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Diarrhoea of famine and malnutrition - investigations using a rat model. 2 - Ileal hypersecretion induced by starvation

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Abstract

The effects of progressive starvation for up to three days on the basal and secretagogue stimulated secretory functions of the rat ileum were investigated *in vitro* and *in vivo*. The secretagogues used included agents acting *via* cyclic AMP (dibutyryl cyclic AMP, theophylline, forskolin, and PGE_2) and those acting *via* Ca^{++} (acetylcholine, bethanecol, carbachol, 5-hydroxytryptamine, and A23187). Starving rats for 24 h (day 1) had no effect on the basal electrogenic secretion (measured as the short circuit current, Isc μamps/cm^2) or on the stimulated maximum electrogenic secretion (measured as the ΔIsc where ΔIsc=maxIsc−basal Isc). By day 2 of starvation, however, both the basal Isc and the ΔIsc induced by all the secretagogues were significantly greater than in the fed and increased even more on day 3. Replacement of all the chloride ions and inhibition by furosemide indicated that the enhanced secretion was due mainly to chloride ions. Cholinergic stimulation was blocked by atropine, indicating the stimulation was *via* muscarinic receptors while cholinergic dose - ΔIsc response curves for fed and starved ilea showed significantly increased maximum electrogenic secretory response in the latter but no evidence of any change in the affinity (ED_{50}) of the receptors mediating the response. The basal secretion and the secretory response to acetylcholine in both fed and starved ilea was unaffected by tetrodotoxin, revealing that the enhanced secretory response could be expressed *via* the muscarinic receptors on the enterocytes without the enteric neural network. Measurement of ileal fluid movement *in vivo* showed that in fed and day 1 starved rats the basal, unstimulated 'tone' of the ileum was absorptive. On day 2, however, the basal 'tone' had reversed to one of secretion which increased further on day 3. Stimulation of fluid secretion *in vivo* by bethanecol, carbachol, or PGE_2 induced larger increases in the starved ilea by day 2 which increased even further on day 3. Lumenal chloride and bicarbonate concentrations were greater in the starved ileal fluid than in the fed. The studies in rat ileum confirm and extend those on rat jejunum and indicate that starvation creates a hypersensitive small bowel that responds to secretagogues and cholinergic neurotransmitters with a greatly enhanced secretory response.

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After 24 hours, continued starvation in the rat induces a secretory hypersensitivity of the jejunum to a variety of secretagogues and bacterial toxins.[1] The ileum is known to have absorptive and secretive properties that make it functionally distinct from the upper intestine. Compared with the jejunum, the ileum shows a greater glucose independent fluid transfer,[2] an ability to actively transfer bile salts[3,4] and vitamin B_{12},[5] an active bicarbonate secretion creating an alkaline lumenal pH,[5-8] a lower 'real' Km for valine and glucose absorption[9] and is the specific site for the storage and release of neurotensin, a gastrointestinal regulatory peptide.[10] Because of these differences, the secretory response of the ileum to progressive starvation was investigated *in vitro* and *in vivo*. The results showed that the response to progressive starvation had many similarities to those of the jejunum but there were a number of important differences. Various aspects of the work have been communicated to the Physiological Society[11,12] and the Nutrition Society.[13]

Methods

ANIMALS AND DIET

Male albino rats, weight 220-250 g, of the Sheffield strain were allowed *ad lib* water and food (Diet CRM, Labsure, London). In the experimental groups, water was given *ad lib* but the food was removed for periods of 24, 48, or 72 hours before the animals were used. The animals were housed in plastic cages with raised wire mesh bottoms to reduce coprophagy. The lights were on from 5 30 am until 6 30 pm and the humidity (72%) and temperature (20±1°C) were controlled. On the day of use, the animals were anaesthetised with ip pentobarbitone sodium (Sagatal, May and Baker; 60 mg/kg body weight). On achieving surgical anaesthesia, a midline incision was made and the ileum identified as the last 20 cm of the small intestine. A 5 cm segment of ileum was removed (some 15 cm from the ileocaecal junction) and cut open along the mesenteric border. It was mounted as a flat sheet and incubated in bicarbonate saline. The tissue potential difference (pd in mV), resistance (R on Ohms), and short circuit current (Isc in μamps) were obtained as described for the jejunum in the previous paper.[1] As with the jejunum, in a number of experiments the external smooth muscle of the ileum was removed by placing the intact segment onto a glass rod and carefully cutting along the mesenteric border with a blunted scalpel blade. The muscle layers could then be peeled away to leave a stripped segment which was mounted as a

flat sheet. After use, the stripped (or unstripped) tissue was removed and wet and dry weights measured.

Electrogenic secretory activity of the ileal sheets were obtained for the basal state and after addition of a variety of secretagogues as described previously.[1] In brief, these were the cholinergic agonists acetylcholine (as chloride or bromide), carbachol (carbamyl choline chloride), and bethanecol (carbamyl-β-methyl choline chloride). Theophylline (10 mmol/l) was added to both mucosal and serosal fluids in buffer that replaced the normal mucosal and serosal fluids. In the stripped ileum, prostaglandin E_2, 5-hydroxytryptamine, A23187, forskolin, and dibutyryl cyclic AMP were used as secretagogues. In some experiments tetrodotoxin, atropine, or furosemide were added to the serosal solution 10 minutes before the addition of the secretagogue acetylcholine.

As in the previous study[1] the index of electrogenic secretory activity used was the maximum change in the Isc induced by the secretagogue (ΔIsc) obtained by monitoring the Isc every 30 seconds throughout the action of the secretagogue and then subtracting the basal Isc (usually that immediately before the addition of the agent) from the calculated induced peak of the Isc – namely, ΔIsc=max Isc−basal Isc. This was then recalculated per cm^2 area of exposed tissue. In some cases the graphic area under the Isc-time response curve induced by the secretagogue, over and above that secreted in the basal state, was obtained as described previously.[1] This was used as an index of total charge secreted (net ion secretion).

In experiments investigating the ion responsible for the secretory currents induced by the secretagogues the chloride ions in the incubating bicarbonate buffer were replaced by isosmolar solutions of sodium, potassium, and calcium gluconate.[1]

IN VIVO MEASUREMENTS OF FLUID

Fluid movements were measured *in vivo* as described in the previous paper[1] for the jejunum except that 15–20 cm of the ileum were cannulated 8–10 cm from the ileocaecal junction.

Measurements of chloride in the lumenal fluids obtained from *in vivo* experiments were made using a Buchler digital chloridometer (G D Searle, New Jersey, USA) and for bicarbonate by Corning 178 pH blood gas analyser (Corning Medical and Scientific Glassworks, Medfield, Mass, USA).

MATERIALS

All chemicals were purchased from Sigma Chemical Company Ltd, Poole, England apart from the prostaglandin E_2 supplied by Upjohn Ltd, Crawley, England.

STATISTICAL ANALYSIS

All results are shown as the mean (SE). Statistical comparison were accomplished using the Student's unpaired *t* test with 0·05 as the level of significance. When multiple comparisons were needed the Kruskal-Wallis analysis of variance was used followed by Conover's multiple *t* test to identify specific differences.[14]

Results

EFFECTS OF PROGRESSIVE STARVATION ON STRUCTURAL AND BASAL ELECTRICAL PARAMETERS OF THE ILEUM (UNSTRIPPED)

The changes that take place in a number of structural and basal electrical parameters of the ileum (unstripped) during 3 days of starvation are shown in Table I. After three days of starvation, the fall in wet and dry weight was 45% ($p<0·01$) and 41% ($p<0·001$) respectively. There was now a large increase in the basal Isc both on an area (+82%, $p<0·001$) and 100 mg dry weight basis (+199%, $p<0·001$). The tissue resistance decreased by 31% ($p<0·001$) and there was a significant elevation in the basal pd (+31%, $p<0·001$).

Regression analysis was undertaken to determine if there were any correlations between the wet and dry weights of the unstripped sections of the ileum and their basal electrical parameters of Isc, pd, and tissue resistance. None were found, however, for any of the nutritional states.

ELECTROGENIC SECRETORY RESPONSES TO SECRETAGOGUES DURING PROGRESSIVE STARVATION FOR UP TO 72 HOURS

The electrogenic secretory responses of the unstripped ileum to acetylcholine, bethanecol, and theophylline in fed controls and during three days of progressive starvation are shown in Table II together with the effect of mucosal glucose (28 mmol/l). Statistically significant increases in the response to the bethanecol and theophylline, on a unit area basis, was not observed until the second day of starvation. Both responses were further enhanced on day 3 of starvation, bethanecol to 102% ($p<0·001$) and theophylline

TABLE I *Various structural and basal bioelectric parameters of the unstripped ileum in fed controls and during three days of progressive starvation*

	Fed	*Day 1 (24 h)*	*Day 2 (48 h)*	*Day 3 (72 h)*
n	13	11	10	29
Dry wt (mg/cm^2)	22 (1)	20 (2)	16 (1)†	13 (1)‡
Wet wt (mg/cm^2)	84 (2)	76 (4)	63 (2)‡	54 (2)‡
Basal Isc ($\mu A/cm^2$)	44 (6)	54 (5)	64 (6)*	80 (5)‡
Basal Isc (μA/100 mg dry wt)	206 (11)	270 (13)	412 (22)‡	617 (14)‡
Basal pd (mV)	2·9 (0·3)	3·1 (0·3)	3·0 (0·3)	3·8 (0·2)*
R ($ohms/cm^{-2}$)	67 (2)	58 (2)	48 (2)‡	47 (2)‡

Results are given as the mean (SE), n is the number of animals used. The levels of significance are *$p<0·05$, †$p<0·01$, ‡$p<0·001$) and refer to comparisons with the fed control group.

TABLE II *Effects of acetylcholine (1 mmol/l), bethanecol (1 mmol/l), theophylline (10 mmol/l), and glucose (28 mmol/l) on the Isc of unstripped sheets of ileum removed from fed control rats and rats starved for 24 h, 48 h, or 72 h*

	ΔIsc ($\mu A/cm^2$)			
	Acetylcholine	*Bethanecol*	*Theophylline*	*Glucose*
Fed	48 (3) [10]	56 (6) [13]	84 (6) [18]	95 (7) [23]
Day 1 (24 h starved)	50 (2) [6]	57 (8) [11]	90 (3) [10]	100 (6) [17]
Day 2 (48 h starved)	89 (5) [9]†	75 (6) [10]*	124 (4) [10]*	141 (5) [19]†
Day 3 (72 h starved)	104 (6) [11]†	113 (5) [29]†	137 (3) [24]†	177 (4) [40]†

Results given as the mean (SE). The levels of significance are *$p<0·01$, †$p<0·001$) and are given as superscripts and refer to comparisons with the fed control group. Mean number of animals in square brackets.

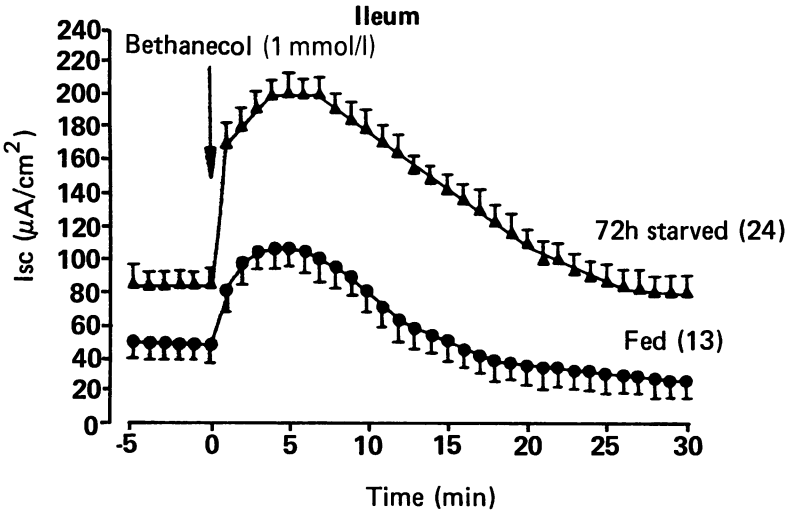

Figure 1: Increase in Isc (ΔIsc) induced across unstripped ilea from fed and 72 h starved rats by addition of 1 mM bethanecol to the serosal solution. The Isc is calculated on a unit serosal area basis. Results are plotted as mean (SE) with the number of animals in brackets.

to 63% ($p<0.001$). If the data were placed on a 100 mg dry weight basis the responses for day 2 (bethanecol +106%, $p<0.001$; theophylline +117%, $p<0.001$) and day 3 of starvation (bethanecol +216%, $p<0.001$; theophylline +299%, $p<0.001$) became greatly enhanced. The response to serosal acetylcholine (1 mmol) on day 3 was 117% greater ($p<0.001$) than the fed ilea on an area basis. The addition of glucose (28 mmol) to the mucosal fluid did not give a significant increase until day 2 (+48%, $p<0.001$) and an 86% increase in the induced absorptive current on day 3 ($p<0.001$).

TIME COURSES OF CHANGE IN ISC INDUCED BY SECRETAGOGUES

The time course of the changes in the Isc in fed and 3 day starved ilea (unstripped) are shown in Figure 1 for the serosal addition of bethanecol and in Figure 2 for the addition of theophylline (10 mmol/l) to the mucosal and serosal solutions. The greater secretory responses of the Isc in the starved ilea are obvious for both secretagogues in relation to greater peak heights (ΔIsc max) and also in regard to the duration of response. As described in the methods, the area under the curve (that above the basal level) represents the total charge secreted. The increase in total charge secreted after bethanecol in starved (day 3) compared with fed ilea was 319% ($p<0.001$) and in the case of theophylline challenge it was 403% ($p<0.001$).

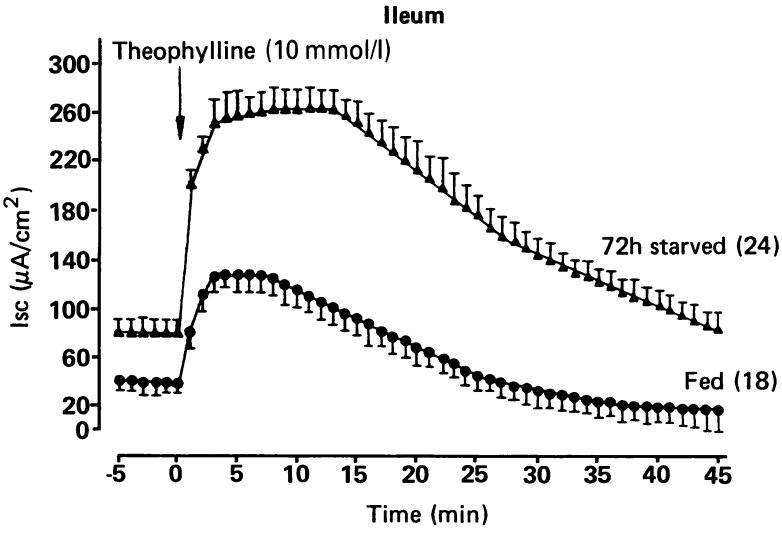

Figure 2: Time course of the response of the unstripped ileal Isc from fed and 72 h starved rats to the mucosal and serosal addition of theophylline (10 mmol/l). Note increased basal Isc of starved ilea compared to fed before addition. Results are plotted as mean (SE) with number of rats in brackets.

ACTION OF VARIOUS SECRETAGOGUES ON STRIPPED ILEA FROM FED AND PROGRESSIVELY STARVED RATS

The basal Isc, pd, and tissue resistance of fed and ilea from day 1, 2, and 3 of starvation, stripped of their external muscle, are given in Table III on a unit area basis and a 100 mg dry mucosal weight. There was little difference between the basal Isc, pd, and tissue resistance between the unstripped (Table I) and stripped ilea (Table III).

The effects of bethanecol, acetylcholine, and theophylline on the Isc of stripped ileum during day 1 to day 3 of the progressive starvation are given in Table IV. No significant changes were observed for the three secretagogues after 24 hours of starvation but after starvation for 48 hours (day 2), the secretagogue challenges gave much greater electrogenic secretory responses in the fasted ileal compared with the fed. The increases were even greater after 72 hours of starvation. Calculating the area under the Isc secretory curve on day 3 of starvation revealed the very large increase in the net charge secreted by the fasted ilea compared with the fed for bethanecol (+301%, $p<0.001$), acetylcholine (+331%, $p<0.001$), and theophylline (+443%, $p<0.001$).

The action of glucose (28 mmol/l) in generating absorptive Isc over the three days of starvation is also shown in Table IV. As with the secretory currents, no significant change in the glucose induced Isc, compared with the fed ilea, was apparent until day 2 when there was a 50% increase ($p<0.001$). The glucose induced Isc was increased even further on day 3 of starvation (+88%, $p<0.001$) compared with the fed controls. It is interesting to note that the glucose induced increases in Isc in stripped ilea are always very much greater than those obtained in the unstripped ilea (Table II) whether for fed controls (+51%), or on day 1 (+54%), day 2 (+52%), or day 3 (+52%) of starvation. The increases, however, are practically identical suggesting a common cause for the effect, probably the more efficient oxygenation of deep mucosal and submucosal tissues.

The effects of various secretagogues on the electrogenic secretion in fed and three day starved ilea (stripped) are listed in Table V. Two groups of secretagogues were used, the first act by raising enterocyte cyclic AMP either directly (addition of dibutyryl cyclic AMP) or indirectly by stimulating adenyl cyclase (forskolin) or inhibiting phosphodiesterase (theophylline) or through PGE_2 addition. The proposed action of the second group is by raising intracellular calcium either directly by the action of an inophore (A23187) or indirectly *via* activation of serosal membrane receptors (acetylcholine, bethanecol, 5-hydroxy-tryptamine). The secretagogues of both groups all gave significant increases in electrogenic secretion (ΔIsc) in the starved compared to the fed ilea. The responses of both the fed and the three day starved ilea to the various secretagogues were much greater in

TABLE III *Effects of progressive starvation for up to 72 h on the basal electrical parameters of stripped sheets of ileum*

	Fed	*Day 1 (24 h)*	*Day 2 (48 h)*	*Day 3 (72 h)*
n	24	18	18	36
Basal Isc (μA/cm²)	47 (3)	48 (4)	68 (2)*	85 (4)†
Basal Isc (μA/100 mg dry wt)	235 (19)	253 (12)	447 (23)†	708 (21)†
Basal pd (mV)	2·8 (0·1)	2·9 (0·1)	3·5 (0·2)†	3·6 (0·1)†
R (ohms/cm⁻²)	60 (2)	62 (3)	51 (2)*	41 (3)†

Results given as the mean (SE). The levels of significance are given as superscripts (*p<0·01, †p<0·001) and refer to comparisons with the fed control group.

TABLE IV *Actions of bethanecol (1 mmol/l), acetylcholine (1 mmol/l), and theophylline (10 mmol/l) on electrogenic secretion (ΔIsc) of stripped sheets of ileum removed from fed control rats and rats starved for 24 h, 48 h, and 72 h. Glucose (28 mmol/l) was added to the mucosal solution after the secretory Isc changes induced by the serosal addition of bethanecol and acetylcholine had returned to unstimulated levels*

	ΔIsc (μA/cm²)			
	Bethanecol	*Acetylcholine*	*Theophylline*	*Glucose*
Fed	140 (10) [9]	142 (5) [19]	137 (8) [9]	143 (3) [28]
Day 1 (24 h starved)	143 (7) [11]	151 (6) [19]	141 (7) [11]	154 (4) [30]
Day 2 (48 h starved)	201 (4) [9]*	216 (5) [11]*	219 (13) [12]*	214 (3) [20]†
Day 3 (72 h starved)	253 (6) [9]†	232 (13) [22]†	276 (16) [9]†	269 (3) [31]†

Results given as the mean (SE) with the number of animals used in square brackets. The statistical comparisons with the fed controls are given as superscripts *p<0·01, †p<0·001).

TABLE V *Action of various secretagogues on the electrogenic secretion (ΔIsc) of stripped sheets of ileum from fed and day 3 (72 h starved) rats*

	ΔIsc (μA/cm²)			
Secretagogue	*Fed*	*Day 3 (72 h starved)*	*% increase*	*p*
db-cAMP (1 mmol/l)	123 (5) [15]	183 (9) [14]	41	<0·001
PGE_2 (3 μmol/l)	59 (6) [9]	79 (13) [7]	34	<0·01
Forskolin (5 μmol/l)	64 (3) [16]	102 (6) [16]	62	<0·001
Theophylline (10 mmol/l)	134 (8) [9]	276 (6) [9]	106	<0·001
Acetylcholine (1 mmol/l)	142 (5) [19]	232 (13) [22]	64	<0·001
Bethanecol (10 mmol/l)	75 (5) [9]	140 (4) [9]	87	<0·001
5-HT (50 μmol/l)	23 (2) [18]	47 (3) [19]	104	<0·001
A23187 (5 μmol/l)	39 (3) [9]	78 (3) [9]	100	<0·001

Results are shown as the mean (SE) with the number of animals used in square brackets. Statistical comparison is of the starved against the fed controls.

TABLE VI *Effects of atropine, tetrodotoxin and furosemide on the responses (ΔIsc) to acetylcholine of stripped sheets of ileum from fed and 72 h starved animals*

	ΔIsc (μA/cm²)	
	Fed	*Day 3 (72 h starved)*
Acetylcholine (1 mmol/l)	142 (5) [19][a]	232 (13) [22][b]
Acetylcholine (1 mmol/l)+atropine (100 μmol/l)	31 (1) [9][c]	28 (2) [9][d]
Acetylcholine (1 mmol/l)+tetrodotoxin (10 μmol/l)	137 (17) [4][e]	227 (21) [5][f]
Acetylcholine (1 mmol/l)+furosemide (1 mmol/l)	32 (3) [9][g]	53 (4) [9][h]

The superscripts facilitate the multiple statistical comparisons using the Kruskal-Wallis ANOVA followed by Conover's multiple *t* test to identify the appropriate results which are statistically significant (a *v* b, p<0·001; e *v* f, p<0·001; g *v* h, p<0·01). Results are given as the mean (SD) with the number of animals used shown in square brackets.

the stripped preparation (Table V) compared with the unstripped (Table II). This is probably because of better oxygenation of the mucosa/crypts from the serosal side and easier diffusional access to the bioactive sites for the secretagogues added from the serosal side.

ACTION OF VARIOUS ANTAGONISTS IN STRIPPED ILEA

A number of experiments using the specific muscarinic blocker atropine and the specific neural blocker tetrodotoxin were undertaken to assess whether the addition of acetylcholine to the serosa of fed and fasted ilea (stripped preparations) acted directly on the enterocyte cells *via* their muscarinic receptors or whether it had any significant activity *via* nicotinic receptors present in the enteric mucosal network (Table VI). Addition of atropine (100 μmol) to the serosal buffer (10 minutes before the addition of acetylcholine) caused dramatic decreases in the electrogenic secretion elicited by the acetylcholine. The same serosal dose of atropine had no significant action *per se* on the basal Isc in either the fed or starved ilea indicating that *in vitro*, cholinergic muscarinic innervation had scant influence on basal secretory 'tone'. Confirmation of the lack of neural influence was shown by the addition of tetrodotoxin to the serosal solution some 10 minutes before acetylcholine which also had no effect on the basal Isc nor on the secretory Isc induced by acetylcholine (Table VI).

In Table VI there are also the results of experiments utilising the addition of serosal furosemide to the solution 10 minutes before the addition of the acetylcholine. Furosemide caused a severe inhibition of the electrogenic response in both fed (−77%, p<0·001) and starved ilea (−77%, p<0·001), but the residual ΔIsc in the starved ilea was still significantly greater (+66%, p<0·01) indicating that ions other than chloride were involved in the hypersecretion. Addition of glucose to the mucosal buffers bathing the ilea after treatment with furosemide still induced increases in the Isc not significantly different from those in control tissues without furosemide indicating that this inhibitor of chloride secretion did not cause a non-specific inhibition of the enterocyte's function but was specific for their secretory response. The result with furosemide strongly suggests that most, but not all, of the secretory current elicited by the cholinergic stimulus is chloride secretion.

TABLE VII *Effects of the replacement of chloride ions by gluconate on basal, unstimulated Isc in fed and day 3 (72 h) starved ilea (unstripped)*

	Chloride	*No chloride*	*% decrease*	*p*
Fed	44 (6) [13]	19 (2) [45]	57	<0·01
Day 3 (72 h starved)	80 (5) [29]	17 (3) [45]	79	<0·001

Results are shown as the mean (SE) with the number of animals used in square brackets. Statistical comparison is with the fed.

EFFECTS OF CHLORIDE REPLACEMENT ON (UNSTRIPPED) ILEAL BASAL ISC AND SECRETORY CURRENTS ELICITED BY ACETYLCHOLINE

In a series of experiments conducted with unstripped ilea from fed and three day (72 h starved) rats the effects of replacement of chloride ions by gluconate in the incubating buffer on the basal Isc (Table VII) and the secretory Isc elicited by acetyl choline bromide were assessed (Fig 3). The absence of chloride reduced the basal Isc in both the fed and starved ilea but the fall was significantly greater in the starved ileum (p<0·001) making its basal Isc equal to that of the fed (Table VII).

The secretory currents elicited by acetylcholine bromide were significantly reduced over the whole range of doses used by approximately 75 to 85% for the fed and by 70 to 83% for the starved ilea (Fig 3). At each dose used, however, the response of the starved ilea in the total absence of chloride was always greater than that of the fed ilea indicating that even in the absence

TABLE VIII *Effect of progressive starvation on ileal fluid movement* in vivo *in the basal state and after bethanecol (60 μg/kg bwt ip). Net absorption is negative, net secretion is positive*

	μg fluid/min/cm	
	Basal	*Bethanecol*
Fed	−750 (27) [9][a]	+979 (46) [7][b]
Day 1 (24 h starved)	−663 (36) [9][c]	+1097 (39) [8][d]
Day 2 (48 h starved)	+197 (36) [8][e]	+1390 (43) [9][f]
Day 3 (72 h starved)	+795 (49) [11][g]	+3200 (97) [13][h]

Results are shown as mean (SE) with number of animals given in square brackets. Superscripts are used to facilitate statistical comparisons undertaken using the Kruskal-Wallis ANOVA and Conover's multiple *t* test to identify specific significant differences. a *v* b, $p<0.001$; a *v* e, $p<0.001$; a *v* g, $p<0.001$; e *v* g, $p<0.001$; c *v* d, $p<0.001$; b *v* f, $p<0.01$; c *v* e, $p<0.001$; b *v* h, $p<0.001$; e *v* f, $p<0.001$; d *v* f, $p<0.05$; g *v* h, $p<0.001$; f *v* h, $p<0.001$.

TABLE IX *Ileal fluid movements in fed and day 3 starved (72 h) rats in the basal unstimulated state and stimulated by carbachol (60 μg/kg bwt) or PGE_2 (10 μg/kg bwt)*

	μg fluid/min/cm		
	Basal	*Carbachol*	*PGE_2*
Fed	−695 (11) [9]	+839 (21) [10]	+697 (12) [10]
Day 3 (72 h starved)	+776 (13) [9]*	+2999 (27) [9]*	+1179 (13) [10]*

Results are shown as mean (SE) with number of animals used in square brackets. Net secretion is shown as positive values and net absorption as negative values (*$p<0.001$ compared against fed controls).

of chloride the starved ilea secreted more than the fed. The increased secretory currents in the absence of chloride in the starved ilea must be due to the electrogenic transport of another ion or ions. What species these are is as yet unknown.

DOSE RESPONSE CURVE FOR BETHANECOL IN FED AND STARVED ILEA (UNSTRIPPED)

The ΔIsc dose-response to various concentrations of bethanecol are shown in Figure 4 for fed and three day starved ilea (unstripped). The increase in electrogenic secretion (ΔIsc) for the starved ilea are significantly greater than the fed responses over the range of concentrations used. At 1 and 10 μmol/l, however, the ΔIsc induced in both fed and starved intestine were transient with a duration of less than seven minutes. Above 10 μmol/l, the duration of the response of the starved ilea was usually double that of the fed. Examination of the Figure shows that there is an unequivocal increase in the maximum Isc induced by bethanecol in the starved compared with the fed ileum but the mean ED_{50} (effective dose, 50%) for the fed (69 μmol/l) and the starved (61 μmol/l) ilea were not substantially different. This indicates that starvation does not induce a change in the affinity of the muscarinic site for the muscarinic agonist bethanecol.

FLUID AND ELECTROLYTE MOVEMENT IN FED AND STARVED ILEA IN VIVO

The effect of progressive starvation for three days on ileal fluid movements was investigated *in vivo* in the basal, unstimulated state and when bethanecol (60 μg/kg bwt ip) was used to stimulate fluid secretion (Table VIII). In the fed, unstimulated ileum there was a clear cut absorption of fluid which was unchanged after the first

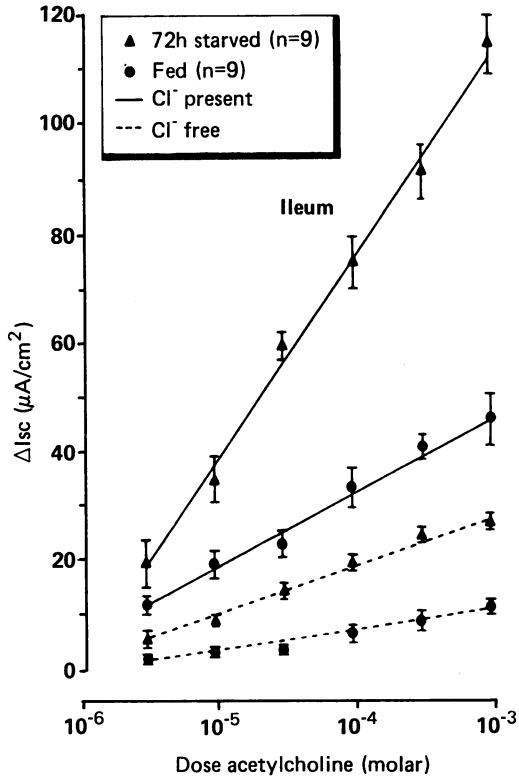

Figure 3: Responses of ΔIsc at different doses of serosal acetylcholine in unstripped ilea from fed and 72 h starved rats in the presence (solid line) and absence (dotted line) of chloride ions using gluconate as replacement solute. Results are plotted as the mean (SE) with the number of rats in brackets.

day of starvation. By the second day of starvation the unstimulated ileum showed a significant secretion of fluid (a *versus* e, $p<0.001$) compared with the fed ileum. This secretion was dramatically increased on day 3 of starvation (+303%, e *versus* g, $p<0.001$). With bethanecol stimulation there was no significant difference between that

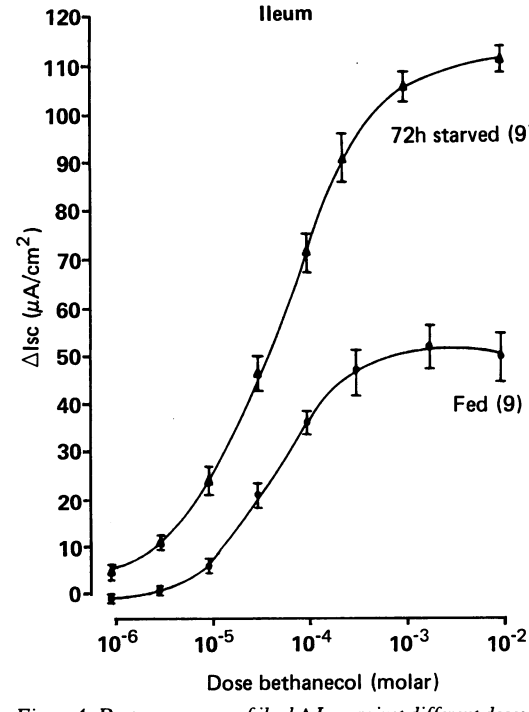

Figure 4: Response curves of ileal ΔIsc against different doses of serosal bethanecol in fed and 72 h starved rats. Results are plotted as the mean (SE) with the number of animals in brackets.

evoked in the fed and the day 1 starved ilea. On day 2, however, the secretion elicited was significantly increased (+42%, b *versus* f, $p<0.01$). A further, much larger increase was observed on day 3 (+130%, f *versus* h, $p<0.001$) compared with day 2.

Fluid movements were measured in the ilea of fed and three day starved rats on an absolute μg fluid/cm/min basis in the basal state and when the animals were injected with either ip carbachol or PGE_2 (Table IX).

Starvation for 72 h (day 3) dramatically reversed the normal basal absorptive tone monitored in the fed ilea to one of frank secretion. Furthermore, both secretagogues not only caused the basal absorptive tone measured in the fed ileum to be converted to secretion, but also induced a much greater secretion in the day 3 starved ilea compared with those in the fed rats (carbachol +125%, $p<0.001$; PGE_2 +83%, $p<0.001$). As the three day starvation period causes significant decreases in both the wet and dry weights of the ileum on a unit length basis (wet −23%, $p<0.001$; dry −29%, $p<0.001$) conversion of the fluid movements from a unit length basis to a unit tissue weight basis (mg fluid/g tissue weight/min) enhances greatly the values of the increases in secretion in the starved compared with the fed ilea (carbachol +573%, $p<0.001$; PGE_2 +411%, $p<0.001$).

The concentration of chloride in the lumenal fluid (0·9% NaCl) instilled into the ileal loops in the fed and three day starved ilea and at the end of the 20 minute basal unstimulated or secretagogue stimulated periods are shown in Table X. Under basal or unstimulated conditions there is net absorption of chloride (decreased chloride concentration in the lumenal fluid) of the fed ilea but there was a highly significant ($p<0.001$) net chloride secretion (gain in chloride concentration in the lumenal fluid) of the starved ilea. After secretagogue stimulation, there was an increased chloride concentration in both fed and starved ilea but the values for the latter were all significantly higher than the former (bethanecol +6%, $p<0.001$; carbachol +6%, $p<0.001$; PGE_2 +6%, $p<0.001$). Thus, in respect to ileal chloride ion movements starvation for three days reversed the basal chloride absorption to secretion and brought about a significant increase in chloride concentration after secretagogue challenge.

Concentrations of bicarbonate were similarly monitored in the instilled ileal fluid (0·9% NaCl), in the final ileal lumenal fluid (collected anaerobically) and in blood obtained by heart puncture in unstimulated and secretagogue injected fed and three day starved rats (Table XI). The concentration of bicarbonate in the 0·9% NaCl never exceeded 0·1 mmol/l and was thus assumed to be negligible while that of the blood was 22·9 (0·4) mmol/l (n=77). Under basal, unstimulated conditions there was an apparent lumenal alkalisation equivalent to a net movement of bicarbonate into the lumenal fluid both in the fed and three day starved ilea but this was significantly greater in the starved condition (+57%, $p<0.001$). After secretagogue challenge there was an apparent increase in the lumen bicarbonate concentration but again this was significantly greater in the starved ilea compared with the fed (bethanecol +40%, $p<0.001$; carbachol +49%, $p<0.001$; PGE_2 +43%, $p<0.001$). If the concentration data were converted to the total amount of bicarbonate in the lumenal fluid there was still a greater amount present in the lumenal fluid of the starved rat under basal conditions (+105%, $p<0.001$) and following secretagogue stimulation (bethanecol +121%, $p<0.001$; carbachol +131%, $p<0.001$; PGE_2 +89%, $p<0.001$).

Thus starvation for three days changes the basal 'tone' of the ileum from an absorptive one to a secretive one and produces a lumenal fluid with a higher concentration of chloride and bicarbonate while secretagogue stimulation gives a greater fluid secretion and lumenal amounts or concentrations of chloride and bicarbonate.

TABLE X *Final concentrations of chloride (mmol/l) of ileal lumenal fluid in fed and three day starved rats in basal and secretagogue-stimulated states (bethanecol, 60 μg/kg bwt; carbachol, 55 μg/kg bwt; PGE_2 10 μg/kg bwt) after instillation of 0·9% NaCl for 20 min*

	Chloride concentration (mmol/l)			
	Basal	*Bethanecol*	*Carbachol*	*PGE_2*
Initial fluid	151·5 (0·4) [77][a]	151·5 (0·4) [77][a]	151·5 (0·4) [77][a]	151·5 (0·4) [77][a]
Fed	140·2 (0·8) [10][b]	161·7 (0·5) [8][c]	162·3 (0·9) [10][d]	159·5 (0·7) [10][e]
Day 3 (72 h starved)	160 (1) [8][f]	170·1 (0·4) [11][g]	171·3 (0·4) [10][h]	169·7 (0·7) [10][i]

Results are shown as mean (SE) with number of animals given in square brackets. Superscripts are used to facilitate comparisons using the Kruskal-Wallis ANOVA and Conover's multiple *t* test to identify specific significant differences. a v b, $p<0.01$; a v c, $p<0.001$; a v d, $p<0.001$; a v e, $p<0.01$; a v f, $p<0.001$; a v g, $p<0.001$; a v h, $p<0.001$; a v i, $p<0.001$; b v f, $p<0.001$; b v c, $p<0.001$; b v d, $p<0.001$; b v e, $p<0.001$; c v g, $p<0.001$; d v h, $p<0.001$; e v i, $p<0.001$; f v g, $p<0.001$; f v h, $p<0.001$; f v i, $p<0.001$.

TABLE XI *Final concentrations of bicarbonate (mmol/l) (measured as lumenal alkalinisation) in lumenal fluid of ilea in fed and three day starved rats in basal and secretagogue stimulated states (bethanecol and carbachol, 60 μg/kg bwt and PGE_2 10 μg/kg bwt)*

	Final lumenal bicarbonate concentration (mmol/l)			
	Basal	*Bethanecol*	*Carbachol*	*PGE_2*
Fed	2·1 (0·4) [10][a]	4·2 (0·2) [8][b]	4·1 (0·3) [10][c]	3·7 (0·4) [10][d]
Day 3 (72 h starved)	3·3 (0·1) [8][e]	5·9 (0·1) [11][f]	6·1 (0·2) [10][g]	5·3 (0·2) [10][h]

Results are shown as mean (SE) with number of animals given in square brackets. Superscripts are used to facilitate comparisons using the Kruskal-Wallis ANOVA and Conover's multiple *t* test to identify specific significant differences. a v e, $p<0.001$; a v b, $p<0.001$; a v f, $p<0.001$; a v c, $p<0.001$; c v g, $p<0.001$; a v d, $p<0.001$; e v f, $p<0.001$; e v g, $p<0.001$; e v h, $p<0.001$.

Discussion

Starvation for longer than 24 hours induced the ileum of the rat to exhibit a hypersensitivity to a variety of secretagogues that elicit electrogenic secretion *in vitro*. Progressive increases in the duration of the starvation up to 72 hours not only caused increases in the maximal response of the ileum to secretagogue stimulation compared to the fed condition, but also in its duration. Thus the net charge transfers secreted by starved ilea were significantly greater than those of the fed ilea. These increases in secretory response were normalised on a unit serosal area basis. As there was a significant decrease in the weight (wet or dry) of the ileum on day 2 and day 3 of starvation, calculating the secretory responses on a weight basis creates even larger responses compared with the fed ilea. Thus a reduction in enterocytes increases the secretory output of those remaining, strongly suggesting that either a greater proportion of the ileal enterocyte population in the starved animal is involved in the secretory response and/or the enterocytes are more effec-

tive in secreting ions and fluid than those in the fed ileum. In this respect the ileum behaves in a manner 'identical to that of the jejunum'. The ileum, however, also shows a behaviour during starvation not observed in the jejunum. On day 2 (48 h) of starvation, the ileal basal Isc or electrogenic ion transfer is significantly greater than that of the fed on either an area or weight basis. The experiments with chloride replacement showed that this basal Isc in the fed ilea was reduced by 57%. This reduction in the basal Isc in a chloride free gluconate medium agrees remarkably well with a previously found 70% reduction of fed jejunal Isc in chloride-free sulphate medium[15] and with our previous data on the jejunum.[1] The basal Isc of the three day starved ileum fell by 79%, in chloride free media, indicating that most of its basal electrogenic ion transfer was chloride secretion. It is clear that on day 2 and day 3 of starvation the ileum exhibits an increased unstimulated or basal electrogenic chloride secretion. Interestingly, even in the absence of chloride, the secretory ΔIsc of the starved ileum induced by acetylcholine were still significantly greater than those of the fed, indicating an electrogenic movement of ion(s) other than chloride were involved. What are the possible species of ions that could be the basis of this enhanced secretory current observed in the absence of chloride? From the experiments conducted *in vivo* it appears that there was an increased movement of bicarbonate ions into the lumenal fluid of the starved ilea in both the basal state and after secretagogue stimulation. Thus starvation for two or three days induces increased chloride secretion *in vitro* and *in vivo* and bicarbonate (measured *in vivo*). It is possible that this bicarbonate secretion is electrogenic and that it represents the larger secretory currents in the starved ilea observed when chloride is removed. Previous *in vivo* work with ileal secretion in fed rats has shown that bicarbonate secretion only takes place in the presence of lumenal chloride.[6] These *in vivo* experiments, however, did not explore the role of electrogenic bicarbonate secretion.[8] It is interesting to note that the loss of bicarbonate in the stool is a prominent feature of diarrhoeal disease[16] and that in experimental cholera in the rabbit the predominant ileal secretion is sodium bicarbonate.[17]

The increased capacity of the stripped ilea to respond to both serosal secretagogues and mucosal glucose was obvious whether the ileum came from fed controls or from ilea removed from rats starved for one, two, or three days. Neither the resistance of the unstripped and stripped intestine nor the basal unstimulated Isc are significantly different either in the fed or during the starvation period. Thus the mechanism(s) that enhanced the secretory and absorptive currents had no influence on these two bioelectric parameters. It is likely that the enhancement of the secretory and absorptive currents is the result of better oxygenation of the deep mucosa and submucosal tissues that can be affected by removing the thick muscle coats.[18] In the case of the secretagogues added to the serosal solution there is also the possibility that there is an increased bioavailability. Stripping muscle layers has been shown to allow lower doses of serosal acetylcholine to stimulate secretion in rat,[19] but stripping guinea pig ileum did not change histamine's action on the Isc.[20] Obviously the stripped rat preparation is more sensitive to bioactive agents and should be the preparation of choice but it must be used with some caution as the stripping may remove or damage neural innervation that can effect epithelial function.

As in the jejunum,[1] the increased ileal secretory current observed on day 2 and day 3 of starvation can be induced by secretagogues known to act by raising the cyclic AMP levels (theophylline, PGE_2, forskolin, db-cAMP) and those presumed to act by raising intracellular calcium (acetylcholine, bethanecol, 5-HT, and A23187). Acetylcholine increased electrogenic secretion on day 3 of starvation even in the presence of tetrodotoxin, indicating that the enhanced secretion could occur independently of neural innervation, as reported for the jejunum.[1] Atropine severely reduced the action of acetylcholine both in fed and starved ilea to the same level indicating that the cholinergic effect was mediated through activation of muscarinic rather than nicotinic receptors.

The bethanecol dose response curves in fed and three day starved ilea showed a clear cut maximum increase in the ΔIsc for the starved ilea but no apparent change in the mean ED_{50}. Thus starvation is unlikely to cause the enhanced secretion by changing the affinity of the enterocyte's muscarinic receptors[21] but until binding or autoradiographic studies are undertaken we cannot say whether there is an increase in their numbers on the cell.

The fluid movements in the ileum measured *in vivo* showed strong similarities with the electrogenic secretion data obtained *in vitro*. In the fed animals and those on day 1 of starvation net fluid movement in the unstimulated state was clearly absorptive. On day 2 and 3 of starvation, however, net fluid movement became increasingly secretive, matching the increasing basal Isc observed *in vitro*. Bethanecol, when given *in vivo*, induced fluid secretion in fed controls that was not significantly different from that on day 1 of starvation, a result identical to the *in vitro* electrogenic data where the bethanecol secretory currents induced on day 1 (ΔIsc's) were not significantly increased compared with those of the fed. By day 2 of starvation, however, bethanecol induced a large increase in fluid secretion which was even greater on day 3, both results again matching the *in vitro* electrogenic secretion data for bethanecol on days 2 and 3. On day 3, carbachol and PGE_2 when given *in vivo*, said produced very large increases in fluid secretion matching their increased electrogenic secretion *in vitro* on day 3. Thus in the ileum, as in the jejunum,[1] secretagogue induced changes in electrogenic secretion measured *in vitro* are an excellent index of secretagogue-induced fluid secretion *in vivo*. The difference between the ileum and jejunum, however, is that in the ileum even the basal electrogenic current measured *in vitro* act as indices of basal fluid secretion *in vivo*. The enhanced secretion of fluid *in vivo* on day 3 of starvation was concomittant with significant increases in the concentration of bicarbonate and

chloride ions both in the basal, unstimulated state and after secretion was stimulated by bethanecol, carbachol, or PGE_2.

All the available experimental evidence is consonant with the concept that starvation induces in the ileum a basal, hypersecretion of chloride, fluid and bicarbonate after three days of starvation. Starvation, for 24–48 hours, also hypersensitises the ileum to the action of secretagogues that act putatively either *via* cyclic AMP or Ca ions, creating increased electrogenic chloride ion secretion *in vitro* and increased fluid, bicarbonate and chloride ion secretion *in vivo*. Other observations have shown that *Escherichia coli* STa enterotoxin, when applied to the mucosal fluid *in vitro*, induces a greatly enhanced electrogenic secretory response in day 3 starved compared with fed ilea.[22] Similar results have been observed with staphylococcus aureus enterotoxin B.[23]

The studies in rat ileum, as with those of the previous paper with the jejunum,[1] strongly suggest that starvation creates a hypersensitive small bowel that responds to cholinergic neurotransmitters, secretagogues, and bacterial enterotoxins with an enhanced secretory response and thus point to a possible mechanism for the phenomenon of famine[24–26] or malnutrition[27 28] diarrhoea observed in man.

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