Intestinal Repair in Chronically Undernourished Rabbits

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ABSTRACT. To assess the effect of chronic undernutrition on intestinal epithelial repair after injury, we studied 10-wk-old rabbits, nutritionally deprived for 6 wk (body weight 1.95 ± 0.2 kg versus controls, 2.68 ± 0.7 kg, $p < 0.001$). Intestinal structure and function were assessed 4, 24, 48, 72, or 96 h after a 90-min vascular occlusion of a 20-cm segment of midintestine in undernourished and control-diet animals. Tritiated thymidine autoradiography showed that movement of epithelial cells along the crypt-villus axis was significantly curtailed in undernourished rabbits after sham operation or after ischemic injury. In sham-operated rabbits, no significant differences were observed in mucosal structure, disaccharidase and Na-K-ATPase activities, or Na transport (Ussing chambers) between nutritionally deprived and control diet rabbits. Four and 24 h after ischemic injury, significant but similar structural and functional small intestinal abnormalities occurred in both diet groups; recovery of enzyme function was not significantly delayed but crypt-villus length recovery was minimally delayed in undernourished rabbits. Glucose-stimulated Na absorption diminished in short circuited jejunum from malnourished but not from control diet animals 24 h after injury and was normal in both diet groups at 72 h. After transient small intestinal villus cell injury we observed suppressed epithelial proliferation but no consistent impact on epithelial differentiation in nutritionally deprived rabbits. (Pediatr Res 20: 1301–1304, 1986)

Abbreviations

Isc, short circuit current
Na-K-ATPase, sodium-potassium-dependent ATPase
PD, potential difference
ΔIsc, transmucosal Isc

Chronic dietary deprivation has been shown in several species to inhibit epithelial renewal in the small intestine (1). We postulated that in the chronically undernourished subject, epithelial repair might be delayed after injury. In fact, a recent study in our laboratory found that chronically under nourished piglets exhibited relatively prolonged recovery from the extensive lesion of experimental viral enteritis (2). The present experiments examine functional and structural intestinal recovery after transient ischemic injury to short segment of mid-small intestine in rabbits subjected to chronic dietary deprivation.

MATERIALS AND METHODS

Beginning at 4 wk of age, when they weighed 1.2 ± 0.2 kg (mean ± SEM), New Zealand White rabbits, each day, received 50% of the daily intake of a control ad libitum fed group, for 6 wk. Preliminary experiments had established that any dietary restriction in excess of 50% of control diet caused restlessness, aggressive behavior, and a significant death rate. All rabbits received conventional Rabbit Chow (Ralston Purina Company, Raleigh, NC) and had free access to water. As shown in Figure 1, the deprived diet group gained less weight, 1.95 ± 0.2 kg, than the ad libitum fed controls, 2.68 ± 0.7 kg (mean ± SEM) over the 6-wk study period ($p < 0.001$).

After this 6-wk period, rabbits from both dietary groups, at random, underwent general anaesthesia and either a sham laparotomy or mesenteric arterial clamping in which the vascular supply to a 20-cm segment of midintestine was occluded for 90 min using techniques similar to those described for the rat (3). Preliminary experiments had determined that longer periods of vascular occlusion caused irreversible necrosis while shorter periods failed to produce significant injury. Serosal sutures were placed at the proximal and distal ends of the occluded segment for later identification. The sham laparotomy procedure consisted of an abdominal incision and manipulation but no occlusion of mesenteric vessels. Animals were killed 4, 24, 48, 72, or 96 h after operation by parenteral Na pentobarbital. For microscopy and enzyme assays we used 52 animals, for ion flux studies, 32, and for autoradiographic measurements, 16 animals.

For structural measurements by light microscopy, 2-cm sections were fixed first in Bouin's solution, then transferred to 70% alcohol, blocked in paraffin, and stained with hematoxylin and eosin. We measured a minimum of 10 crypt-villus units in
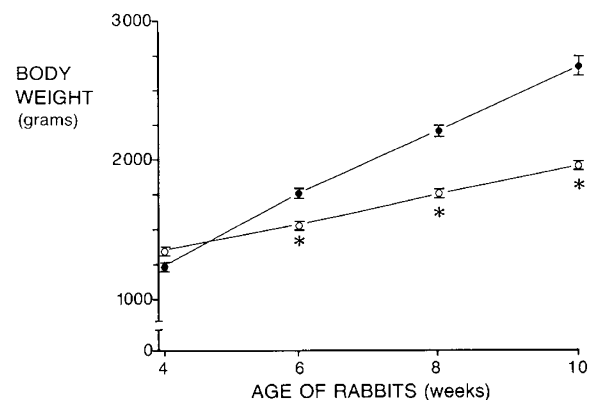

Fig. 1. Body weights, control (●) and undernourished rabbits (O) during a 6-wk period (mean ± SEM). Undernourished group weighed less at ages 6, 8, and 10 wk. *$p < 0.001$.

properly oriented sections using a calibrated micrometer eye piece.

For autoradiographic cell migration studies, rabbits were injected intraperitoneally with ^{3}H thymidine (New England Nuclear, Boston, MA, specific activity 78 Ci/mmol) 0.5 mCi/g of body weight, at the time of the vascular clamping or sham procedure, and killed 4 or 24 h later. Jejunal tissue sections, fixed as described above, were dip-coated with Kodak NTB-2 Nuclear Tract emulsion, and exposed for 3 wk (4). For measurements of cell migration a cell was considered labeled when at least 4 grains were seen over the nucleus. We used a calibrated eye piece to measure distance between the crypt base and the foremost labeled cell along the crypt villus unit and calculated enterocyte migration rates from cell positions at 4 and 24 h.

For mucosal enzyme assays, the ischemic segment of small intestine or the corresponding segment in sham-operated animals was removed quickly, flushed with ice cold normal saline and opened; the mucosa was scraped off with a glass slide and homogenized with 2.5 mM EDTA, 100 mg/ml. Sucrase and lactase activities were measured by the method of Dahlqvist (5), thymidine kinase by a modification (6) of the method of Klemperer and Haynes (7), and Na-K-ATPase as described by Kelly et al. (8).

In Ussing chambers we measured glucose-facilitated Na flux under short-circuited conditions in mucosa from injured and control segments of mid-small intestine. Segments were removed, flushed with ice-cold normal saline, placed in oxygenated chamber buffer (Krebs Ringer bicarbonate), and slipped over a glass rod where muscular and visceral peritoneal layers were removed. The remaining mucosa was opened into sheets and mounted in conventional Ussing chambers, exposing an area of 1.29 cm^2 to buffer. Both sides of the tissue were bathed with equal volumes of Krebs bicarbonate buffer, pH 7.4, which was gassed with 95% O_2 and 5% CO_2 and kept at 37° C. This buffer was constituted as follows (mmol/liter): Na 140, K 10, Mg 0.1, Ca 1.25, Cl 127.5, HCO_3 25, and H_2PO_4 2. After preliminary experiments established that tissue viability was enhanced by the inclusion of 10 mM glucose in the serosal buffer, this concentration of serosal glucose was used, osmotically balanced with mannitol in the mucosal buffer. PD, total conductance, and ISC were measured at 10-min intervals and transmucosal PD neutralized from an appropriate current source as previously described (9). All tissues were studied first under basal conditions for 45 min, and then in the presence of glucose 30 mM added to both mucosal and serosal chambers during 55 min.

After equilibration periods of 15 min in the basal state and in the presence of glucose, unidirectional and net ion fluxes were measured with tracer quantities of ^{22}Na added either to the serosal or mucosal side of paired tissues. Reservoirs were sampled at 10-min intervals and ion fluxes were circulated during standard equations (9). Statistical comparisons were made using Student's unpaired t test.

RESULTS

No apparent clinical differences were seen between dietary groups in their general or their intestinal response to intestinal injury.

Light microscopy. In both dietary groups, vascular occlusion caused a typical ischemic lesion in the intestine of the injured segment with submucosal and subserosal hemorrhage seen at 4 and 24 h. Small intestinal mucosal measurements for each group are summarized in Figure 2. In sham-operated animals, dietary deprivation had no effect on total crypt-villus length. Four hours after ischemic injury, crypt-villus lengths were similarly reduced in both diet groups ($p < 0.05$). By 24 h, crypt-villus length had returned to normal in control diet animals but in the malnourished group, it remained blunted. By 48 h after injury, these microscopic measurements were normal in both dietary groups.

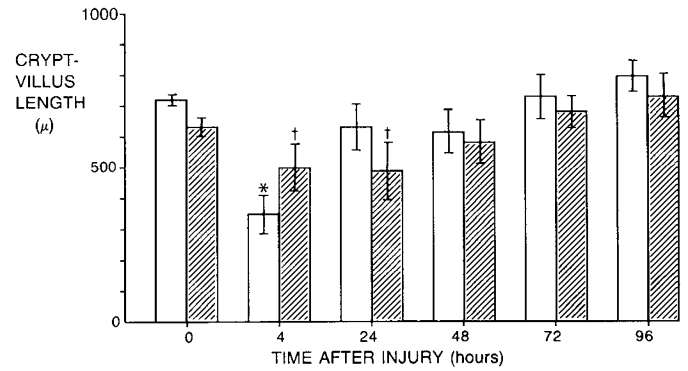*Autoradiography.* Measurements of epithelial cell position at

Fig. 2. Crypt-villus length in control diet (*open bars*) and undernourished rabbits (*shaded bars*) after ischemic injury (mean ± SEM). After significant decrease in both diet groups at 4 h, control group returned to normal at 24 h, undernourished at 48 h. *$p < 0.05$ compared with control diet sham-operated group; †$p < 0.05$ compared with sham undernourished.

intervals after ^{3}H-thymidine injection are summarized in Table 1 for the different study groups. In sham-operated animals, labeled cells were located in the crypts 4 h after injection of the label, administered at the time of operation in both diet groups; the position of the foremost labeled cell was 52 ± 2 μ (mean ± SEM) from the crypt base in control diet rabbits *versus* 43 ± 2 μ in the deprived-diet group ($p < 0.01$). Twenty-four h after thymidine injection, foremost labeled cells in undernourished animals were 120 ± 3 μ from the crypt base, a lesser distance than those in sections from control diet rabbits (158 ± 4 μ) ($p < 0.001$). After ischemic injury foremost labeled cells had traveled significantly further than those in sham-operated animals (control diet, injured *versus* sham, 231 ± 7 *versus* 158 ± 5, undernourished, 201 ± 5 *versus* 120 ± 3, $p < 0.001$) but the distances traveled by these cells at 24 h were significantly less in undernourished animals compared with control diet rabbits, for the sham-operated groups ($p < 0.001$) and for the ischemic injury groups ($p < 0.005$). Migration rates calculated from these means were greatly reduced in undernourished rabbits, 3.9 μ/h for sham operated, and 7.4 μ/h for ischemic injury animals, compared with 5.3 and 9.1 μ/h, respectively, in control diet animals.

Mucosal enzymes. In sham-operated animals, jejunal sucrase specific activities did not differ between dietary groups (138 ± 12 *versus* 113 ± 14 μ/g protein, control *versus* deprived diet), in animals 4 h after ischemia, when levels were less (27 ± 9 *versus* 48 ± 8 μ/g protein $p < 0.05$) than those of sham-operated controls, and in animals 72 h after ischemia when activities were normal (Fig. 3). Lactase activities too, were similar in both dietary groups, although in the undernourished group, normal levels were achieved at 48 h and not until 72 h in control diet animals (Fig. 3). Na-K-ATPase activities followed the same pattern as sucrase activities. Levels of this basolateral enzyme activity in undernourished animals did not differ from those in control diet animals for sham-operated groups (74 ± 8 *versus* 68 ± μ/g protein, control *versus* malnourished). Four h after injury, Na-K-ATPase specific activity was diminished ($p < 0.05$) in both dietary groups but recovered by 72 h in control and deprived diet rabbits (Fig. 3). Thymidine kinase activity, a marker of cell proliferation, increased in control diet rabbits 4 h after ischemia (4.7 ± 0.6 *versus* 8 ± 1 μ/g protein) with further increases at 24 h, returning to normal by 48 h. This rise was delayed in the malnourished animals; it was not apparent until 24 h, and it returned to normal by 48 h.

Sodium transport. Table 2 summarizes unidirectional and net intestinal sodium fluxes for each study group measured under basal conditions and in the presence of 30 mM D-glucose. Glucose-Na cotransport, like the disaccharidases, is a feature of

Table 1. ^3H-thymidine autoradiography, small intestinal epithelium, before and after ischemic injury in control diet and deprived diet rabbits* (mean ± SEM)

	Sham operated				Ischemic injury		
	Control diet	Deprived diet	p	Time after injury (h)	Control diet	Deprived diet	p
Distance traveled by foremost labeled cell (μ)†	52 ± 2	43 ± 2	0.001	4	49 ± 3	53 ± 3	NS
	158 ± 5	120 ± 3	0.001	24	231 ± 7	201 ± 5	0.005
Migration rate (μ/h)	5.3	3.9			9.1	7.4	

* Measurements based on a minimum of 10 crypt-villus units.
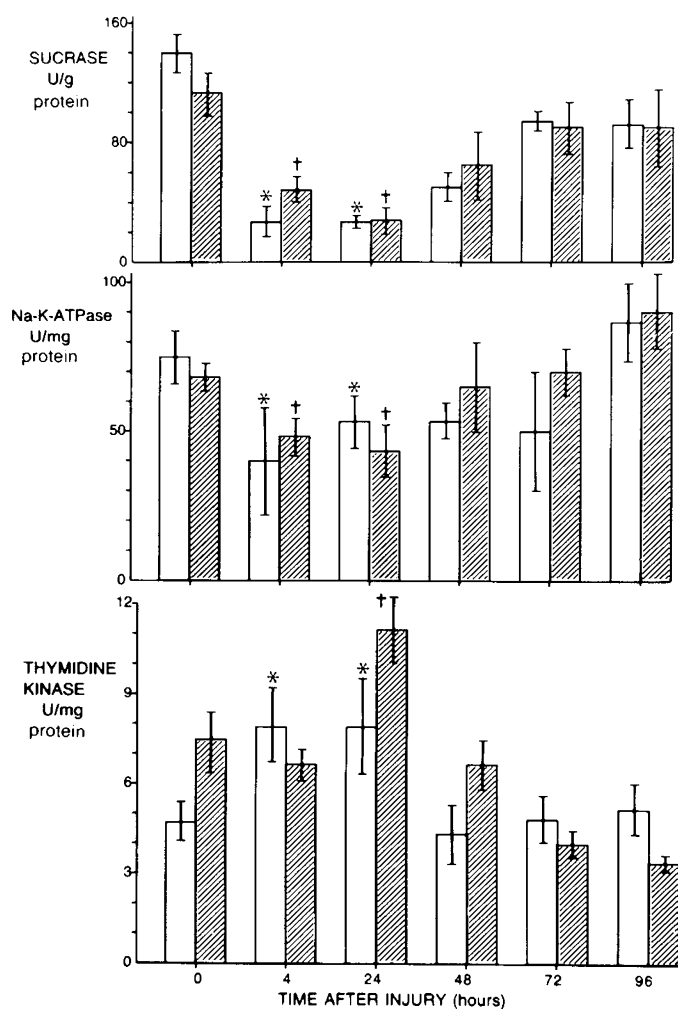† ^{3}H-thymidine injected at time of mucosal injury.

Fig. 3. Enzyme specific activities in small intestinal mucosal homogenates, control diet (open bars) and undernourished rabbits (shaded bars) after ischemic injury (mean ± SEM). Sucrase and Na-K-ATPase activities similar in control diet and undernourished rabbits under all experimental conditions. Thymidine kinase activity increased in control diet rabbits at 4 and 24 h, normal at 48 h; in undernourished group, thymidine kinase increased at 24 h, not at 4 h, normal by 48 h. *p < 0.05 compared with control diet sham-operated group; †p < 0.05 compared with sham undernourished.

luminal membrane function of the villus cell (10, 11). In sham-operated rabbits neither basal nor glucose-stimulated Na flux differed between diet groups. In rabbits fed the control diet, basal and glucose-stimulated Na fluxes, 24 and 72 h after ischemic

injury, did not differ from sham-operated controls. In the malnourished rabbits glucose-stimulated Na absorption, blunted at 24 h compared with sham-operated animals, had recovered when measured 72 h after injury. Presumably, with the loss of villus cells, reflected by diminished disaccharidase activities 4 h after injury, the Na absorptive response to glucose did diminish even in control diet animals immediately after ischemic injury; therefore our normal 24 and 72 h control diet data probably represent recovery. Increments in ΔIsc after the addition of glucose did not differ between dietary groups. This ΔIsc in sham-operated groups (78 versus 84 μA, control versus malnourished) decreased at 24 h (39 versus 21 μA) but returned to the level of sham-operated rabbits 72 h after injury.

DISCUSSION

A 6-wk period of dietary deprivation, which did alter intestinal epithelial proliferation in 10-wk-old rabbits subjected to sham laparotomy, failed to affect structure or enzyme and transport function of uninjured jejunal epithelium. Preliminary studies had shown this deprivation to be as severe as these young animals could tolerate. In sham-operated rabbits, normal levels of mucosal disaccharidases and Na-K-ATPase activities and intact glucose-stimulated Na transport indicate that intestinal epithelial cell differentiation (11, 12) was preserved during a period of nutritional restriction sufficient to reduce body weight gain, and delay epithelial migration. This apparent functional resilience of the gut to semistarvation is suggested by earlier animal studies (13), although the response to starvation can differ depending on the species studied and the nature of the dietary deprivation. For example, in suckling animals during postnatal maturation of the gut, the functional impact of undernutrition seems greater than in mature animals because of its effect in delaying normal postnatal development patterns (1). Studies in man have shown marked structural and functional damage to the gut (14) in malnourished patients but these clinical findings may result, not from direct nutritional damage to the gut, but from coexistent disease such as enteric infestations or infections. Our current data reflect only qualitative characteristics of function not total functional capacity since the findings are related to the protein content of a segment of intestine.

The small intestinal injury in our rabbits by transient vascular injury was in keeping with previous experience using the technique in rats (3). After early loss of viable villus cells there was a relative decrease in a range of functions normally associated with differentiated villus cells (disaccharidases, Na-K-ATPase, glucose-stimulated Na transport). These findings, 4 h after clamping, were the same in control and undernourished groups. Not only were the initial mucosal injuries comparable, but no major differences between dietary groups were seen in the pattern of repair after this segmental lesion. Our data on cell migration show that enhanced migration rates seen in normally nourished rabbits after vascular injury were suppressed in the starved group

Table 2. *Small intestinal mucosa from control diet and deprived diet rabbits, studied in vitro; transmucosal Na fluxes, PD, Isc in sham-operated group and in groups 24 or 72 h after ischemic injury**

			Sham		24 H		72 H	
			Mean	SEM	Mean	SEM	Mean	SEM
Control diet								
Na flux (μEq·cm⁻²·h⁻¹)								
	$J_{m \to s}^{Na}$	Basal	8.2	0.5	8.6	0.5	8.2	0.3
		Glucose	10.7	0.6	9.3	0.5	10.5	0.5
		p	<0.05		NS		<0.05	
	$J_{s \to m}^{Na}$	Basal	8.6	0.3	8.8	0.3	7.8	0.4
		Glucose	9.3	0.5	8.2	0.3	8.8	0.4
		p	NS		NS		NS	
	J_{net}^{Na}	Basal	−0.4	0.4	−0.2	0.2	0.4	0.4
		Glucose	1.4	0.5	1.1	0.4	1.7	0.9
		p	<0.05		<0.05		<0.05	
PD (mV)		Basal	−1.8	0.1	−1.4	0.2	−1.8	0.1
		Glucose	−4.5	0.2	−2.8	0.2	−3.5	0.2
		p	<0.001		<0.001		<0.001	
Isc (μA·cm⁻²)		Basal	39	1.4	29.4	4.0	49	3.5
		Glucose	117	3.8	69.1	6.3	111	13
		p	<0.001		<0.001		<0.001	
Deprived diet								
Na flux (μEq·cm⁻²·h⁻¹)								
	$J_{m \to s}^{Na}$	Basal	9.8	0.4	7.3	0.3	8.7	0.4
		Glucose	11.7	0.4	8.6	0.6	10.9	0.4
		p	<0.05		NS		<0.05	
	$J_{s \to m}^{Na}$	Basal	9.8	0.5	8.3	0.2	8.8	9.3
		Glucose	10.3	0.5	8.2	0.4	0.2	0.2
		p	NS		NS		NS	
	J_{net}^{Na}	Basal	−0.1	0.4	0.9	0.4	−0.1	0.4
		Glucose	1.4	0.4	0.4	0.4	1.6	0.4
		p	<0.05		<0.05		<0.05	
PD (mV)		Basal	−2.6	0.3	−1.8	0.2	−2.0	0.2
		Glucose	−5.4	0.2	−2.9	0.4	−4.2	0.2
		p	<0.001		<0.01		<0.001	
Isc (μA·cm⁻²)		Basal	63.2	8.7	40	5.6	48.3	5.5
		Glucose	147.6	11	61.2	7.8	123.9	31
		p	<0.001		<0.001		<0.001	

* $J_{m \to s}^{Na}$, mucosa to serosa sodium flux; $J_{s \to m}^{Na}$, serosa to mucosa Na flux; J_{net}^{Na}, net sodium flux.

probably reflecting reduced proliferative activity in the crypt epithelium. However, there was little evidence of a consistent functional impact of this altered cell renewal. Enzyme markers of differentiation (disaccharidases, Na-K-ATPase) were not altered by dietary deprivation during recovery from injury and only minor transport differences, with a slight delay in recovery of glucose-stimulated Na absorption, were observed. The delayed rise of thymidine kinase activity in the undernourished groups probably reflects the slow proliferative response apparent from our autoradiographic measurements. We conclude that delayed intestinal epithelial cell proliferation was not associated with altered cell differentiation as reflected by enzyme function.

In a previous study from our laboratory, structural and functional recovery of the small intestinal mucosa was delayed in chronically undernourished piglets after experimentally induced viral enteritis (2). This viral lesion, like that caused by ischemia, is one of villus cells primarily; unlike the ischemic lesion, the virus involves most of the small intestine rather than the short segment we exposed to vascular occlusion in the current experiments. We suspect that the chronically malnourished animal has sufficient endogenous reserves to cope efficiently with the increased demands of repair after a limited intestinal lesion whereas a more extensive injury may overwhelm those reserves.

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