Effects of Caffeine on Transport, Metabolism and Ultrastructure of Isolated Rat Colon

T. J. SERNKA[1] and C. H. TSENG[2]

1 *Program in Physiology, School of Medicine, Department of Physiology, College of Science and Engineering, Wright State University, Dayton, Ohio 45435, USA*
2 *Department of Pathology, Medical College of Pennsylvania, Laboratory Service, Veterans Administration Medical Center, Philadephia, Pennsylvania 19104, USA*

Abstract. The effect of caffeine on the transport, metabolism and ultrastructure of the colon were determined. Segments of proximal colon were excised from the anesthetized rat and prepared for radioisotopic tracing of ion transport in the flux chambers or oxidative metabolism in an incubator. Other segments were fixed before or after caffeine administration for electron microscopy. The isolated rat colon actively transported both Na^+ and Cl^- in the absorptive direction, mucosa to serosa. Serosal addition of 10 mmol/l caffeine abolished the smaller Na^+ transport but did not significantly affect the larger Cl^- transport. The electrical potential difference and the short-circuit current rose accordingly. Although the oxidation of glucose was inhibited by 35%, caffeine had no significant effect on the oxidation of the fatty acid, butyric acid. Comparable metabolic responses were obtained using the isolated terminal ileum of the rat. Neither the height nor the density of the microvilli in the proximal colon were affected significantly by caffeine. It may be concluded that caffeine, unlike theophylline, effectively preserves the normal absorptive condition of the colon. Thus, caffeine may have actions other than inhibition of phosphodiesterase in the distal intestine.

Key words: Caffeine — Colon — Transport — Metabolism — Ultrastructure

Introduction

Methyl xanthines like theophylline have been found to mimic cyclic AMP in stimulating secretions in the stomach (Harris et al. 1969), ileum (Field 1971) and colon (Binder and Rawlins 1973). Theophylline appears to act by inhibiting the degradative enzyme, phosphodiesterase, for cyclic AMP breakdown (Sutherland and Rall 1960). Although another methyl xanthine, caffeine has not been as well studied in the gastrointestinal tract. Since it is such a common drug in our beverages, special attention to the intestinal effects of caffeine seemed warranted. When investigated in the same tissue, the isolated rat colon, caffeine was found to have some transport responses that differed from those found for theophylline

(Binder and Rawlins 1973). Whereas theophylline brought about a net secretory state, caffeine effectively preserved the normal absorptive condition of the colon. A preliminary report has been presented (Sernka 1984).

Materials and Methods

Male adult rats weighing 300—500 g were fasted overnight and anesthetized with intraperitoneal injections of sodium pentobarbital (60 mg/kg). Following laparotomy and using the cecum as an anatomical marker, about 4—6 cm of proximal colon (or terminal ileum) was excised. The isolated colon wall was cut longitudinally and bisected into equal portions for transport and histopathological studies. For metabolic studies, the isolated length of intestine was cut cross-sectionally into equal segments.

The two sheets of isolated colon wall were mounted in identical thermoregulated flux chambers having exposed tissue areas of 1 cm^2. The two flux chambers were connected to a voltage clamp system (Cabler Biomedical) controlled by a microcomputer. The microcomputer generated an opposing short-circuit current (*Isc*) that nullified the measured electrical potential difference (*PD*) generated by the tissue. Further details of this system are described elsewhere (Sernka et al. 1982).

Mucosal and serosal surfaces of the chambered tissues were bathed with 10 ml of isosmotic Ringer solution containing 25 mmol/l glucose. For buffer, 10 mmol/l N-tris (hydroxymethyl) methyl--2-aminoethane-sulfonic acid (TES, Sigma) was added and the pH adjusted to 7.4 with 100% O_2 aeration. (The measured pH value of proximal colonic contents solubilized in 5 ml of neutralized distilled water was 7.37.) The other components of the Ringer solution were (in mmol/l): 133 NaCl, 5 KCl, 1 $MgSO_4$, 1 Na_2HPO_4, 1 $CaCl_2$ and 10 mannitol (Mallinckrodt) or 10 caffeine (Sigma). The osmolality (Osmette Precision) was 305 mosmol/l for mannitol-containing and 303 for caffeine-containing Ringer solutions. The Cl^- concentration (Corning) of the latter was 146 mequiv/l. All Ringer solutions also contained 8 mg/100 ml of penicillin (Sigma) and 16 mg/100 ml of streptomycin sulfate (Sigma) to minimize bacterial growth. All chamber solutions were maintained at 37 °C.

Radioisotopic tracer was added to the serosal half-chamber of one chamber set-up and to the mucosal half-chamber of the other. In Na^+ flux experiments, 3 μCi of ^{22}Na (New England Nuclear) in 0.05 ml of Ringer solution was added; in the Cl^- flux experiments, 2.5—5 μCi of ^{36}Cl (New England Nuclear) in 0.05—0.10 ml of Ringer solution was added. After steady state was achieved, aliquots were sampled periodically from the unlabelled half-chamber to determine ion fluxes. The colon tissues were kept short-circuited during the experiment except for brief determinations of *PD* and electrical resistance (*R*), calculated automatically by the microcomputer as *PD*/*Isc*. More detail of the protocol is found elsewhere (Sernka et al. 1982).

In metabolic studies, serial sections of isolated colon or ileum were prepared for incubation in Ringer solution containing antibiotics and TES buffer, pH 7.4, as previously described (Rollin et al. 1979). Solutions contained 25 mmol/l substrate, glucose or butyrate, and the corresponding radioisotope, 0.25 μCi of (U—^{14}C) glucose or (1—^{14}C) butyric acid. Flasks were gassed with 100% O_2 and sealed for 1 h incubation of tissue at 37 °C. Oxidation of substrate was traced by the production of $^{14}CO_2$, which was liberated by acidifying the flasks at termination. The freed $^{14}CO_2$ was trapped in base (Hyamine, Amersham) added to circles of filter paper suspended in the flasks. The rate of oxidation was expressed as dpm radioactivity corrected with a blank control and normalized for tissue wet weight and time of incubation.

In histopathological studies, the isolated colon tissues were mounted in flux chambers and incubated for 45 min in Ringer solution with 10 mmol/l of mannitol or caffeine. Exactly the same protocol as for the transport studies was followed. After dismounting the tissue, it was fixed immediately

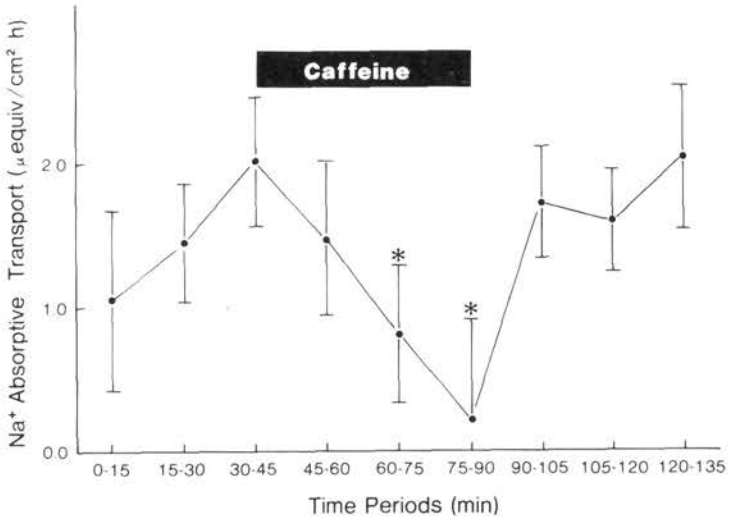

Fig. 1. Effect of 10 mmol/l serosal caffeine on active transport of Na^+ from mucosa to serosa of isolated rat colon. Mean values ± SE from 6 animals. *Significant difference ($P < .05$) from paired values at last pre-control period, 30—45 min.

in 10% formaldehyde-containing phosphate buffer, pH 7.0. These specimens were single-blind coded to eliminate bias and sent to the pathologist (C.H.T.) for electron microscopy. The physiologist (T.J.S.) measured the relative height of microvilli (in mm) from the high-power (×38,000) and the relative density of microvilli (per 25 mm length) from the low-power (×7800) micrographs.

All values reported are means ± SE. Significance of the differences was determined from the P value calculated from the Student t test being less than 0.05 for transport and histopathological studies. For metabolic studies, statistical significance was sought by an analysis of variance followed by the Studentized range test (Sokal and Rohlf 1969).

Results

Active transport of Na^+ from mucosa to serosa across the isolated rat colon was progressively reduced and finally abolished by 10 mmol/l caffeine on the serosal side (Fig. 1). Compared with the pretreatment control rate of 2.02 ± 0.45 μequiv/$/cm^2h$, Na^+ absorptive transport fell significantly by 60% after 30 min and by 88% after 45 min exposure to caffeine. The inhibition of Na^+ transport appeared to have been brought about by an increase in tissue permeability, since the passive unidirectional flux of Na^+ from serosa to mucosa rose from a pretreatment control rate of 5.5 ± 0.3 μequiv/cm^2h to a final rate with caffeine of 7.8 ± 1.1 μequiv/cm^2h. (The opposite unidirectional flux remained relatively constant.)

Removal of the caffeine fully restored the active absorptive transport of Na^+ (Fig. 1). Within 15 min the transport rate was 86% of the pretreatment value and after 45 min the post-control rate was 101% the maximal pre-control rate, the

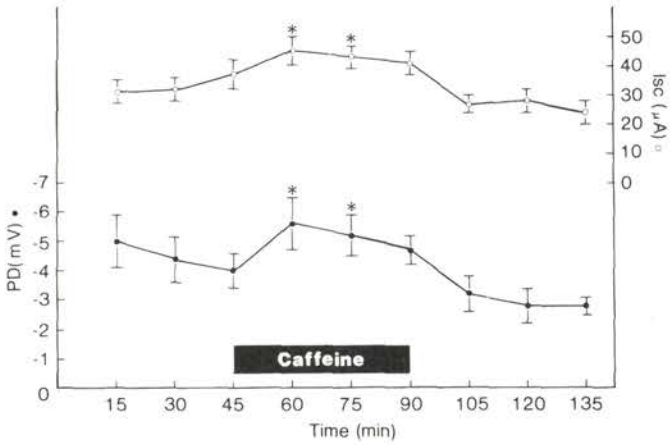

Fig. 2. Responses of *Isc* and *PD* to 10 mmol/l serosal caffeine in isolated rat colon. Mean values ± SE from same 6 animals in Fig. 1. *Significant difference ($P<.05$) between treatment and control paired values at 45 min.

pretreatment value. The recovery was mediated by a decline in the passive unidirectional flux of Na^+ from serosa to mucosa to a value of 5.6 ± 0.5 μequiv/ /cm^2h 45 min after removal of the caffeine. The opposite unidirectional flux was constant once again.

Both the *Isc* and the *PD* rose significantly during caffeine exposure and both parameters fell again upon removal of the caffeine (Fig. 2). Compared with the pretreatment current of 36.8 ± 4.7 μA (1.37 μequiv/cm^2h), net ionic movement rose significantly by 23% after 15 min and remained above the control value throughout caffeine exposure. The *PD* likewise increased significantly by 40% over the control voltage of -4.0 ± 0.6 mV (mucosal side negative) within 15 min of the caffeine administration and remained high until the caffeine was removed. The *R* did not change significantly from the pre-caffeine value of 171 ± 22 Ω-cm^2 throughout the experiment.

Since the *Isc* represents the algebraic sum of net ionic movements, the fall in Na^+ absorptive transport concomitant with the rise in *Isc* brought about by caffeine indicated the active transport of another ion. As shown in Fig. 3, the active absorptive transport of Cl^- was several times the magnitude of Na^+ absorptive transport. At a serosal concentration of 10 mmol/l, caffeine did not significantly affect the net flux of Cl^- from mucosa to serosa. The pretreatment control rate of 10.1 ± 0.5 μequiv/cm^2h was not significantly greater than the treatment rates. Unidirectional fluxes of Cl^- were also unaffected by caffeine. Removal of caffeine likewise had no significant effect on the active absorptive transport of Cl^-.

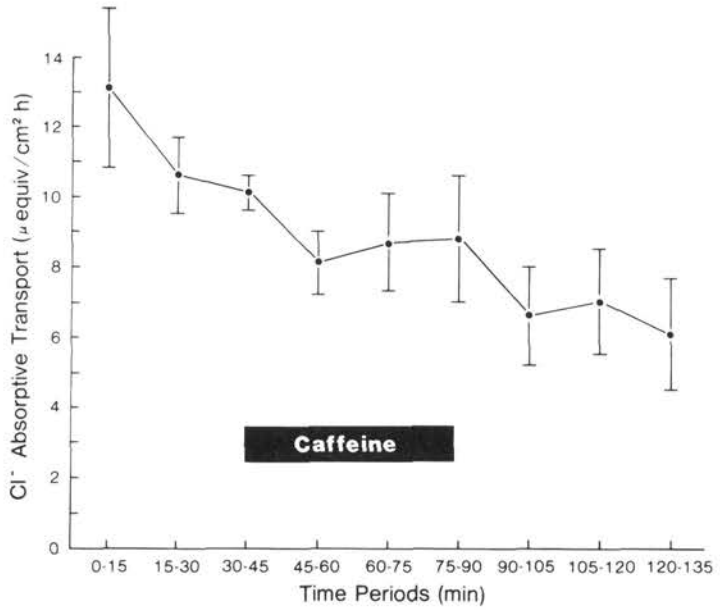

Fig. 3. Effect of 10 mmol/l serosal caffeine on active transport of Cl^- from mucosa to serosa of isolated rat colon. Mean values ± SE from 3 animals.

The algebraic sum of net Na^+ and Cl^- movements exceeded the average *Isc* throughout the experiments, indicating that substantial movement of residual ions was also involved.

The oxidation of glucose by slices of proximal colon and terminal ileum of the rat is shown in Fig. 4. Incubation with 10 mmol/l caffeine significantly reduced glucose oxidation by 35% in the colon. The effect on ileal oxidation of glucose was not significant. To test the possible role of cyclic AMP in mediating the response to caffeine, paired colonic and ileal segments were treated with 2.6×10^{-4} mol/l dibutyryl cyclic AMP (dbcAMP). This agent had no effect on glucose oxidation in either tissue (Fig. 4).

Butyrate oxidation was not changed by caffeine or dbcAMP in the colon or ileum (Fig. 5). In contrast to the oxidation of glucose, butyrate oxidation was greater in the colon than in the ileum.

The density of microvilli was determined from a defined length along a low-power electron micrograph. The control colon tissues incubated in mannitol solution had a microvillous density of 6.9 ± 0.5 per μm of length measured from the micrograph. The density of colon tissues incubated in caffeine solution was 6.2 ± 0.7 per μm. The tissues were paired for statistical analysis, and the paired difference as a percent, $-7 \pm 12\%$, was not significant. Figs. 6*A* and 7*A* show

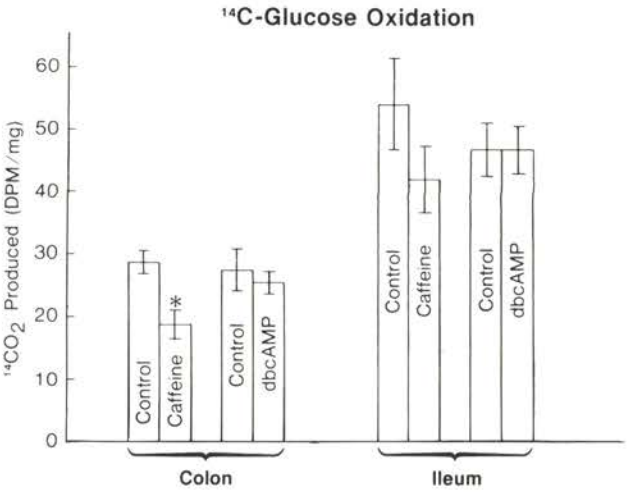

Fig. 4. Glucose oxidation in slices of rat colon or ileum incubated for 1 h at 37 °C in the presence or absence of 10 mmol/l caffeine or 2.6×10^{-4} mol/l dibutyryl cyclic AMP (dbcAMP). In the controls for caffeine treatment, 10 mmol/l mannitol was added for osmotic balance. Mean values ± SE from 6 animals. *Significant difference ($P < .05$) between caffeine treated and mannitol control in the colon.

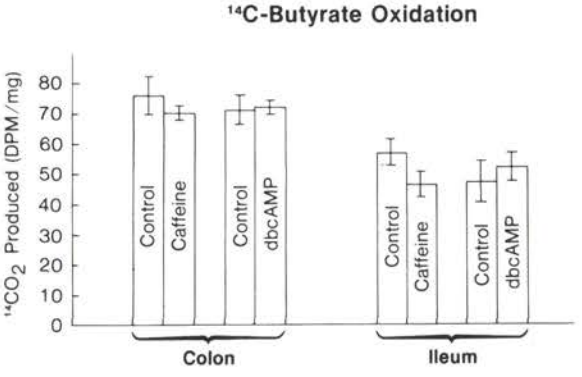

Fig. 5. Butyrate oxidation in slices of rat colon or ileum incubated for 1 h at 37 °C in the presence or absence of 10 mmol/l caffeine or 2.6×10^{-4} mol/l dibutyryl cyclic AMP (dbcAMP). Mannitol was added to controls of caffeine studies for osmotic balance. Mean values ± SE from 6 animals.

a typical pair of colon tissues from the same rat incubated in mannitol- or caffeine-containing Ringer solution, respectively. The density of microvilli in the caffeine-treated tissue was 13% lower than in the paired control in this particular experiment.

The height of the microvilli was measured in triplicate from a high-power electron micrograph of the same group of paired colon tissues. The microvillous

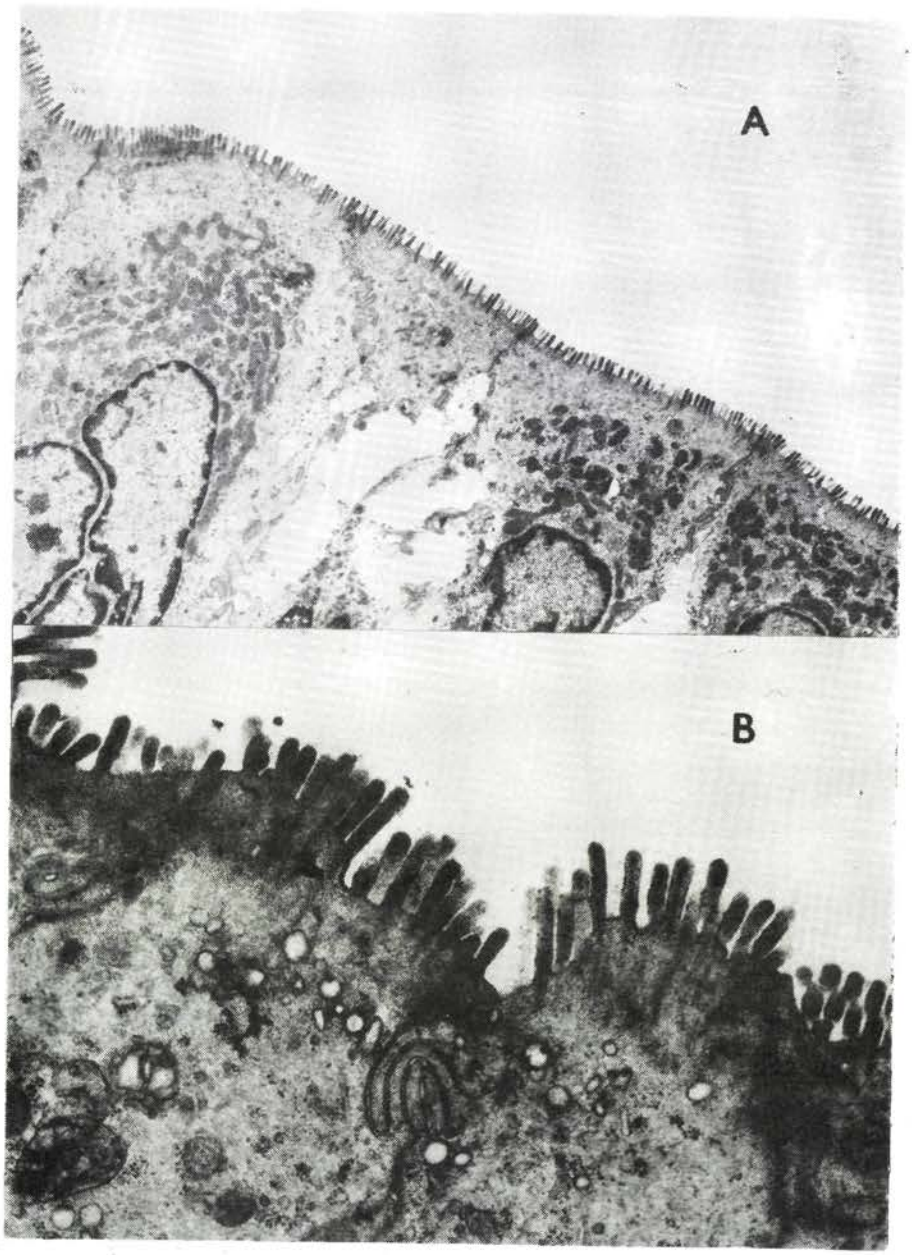

Fig. 6. Electron micrographs of rat colonic epithelial border showing density (*A*, ×7800) and height (*B*, ×38,000) after a 45 min incubation in Ringer solution containing 10 mmol/l mannitol.

height of tissues incubated in mannitol solution was 0.40 ± 0.05 μm and that of tissues incubated in caffeine solution, 0.35 ± 0.04 μm. The paired difference as a percent, $-17 \pm 17\%$, was not significant. Figs. 6*B* and 7*B* show a typical pair in which the caffeine-treated microvillous height was 33% lower than the control.

The single-blind assessment of the electron micrographs by the pathologist was as follows: tall and very regular microvilli for 2 of the 6 control, none of the caffeine-treated; low and irregular microvilli for 2 of the 6 caffeine-treated, one irregular control; and intermediate microvilli for the remainder of both groups.

Discussion

Although previous investigations had shown quantitative differences in the effects of the methyl xanthines, the present results are the first to show a qualitative difference. Butcher and Sutherland (1962) demonstrated that theophylline inhibited purified phosphodiesterase to a much greater extent than did caffeine, and Harris et al. (1969) discovered that theophylline produced a greater increase in cyclic AMP content of bullfrog gastric mucosa than did caffeine. Alonso and Harris (1962) had previously found that theophylline was a more effective stimulant of acid secretion than was caffeine in the gastric mucosa. These studies suggested that the effects of caffeine in the isolated colon might be similar but less pronounced than those of theophylline.

Binder and Rawlins (1973) determined that addition of 10 mmol/l theophylline to the solutions bathing the isolated colonic mucosa of the rat abolished the absorptive transport of Na^+ and reversed the absorptive transport of Cl^-. Theophylline caused increases in the *Isc* and *PD* in much the same way as did dbcAMP. Using the same concentration of caffeine on the serosal side as did Binder and Rawlins, we found contrasting results with regard to Cl^- transport. Although caffeine, like theophylline, abolished absorptive transport of Na^+ and stimulated *Isc* and *PD*, the effect of caffeine on absorptive transport of Cl^- was not to inhibit and certainly not to reverse. This single difference is important in that it determines whether the tissue will be converted to a net secretory state as after theophylline or be effectively maintained in the absorptive state as after caffeine. Since Cl^- transport was about five times as large as Na^+ transport, overall active absorption of these ions was reduced only 25 % by caffeine.

Theophylline most likely acts solely through elevation of mucosal cyclic AMP in the colon (Binder and Rawlins 1973) as well as in the ileum (Field 1971), since exogenously added cyclic AMP or its analogues mimics theophylline in bringing about a secretory state. Vasoactive intestinal peptide acts identically to theophylline in the rat colonic mucosa (Racusen and Binder 1977). The association of this peptide hormone with certain types of watery diarrhea (Verner and Morrison 1974) serves to reinforce the cyclic AMP-mediated secretory state stimulated by

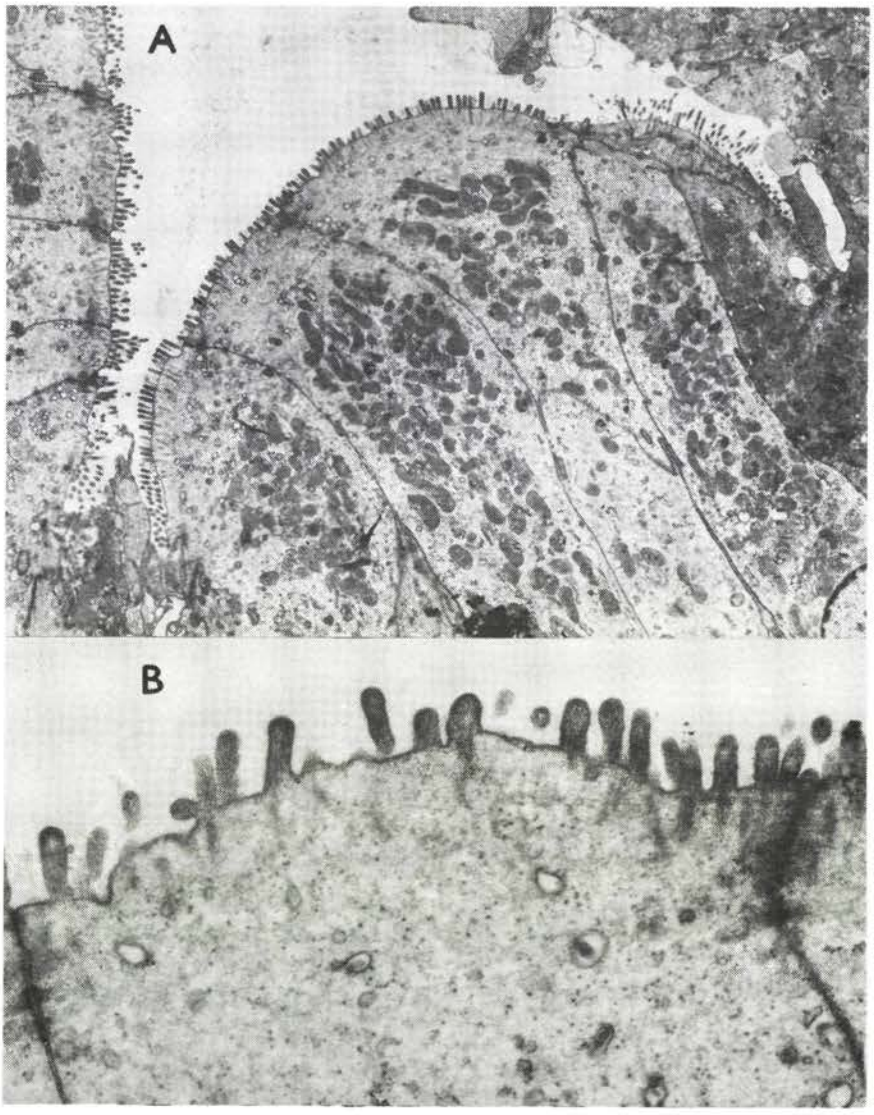

Fig. 7. Electron micrographs of epithelial border from paired rat colon (see Fig. 6) showing density (*A*, ×7800) and height (*B*, ×38,000) after a 45 min incubation in Ringer solution containing 10 mmol/l caffeine.

theophylline. Vasoactive intestinal peptide and theophylline act synergistically to stimulate net water flow in everted sacs of rat colonic mucosa (Waldman et al. 1977). Similar arguments have been advanced to identify theophylline action with secretion stimulated by conjugated dihydroxy bile salts in rat colonic mucosa (Binder and Rawlins 1973). We found that caffeine, in contrast, reduces glucose oxidation in the rat colon whereas dbcAMP does not. This may indicate that caffeine does not act solely through cyclic AMP mediation.

Although no previous study has examined how caffeine may affect electrolyte movements in the colon, several studies of caffeine's effects on colonic motility have been conducted. In the quinea-pig taenia coli, caffeine progressively inhibited the phasic and tonic components of K^+-induced contracture (McFarland and Pfaffman 1972). These effects of caffeine could be reversed by raising the level of extracellular Ca^{2+} (Sunano and Miyazaki 1973). In the cat colon, caffeine as well as theophylline and dbcAMP reduced the frequency and amplitude of slow waves and the duration of migrating spike bursts (Anuras 1982). The former but not the latter response could be partially reversed by raising the extracellular Ca^{2+} concentration. These studies suggest that caffeine may be inhibitory to motility in the colon. Whether such hypomotility brings about an altered transit rate for passage of material through the colon was not determined.

The changes in colonic metabolism and ultrastructure that we observed with caffeine treatment were small and mostly insignificant. This might be expected if the principal effect of caffeine is to inhibit one ion transport process while not inhibiting another larger ion transport process. In the bullfrog gastric mucosa, Alonso and Harris (1965) found that caffeine stimulated oxygen consumption only when the bathing medium contained a transportable anion like Cl^-. A possible stimulatory effect of caffeine on Cl^- transport is more apparent in the overall oxidative metabolism of the gastric mucosa, because the amphibian gastric mucosa does not transport Na^+ (Hogben 1955), at least not under aerobic conditions (Flemström 1971).

Finally, the question of concentration needs to be addressed. In the present studies, 10 mmol/l caffeine was used because all previous studies on the transport and metabolic effects of methyl xanthines in gastric, ileal and colonic mucosae were conducted at this concentration. It is difficult to relate the effective concentration of a substance *in vitro* to that *in vivo*, since the former has no blood supply. However, if we assume that a cup of coffee contains about 125 mg caffeine, then it would take some 76 cups of coffee to achieve a caffeine concentration of 10 mmol/l in the blood of an average man! Although this seems pharmacologically high for the colon if not the stomach, the relatively mild responses that we obtained become correspondingly more cogent arguments for the hypothesis that caffeine effectively maintains the normal absorptive state of the colon. In contrast, neuropharmacological effects of caffeinism such as anxiety, headaches and tremulousness were

common in hospital subjects who were low consumers of caffeine (0—249 mg/day) as well as in those who were high consumers of 750 mg/day and above (Victor et al. 1981). The calculated caffeine concentration in the blood of the high consumers in this study would be 0.79 mmol/l or above. Somewhat less common were gastrointestinal symptoms such as stomach pain and diarrhea. The diarrhea was determined by an answered questionnaire rather than by quantitative measurements of stool size and frequency, and so it cannot be concluded that this manifestation of caffeine consumption was a true diarrheal or merely a cathartic action of the caffeinated beverages ingested. Gastrointestinal manifestations of theophylline toxicity, such as vomiting and diarrhea, on the other hand, were apparent at relatively low blood concentrations (below 0.08 mmol/l) and common above 0.14 mmol/l (Jacobs et al. 1976). Such dose responses would be expected in view of the present results indicating that caffeine does not predispose the colon to secretion as does theophylline.

In conclusion, caffeine exerts an inhibitory effect on only the smaller of two principal ion transport processes of the colon, thereby effectively preserving the normal absorptive state of its mucosa. The occasional appearance of diarrhea in subjects who have consumed caffeinated beverages may be explained by other observed actions of caffeine, such as an inhibition of motility in colonic smooth muscle or even by central nervous system excitation.

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