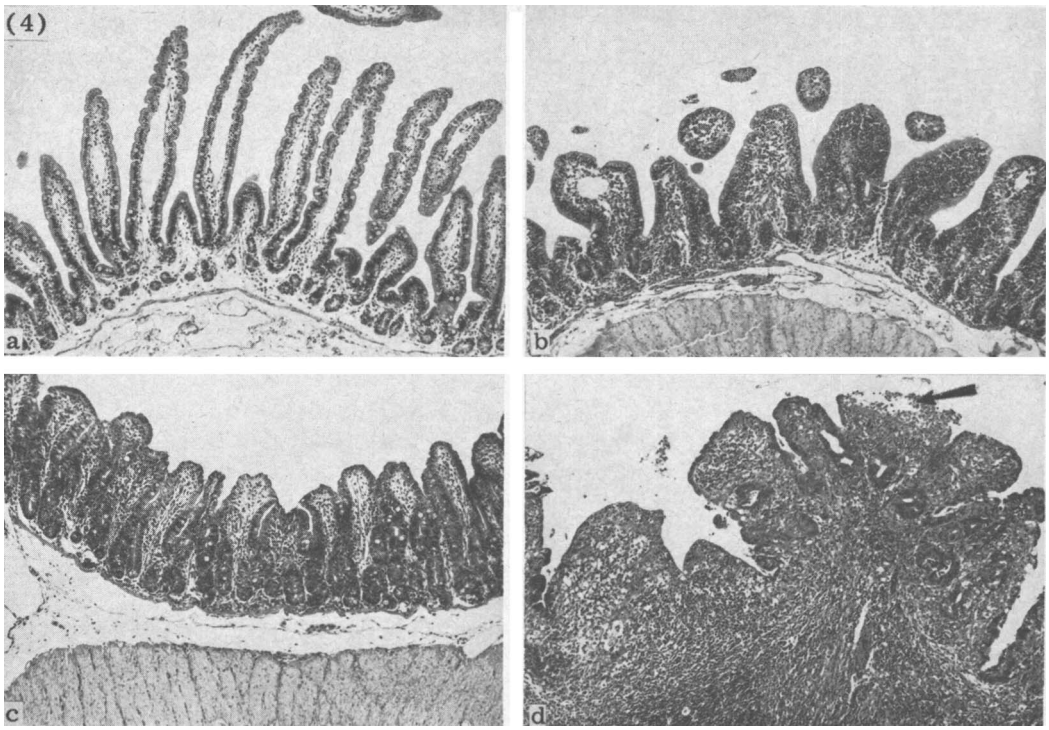

FIG. 4. Rabbit ileum, H & E stain, 64×:
(a) normal; long, slender, tapering, uniform villi.
(b) atrophic enteritis; short, blunt villi; regular thickening of epithelial lining; cellular infiltration of lamina propria.
(c) atrophic enteritis; short, clubbed villi with intervillous adhesions; epithelial hyperplasia particularly involving the crypts; cellular infiltration of the lamina propria.
(d) ulcerative-granulomatous enteritis; complete disorganization of villous pattern; focal ulceration (arrow) and hyperplasia of epithelium; markedly thickened mucosa and submucosa, heavily infiltrated by inflammatory cells.

high prevalence of intestinal disease in rabbit colonies it is imperative that judgment be made as to the presence or absence of pathology when carrying out experiments on the transport of ions, water, and probably many other substances. Our observations suggest that a tissue resistance of more than 50 ohms/cm^2 is always associated with structural damage. Another sensitive test of normality seems to be the response of the short-circuit current to glucose. All abnormal tissues in the present series exhibited a response of less than 20 μA/cm^2 to mucosal glucose.

The results show that sodium is absorbed in the absence of electrochemical gradients in the histologically normal ileal mucosa. This net movement is absent both in cholera and in tissues with histological abnormalities. Theoretically, there can be four ways in which this can come about: (a) the sodium "pump" is inhibited, (b) the rate of delivery of ions to the pump can be so reduced as to result in no appreciable movement, (c) the passive permeability of the membrane can increase to such a level as to make it impossible to detect statistically the existence of a small net flux, and (d) the s→m flux can be stimulated to a magnitude equal to the m→s so that the net effect will be no sodium movement. The latter two possibilities can be dismissed immediately for the serosa-to-mucosa flux remains unchanged (Table I). Hence, (d) does not apply and since the s→m flux is a measure of the passive permeability of the tissue (4), then the fact that they have decreased (Table I) rather than increased sug-

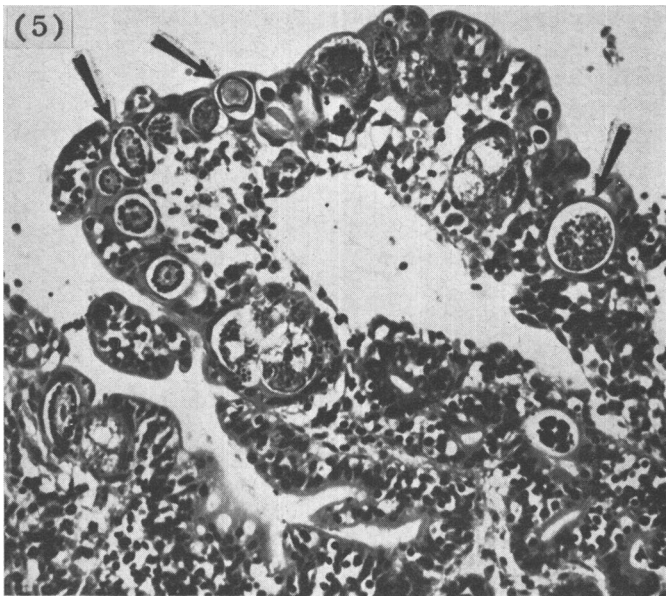

FIG. 5. Rabbit ileum; coccidia infection; numerous cocysts, schizogonts, and sporocytes of Eimeria (arrows), within the cytoplasm of intestinal epithelial cells; Cellular infiltration of lamina propria; H & E stain; 245×.

gest that (c) is untenable. In order to dissect out the other two possibilities, use was made of Crane's hypothesis (13) of glucose–sodium interaction. He showed that there is a one-to-one coupling of sodium and glucose at the brush border, so that addition of glucose to the mucosal surface of the intestine results, in the presence of sodium, in movement of glucose and sodium into the intracellular compartment. If the "sodium pump" is intact, then sodium absorption will be stimulated and the short-circuit current will increase. Figure 2 shows that in cholera there is an appreciable rise in short-circuit current while in pathologic tissues these is none. Hence we can conclude that in both cholera and ileal inflammatory disease net sodium absorption is inhibited, but that in cholera the "sodium pump" and glucose:sodium interaction are intact. In inflammatory disease the situation is less clear, inhibition of either the sodium pump or the glucose absorptive mechanism could explain the results. In idiopathic steatorrhea in man, glucose absorption is diminished (14).

There is significant net absorption of chloride in the histologically normal ileum while in cholera there is net secretion of chloride (Fig. 1). In the pathological ileum there was no net movement of chloride (Table I). Field and McColl (15) have shown that theophylline increases the short-circuit current in this preparation and that this increase can be partially explained by chloride secretion. There was no difference between the normal and pathological intestine in this response suggesting that the structurally abnormal ileum retains its secretory response, although its "absorptive" capacity has been lost. In cholera, however, there was marked decrease of the theophylline response. This can be explained by the observation (5) that both cholera and theophylline produce the same changes, hence in a tissue almost maximally stimulated by cholera, only a small or no additional effect will be seen with theophylline.

Diarrhea can be brought about by inhibition of intestinal absorption, stimulation of secretion or a combination of the two. The above data show that in the pathological ileum there is inhibition of absorption while in cholera there is both inhibition of absorption and production of secretion. The mechanisms of these changes must remain conjectural at the present time. In the pathological tissue

the production of inhibitors of the sodium pump or of the glucose absorptive mechanisms may occur. Also, as can be seen from Fig. 4b, c, and d, a decrease in the absorptive surface may explain these findings. In cholera, there seems to be a relation between the action of exotoxin and cyclic AMP in the gut mucosa (5). Field *et al.* have shown (16) that the effect of theophylline and cyclic AMP on the ileum is to stimulate the secretion of chloride ions and decrease the permeability of the brush border to sodium thus inhibiting the net sodium movement.

Pathologically, the brunt of the rabbit small intestinal abnormality seems to have fallen on the villi, and the crypts appear to be relatively preserved. Serebro *et al.* (17) and Elliott *et al.* (3) have postulated that crypt cells are the cell types responsible for secretion. If the change in short-circuit current following theophylline, is indeed a measure of the ability of the mucosa to secrete, then the fact that it is preserved in pathological tissues with relatively normal crypts may lend some support for the above-mentioned hypothesis. In the presence of gross disorganization of the architecture of the mucosa, it is, however, difficult to draw conclusions regarding differential function among cell types.

Summary. Histologically normal isolated, short-circuited rabbit ileum absorbed both sodium and chloride ions. Structurally damaged mucosa did not absorb either ion. Glucose mediated short-circuit current effect was present in normal but was much reduced in abnormal tissues. These findings in tissue with altered structure were compared to tissue exposed to cholera toxin, where no net sodium absorption occurs yet net chloride movement from serosal-to-mucosal surfaces occur. In contrast to the pathological tissues the absent net sodium absorption in cholera is reversible with the addition of glucose to the mucosal medium.

1. Fordtran, J. S., Rector, F. C., Locklearn, T. W., and Ewton, M. F., J. Clin. Invest. **46**, 287 (1967).

2. Gangarosa, E. J., Beisel, W. R., Benyajati, C., Sprinz, H., and Pityaratn, P., Amer. J. Trop. Med. Hyg. **9**, 125 (1960).

3. Elliott, H. L., Carpenter, C. C. J., Sack, R. B., and Yardley, J. H., Lab. Invest. **22**, 112 (1970).

4. Schulz, S. G., and Zalusky, R., J. Gen. Physiol. **47**, 567 (1964).

5. Field, M., Fromm, D., Wallace, C. K., and Greenough, W. B., III, J. Clin. Invest. **48**, 24a (1969).

6. Bray, G. A., Anal. Biochem. **1**, 279 (1960).

7. Schulz, S. G., and Zalusky, R., J. Gen. Physiol. **47**, 1043 (1964).

8. Tabaqchali, S., and Booth, C. C., Brit. Med. Bull. **23**, 285 (1967).

9. Banwell, J. B., Gorbach, S. L., Mitra, R., and Pierce, N. F., (Abstr.) Gastroenterology **58**, 925 (1970).

10. Gyles, C. L., and Barnum, D. A., J. Infec. Dis. **120**, 419 (1969).

11. Smith, H. A., and Jones, T. C., "Veterinary Pathology," 3rd ed. p. 549. Lea and Febiger, Philadelphia (1966).

12. McPherson, C. W., Haberman, R. T., Every, R. R., and Pierson, R., Proc. Anim. Care Panel. **12**, 133 (1962).

13. Crane, R. K., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **24**, 1000 (1965).

14. Hardsworth, C. D., and Dawson, A. M., Gut **6**, 387 (1965).

15. Field, M., and McColl. I., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **27**, 603 (1968).

16. Field, M. Fromm, D., and Silen, W., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **28**, 651 (1969).

17. Serebro, H. A., Iber, F. L., Yardley, J. H., and Hendrix, T. R., Gastroenterology **56**, 506 (1969).

Received June 8, 1970. P.S.E.B.M., 1970, Vol. 135.