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*EFFECTS OF DIETARY DEPRIVATION ON SMALL AND LARGE INTESTINAL ION TRANSPORT IN THE MOUSE**

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Farede, açlığın ince ve kalın bağırsaktaki iyon transferi üzerine etkileri

Özet: *Bu çalışmanın amacı, farede beslenme düzeylerinin ince ve kalın bağırsağın salgılatıcı fonksiyonları üzerine etkilerini araştırmaktır.*

Araştırmada 20-40 gr. ağırlığında yetişkin, "swiss", erkek fareler (yaklaşık olarak 700 adet) kullanıldı. Fareler 48 saat aç bırakıldı ve 48 saat açlıktan sonra tekrar yem verildi Farelere ayrıca 4 gün için günlük yem tüketimlerinin %50'si (3 gr/her fare/her gün) verildi. Farelerin ince (medial jejunum ve proksimal ileum) ve kalın bağırsağından (proksimal, medial ve distal kolon) elde edilen veriler değerlendirildi.

Medial jejunumda ve proksimal ileumda potansiyel farklılık (P.D.) doku direnci (R) ve net iyon transferi (Isc) gibi biyoelektrik değerlere göre fonksiyonel olarak farklılıklar görüldü. Medial jejunumda bu değerler açlık durumunda değişti, fakat proksimal ileumda değişiklik yoktu. Kalın bağırsakta da fonksiyonel olarak farklılık gösteren 3 bölge tesbit edildi (proksimal, medial ve distal kolon) ve toklukta elde edilen değerler açlıkta değişti.

48 saat aç ince bağırsakta, etkisini hücre için Ca^{2+}'u arttırarak gösteren agonistler (bethanechol, 5-HT) ve DbcCAMP ile tok bağırsağa göre daha farklı miktarda sekresyon oluşmadı.

Kolonda ise, 48 saat açlık sonucu "bethanechol" toklukta görülenden daha fazla sekresyon doğurdu. Distal kolonda "bethanechol" önce düşüş hemen ardından yükselme gösteren bir "Isc" oluşturdu. Proksimal ve medial kolonda açlık durumunda siklik AMP yoluyla etkisini gösteren uyarıcılar (DbcAMP, theophylline) tok kolonla kıyaslandığında fazla sekresyon oluşturmadılar. Aç proksimal kolonda atropin. "5-HT" tarafından oluşturulan Isc'de artışa neden oldu, bu da toklarda elde edilenden istatistiksel olarak önemli bir şekilde farklıydı.

İnce ve kalın bağırsakta in vitro olarak Cl^-'un yerine glukonat koyarak yapılan deneyler, kolinerjik sekresyon doğuran maddeler kullanmadan önce ve kullandıktan sonra oluşan sekresyonun asıl kaynağının Cl^- iyonu olduğunu gösterdi.

İnce bağırsakta in vivo koşullarda açlık durumunda, "basal" ve "bethanechol" tarafından uyarılan sıvı transferinde sekresyonun olmaması in vitro sonuçları doğruladı. Fakat, kolonda açlıkta in vitro "bethanechol" ile elde edilen fazla sekresyon in vivo sonuçlar ile doğrulanmadı.

Summary: *The aim of this work is to investigate the effects of nutritional levels on the secretory functions of the small and large intestine in the mouse.*

The study was carried out on male swiss strain adult mice (of approximate 700) weighing 20-40 gr. Mice starved for 48h refed after 48h starvation allow-

* Bu çalışma aynı adlı doktora tezinden özetlenmiştir.
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ing them food ad lib. Mice were also undernourished for 4d with 50% (3gr/per mouse/per day) of the fed control adult daily food intake. Experimental data was collected from mice small (mid jejunum and proximal ileum) and large intestine (proximal, mid and distal colon).

In mid jejunum and proximal ileum, functionally distinct differences were observed by their bioelectric properties [i.e potential difference (P.D.), tissue resistance (R) and basal short-circiuit current (Isc)]. These were altered by changes in nutritional status in the mid jejunum, while those of the proximal ileum remained unchanged. Three functionally distinct colonic segments (proximal, mid and distal) were identified by their bioelectric properties (P.D., R and basal Isc) which varied along the colon. These properties were altered by changes in nutritional status.

When stimulated to secrete the 48h starved mid jejunum and proximal ileum did not exhibit a hypersecretory response to the Ca^{2+} acting secretory agonists (bethanechol, 5-HT) and DbcAMP.

In the colon, after 48h starvation bethanechol activated a higher electrogneic secretion that in the fed controls. The response of the distal colon to bethanechol was biphasic. In the proximal and mid colon, in the case of food deprivation, cyclic nucleotide acting secretory agonists (DbcAMP, theophylline) did not activate a hypersecretory response compared to the fed colons. In the starved proximal colon, atropine potentiated 5-HT-induced increase in Isc and this was significantly larger than that in the fed.

In the small and large intestine, Cl^- replacement by gluconate in vitro, indicated that the basal and cholinergic secretagogue stimulated secretion were mainly carried by the Cl^- ion.

In the case of starvation, in the small intestine in vivo, the basal and bethanechol-stimulated fluid transport confirmed the lack of secretion obtained in vitro. In the colon, however, the in vivo results did not confirm the hypersecretion induced by bethanechol in vitro.

Introduction

The intestine plays a major role in preventing a variety of intraluminal substances such as food antigens, micro-organisms and toxins from penetrating the mucosal barrier and entering the systemic circulation (35). Starvation and undernutrition result in intestinal mucosal thinning, damage and loss of its protective barrier function. For examle, fasting over a period of two to three days can result in significant decreases in total villous volume and villous surface area as well as an apparent thinning of villi (1). Fasting has also been reported to cause a reduction in the primary mucosal surface area and an apparent increase in the cell cycle time of enterocytes (1, 2, 31). The major effects of dietary restriction (fasting) on selected aspects of small intestine in relation to fed controls in various species were reviewed by Levin (20).

In rat, starvation for 48h and 72h creates a hypersensitive duodenum (27), jejunum (25, 29) and proximal ileum (28, 30, 49), but not terminal ileum (28), that react to secretagogues and neurotransmitters with a greatly enhanced secretory response *in vitro* and *in vivo*. More over, in the case of the ileum, even the fed basal absorptive tone was converted to a secretory one after 24h of food withdrawal and its magnitude increased on continued food deprivation (49). Young and Levin (48) also showed that 48 to 72h starvation make the rat jejunum hyperreactive to a variety of stimuli that elicit intestinal secretion despite the decreasing cell population of crypts and vill. Confirmation of the hypersecretory action of starvation on small intestinal secretory function *in vitro* has been reported in the jejunum of starved piglets (9).

Although, acute undernutrition has no effect on basal Isc along the small intestine (51), acute and chronic undernutrition hpyersensitize jejunum and ileum to secretagogues (40, 51). Young et al (52) also reported a prolonged activation of both Isc and area under the curve to E. Coli heat-stable enterotoxin, *in vitro*, of the jejunal and ileal response in the chronically undernourished rats.

Levin (21) discussed the possible reasons for hypersecretion in case of starved small intestine. It has been shown by Debnam and Thompson (12) that the P.D. across the luminal membranes of the starved enterocytes in the both jejunum and the ileum becomes hyperpolarised after only 24h of fasting and is maintained for at least 72h of starvation. Levin (21) suggested that the membrane hyperpolarisation creates an increased driving force on the intracellular Cl^- ions when the Cl^- channels of the luminal membrane are opened by neurotransmitters or secretagogues. Thus the hyperpolarisation of the starved entrocyte's luminal membrane can account for the hypersecretory activity shown by the small intestine in the food deprived state.

Further studies showed that the proximal colon (23), and even the rectum (24) from rats starved for three days responded to cholinergic agonists by electrogenic Cl^- hypersecretion, not the agents those acting through increases in intracellular cAMP or in mid colon through cyclic GMP (22, 36). Undernutrition (9d and 21d) causes hypersecretory response to the C^{2+}acting agonists in the stripped proximal, mid and distal colon, but not in the unstripped mid colon (35, 38).

The rat model is used to mimic the starvation and malnutrition diarrhoea seen in humans exposed to these conditions. The present study uses the mouse to:-assess whether the effects observed previously are exclusive to the rat or have wider scope and create and animal model to allow bacterial/viral diarrhoea to be studied (as the mouse employs much smaller amounts of toxins, chemicals etc.).

Materials and Methods

Male Swiss strain adult mice (of approximate 700 and body weight 20-40 gr) were used in all the experiments described. All mice were maintained until required on standard commercial diet with free access to tap drinking water. All were housed in a room with a 12h light/dark cycle with controlled humidity (72%) and temperature 23±1°C.

Mice were starved for 48h and refed after 48h starvation allowing them food *ad lib.* The food intake was checked for 24h, 48h and 72 h refeeding process. Mice were undernourished for 4d with 50% (3 gr/per mouse/per day) of the fed control adult daily food intake. All starved and undernourished mice were supplied throughout with tap drinking water *ad lib.*

In vitro studies: In vitro studies employed the method of estimating the electrogenic ion transfer (4, 43) using the short-circuit current technique. Experimental data was collected from mice small and large intestinal segments. The segments of small intestine included: -i) the mid jejunum, a 2 cm length of tissue 25-27 cm proximal to the ileo-caecal junction. The segments of large intestine included:-i)proximal colon located immediately distal to the ileocaecal junction. This segments in readily identified by its relatively thin and striated luminal surface, ii) mid colon located approximately 4-5 cm distal to the ileo-caeacal junction. Identified by its typically thick external muscle layer and smooth luminal surface, and iii) distal colon located approximately 6-8 distal to the ileocaecal junction. It is characterised by its relatively thin external muscle layer, smooth luminal surface and inclusive of the caudal lymph node.

In vivo studies: A modification of the gravimetric method of Strombeck (45) by Young and Levin (48, 49) was used to assess intestinal fluid transport *in vivo.*

The luminal fluid collected after centrifugation was analysed for Cl^- concentration (with a digital chloridometer, Model 4-2500, Buchler Instruments).

Expression of results, on which parameter, and interpretation of experimental data: Levin (19) and Nzegwu (35) reviewed the varies parameters used to characterize intestinal function. The choice can be crucial as different bases for calculating the data can change the interpretation placed on the assessment.

Statistical tests: In all cases results have been expressed as mean ±S.E. Statistical tests include: -a) Student's unpaired t-test, b) Student's unpaired t-test with Bonferroni correction (14, 15), and c) Kruskal-Wallis ANOVA followed by Conover's multiple comparison t-test (47).

Results

Changes in body weight: Starvation for 48h and acute undernutrition for 4d caused a continuous fall in body weight. At the end of the study period this was 22% ($p<0.001$) and 26% ($p<0.001$) respectively on the first day compared to the initial weight.

Changes in small and large intestinal lengths: In the fed Swiss adult male mouse (of approximate 35 gr), the entire length of small intestine (from pylorus to ileocaecal jnuction) is approximately 47 cm (46.8±0.6, n=12) and the large intestine (from caecum to caudal lymph

Table 1: Bioelectric parameters of mid jejunum from fed and 48h starved mice. Data show basal P.D. (mV), R (Ω/cm^2) and Isc ($\mu A/cm^2$ serosal area) given as mean ±SE with number of animals used in parentheses.

Nutritional State	P.D. (mV)	R (Ω/cm^2)	Isc ($\mu A/cm^2$)
Fed (Control)	2.9 ± 0.1 (53)	38 ± 2 (53)	81 ± 5 (53)
48h Starved	1.0 ± 0.1 (58)	28 ± 1 (58)	37 ± 5 (58)
P	<0.001	<0.001	<0.001

Table 2: Bioelectric parameters of proximal (A) and mid colon (B) from fed and 48h starved mice. Data show basal P.D. (mV), R (Ω/cm^2) and Isc ($\mu A/cm^2$ serosal area) given as mean ± SE with number of animals used in parentheses.

A

Nutritional State	P.D. (mV)	R (Ω/cm^2)	Isc ($\mu A/cm^2$)
Fed (Control)	4.1 ± 0.1 (151)	56 ± 1 (151)	72 ± 2 (151)
48h Starved	5.4 ± 0.1 (117)	61 ± 1 (117)	92 ± 3 (117)
P	<0.001	<0.001	<0.001

B

Fed (Control)	2.6 ± 0.1 (152)	56 ± 1 (152)	45 ± 2 (152)
48h Starved	4.7 ± 0.2 (109)	48 ± 2 (109)	81 ± 3 (109)
P	<0.001	<0.001	<0.001

Table 3: Bioelectric parameters of distal colon from fed, 48h and 4d acute undernourished (50% normal food intake) mice. Data show basal P.D.(mV), R (Ω/cm^2) and Isc ($\mu A/cm^2$ serosal area) given as mean ±SE with number of animals used in parentheses.

Nutritional State	P.D. (mV)	R (Ω/cm^2)	Isc ($\mu A/cm^2$)
Fed (Control)	2.5 ± 0.2 (90) [1]	80 ± 3 (90) [a]	33 ± 3 (90) [A]
48h Starved	6.1 ± 0.4 (92) [2]	79 ± 2 (92) [b]	77 ± 4 (92) [B]
4d acute U.N.	5.3 ± 0.3 (80) [3]	86 ± 3 (80) [c]	63 ± 4 (80) [C]

$p<0.01$: B vs C
$p<0.001$: 1 vs 2, 1 vs 3, A vs B, A vs C

node) is approximately 8 cm (8.1±0.2, n=12). In the small intestine, 48h starvation and 4d acute undernutrition caused similar, significant decreases (5%, $p<0.02$). In the large intestine, 48h starvation and 4d acute undernutrition caused significant decreased of 119 ($p<0.01$) and 12% ($p<0.002$) respectively.

In vitro bioelectric properties of the small and large intestine in case of starvation: Starvation caused decreases in the basal P.D. and Isc of the mid jejunum (Table 1). The P.D. decrease for 48h starvation was 66% ($p<0.001$). The decrease in basal Isc was 54% ($p<0.001$) compared to the fed control. In the proximal ileum, starvation for 48h caused small decreases in all values compared fed group but these changes were not significant.

In the proximal colon, a significant increase 32% ($p<0.001$) in the P.D. occured in 48h starved group relative to the fed control (Table 2A). A significant increase in the tissue R (9%, $p<0.001$) and Isc (28%, $p<0.001$) were seen in the 48h starved group relative to the fed control.

In the mid colon, starvation for 48h gave highly significant increases ($p<0.001$) in both P.D. (81%) and Isc (80%) while the R decreased significantly (14%, $p<0.001$) compared to fed group (Table 2b).

In distal colon beside the fed and 48h starved groups, the effects of undernutrition was also investigated. Starvation for 48h and 4d acute undernutrition (50% of food), when com-

Table 4: Comparison of bioelectric parameters in Cl^- present and absent media in mid jejunum (I) and proximal ileum (II) of fed (A) and 48h starved (B) mice. Data show basal P.D. (mV), R (Ω/cm^2) and Isc ($\mu A/cm^2$ serosal area) given as mean ±SE with number of animals used in parentheses.

(I)-A

Media	P.D. (mV)	R (Ω/cm^2)	Isc ($\mu A/cm^2$)
Cl^- present	3.2 ± 0.2 (7)	61 ± 7 (7)	54 ± 5 (10)
Cl^- absent	-1.8 ± 0.5 (6)	136 ± 9 (6)	-15 ± 5 (6)
P	<0.001	<0.001	<0.001

B

Cl^- present	0.4 ± 0.2 (7)	34 ± 2 (7)	10 ± 7 (7)
Cl^- absent	-0.2 ± 0.5 (6)	125 ± 8 (6)	-1 ± 4 (6)
P	N.S.	<0.001	N.S.

(II)-A

Media	P.D. (mV)	R (Ω/cm^2)	Isc ($\mu A/cm^2$)
Cl^- present	2.3 ± 0.6 (7)	27 ± 1 (7)	81 ± 19 (7)
Cl^- absent	-1.8 ± 0.8 (6)	152 ± 12 (6)	-13 ± 5 (6)
P	<0.001	<0.001	<0.001

B

Cl^- present	0.4 ± 0.2 (7)	34 ± 2 (7)	10 ± 7 (7)
Cl^- absent	-0.2 ± 0.5 (6)	125 ± 8 (6)	-1 ± 4 (6)
P	N.S.	<0.001	<0.02

pared to fed and 91% compared to the fed control group respectively. The Isc of undernourished group was less (18%, $p<0.01$) than of the starved group (Table 3).

Effect of the Cl^- ion on small and large intestinal bioelectric parameters: Replacement studies of Cl^- with gluconate in the mid jejunum were undertaken in fed and 48h starved mice. Table 4- (I) shows comparisons between Cl^- present and absent results both in fed (A) and 48h starved (B) animals. In the fed group without Cl^-, the P.D. and Isc decreased by 156% ($p<0.001$) and 128% ($p<0.001$) but the R value increased by 123% ($p<0.001$) compared to the values with Cl^-. In the 48h starved group, in the absence of Cl^- the P.D. and Isc values were reduced but not significantly again R value increased by 268% ($p<0.001$) compared to the results obtained in the presence of Cl^-. In the Cl^- free media, the P.D. and Isc polarity reversed from + ve (serosa) to -ve in both fed and 48h starved groups. Compared to the fed control, 48h starvation gave an increase for the both P.D. (89%, $p<0.05$ and for the Isc (93%, $p<0.05$).

Replacement studies of Cl^- with gluconate were also made in proximal ileum from fed and 48h starved mice. Table 4- (II) shows comparisons between Cl^- present and absent both in fed (A) and 48h starved (B) animals. In the fed group without Cl^-, the polarity of the P.D. and Isc reversed to -ve (serosa) 178% and 116% (both $p<0.001$). The tissue R rose by 463% ($p<0.001$). After starvation for 48h, the Isc was reduced (91%, $p<0.02$) and the tissue R increased with Cl^- absence (729%, $p<0.001$) respectively. In contrast to mid jejunum, in the absence of Cl^-, with 48h starvation the P.D. and Isc of the proximal ileum shifted from -ve to +ve of 139% for the P.D. and 138% for the Isc (both $p<0.05$). A further comparison in fed and starved small intestine was made between mid jejunum and proximal ileum of the values obtained in the Cl^- free media. No significant changes were noted in any of the measurements.

In the fed proximal colon (Table5-I A), removal of Cl^- caused decreases in P.D. and Isc of 122% ($p<0.001$) and 111% ($p<0.001$) while the R increased by 116% ($p<0.001$). Similarly in the 48h starved group (B), removal of Cl^- decreased the P.D. and Isc by 131% and 118% (both $p<0.001$) while the R increased by 77% ($p<0.001$). Removal of Cl^- in both fed and 48h starved mice produced a reversal of the polarity

Table 5: Comparison of bioelectric parameters in Cl^- present and absent media in proximal colon (I) and mid colon (II) of fed (A) and 48h starved (B) mice. Data show basal P.D. (mV), R (Ω/cm^2) and Isc ($\mu A/cm^2$ serosal area) given as mean ±SE with number of animals used in parentheses.

(I)-A

Media	P.D. (mV)	R (Ω/cm^2)	Isc ($\mu A/cm^2$)
Cl^- present	4.1 ± 0.3 (10)	64 ± 2 (10)	63 ± 4 (10)
Cl^- absent	-0.9 ± 0.4 (9)	138 ± 7 (9)	-7 ± 3 (9)
P	<0.001	<0.001	<0.001

B

Cl^- present	4.9 ± 0.3 (10)	70 ± 3 (10)	72 ± 5 (10)
Cl^- absent	-1.5 ± 0.3 (9)	124 ± 4 (9)	-13 ± 3 (9)
P	<0.001	<0.001	<0.001

(II)-A

Media	P.D. (mV)	R (Ω/cm^2)	Isc ($\mu A/cm^2$)
Cl^- present	2.3 ± 0.3 (21)	63 ± 3 (21)	34 ± 3 (21)
Cl^- absent	0.1 ± 0.1 (9)	198 ± 9 (9)	0.4 ± 0.7 (9)
P	<0.001	<0.001	<0.001

B

Cl^- present	4.1 ± 0.5 (9)	67 ± 5 (9)	59 ± 5 (9)
Cl^- absent	0.3 ± 0.4 (9)	173 ± 20 (9)	0.6 ± 2 (9)
P	<0.001	<0.001	<0.001

Table 6: Comparison of bioelectric parameters in Cl^- present and absent media in distal colon of fed (A), 48h starved (B) and 4d acute undernourished mice. Data show basal P.D. (mV), R (Ω/cm^2) and Isc ($\mu A/cm^2$ serosal area) given as mean ±SE with number of animals used in parentheses.

A

Media	P.D. (mV)	R (Ω/cm^2)	Isc ($\mu A/cm^2$)
Cl^- present	2.5 ± 0.5 (8)	63 ± 5 (8)	48 ± 6 (8)
Cl^- absent	2.8 ± 0.4 (8)	145 ± 10 (8)	20 ± 3 (8)
P	N.S.	<0.001	<0.002

B

Cl^- present	6.4 ± 0.7 (8)	68 ± 6 (8)	93 ± 5 (8)
Cl^- absent	5.1 ± 0.5 (8)	129 ± 10 (8)	39 ± 3 (8)
P	N.S.	<0.001	<0.001

C

Cl^- present	8.4 ± 1 (7)	80 ± 4 (7)	105 ± 12 (7)
Cl^- absent	4.9 ± 1 (7)	118 ± 9 (7)	40 ± 9 (7)
P	N.S.	<0.002	<0.001

Table 7: Response to serosal bethanechol (1 mM) in mid jejunum and proximal ileum from fed, 48h starved followed by refeeding for 24h, 48h and 72 h mice. Data show ΔIsc (μA/cm² serosal area) given as mean ±SE with number of animals used in parentheses.

	ΔIsc (μA/cm²)	
Nutritional State	Mid Jejunum	Proximal Ileum
Fed (Control)	92 ± 5 (10)[a]	112 ± 10 (7)[f]
48h Starved	96 ± 10 (9)[b]	65 ± 8 (7)[g]
24h Refed	120 ± 11 (9)[c]	109 ± 10 (7)[h]
48h Refed	123 ± 12 (7)[d]	94 ± 6 (7)[i]
72h Refed	116 ± 15 (8)[e]	121 ± 15 (5)[j]

p<0.05 : a vs c, a vs d, b vs g, d vs i
p<0.01 : f vs g, g vs h
p<0.001: g vs j

Table 8: Response to serosal 5- hydroxytryptamine (5-HT, 50μM) in presence and absence of serosal atropine (10 μM) by mid jejunum (A) and proximal ileum (B) from fed, 48h starved mice. Data show ΔIsc (μA/cm² serosal area) given as mean ±SE with number of animals used in parentheses.

A

	ΔIsc (μA/cm²)	
Nutritional State	5-HT	5-HT + Atropine
Fed (Control)	55 ± 4 (9)[a]	72 ± 8 (6)[c]
48h Starved	50 ± 9 (8)[b]	29 ± 4 (6)[d]
B		
Fed (Control)	91 ± 18 (8)[1]	64 ± 10 (6)[3]
48h Starved	40 ± 9 (9)[2]	37 ± 6 (8)[4]

p<0.05 : b vd d, 3 vs 4
P<0.01 : c vs d, 1 vs 2

of the P.D. and Isc from +ve to -ve. The differences between fed and starved groups in basal parameters (P.D., R, Isc) were not significant.

In the mid colon, when Cl^- was removed the P.D. and Isc fell significantly (p<0.001) in the fed group by 96% and 99%, in the 48h starved group by 93% and again 99%. The R on the other hand significantly increased both in the fed group (214%, p<0.001) and in the 48h starved group (158%, p<0.001) (Table 5-II A, B). Comparison between fed and 48h starved mice in the Cl^- free media showed no significant differences in the P.D., R and Isc values.

In the fed distal colon, removal of Cl^- increased R by 179% (p<0.001) and decreased Isc by 58% (p<0.01) (Table 6A). In the 48h starved group, R was increased by 90% and the Isc was decreased by 58% (p<0.001) (Table 6B). With the undernourished group although there was decrease in P.D. in Cl- free media, this was not significant. The R increased by 48% (p<0.002) while the Isc decreased by 62% (p<0.001) (Table 6C). Unlike the other parts of the colon, removal of Cl- in starvation significantly increased the distal P.D. and the distal Isc by 82% (p<0.01) and 95% (p<0.001) compared to the fed control group.

Studies on small intestinal function in vitro and in vivo: In mid jejunum, bethanechol (1 mM) induced in 48h starved segment an insignificant increase in the ΔIsc and a significant decrease in 48h starved proximal ileum (42%, p<0.01) compared to the fed (Table 7). These responses in the mid jejunum and proximal ileum after 48h starvation were increased by progressive refeeding. In the mid jejunum the increases were 30% and 34% (both p<0.05) after 24h and 48h refeeding compared to fed control. In the proximal ileum these were 68% (p<0.01)

Table 9: Fluid and Cl^- transport in vivo in unstimulated (basal) and bethanechol stimulated (bch, 60 µg/kg b. wt.) state by mid jejunum (A) and proximal ileum (B) from fed and 48h starved mice. Data given as mean±SE with number of animals used in parentheses. Positive values denote gain of luminal fluid and Cl^-; negative values denote loss of fluid and Cl^- from lumen.

A

Nutritional State	Fluid (mg/cm/15min) Basal	+Bch	Fluid (mg/100mg dry w) Basal	+Bch	Cl^- (mmol/L/15 min) Basal	+Bch
Fed	+0.3±1.6 (23)[1]	+21±2 (13)[3]	+5±15 (23)[a]	+216±22 (13)[c]	-2.2±1.4 (23)[A]	+3.2±1.6 (13)[C]
48h Starved	-9±3 (19)[2]	+15±2 (10)[4]	-136±19 (19)[b]	+251±46 (10)[d]	-2.8±1.6 (19)[B]	-2.6±1.4 (10)[D]

P<0.05 : 1 vs 2, a vs b
p<0.001 : 1 vs 3, 2 vs 4, a vs c, b vs d

B

Fed	-7±3 (20)[1]	+12±2 (13)[3]	-76±39 (20)[a]	+148±22 (13)[c]	-3±2 (20)[A]	-3±1 (13)[C]
48h Starved	-4±3 (18)[2]	+8±1 (10)[4]	-89±50 (18)[b]	+179±34 (10)[d]	+0.3±1.6 (18)[B]	-3±1 (10)[D]

P<0.01 : 2 vs 4
p<0.001 : 1 vs 3, a vs c, b vs d

Table 10: Response to serosal bethanechol (1 mM) by proximal and mid colon from fed and 48h starved (A) and also from fed and 48h starved followed by refeeding for 24h, 48h and 72h (B) mice. Data show ΔIsc (µA/cm² serosal area) given as mean±SE with number of animals used in parentheses.

A

Nutritinal State	ΔIsc (µA/cm²) Proximal Colon	Mid Colon
Fed (Control)	44 ± 3 (14)[a]	33 ± 4 (15)[c]
48h Starved	92 ± 11 (9)[b]	41 ± 4 (10)[d]

B

Fed (Control)	83 ± 7 (9)[e]	27 ± 3 (11)[j]
48h Starved	120 ± 9 (8)[f]	63 ± 8 (8)[k]
24h Refed	95 ± 5 (7)[g]	55 ± 7 (6)[l]
48h Refed	131 ± 15 (7)[h]	36 ± 4 (7)[m]
72h Refed	94 ± 8 (6)[i]	36 ± 5 (5)[n]

The following are significant at:
p<0.05 : a vs c, f vs i, g vs h, l vs m, l vs n
p<0.01 : k vs m, k vs n
p<0.001 : a vs b, b vs d, e vs f, e vs h, e vs j, j vs o, g vs l, h vs m, i vs n, i vs k, j vs l

and 86% (p<0.001) by 24h and 72h refeeding compared to the 48h starved group respectively.

In mid jejunum (Table 8A), serosal 5-HT (50µM) induced increases in ΔIsc in the fed and 48h starved groups but they were not significantly different. In the presence of serosal atropine (10µM), in the 48h starved group, the 5-HT-induced Isc was significantly less (60%, p<0.001) than that in the fed group, In proximal ileum (Table 8B), the 5-HT-induced secretion was reduced in the 48h starved groups both in the absence (56%, p<0.01) and presence of atropine (42%, p<0.05) when compared to the fed group.

Starvation for 48h caused decreases in ΔIsc induced by serosal DbcAMP (1 mM) in both mid jejunum and proximal ileum but these were not significant compared to the fed values.

Role of Cl^- in the secretory response of the small intestine: In the mid jejunum the ΔIsc in response to bethanechol was significantly reduced in the Cl^- free bicarbonate saline compared to the Cl^- present controls by 91% (p<0.001) in the 48h starved groups. Similarly in the proximal ileum, removal of Cl^- from the bathing medium reduced the ΔIsc in response to bethanechol by 95% (p<0.001) in the fed and

by 91% ($p<0.001$) in the 48h starved group. There was no significant difference in the ΔIsc between fed and 48h starved groups in Cl^- free medium either in the mid jejunum or the proximal ileum.

Fluid and ion tranport in the small intestine: Measurement of jejunal fluid movement *in vivo* showed that (Table 9A), the basal tone in the fed intestine was neutral (0.3±1.6) and the 48h starvation induced a significant increase in the basal absorption of fluid both per cm and per 100 mg dry weight. Bethanechol caused a net secretion in both fed and starved groups this was significantly different (both same on a length and dry weight basis, $p<0.001$) from fed and starved basal states, unstimulated, but not significantly different from each other whether placed on a length basis or on a dry weight basis. Basal and bethanechol-induced final Cl^- concentration values were not significantly different from each other and also from zero.

Fluid and Cl^- transport in the ileum are also shown in Table 9B. Basal absorptive tone in the fed and starved basal states of the ileum was significantly reversed by bethanechol to the secretory tone in the fed (both length and dry weight bases, $p<0.001$) and 48h starved groups (on a length basis, $p<0.01$; on a dry weight basis, $p<0.001$) but there was no significant difference between them either on the length or weight basis of expressing the results. Basal final Cl^- concentrations in the fed and in the 48h starved groups were not significantly different from each other and also from zero. There was the same amount of bethanechol-induced Cl^- absorption in fed and 48h starved groups.

Studies on large intestinal function in vitro and in vivo: The response to secretory stimulation was examined in porximal and mid colon taken from mice in the two main nutritional groups; fed control and 48h starved.

The results (Table 10A). show that in the proximal colon, progressive starvation resulted in a hypersecretory response to bethanechol (1 MM, serosal). There was a significant elevation in ΔIsc above that of the fed control after 48h starvation (109%, $p<0.001$). In the mid colon, compared to the fed control 48h starvation did not cause a significant increase. Comparison between these two segments of the large intestine showed that the proximal colon had larger increases in bethanechol induced ΔIsc than the mid colon (fed, $p<0.05$; 48h group, $p<0.001$). The response to bethanechol was also examined in proximal and mid colon from mice starved for 48h and in a group refed up to 72h. The results (Table 10B) show that starvation for 48h resulted in a hypersecretory response to bethanechol (1 mM, serosal), significant increases in ΔIsc by 45% ($p<0.001$) in the proximal colon and by 133% ($p<0.001$) in the mid colon compared to the fed controls. This ΔIsc induced by bethanechol in the 48h starved proximal colon was not shown after 24h refeeding, however, in the mid colon, after 24h refeeding the ΔIsc induced by bethanechol was still significantly larger (104%, $p<0.001$) than that of the fed group. In the proximal colon after 48h refeeding, increased ΔIsc was restored to 58% ($p<0.001$) but 72h of refeeding returned the ΔIsc to the fed level. Proximal colon had significantly larger ΔIsc induced by bethanechol than mid colon in all nutritional conditions (same for all, p+0.001). Typical time course response of the proximal colon to bethanechol (similar observed in the mid colon) shows that adding serosal bethanechol resulted in an instantaneous increase in Isc, which reached a peak within 2-3 followed by a gradual fall back to the basal level after 10-15 min.

The response of distal colon to 1 mM (serosal) bethanechol was different from proximal and mid colon. Initially, there was a transient rapid decrease lasting about 15 sec which was

Table 11: Biphasic response to 1 mM serosal bethanechol, -ΔIsc (maximal fall below basal level over 15 sec) and +ΔIsc by distal colon from fed, 48h starved and 4d acute undernourished mice. Data show ΔIsc ($\mu A/cm^2$ serosal area) given as mean±SE with number of animals used in parentheses.

Nutritional State	Bethanechol	
	-ΔIsc	+ΔIsc
Fed (Control)	-14 ± 4 (19)[1]	31 ± 3 (26)[a]
48h Starved	-30 ± 6 (23)[2]	49 ± 5 (29)[b]
4d Acute U.N.	-13 ± 4 (14)[3]	31 ± 5 (15)[c]

The following are significant at:
$p<0.05$: 1 vs 2, 2 vs 3
$p<0.01$: a vs b, b vs c

Table 12: Response to serosal bethanechol (1 mM) firstly maximal fall below basal level over 15 (A) and and increase (B) by distal colon from fed, 48h starved and 4d acute undernourished mice in the absence (control) and prence of serosal tetrodotoxin (TTX, 1μM) and diphenylamine-2-carboxylic acid (DPC, 2.5 mM). Data show ΔIsc (μA/cm^2 serosal area) given as mean±SE with number of animals used in parentheses.

A

Nutritional State	ΔIsc (μA/cm^2) Control	TTX	DPC
Fed (Control)	-17 ± 6 (9)[a]	-0.6 ± 0.3 (7)[d]	-3 ± 2 (6)[g]
48h Starved	-37 ± 9 (12)[b]	-0.6 ± 0.4 (7)[e]	-8 ± 3 (6)[h]
4d Acute U.N.	-15 ± 5 (7)[c]	-3 ± 2 (6)[f]	-4 ± 2 (7)[i]

B

Fed (Control)	38 ± 7 (9)[1]	13 ± 2 (7)[4]	7 ± 3 (6)[7]
48h Starved	59 ± 7 (12)[2]	20 ± 2 (6)[5]	21 ± 5 (6)[8]
4d Acute U.N.	37 ± 8 (7)[3]	24 ± 4 (6)[6]	16 ± 2 (7)[9]

The following are significant at:
$p<0.05$: a vs g, e vs h, 1 vs 2, 2 vs 3, 4 vs 8
$p<0.01$: b vs h, c vs f, c vs i, 3 vs 9, 7 vs 8
$p<0.001$: a vs d, b vs e, 1 vs 4, 1 vs 7, 2 vs 5, 2 vs 8

followed by an immediate rise in the Isc and plateaued at least 10 min. The maximum decreases and increases are shown in Table 11. The responses to bethanechol indicated that 48h starved distal colon had significantly greater decrease by 114% and 131% (both, $p<0.05$) and increase by same 58% (both, $p<0.001$) when compared to the fed and 4d acute undernourished groups respectively.

In order to investigate if there was any neural mediation of the basal Isc and of the decrease caused by serosal bethanechol, TTX (1μM) was added serosally after recording the basal Isc. It caused a rapid decrease in Isc especially in the food deprived intestine. Moreover, in the presence of TTX, the bethanechol-induced decreases (-ΔIsc) were practically abolished in the fed (97%, $p<0.001$), in the 48h starved (98%, $p<0.001$) and in the 4d acute undernourished groups (80%, $p<0.01$) compared to those in the control groups (Table 12A). The action of the Cl^- channel blocker DPC (2.5 mM, mucosal) was also monitored. DPC caused a rapid fall especially in the food deprived intestine and a decrease in the bethanechol-induced ΔIsc as it was expected but also it reduced the -ΔIsc caused by bethanechol. The reductions caused by DPC in the -ΔIsc induced by bethanechol were 82% ($p<0.5$) in the fed, 78% ($p<0.01$) in the 48h starved and 73% ($p<0.01$) in the 4d acute undernourished groups compared to the control groups. All these percentage reductions caused by DPC were less than that caused by TTX but there was only significant difference between TTX and DPC treated starved groups ($P<0.05$). TTX and DPC reduced the ΔIsc caused by bethanechol (Table 12B). In the presence of TTX, the bethanechol-induced ΔIsc was reduced in the fed group by 66% ($p<0.001$) and in the 48h starved group by 66% ($p<0.001$) compared to the control groups respectively. There was no significant reduction in ΔIsc of the 4d acute undernourished group compared to that of the control undernourished group but it was significantly greater than the reduction in ΔIsc of TTX treated fed group (85%, $p<0.05$). In the presence of TTX, the ΔIsc induced by bethanechol was the direct effect of bethanechol on cells not the neural component. In the 4d acute undernourished intestine, it seemed the neural mechanism did not play big role in the bethanechol-induced secretion as it did in fed and starved groups. In the presence of DPC, the bethanechol-induced ΔIsc was reduced in the fed (83%, $p<0.001$) 48h starved (64%, $p<0.01$) and 4d acute undernourished groups (57%, $p<0.01$). In the presence of DPC, there was still a significant increase (200%, $p<0.01$) in ΔIsc caused by bethanechol with 48h starvation compared to the fed group.

Neural involvement in proximal and mid colonic secretion: Food deprivation (starvation for 48h) did not change the ΔIsc induced by 5-HT in the proximal colon (Table 13). In the

Table 13: Response to serosal 5-HT (5 μM) by proximal colon in the absence (control and presence of serosal atropine (10 μM), pirenzepine 50 μM) and hexamethonium (100 μM) from fed and 48h starved mice. Data show ΔIsc (μA/cm² serosal area) given as mean±SE with number of animals used in parentheses.

Nutritional State	ΔIsc (μA/cm²)			
	Control	Atropine	Pirenzepine	Hexamethonium
Fed (Control)	39 ± 6 (8)[a]	32 ± 3 (12)[c]	21 ± 5 (6)[e]	21 ± 3 (8)[g]
48h Starved	37 ± 7 (8)[b]	64 ± 6 (14)[d]	39 ± 5 (8)[f]	32 ± 5 (7)[h]

The following are significant at:
p<0.05 : a vs e, c vs g, d vs f
p<0.01 : a vs g, e vs f
p<0.001 : b vs d, c vs d, d vs h

presence of serosal atropine (10 μM), 5-HT-induced secretion was elevated in 48h starvation by 100% (p<0.001) compared to the fed group. In the presence of serosal atropine, starvation for 48h showed significantly larger response to 5-HT (73%, p<0.001) than that in the absence of atropine, although fed response was similar. In the presence of pirenzepine (50 μM, serosal), 5-HT ΔIsc was significantly reduced (46%, p<0.05) in the fed group but in the 48h starved group it was unaffected, Starvation for 48h caused a significand increased response to serosal 5-HT in the presence of pirenzepine (86%, p<0.01) when compared to the fed group. Similar to pirenzepine, hexamethonium (100 μM, serosal) significantly reduced the 5-HT-induced secretion in the fed group 46%, p<0.01), but hexamethonium did not give a significant increase in the 5-HT ΔIsc in the 48h starved group compared to the fed group. Comparison of 5-HT-induced ΔIsc with the various antagonists indicated that 5-HT caused more secretion in the presence of atropine than pirenzepine and hexamethonium in both fed and 48h starved groups. In the fed group, atropine insignificantly increased the effect of 5-HT by 52% compared to pirenzepine and same by 52% (p<0.05) compared to the hexamethonium respectively. In the 48h starved group, these differences were 64% (p<0.05) compared to pirenzepine and 100% (p<0.001) compared to hexamethonium respectively.

In the mid colon, similar studies showed that in the presence of atropine the fed tissue gave the highest response to 5-HT, but in the 48h starved group, the biggest ΔIsc was obtained in the presence of hexamethonium. Although in the presence of antagonists 5-HT ΔIsc was greater than in their absence both in fed and 48h starved groups these responses were not significantly different from each other and the control groups.

Comparison between proximal and mid colon in the fed groups showed that in the absence and presence of antagonists, 5-HT induced more secretion (ΔIsc) in the mid colon than in the proximal colon. These significant differences were: in the presence of atropine 153% (p<0.002), pirenzepine 200% (p<0.05) and hexamethonium 229% (p<0.001) respectively. The same comparison in 48h starved groups indicated that the mid colon again showed larger 5-HT-induced secretion in all groups but the only significant ΔIsc was seen in the presence of hexamethonium (184%, p<0.01).

Colonic response to cyclic nucleotide secretory agonists: In the proximal and mid colon from fed and 48h starved mice, the effects of serosal DbcAMP (1 mM) and theophylline (10 mM), a phosphodiesterase inhibitor, added to both mucosal and serosal solutions to raise the intracellular level of cAMP, were assessed on the Isc. In the case of proximal colon, DbcAMP did not cause a significant increase in the short circuit current in the starved colon compared to the fed colon. Theophylline treatment showed an increase but this was not significant. In the mid colon, DbcAMP gave a small increase in the starved (25%), but this was not significant. Theophylline did not change ΔIsc significantly in the starved colon. In distal colon in the presence of serosal hexamethonium (100 μM) and serosal and mucosal indomethacin (1 μM), DbcAMP (1 mM) did not cause any significant increases with food deprivation.

Role of Cl- in secretory response of the colon: In the proximal colon, removal of Cl- from bathing medium reduced the bethanechol-induced ΔIsc by 80% (p<0.001) in the fed and by 90% (p<0.002) in the 48h starved groups. The significant increase in ΔIsc induced by bethanechol with starvation of 173% (p<0.001) with Cl^- present was not seen in absence of Cl^-. In the mid colon the absence of Cl^- caused decreases in bethanechol induced ΔIsc by 91% (p<0.001) in the fed and by 93% (p<0.001) in the 48h starved groups compared to the Cl^-present controls. In the presence of Cl^-, star-

Table 14: Fluid and Cl^- transport *in vivo* in unstimulated (basal) and bethanechol stimulated (bch, 60µg/kg b.wt.) colons in fed and 48h starved mice. Data given as mean±SE with number of animals used in parentheses. Positive values denote gain of luminal fluid and Cl^-; negative values denote loss of fluid and Cl^- from lumen.

Nutritional State	Fluid (mg/cm/15min) Basal	+Bch	Fluid (mg/100mg dry w) Basal	+Bch	Cl^- (mmol/L/15 min) Basal	+Bch
Fed	0 ± 2 (17)[1]	+12 ± 2 (10)[3]	-11±14 (17)[a]	+137±20 (10)[c]	-6 ± 1.5 (17)[A]	-7 ± 2 (10)[C]
48h Starved	-2 ± 2 (18)[2]	+4 ± 1 (10)[4]	-12±22 (18)[b]	+46±14 (10)[d]	-3 ± 2 (18)[B]	0.4 ± 2 (10)[D]

P<0.05 : 2 vs 4, 3 vs 4, c vs d
p<0.01 : b vs d
p<0.001 : a vs c

vation for 48h gave a significant increase of 67% (p<0.05) in the bethanechol-induced ΔIsc compared to the fed group but this was not observed in the absence of Cl^-. Comparisons between proximal and mid colonic ΔIsc induced by bethanechol in the absence and presence of Cl^- showed that in the fed groups in Cl^- -free medium, the proximal colon had significantly larger ΔIsc (+200%, p<0.001) than the mid colon. In the 48h starved groups the proximal colonic ΔIsc in response to bethanechol was larger than in the mid colonic ΔIsc in both Cl^- -containing (+119%, p<0.01) and Cl^- free medium (+200%, p<0.001). In the distal colon, the increases in Isc (ΔIsc) caused by behanechol were also reduced in the Cl^- -free medium by 85% (p<0.001) in the fed group, by 76% (p<0.001) in the 48h starved group and by 60% (p<0.05) in the 4 d acute undernourished group.

Fluid and ion (Cl^-) transport in the colon: Fluid and ion (Cl^-) transport were measured in the whole large intestine. No division with three segments could be accomplished because of short lengths involved. The net fluid transport was calculated in both per cm intestine and per 100 mg dry weight of intestine in both unstimulated (basal) and secretagogue stimulated states. Secretion was induced by i.p. bethanechol (60µg/kg b. wt. in 50µl KBS) (Table 14). The results indicated that there was no basal fluid transport in either the fed or starved colon but bethanechol-induced secretion (mg/cm) was significantly reduced by 48h starvation (67%, p<0.05) compared to the secretion in the fed group. The second comparison of fluid transport (mg/100mg dry. wt.) confirmed the previous decrease and this time decrease in the bethanechol-induced secretion was reduced by starvation again significantly 66% (p<0.05) compared to the fed group. In the case of the bethanechol-stimulation in the fed colon, the final concentration of Cl^- was negative although an increase of fluid transport was observed. In the starved colon also final Cl^- concentration was not consistent with the fluid transport induced by bethanechol. One explanation, there are other ion (s) (like HCO3-) and in the case of bethanechol-stimulation they come to the luminal fluid, not Cl^-.

Discussion

In was shown in this study that dietary deprivation (48h starvation and 4d acute undernutrition) effects the basal and stimulated bioelectric properties of the mouse small and large intestine *in vitro* and *in vivo*. Starvation for 48h caused 22% fall in body weight, the decrease in body weight caused by 4d acute undernutrition was 26%. The largest decreases (11-13%) during the 4d acute undernutrition and starvation for 48h happened on the first day of their period. There were also changes in the lengths of mouse intestine after food deprivation. These findings are consistent with the starvation induced mucosal thinning and reduction in cell proliferation also loss of body weiht previously reported in several studies (5, 8, 17, 35, 41, 46). It was also reported by Goodlad et al. (16) that after 4d starvation the percentage of the loss in body weight was 23% in the rats which was lost by mice only after 1d starvation. That is probably due to different surface/volume ratio. Mouse is smaller and metabolism has to function, especially in the case of starvation, at higher level because of increased heat loss.

In the case of starvation, basal electrogenic secretion of the mid jejunum was significantly reduced but the bethanechol-stimulated secretion was not significantly different from the fed control. In the proximal ileum, however, starvation did not reduce the basal secretion, but bethanechol-stimulated secretion was significantly reduced. In the fed condition, basal secretion of mid jejunum was significantly larger than that of the proximal ileum, but not in the starvation.

Experiments with mouse intestine showed that after starvation the mid jejunum and proximal ileum did not exhibit a hypersecretory response to bethanechol and 5-HT. In contrast to the mouse, in rat jejunum and ileum, starvation for 48h and 72h increased the electrogenic secretion induced by bethanechol and 5-HT *in vitro* (25, 26, 48, 49). There might be several reasons for the decreased response to bethanechol and 5-HT in case of starvation by mouse small intestine compared to the rat, apart from just saying "species difference".

In the fed and 48h starved mid jejunum and proximal ileum, atropine produced no significant effect on the basal Isc. Sheldon *et al.* (42) also reported that in fed mouse jejunum, atropine did not cause any significant change. The 5-HT-induced ΔIsc was qualitatively similar to bethanechol-induced ΔIsc. Atropine did not block the effect of 5-HT in the starved proximal ileum but interestingly blocked it in the starved mid jejunum. Atropine inhibits the response to 5-HT in the guinea-pig ileum *in vitro* in the fed condition (10). One possibility for this is that in starved mouse mid jejunum and in the guinea-pig ileum, the stimulatin of 5-HT might be via the release of acetylcholine which then stimulates secretion by muscarinic receptors. In contrast to mouse and guinea-pig ileum, in the rat ileum 5-HT -induced electrogenic secretion potentiated by atropine (6). The explanation by Beesley and Levin, (6) was presence of a local enteric neural pathway in rat small intestine, like rat colon (37), and that inhibits electrogenic ion secretion induced by 5-HT and various secretagogues.

In response to DbcAMP, starved mid jejunum and proximal ileum again failed to show any enhanced secretion compared to fed control. This indicates that hypersecretory phenomena of the mouse small intestine can not be elicited by raising the cylic AMP concentrations of the starved enterocyte. Whereas in rat jejunum and ileum, beside various secretagogues whose action are mediated through cyclic AMP, DbcAMP *per se* caused significant increases after 72h starvation compared to fed condition (29, 48, 49).

The ion replacement studies suggest that in the fed and starved mid jejunum and proximal ileum, the bethanechol-stimulated Isc could be accounted for almost entirely by the Cl^- ion. The significant reduction in bethanechol-induced ΔIsc seen in the 48h starved proximal ileum in Cl^- containing saline, was not observed in the Cl^- free media. The major role Cl^- in rat small intestinal secretion was also shown by Young and Levin (48, 49). Moreover, *in vivo*, the final Cl^- concentration values did not confirm that the bethanechol-induced secretion in both fed and starved jejunum and ileum was carried by Cl^-. What are the possible species of ions that could be the basis of the secretory movement observed *in vivo*? Lack of fluid did not make possible to measure other ions. Possibly, *in vivo* bethanechol like some secretagogues (7, 11, 18) induces secretion by inhibiting electroneutral NaCl absorption without changing electrogenic Cl^-secretion.

The increases in the mid and distal colonic basal Isc after starvation were consistent those in the rat (35), but in rat the proximal colonic basal Isc was reduced after 72h starvation although it was increased in mouse after 48h starvation.

The biphasic response was also reported in rat stripped distal colon, but not in unstripped one, by Nzegwu (35). Serosal TTX and mucosal DPC reduced the basal Isc and both the decreases and increases induced by bethanechol. In the presence of TTX, the increases in Isc induced by bethanechol decayed after the maximum had been reached unlike the plateaus observed in the absence of TTX. These findings suggested that bethanechol activates electrogenic ion transport in fed and dietary-deprived distal colon by neural and non-neural mechanisms. The initial decrease in the basal Isc appears to be neurally mediated while the increase in Isc has both non-neural (direct action on colonocytes?) and neural components. The latter influences not only the maximum response but also its duration. The findings observed in case of refeeding were similar to those found in rat proximal colon after starvation and following refeeding, only difference increased Isc was not reduced by 24h refeeding (39).

The increased secretion in the starved mouse colon is interesting as this condition causes a decrease in colonic crypt cell production rate (18, 40). Crypts are generally accepted as the secretory cells, although there is some evidence against the view of compartmentalization of all absorptive cells to the villus and all secretory cells to the crypts (13, 44).

In the case of food deprivation, the basal Isc of the distal colon was decreased by various antagonists, this was especially so by TTX, a nerve toxin blocking neural transmission and by DPC, a Cl^- channel blocker. This indicates that there is a neural control mechanism of the basal distal colonic secretion which appears composed mainly of electrogenic Cl^- secretion.

TTX- induced decreases in the basal Isc of fed rat distal colon was also reported by Andres et al. (3) and Nobles et al. (33). In their work the magnitude of the decrease was larger but similarly, a plateau was reached in less than 5 min.

In the starved proximal colon, atropine potentiated 5-HT-induced increase in Isc and this was significantly larger than that in the absence of atropine. In the starved distal colon, hexamethonium potentiated bethanechol-induced increase in Isc. These potentiated increases in the starved colons were significantly larger than those in the fed tissues. This result suggests that there may be an ENCAP (enteric neural cholinergic adrenergic pathway) in mouse colon which becomes effective especially in the starved condition. The results appear very similar to the mechanism said previously in the rat colon (35, 36).

The mechanism(s) of the secretory responses induced by the cyclic nucleotide acting agonists during starvation and undernutrition are not known. In the proximal colon the secretory responses to the secretagogue action of DbcAMP and of theophylline were generally lower than those produced by the Ca2+ acting agonist bethanechol. This was also found in rat colon (35, 36).

The reasons for the differences between *in vitro* and *in vivo* results are not known, but possible explanations are: -) differences in neural and/or hormonal influences on fluid transfer and electrogenic secretion. For example the *in vitro* colonic preparation is removed from its normal hormonal and neural interactions, 2) although, in the case of starvation, proximal, mid and distal colon showed hypersecretion induced by bethanechol above the fed control, using whole colon *in vivo* any cause different response to bethanechol because of different transfer mechanisms in different parts of colon. The final concentration of Cl^- in the luminal fluid were not significantly different in the fed and 48h starved colon in both the basal and bethanechol-stimulated states. Bethanechol did not increase the final Cl^- concentration in both fed and starved colon unlike the luminal fluid volume. It is likely that these discrepancies between fluid volume and Cl^- concentrations are due either to the net movement of other ionic species (e.g. Na^+, K^+, HC_3^- and H^+) or to large amounts of water passing into the lumen.

Basal parameters of electrogenic ion transport and the responses to the different secretagogues under fed and food deprived conditions vary from the proximal to the distal end of the mouse colon (summarized previously). Similar findings were also reported for rat colon under the fed condition (32, 33, 34, 50) and under the fed and food deprived conditions (35).

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