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LOCALIZATION OF THE EFFECT OF ACETYLCHOLINE IN REGULATING INTESTINAL ION TRANSPORT

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SUMMARY

1. The location of the site involved in the secretory response of rat jejunum and colon to ACh was investigated by selectively damaging either the villi of the jejunum and the surface epithelium of the colon or the crypts.

2. The secretory response induced by ACh was measured both in terms of changes in electrical activity and chloride fluxes.

3. Exposure of the mucosa to 2 M-Na_2SO_4 for 30 min selectively damaged the jejunal villi and colonic surface epithelium but did not reduce the increased potential difference and current generated by ACh.

4. When resistance changes were taken into account the colonic response was markedly increased after Na_2SO_4 treatment although the jejunal response was unchanged. Under control conditions ACh reduced net Na absorption and stimulated Cl secretion by the colon. After exposure to Na_2SO_4 only the Cl secretory component of the ACh response remained, thus accounting for the enhanced effect.

5. Cycloheximide, administered I.V. at a dose of 12 mg/kg, damaged the crypts after 2 hr without affecting the villi of the jejunum or the surface epithelium of the colon. After cycloheximide treatment the increased potential difference, current and net Cl secretion induced by ACh were significantly reduced.

6. The crypts therefore appear to be the site primarily involved in the secretory response of rat jejunum and colon to ACh, although in the colon an inhibitory effect on the Na transport process located in the surface epithelium was observed.

INTRODUCTION

Acetylcholine (ACh) has been shown to induce secretion in both the small intestine (Hardcastle & Eggenton, 1973; Hubel, 1976) and the colon (Browning, Hardcastle, Hardcastle & Sanford, 1976) of the rat but the location of the site involved in this response has not yet been established. The intestine is a complex multilayer structure consisting of many different cell types. Although ACh is known to influence muscle activity in the intestine it is evident that the secretory response does not originate from this area, as removal of the muscle layers does not abolish its secretory effects (Hardcastle & Eggenton, 1973; Browning *et al.* 1976). Therefore it seems more likely that the locus of the ACh response is the epithelial layer. This consists predominantly of two cell types: immature crypt cells and mature villous or surface epithelial cells.

Workers seeking the origin of the secretion induced by cholera toxin have shown that it is possible to damage selectively either the villi or the crypts of the small intestine. The villous cells are susceptible to osmotic shock induced by making the luminal contents grossly hypertonic. This can be achieved using a hypertonic solution of Na_2SO_4 (Roggin, Banwell, Yardley & Hendrix, 1972). Crypt cell function can be preferentially impaired by cycloheximide, an inhibitor of protein synthesis (Serebro, Iber, Yardley & Hendrix, 1969). This approach has now been used in the present investigation to define more precisely the site of action of ACh in stimulating intestinal secretion. A preliminary report of the results obtained has been presented (Browning, Hardcastle, Hardcastle & Redfern, 1977).

METHODS

Experiments were carried out on male albino rats weighing between 230 and 250 g. These were obtained from the Sheffield Field Laboratories and were allowed free access to food (diet 86, Oxoid, London) and water.

Measurement of transintestinal potential difference in vivo

The transmural potential difference was used to monitor the secretory response to ACh. This was measured *in vivo* using a modification of the preparation described by Hardcastle & Eggenton (1973). Rats were anaesthetized with pentobarbitone and 5 cm segments of jejunum and proximal colon were cannulated at each end to allow the luminal contents to be changed. Each sac was initially filled with 154 mM-NaCl. The transmural potential differences were recorded using two pairs of salt bridge electrodes, one of each pair in contact with the luminal fluid and the other in contact with saline in the peritoneal cavity by means of a wick electrode. The electrodes were connected via calomel half cells to two Vibron electrometers and a visual record was obtained on a Telsec two channel chart recorder. Drugs were administered through a cannula in the jugular vein and each dose was washed in with 0·2 ml. 154 mM-NaCl. Two doses of ACh were used: 3 μg, representing the steep part of the sigmoid dose–response curve and 7 μg, which is on the plateau region. The glucose transfer potential in the jejunum was measured by adding glucose to the luminal fluid to give a final concentration of 28 mM. Under control conditions this concentration of glucose produced a transfer potential that was 65% of the maximum. The responses to ACh and glucose were tested before and after the treatment employed to damage selectively either the villi and surface epithelium or the crypts.

Damage to the jejunal villi and the colonic surface epithelium was achieved by replacing the luminal fluid with 2 M-Na_2SO_4 which was left in contact with the mucosa for 30 min. At the end of this period it was washed out and replaced with 154 mM-NaCl. In control experiments the same procedure was followed but 154 mM-NaCl was used instead of 2 M-Na_2SO_4.

The crypts were damaged selectively by cycloheximide, which was administered I.V. at a dose of 12 mg/kg. After 2 hr the responses to ACh and glucose were tested again. In control experiments an equivalent volume of 154 mM-NaCl was injected I.V. and after 2 hr the responses measured again.

Measurement of tissue resistance

Tissue resistance was measured in everted sacs of rat jejunum and colon using the method of Barry, Smyth & Wright (1965). A current of 1 mA was passed across the tissue and the change in potential difference noted. Since both the small intestine (Clarkson & Toole, 1964) and the colon (Edmonds & Marriott, 1968) behave as ohmic resistors the current generated by the tissue can be calculated using Ohm's law.

Measurement of ion fluxes

Na and Cl fluxes were measured across short-circuited sheets of intestine using the method described by Browning, Hardcastle, Hardcastle & Sanford (1977).

Histological assessment of experimental treatments

Segments of jejunum and colon were prepared *in vivo* in the same way as that described for potential difference measurement. At the end of the treatment or control periods the segments were removed from the animal and fixed in formal saline. They were embedded in wax and sections 5 μm thick were cut. These were stained with haemotoxylin and eosin.

Expression of results

Results are expressed as mean values ± S.E. of mean with the number of observations (n) in brackets. The initial increases in potential difference and calculated current induced by ACh and glucose in control and test preparations were compared using an unpaired t test. In all cases these were not significantly different ($P > 0{\cdot}05$). The responses to ACh and glucose before and after the experimental period in control animals were never significantly different ($P > 0{\cdot}05$). Thus since the duration of the experiment did not appear to affect the magnitude of the responses, paired t tests were used to assess the effects of treatment in test preparations.

Chemicals

Acetylcholine chloride and cycloheximide were obtained from the Sigma Chemical Company, P.O. Box 14508, St Louis, Missouri, 63178 U.S.A. For I.V. administration they were dissolved in 154 mM-NaCl. Glucose was obtained from British Drug Houses Ltd., Poole, England. Na_2SO_4 and reagents for Krebs bicarbonate saline (Krebs & Henseleit, 1932) were from May and Baker Ltd., Dagenham, England.

RESULTS

Both the jejunum and the colon generate a transmural potential difference, the serosal side of the tissue being positive with respect to the mucosal side. In the *in vivo* preparation used in this study the jejunal potential difference was 4–7 mV while that in the colon was 8–15 mV. Intravenously administered ACh caused a transient increase in these potential differences, the magnitude of which was dependent on the dose.

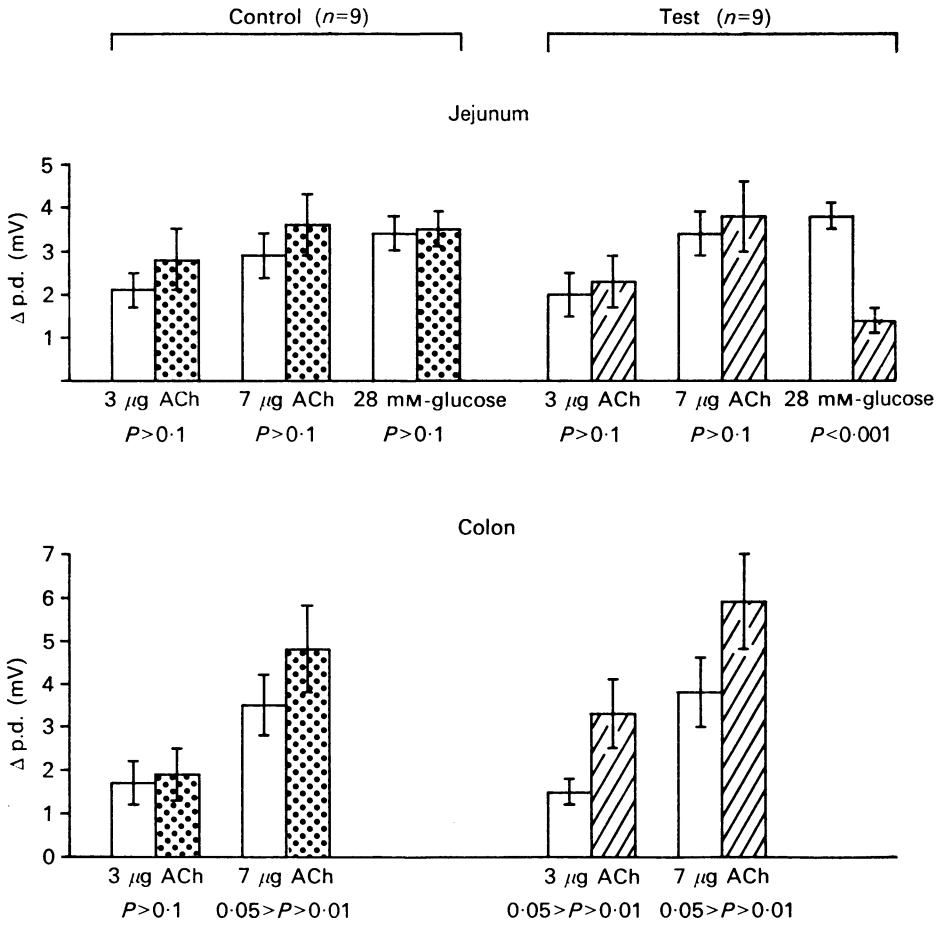
Effect of 2 M-Na_2SO_4

The effect of 30 min exposure of the intestinal mucosa to 2 M-Na_2SO_4 is shown in Text-fig. 1. The increased potential difference in the jejunum caused by glucose was reduced significantly following Na_2SO_4 treatment but the effects of both 3 and 7 μg doses of ACh in the jejunum and colon were unimpaired. In control experiments none of the responses was altered.

It was possible that changes in the response to ACh could have been masked by an alteration in tissue resistance. The effect of Na_2SO_4 treatment on this parameter is shown in Table 1. The resistance of the jejunum was not affected significantly ($P > 0{\cdot}1$) but in the colon there was a significant decrease in this parameter ($P < 0{\cdot}001$). To assess the effects on net ion movement the current generated by ACh was calculated from potential difference and resistance values (Text-fig. 2). The calculated current changes induced by ACh and glucose in the jejunum followed the same pattern as the potential difference changes. However, in the colon it can be seen that the ACh response was significantly increased.

The mechanisms involved in the increased colonic response to ACh following Na_2SO_4 treatment were investigated by measuring ion fluxes in this tissue before and after exposure to Na_2SO_4 (Table 2). Under control conditions the colon actively absorbs Na while there is no net movement of Cl. ACh inhibits Na absorption and

stimulates Cl secretion. In the Na_2SO_4-treated tissue there was no net absorption of Na, and ACh did not alter this ($P > 0{\cdot}1$) although it still stimulated Cl secretion ($0{\cdot}05 > P > 0{\cdot}01$). In the jejunum however, ACh had no effect on Na movement but, as in the colon, it induced a net secretion of Cl.

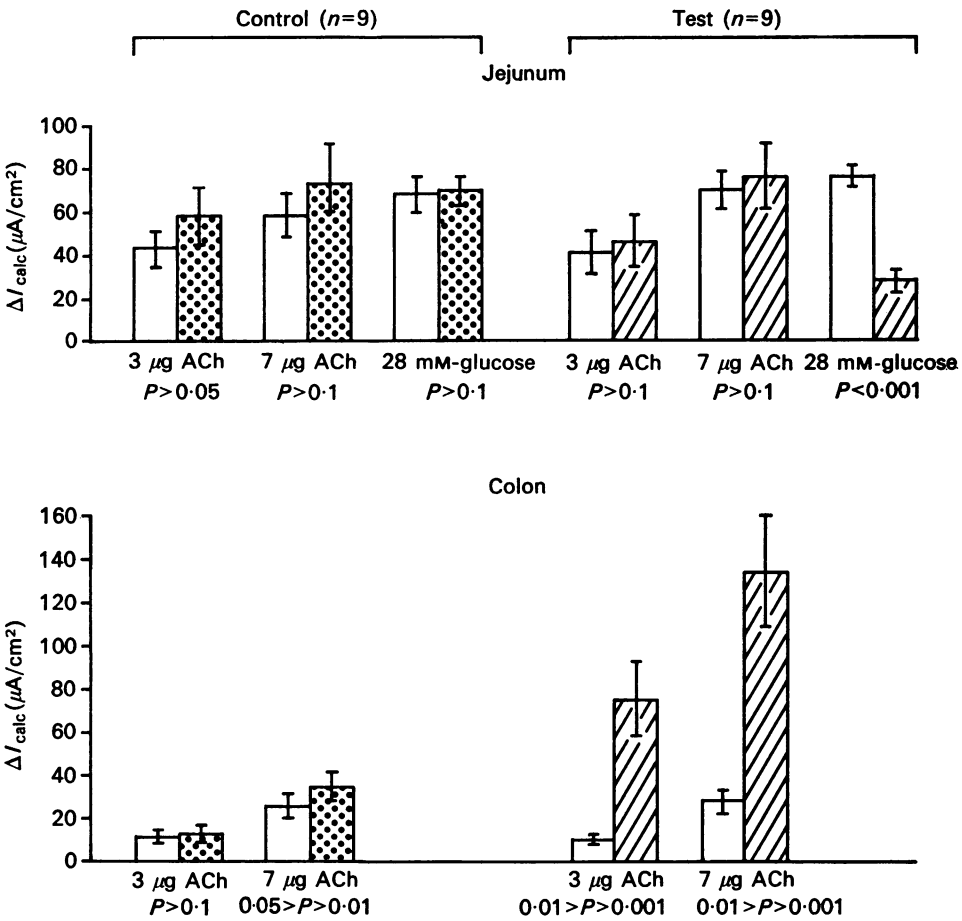


Text-fig. 1. Increased potential difference (Δ p.d.) across rat jejunum and colon *in vivo* in response to I.V. ACh before and after exposure of the mucosa to 2 M-Na_2SO_4 for 30 min. In control experiments the intestinal segments were exposed to 154 mM-NaCl for 30 min. The transfer potential caused by 28 mM-glucose in the lumen of the jejunum was also measured. The p.d.s. are expressed in mV. □, Before treatment; ▦, after control treatment; ▨, after Na_2SO_4 treatment.

The normal histological appearance of the jejunum and colon is shown in Pl. 1*A* and 1*C*. Treatment with Na_2SO_4 caused the villi of the jejunum to become blunted and there were areas of erosion around their tips (Pl. 1*B*). The epithelial cells at the tips of the villi have shrunk. Similarly in the colon Na_2SO_4 damaged the surface epithelium where the mucosal layer has been almost completely eroded away (Pl. 1*D*). In both regions of the intestine the crypts appeared normal.

TABLE 1. Effect of Na_2SO_4 and cycloheximide treatments on the resistance of everted sacs of rat jejunum and colon. Na_2SO_4 treatment: intestinal segments were filled *in vivo* with 2 M-Na_2SO_4 and after 30 min were removed from the animal and the resistance determined. Cycloheximide treatment: cycloheximide was administered I.V. at a dose of 12 mg/kg and after 2 hr the intestinal segments were removed and the resistance measured. In control experiments the jejunum and colon were removed from the animal without treatment. The bathing medium used was Krebs bicarbonate saline and results are expressed as ohm/cm² serosal area. The number of observations is given in parentheses.

	Jejunum	Colon
Control	50·4 ± 6·0 (8)	140·4 ± 3·6 (8)
Na_2SO_4	51·6 ± 5·4 (8)	44·1 ± 5·4 (8)
Cycloheximide	42·6 ± 4·2 (6)	84·6 ± 6·3 (6)



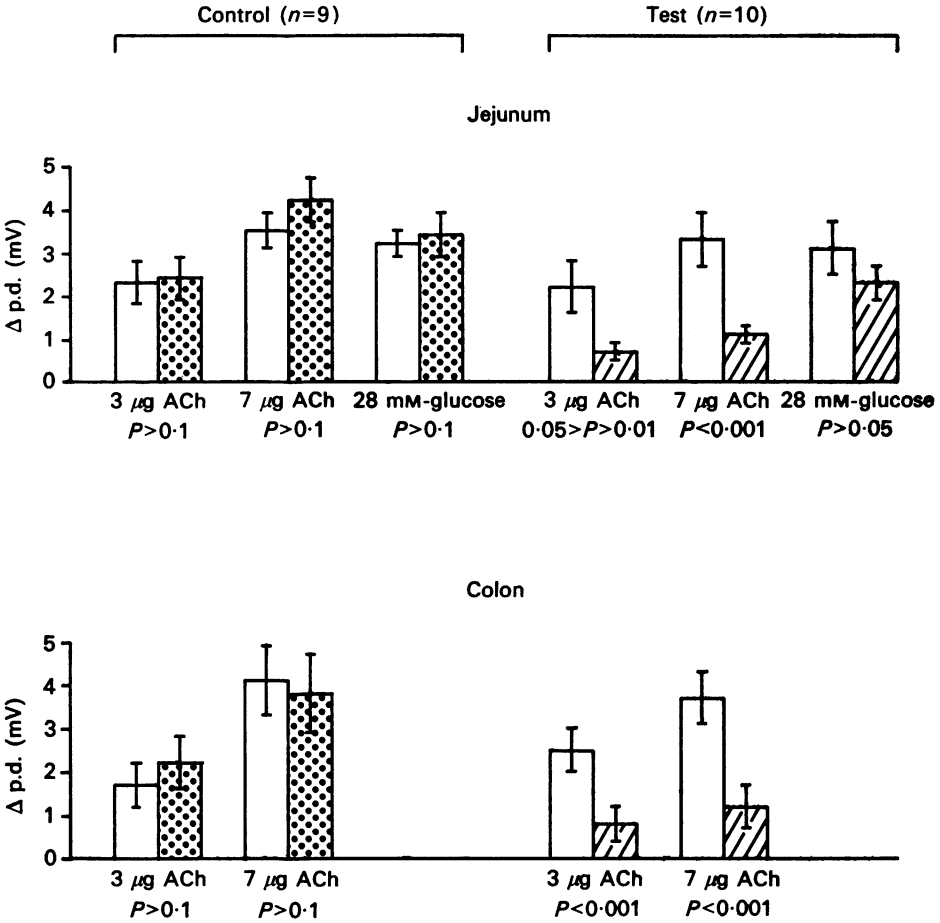
Text-fig. 2. Calculated increases in current generated (ΔI_{calc}) by rat jejunum and colon in response to ACh before and after exposure of the mucosa to 2 M-Na_2SO_4 for 30 min. In control experiments the intestinal segments were exposed to 154 mM-NaCl for 30 min. The jejunal response to 28 mM-glucose in the lumen was also measured. The results are expressed as μA/cm² serosal area. □, Before treatment; ▦, after control treatment; ▨, after Na_2SO_4 treatment.

TABLE 2. Effect of 10 mM-ACh in the serosal fluid on Na and Cl fluxes across sheets of rat jejunum and colon. Mucosal to serosal (J_{ms}) and serosal to mucosal (J_{sm}) fluxes were determined simultaneously in adjacent segments of tissue and the net flux (J_{net}) calculated from the difference between the two unidirectional fluxes. In the Na_2SO_4-treated colon the lumen was filled with 2 M-Na_2SO_4 *in vivo* and after 30 min the tissue was removed and the fluxes measured. Results are expressed as μmole/cm^2.hr, with the no. of observations in parentheses.

		Jejunum		Colon		Na_2SO_4-treated colon	
	Flux	Na	Cl	Na	Cl	Na	Cl
Control	J_{ms}	14·08 ± 2·09 (8)	5·80 ± 0·64 (7)	5·69 ± 0·35 (6)	8·11 ± 1·25 (7)	6·80 ± 0·54 (9)	9·94 ± 0·46 (9)
	J_{sm}	9·46 ± 0·90 (8)	6·60 ± 1·06 (7)	4·70 ± 0·42 (6)	8·38 ± 0·88 (7)	7·65 ± 0·89 (9)	10·40 ± 0·92 (9)
	J_{net}	4·52 ± 1·31 (8)	−0·80 ± 1·40 (7)	0·97 ± 0·35 (6)	−0·28 ± 1·55 (7)	−0·85 ± 0·56 (9)	−0·46 ± 0·94 (9)
+10 mM-ACh	J_{ms}	12·95 ± 1·20 (8)	5·25 ± 0·48 (7)	5·39 ± 0·36 (6)	7·13 ± 0·85 (7)	7·43 ± 0·74 (6)	9·52 ± 0·57 (6)
	J_{sm}	9·30 ± 1·16 (8)	11·43 ± 1·82 (7)	5·46 ± 0·40 (6)	9·15 ± 0·90 (7)	9·60 ± 1·09 (6)	13·04 ± 1·40 (6)
	J_{net}	3·67 ± 1·51 (8)	−6·17 ± 1·87 (7)	−0·11 ± 0·24 (6)	−2·02 ± 1·41 (7)	−2·17 ± 0·79 (6)	−3·51 ± 1·06 (6)

Effect of cycloheximide

The effect of cycloheximide on the jejunal and colonic responses to ACh and glucose are shown in Text-fig. 3. The jejunal response to glucose was not changed significantly but in both the jejunum and the colon the increased potential differ-



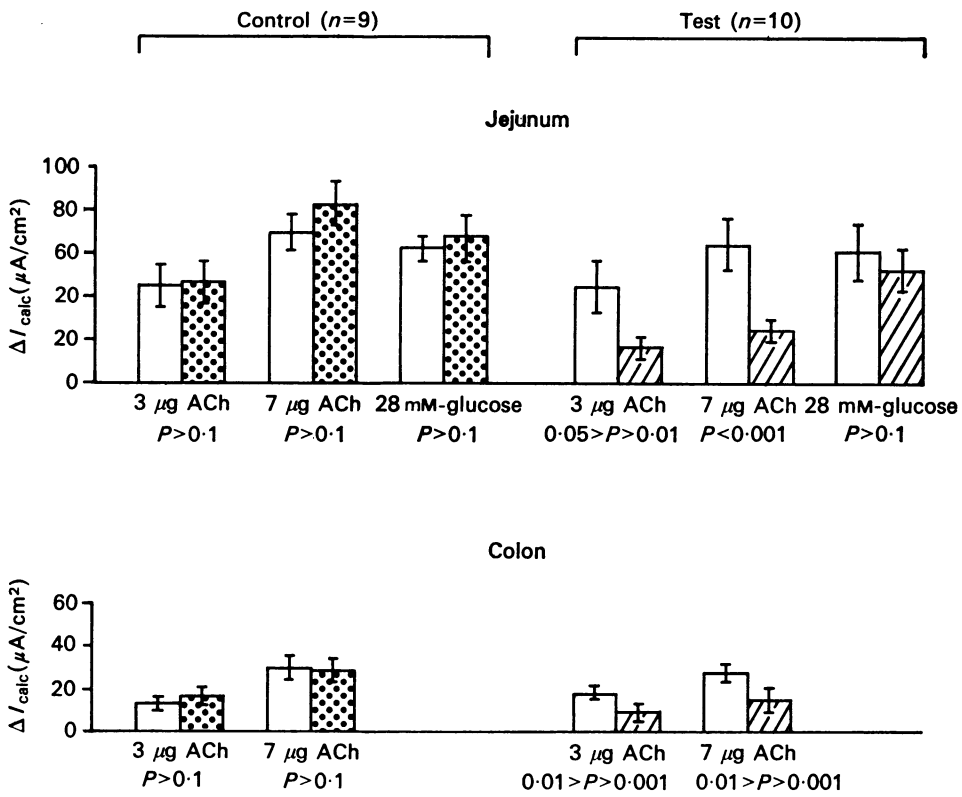
Text-fig. 3. Increased potential differences (Δ p.d.) across rat jejunum and colon *in vivo* in response to I.V. ACh before and 2 hr after I.V. administration of cycloheximide (12 mg/kg). In control experiments an equivalent volume of 154 mM-NaCl was injected and the response tested again after 2 hr. The transfer potential caused by 28 mM-glucose in the lumen of the jejunum was also measured. The p.d.s are expressed in mV. □, Before treatment; ▦, after control treatment; ▨, after cycloheximide treatment.

ences induced by ACh were decreased significantly. In the control experiments none of the parameters was altered.

Cycloheximide did not reduce significantly ($P > 0{\cdot}1$) the resistance of the jejunum but caused a significant decrease ($P < 0{\cdot}001$) in the colonic resistance (Table 1). This did not, however, substantially affect the pattern of the results as can be seen

in Text-fig. 4. The jejunal current change induced by glucose was not decreased significantly by cycloheximide. The current changes generated in response to ACh however, were reduced significantly although there was no change in the control experiments.

Since the electrical response to ACh is primarily due to a stimulation of net Cl secretion (Table 2), the reduced responses after cycloheximide treatment should be



Text-fig. 4. Calculated increases in current generated (ΔI_{calc}) by rat jejunum and colon in response to ACh before and 2 hr after I.V. administration of cycloheximide (12 mg/kg). In control experiments an equivalent volume of 154 mM-NaCl was injected and the responses tested again after 2 hr. The jejunal response to 28 mM-glucose in the lumen was also measured. The results are expressed in $\mu A/cm^2$ serosal area. □, Before treatment; ▦, after control treatment; ▨, after cycloheximide treatment.

reflected by a decrease in the net secretion of this ion. This was confirmed by direct flux measurements. In the jejunum the net Cl secretion induced by ACh was $4{\cdot}73 \pm 0{\cdot}54$ μmole/cm^2.hr ($n = 4$) under control conditions and $2{\cdot}27 \pm 0{\cdot}57$ μmole/cm^2.hr ($n = 4$) after cycloheximide treatment. Similar findings were observed in the colon where ACh induced a net chloride secretion of $3{\cdot}38 \pm 0{\cdot}28$ μmole/cm^2.hr ($n = 4$) in control tissues and $1{\cdot}84 \pm 0{\cdot}15$ μmole/cm^2.hr ($n = 4$) in cycloheximide-treated preparations. These differences were significant in both the jejunum ($0{\cdot}05 > P > 0{\cdot}01$) and the colon ($0{\cdot}05 > P > 0{\cdot}01$).

The effects of cycloheximide on the histological appearance of the jejunum and colon are shown in Pl. 2 and 3. The control tissues are shown in Pl. 2*A* and 3*A* while the tissues exposed to cycloheximide are shown in Pl. 2*B* and 3*B*. Cycloheximide treatment caused the crypt cells of both the jejunum and the colon to become darkly staining, the boundaries between them indistinct and the mucus within the goblet cells in the colonic crypts to disappear. However, the villous and surface epithelial cells appeared normal.

Neither the Na_2SO_4 nor the cycloheximide treatments enhanced the transient hypotensive action of ACh administered intravenously nor did they alter the time from ACh administration to the onset of the electrical response or the time course of this effect. These observations suggest that the treatments did not influence the access of ACh to its site of action.

Intravenous administration of μg doses of carbachol, a choline ester not hydrolysed by cholinesterase, resulted in a transient change in potential difference very similar in character to that obtained in response to ACh. Both Na_2SO_4 and cycloheximide treatments influenced the carbachol response in the same manner as the ACh response, suggesting that neither treatment was exerting its effect by an alteration in cholinesterase activity.

DISCUSSION

The secretion from the mucosa of rat jejunum and colon induced by ACh may originate from either the villous cells and in the case of the colon the surface epithelium, or the crypt cells. By selective damage to each of these cell populations it is possible to localize the region primarily responsible for the secretion stimulated by ACh.

There are a number of ways of damaging preferentially the villous cells of the small intestine and the surface epithelial cells of the colon. These cells are very sensitive to anoxia, so that obstructing their blood supply for a limited period results in gross lesions of the villi and surface epithelium, while the crypts remain intact (Robinson, Antonioli & Mirkovitch, 1966; Rausis & Robinson, 1972). Small intestinal loops that have been subjected to ischaemia have a reduced capacity for absorbing amino acids (Robinson *et al.* 1966) which supports the view that the active transport of nutrients is a function of the villi. Autoradiographic studies in hamster small intestine have also demonstrated that it is the villous cells and not the crypt cells that actively take up sugars and amino acids from the luminal fluid (Kinter & Wilson, 1965). Thus it is possible to use the capacity of the small intestine to absorb such nutrients as a test of villous integrity. Since the transport of sugars is coupled electrogenically to net Na movement (Schultz, Frizzell & Nellans, 1974) their absorption leads to an increase in the transmural potential difference. This transfer potential, together with the resistance of the preparation, allows the increase in current generated to be calculated and it was this parameter which was used to assess the functional integrity of the villous cells in the present study. Unfortunately there is no such simple functional test for the surface epithelium of the colon.

Another method of inducing selective villous damage is to expose the mucosa to a grossly hypertonic solution, since the epithelial cells are susceptible to osmotic

shock. The crypts appear to be undamaged by this procedure, probably because they are not exposed to the luminal contents which have difficulty in penetrating the narrow, mucus-plugged necks of the crypts (Kinter & Wilson, 1965). Initial experiments were carried out using 2·4 M-urea (Iber & McGonagle, 1968) but it was found subsequently that more consistent damage to the villous cells could be produced with a 2100 m-osmolar solution of Na_2SO_4 (Roggin *et al.* 1972). Such procedures reduced glucose absorption from loops of rabbit jejunum but failed to alter the secretory response to cholera toxin, which was therefore assumed to originate from the crypt cells.

In the present study Na_2SO_4 treatment significantly reduced glucose transport in the jejunum, as indicated by using the change in current (Text-fig. 2) as an index of sugar absorption. In view of the location of the hexose transport process this observation suggests that the villi had been injured. Structural damage was evident from the histological appearance of the tissue following exposure to Na_2SO_4 (Pl. 1*B*), the region at the tips of the villi being more severely affected than those nearer the crypts. In the jejunum the response to ACh was not reduced (Text-fig. 1 and 2). If the villi were involved in this response some diminution would have been expected, even if the villous epithelium was not totally destroyed. Thus it appears that the villous cells of the jejunum do not participate in the secretion induced by ACh.

In the colon, Na_2SO_4 treatment resulted in extensive damage to the surface epithelium (Pl. 1*D*), the degree of injury being much more marked than that observed in the small intestine. This was associated with a loss of the ability of the colon to actively absorb Na ions (Table 2), which must therefore be a function of the surface epithelial cells. Support for this view comes from the observation that active Na transport is reduced after ischaemia (Rausis & Robinson, 1972). As in the jejunum, the response to ACh was not diminished (Text-fig. 1) and in fact when resistance changes were taken into account it was enhanced (Text-fig. 2). This finding can be explained in terms of the changes in colonic ion transport induced by ACh. Under normal conditions ACh inhibits Na absorption, which would tend to decrease the potential difference and current, and stimulates Cl secretion, which would increase them. Since Cl secretion is greater than the reduction in Na absorption, an increased potential difference and current is observed in response to ACh. In the Na_2SO_4-treated colon the effect on Na transport is absent due to the destruction of the surface epithelium, but Cl secretion remains and this accounts for the enhanced response. Thus in the colon, Cl secretion does not appear to involve the surface epithelium but this region does respond to ACh, with a decreased rate of Na absorption. In this respect the colon differs from the jejunum where ACh stimulates Cl secretion without altering Na absorption. Hence Na_2SO_4 treatment does not enhance the jejunal response to ACh.

There are several reports of the action of cycloheximide in preventing the secretory response of the small intestine to cholera toxin (Serebro *et al.* 1969; Grayer, Serebro, Iber & Hendrix, 1970; Harper, Yardley & Hendrix, 1970; Moritz, Iber & Moore, 1972; Kimberg, Field, Gershon, Schooley & Henderson, 1973). The crypt cells of the intestine represent a region of rapid cell division, a process that requires protein and DNA synthesis. Cycloheximide reduces protein synthesis by the crypt cells and as a result reduces their proliferative capacity (Verbin & Farber, 1967;

Verbin, Liang, Saez, Diluiso, Goldblatt & Farber, 1971). The primary action of cycloheximide appears to be at the level of the crypts since glucose absorption is unaffected at doses of the antibiotic that prevent the fluid secretion induced by cholera toxin (Serebro *et al.* 1969). In the present study cycloheximide reduced the response to ACh in the jejunum without significantly affecting the change in potential difference and current caused by glucose (Text-figs. 3 and 4). This suggests that the secretory response to ACh originates in the crypts. This region of the mucosa was found to have a damaged appearance after cycloheximide administration although the villi were unchanged (Pl. 2*B*). This localization of cycloheximide action has been questioned as a result of the work of Frizzell, Nellans, Acheson & Schultz (1973). They found that the influxes of Na, Cl, alanine and 3-*O*-methyl-glucose across the brush border of rabbit ileum were significantly reduced by cycloheximide treatment. However, the conditions they used were different from those employed in the present study. The dose of cycloheximide was 20 mg/kg given 3 hr before experiment as compared with the dose of 12 mg/kg and the 2 hr period used here. The fact that the electrical response of the mucosa to glucose was not diminished under the conditions used in this study suggests that cycloheximide primarily damaged the crypts without having any observable effect on the villi. Thus the conclusion that the crypts of the jejunum represent the site of ACh action seems to be a valid one.

In the colon cycloheximide also inhibited the response to ACh (Text-figs. 3 and 4) and this was associated with histological damage to the crypt region (Pl. 3*B*). It would therefore appear that in this region of the intestine ACh also acts on the crypt cells.

The fact that the intestinal crypts represent the locus of the secretory response to ACh supports the work of Trier (1964) in human jejunum. He found that pilocarpine administration caused changes in the morphology of undifferentiated crypt cells which suggested that they produced a secretory response to cholinergic stimulation. ACh has also been shown to accelerate cell proliferation in the jejunal crypts of the rat (Tutton, 1975). These studies, together with the results of the present investigation, all point to the crypts as being the primary site of cholinergic action on the intestinal epithelium. However, whether ACh acts directly on the crypt cells or via some intermediate link has yet to be determined.

There seems to be in the small intestine and the colon a spatial separation of absorptive and secretory processes, a concept that has been advanced by Field (1976). Thus in the small intestine the absorption of nutrients is a function of the villi while in the colon the surface epithelium actively absorbs Na. On the other hand the crypts seem to be primarily concerned with the secretion of ions and water, a function which early physiologists had no hesitation in ascribing to this region of the intestinal epithelium (see Hendrix & Bayless, 1970). This may have several important clinical implications. In coeliac disease the normal balance between villous and crypt cells is altered so that the latter predominate. One of the symptoms is diarrhoea and this is associated with a net secretion of ions and water by perfused intestinal loops in patients with this disease (Fordtran, Rector, Locklear & Ewton, 1967). Similarly diarrhoea is observed in post-gastrectomy dumping syndrome (Smith & Jeffries, 1973). In this situation hypertonic solutions are rapidly introduced into the proximal jejunum and these could osmotically damage the villous cells. Ischaemia

of the intestine can also lead to diarrhoea. Thus any pathological condition that selectively damages the villi will reduce the absorptive capacity of the intestine while leaving its secretory ability unimpaired. This leads to a net loss of fluid into the gut lumen which can result in diarrhoea. The ability to control secretion in these circumstances may be useful in providing effective therapy for the treatment of at least some forms of diarrhoea.

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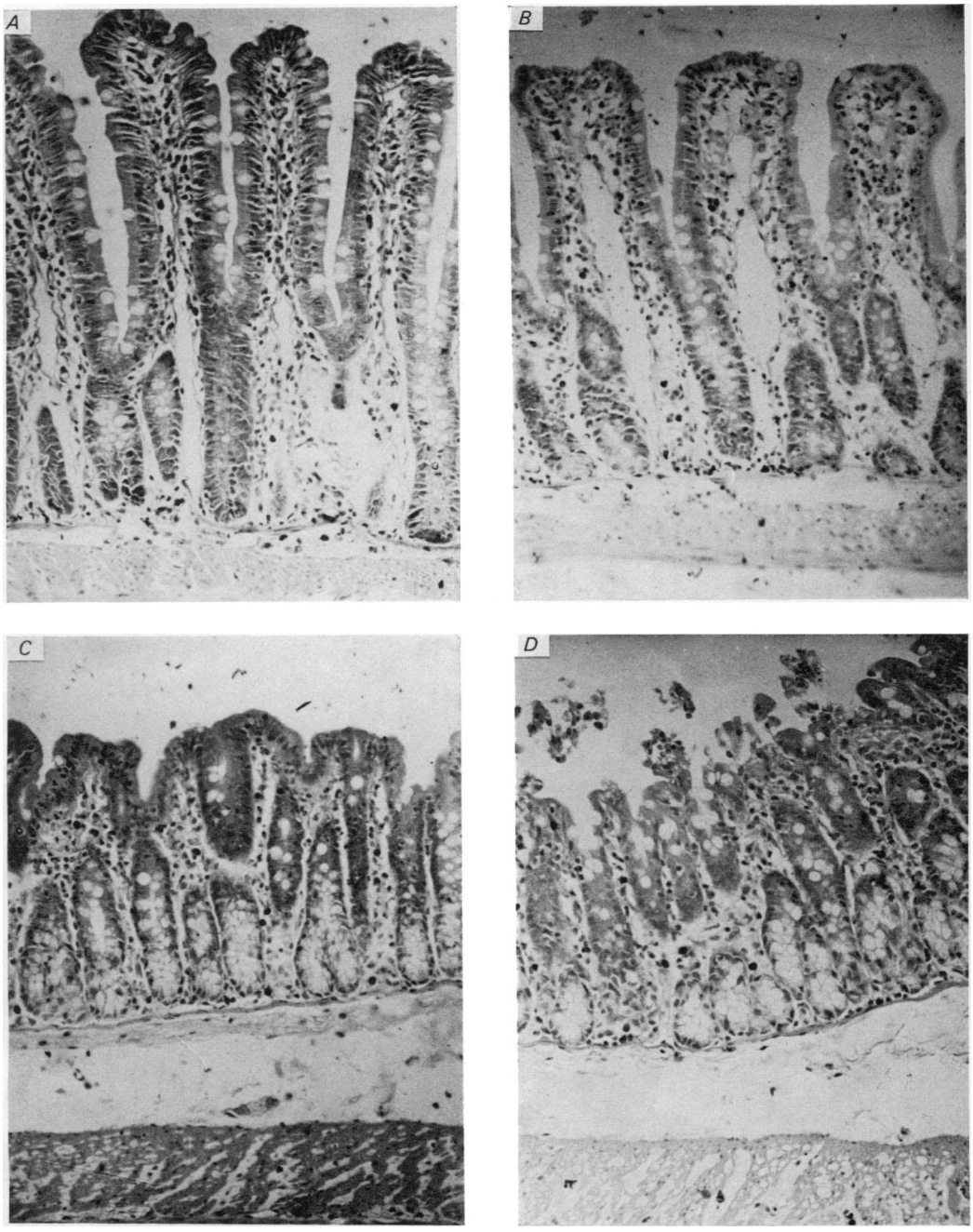
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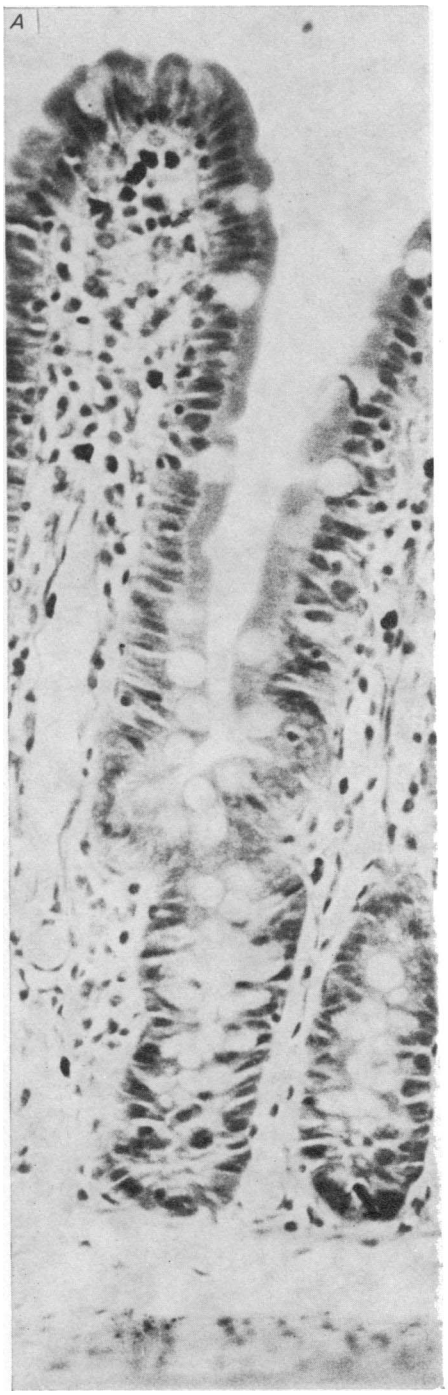

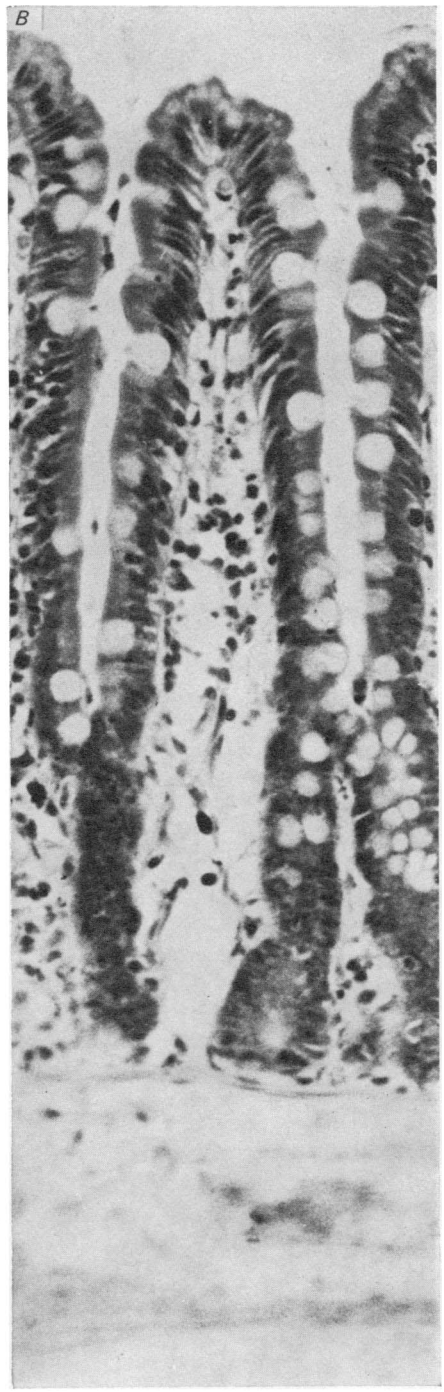

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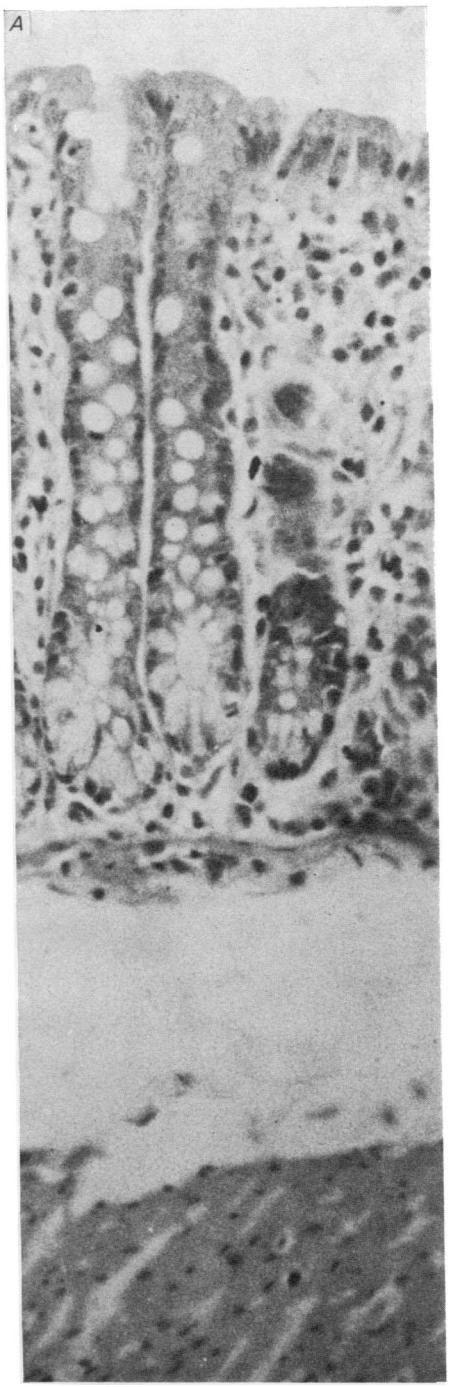
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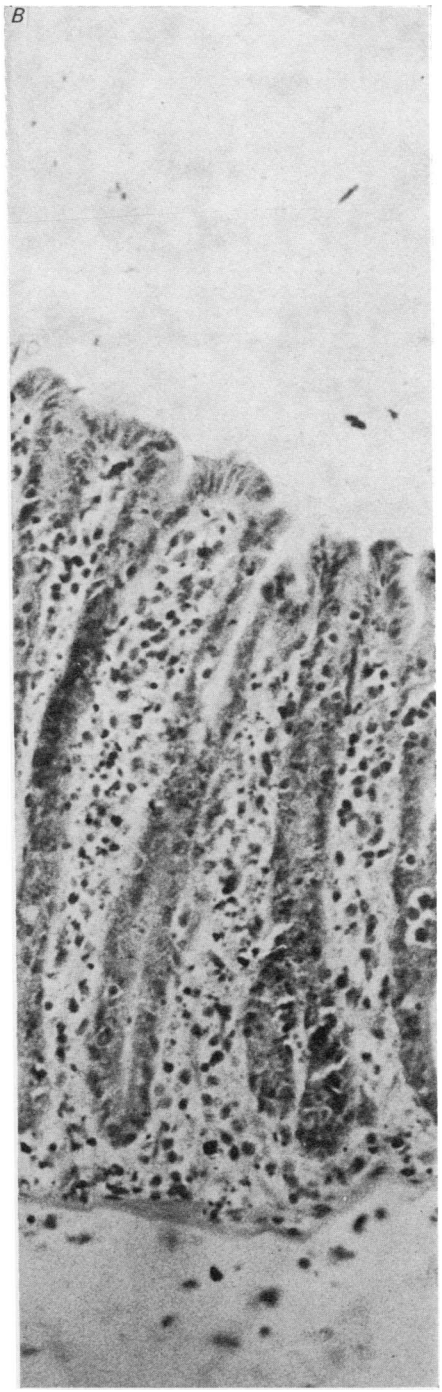
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EXPLANATION OF PLATES

PLATE 1

Effect of Na_2SO_4 treatment on the histological appearance of rat jejunum and colon. Intestinal segments were filled *in vivo* with 2 M-Na_2SO_4 (154 mM-saline in control animals) and after 30 min were removed and fixed in formal saline. They were then embedded in wax, cut into sections 5 μm thick and stained with haemotoxylin and eosin. Magnification × 200. *A*, control jejunum. *B*, Na_2SO_4-treated jejunum. *C*, control colon. *D*, Na_2SO_3-treated colon.

PLATE 2

Effect of cycloheximide treatment on the histological appearance of rat jejunum. Cycloheximide was administered intravenously at a dose of 12 mg/kg (an equivalent volume of 154 mM-NaCl was used in control animals) and after 2 hr segments of jejunum were removed and fixed in formal saline. They were then embedded in wax, cut into sections 5 μm thick and stained with haemotoxylin and eosin. Magnification × 400. *A*, control jejunum. *B*, cycloheximide-treated jejunum.

PLATE 3

Effect of cycloheximide treatment on the histological appearance of rat colon. Cycloheximide was administered intravenously at a dose of 12 mg/kg (an equivalent volume of 154 mM-NaCl was used in control animals) and after 2 hr segments of colon were removed and fixed in formal saline. They were then embedded in wax, cut into sections 5 μm thick and stained with haemotoxylin and eosin. Magnification × 400. *A*, control colon. *B*, cycloheximide-treated colon.