Modulation of small-intestinal secretion and absorption in chronic vitamin E deficiency: studies in rat jejunum
in vitro

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INTRODUCTION

In their historic review of infants with protracted diarrhoeal diseases (PDDs) seen at the Hospital for
Sick Children, Great Ormond Street, London, in the 1960s and 1970s, Larcher et al. [1] proposed that a
vicious cycle of malabsorption and malnutrition was important in the perpetuation of the protracted
diarrhoeal state. This view has long since been upheld, yet the mechanism(s) by which malnutrition
might predispose to malabsorption and so to PDD remain ill defined. Studies of malnourished infants
in the West Indies [2] have demonstrated that many infants, particularly those with protracted
diarrhoea, have low plasma levels of vitamins A and E, and a number of metals, including zinc, copper
and selenium. A prospective study currently in progress at the Hospital for Sick Children, Great
Ormond Street, London, has demonstrated that over half of the children presenting to this hospital
with PDD have a severe deficiency of one or more of these nutrients [2a]. All of these micronutrients
play an important role in defence against oxidative stress. We have previously demonstrated that oxidative
damage to the enterocyte is associated with an increase in electrogenic anion secretion [3] and
have suggested that a depletion of antioxidant defences, as may arise through malnutrition,
coupled with an increased flux of oxygen-derived free radicals leads to a modulation of intestinal
secretion. Vitamn E is the most important lipid-soluble chain-breaking antioxidant in biological
membranes [4]. We have therefore studied the effects of chronic and severe vitamin E deficiency on
small-intestinal function in a rat model.

There were three aims at the outset of this series of studies in vitro: first, to look for evidence of a
change in ionic secretion in the jejunum in association with vitamin E deficiency by performing
steady-state flux experiments; secondly, to examine the secretory response at the enterocyte level [after
pharmacological 'denervation' of the enteric nervous system with tetrodotoxin (TTX)] to gain information
about a number of final common pathways of secretion by the enterocyte and how these are
affected by vitamin E deficiency; and thirdly, to look

1. A vicious cycle of malabsorption and malnutrition has been implicated in the pathogenesis of protracted
diarrhoeal disease in infancy. Vitamin E deficiency is common in malnourished infants with protracted
diarrhoea. We have studied the effects of chronic vitamin E deficiency on small-inestinal secretion and
absorption in the rat.

2. Weanling rats were fed vitamin E-sufficient or -deficient diets for 21 weeks. Jejunal function was
studied in vitro in an Ussing chamber after this period.

3. Steady-state isotopic flux experiments in unstimulated tissues demonstrated net Na⁺ and Cl⁻
secretion in vitamin E-deficient jejunia but net Na⁺ and Cl⁻ absorption in vitamin E-sufficient jejunia.

4. Basal intestinal short-circuit current was the same in both groups.

5. Cyclic nucleotide and maximal non-neuronal acetylcholine-mediated electrogenic secretion were
increased in vitamin E-deficient jejunia.

6. Exogenous 5-hydroxytryptamine (serotonin) induced a smaller increment in electrogenic secretion in
vitamin E-deficient jejunia.

7. Vitamin E-deficient jejunia were less responsive to exogenous noradrenaline, resulting in a smaller
α₂-adrenergic-mediated decrease in intestinal short-circuit current.

8. Fasting for 72 h produced a greater increment in intestinal short-circuit current in vitamin E-deficient
jejunia.

9. Chronic vitamin E deficiency is prosecretory in the small intestine and may predispose to the perpetu-
ation of protracted diarrhoeal diseases.

Key words: jejunum, secretion, vitamin E.

Abbreviations: dNcAMP, dibutyryl cyclic AMP; E+ , vitamin E-sufficient; E-, vitamin E-deficient; EC₅₀,
concentration of secretagogue required for 50% of the maximal elec-
trogenic secretory response; IBMX, isobutylmethylxanthine; p.d., tran-
smucosal potential difference; PDD, protracted diarrhoeal disease; STX, heat-stable toxin; TTX,
tetrodotoxin.

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for evidence of an alteration in amine-mediated secretion and absorption because these neurotransmitters are particularly susceptible to the effects of vitamin E deficiency in neurological tissues [5].

METHODS

Animals

A well-established model of chronic vitamin E deficiency in the rat was used [6]. Twenty-one-day-old weanling male Wistar rats (Charles Rivers) were placed on synthetic diets (Dyets Inc, Bethlehem, PA, U.S.A.), based on tocopherol-stripped lard (10%), vitamin-free casein (20%) and glucose (65%) with a vitamin E-free mineral and vitamin mix (Machlin/Draper HLR No. 814) [vitamin E-deficient (E -)]. In one group [vitamin E-sufficient (E +)] the diet was supplemented with α-tocopheryl acetate (40 mg/kg of feed). All animals were allowed continued free access to food and water. Animals were housed four to a cage on grids to prevent coprophagy on a 12 h light/12 h dark cycle at a temperature of 70°C and a humidity of 70%. Animals from both groups were studied individually in metabolic cages over a 5 day period in the week before the Ussing chamber studies to determine dietary intake.

Preparation of jejunum

After 21–23 weeks on the respective diet animals were fasted overnight (but were allowed continued access to water) and anaesthetized with 60 mg/kg sodium pentobarbitone (intraperitoneally). A 6 cm length of jejunum at a distance 15 cm distal to the ligament of Trietz was quickly removed, flushed gently with ice-cold gassed Ringer-bicarbonate solution and mounted in an Ussing chamber. The external smooth muscle of the intestine was removed by sliding the excised intact intestinal segment on to a cold moist glass rod, carefully cutting along the antimesenteric border with a blunt scalpel blade and then gently peeling away the muscle layers. This preparation was mounted as a rectangular piece of tissue in pins between two Perspex half-chambers as described by Field et al. [7]. An area of 2 cm² of tissue was exposed to 15 ml of Ringer-bicarbonate solution containing (in mmol/l): NaCl, 107; KCl, 4.5; NaHCO₃, 25; Na₂HPO₄, 0.2; NaH₂PO₄, 0.2; CaCl₂, 1.25; MgSO₄, 1.0; pH 7.4. Each solution was maintained at 37°C by heated water jackets and oxygenated and circulated by an O₂/CO₂ (95:5, v/v) gas lift system. Glucose (10 mmol/l) was present in the serosal bathing solution and mannitol (10 mmol/l) in the mucosal bathing solution.

Electrical measurements

Transmucosal potential difference (p.d.) was measured through 3% agar–salt bridges containing 3 mol/l KCl (bridges were soaked in Ringer solution for 1 week before use) positioned close to the mucosal and serosal surfaces of the tissue via matched calomel electrodes (asymmetric potential ≤0.2 mV) immersed in 3 mol/l KCl. p.d. was measured under open-circuit conditions with a DVC 1000 automatic voltage clamp (World Precision Instruments Inc). Combined electrode/bridge junction potentials were always ≤0.3 mV and this was manually offset with a rheostat and voltage source within the DVC 1000 circuitry. The epithelium was automatically short-circuited by a current (Iₑ) passed from the DVC 1000 via Ag/AgCl electrodes and salt–agar bridges at opposite ends of the half-chambers. Corrections were automatically made for the resistance of the fluid gap between the tips of the p.d.-sensing electrodes and the tissue surfaces. The electrical resistance (Rₑ) of the mucosa was determined under open-circuit conditions by passing a current pulse of 100 µA, correcting the subsequent change in p.d. for fluid resistance and then applying Ohm's law.

A fall in Iₑ usually occurred after mounting the tissues followed by a rise to a stable plateau within 15 min of mounting at which time open-circuit p.d. and Rₑ had also reached a stable plateau. These values were taken as the basal electrical values. At the end of each experiment addition of mucosal glucose (10 mmol/l) resulted in a rise in Iₑ, indicating the continued viability of the epithelium.

Radioisotope fluxes

Transmucosal fluxes of Na⁺ and Cl⁻ were measured on paired tissues taken from adjacent segments of jejunum. Tissues were only paired if their electrical resistance differed by less than 25%. Fifteen minutes after mounting, 2.2 µCi of [²²Na] and 2.2 µCi of [³⁶Cl] (Amersham International) were added to the mucosal surface of one tissue and to the serosal surface of the paired tissue under short-circuit conditions. After a further 15 min equilibration period a 2 ml sample was removed from each unlabelled bathing solution and a 100 µl sample from each labelled solution. Samples removed from the unlabelled solutions were replaced with an equal volume of fresh unlabelled Ringer-bicarbonate solution. Duplicate samples were taken 20 min later in order to determine baseline flux rates. At the end of this period, isobutylmethylxanthine (IBMX), final concentration 200 µmol/l, was added to both the mucosal and serosal bathing solutions. A 15 min period under short-circuit conditions was allowed for equilibration, and then fluxes were measured as described above over the following 20 min period.

Half of each sample was counted for [²²Na] in an automatic well-type γ-counter (LKB Wallace). Ten millilitres of scintillant (Ria Luma) was added to the other half of each sample for determination of the combined β-emissions of [²²Na] and [³⁶Cl] in a liquid scintillation counter (LKB Wallace). The activity of
22Na assayed in the γ-counter multiplied by a factor determined by the relative efficiency of the two counters for 22Na, was subtracted from the total β-counts to give the 36Cl counts. Corrections were made for the dilution of the 'unlabelled' solution after the serial removal of aliquots for counting and the replacement of these aliquots with fresh Ringer–bicarbonate solution.

**Calculations**

Unidirectional fluxes of Na⁺ and Cl⁻ were determined by dividing the steady-state rates of radioisotope transfer by the specific radioactivities of the labelled solutions and by the surface area of the exposed tissue (2 cm²) [8]. The net fluxes of Na⁺ and Cl⁻ (J\text{Na net}) were calculated as the difference between the flux from mucosa to serosa (J\text{Na m}) and from serosa to mucosa (J\text{Na s}). The mean Iₘ value of the tissues in each pair were summed and averaged to give single values for each pair. This value was converted from μA/cm² to μmol h⁻¹ cm⁻² by multiplying by a factor of 0.01865 (3.6 × 10³ / AF, where A is the surface area of exposed tissue and F is the Faraday constant). Residual ion flux (J\text{R net}) was calculated as [Iₘ - (J\text{Na net} - J\text{Cl net})].

**Secretagogues**

Cumulative concentration–response curves for secretagogues were constructed by the sequential addition of secretagogue (concentrations increasing by a factor of 10 with successive doses) timed so that each response was elicited before the previous response had started to wane. This approach was validated by comparison of these curves with non-cumulative concentration–response curves for acetylcholine. Cumulative and non-cumulative curves (in the presence of serosal neostigmine and TTX, see below) were comparable. With each of the other secretagogues examined the dose of agonist achieving the maximal increment in Iₘ (ΔIₘ,max) was, in separate experiments, applied to preparations which had not previously been exposed to a secretagogue and the responses found not to differ significantly from those achieved with cumulative increments in dose. The maximal increment in Iₘ and the concentration of secretagogue required for 50% of the maximal electrogenic secretory response (EC₅₀) were derived from the concentration–response curves. No tissue was exposed to more than one secretagogue. The secretagogues acetylcholine, dibutyryl cyclic AMP (dbcAMP) and 5-hydroxytryptamine (5HT, serotonin) were applied to the serosal surface of the preparation. Acetylcholine was added in the presence of 10⁻⁴ mol/l serosal neostigmine to inhibit cholinesterase activity and in the presence of 1.25 μmol/l serosal TTX to block the enteric nervous system. IBMX was applied to both mucosal and serosal surfaces simultaneously, and

**RESULTS**

### Characteristics of animals

The weights and dietary intakes of animals in both groups were comparable at all times. At age 24 weeks weights were E⁺, 523 ± 28 g (n = 35); E⁻, 564 ± 28 g (n = 35); and dietary intakes were E⁺, 29.8 ± 1.5 g/24 h; E⁻, 31.6 ± 0.9 g/24 h (n = 12 in each group).

### Morphological changes

Villus height and mucosal thickness were significantly greater (P < 0.01) in the E⁻ jejuna. The data are given in full in Table 1.

### Basal electrical characteristics and steady-state isotopic fluxes

Basal Iₘ p.d. and Rᵢ were similar in both groups [Iₘ: E⁺, 69 ± 2 μA/cm²; E⁻, 71 ± 2 μA/cm² (n = 55); p.d. E⁺, 1.21 ± 0.05 mV; E⁻, 1.14 ± 0.05 mV; Rᵢ:

| Table I. Morphological characteristics of jejunum from E⁺ and E⁻ rats. Measurements were made on 10 villi/crypts from four animals from each group. Data are presented as mean ± SEM. Abbreviation: NS, not significant. |
|-----------------------------------------------|-----------------|--------|
| Villus height (μm) | E⁺ | E⁻ | P   |
| 587 ± 36 | 684 ± 32 | <0.01 |
| Crypt depth (μm) | 167 ± 14 | 182 ± 12 | NS   |
| Villus/crypt ratio | 3.54 ± 0.39 | 3.80 ± 0.34 | NS   |
| Mucosal thickness (μm) | 755 ± 39 | 867 ± 34 | <0.01 |

Escherichia coli heat-stable toxin (STα) was applied to the mucosal surface.

**Morphometry**

A segment of jejunum adjacent (distal) to that used in the Ussing chamber experiments was fixed in 4% (v/v) formaldehyde in phosphate buffer and sections of this were subsequently stained with haematoxylin and eosin. Computerized morphometric analysis of the sections was performed using an Imagan video system and image analysis software supplied by Kompira. A minimum of 10 villi/crypts were measured on each of four sections from each group of animals.

**Statistics**

Normally distributed data were compared using Student's unpaired t-test. Results are expressed as means ± SEM. EC₅₀ values are expressed as geometric means with the 95% confidence interval for the mean in parentheses. Unpaired t-tests were used to compare these values after logarithmic transformation.
E+, 17.2 ± 0.5 Ω/cm²; E−, 16.6 ± 0.4 Ω/cm²]. Addition of serosal TTX (final concentration of 1.25 μmol/l) had no influence on basal Isc p.d. of Rn.

Values of these basal electrical parameters 40 min after mounting of the tissues (during the ‘basal’ period of the steady-state flux experiments) were also similar (Table 2). The steady-state transmucosal fluxes of Na+ and Cl− are shown in Table 2. There was net Na+ and Cl− absorption in E+ jejuna, but net Na+ and Cl− secretion in E− jejuna. Basal Isc was comparable in the two groups, as was Jnet.

Mucosal and serosal IBMX (200 μmol/l) resulted in Na+ and Cl− secretion in both E+ and E− jejuna. After IBMX, Isc became significantly greater in E− animals (Table 1). JCl net increased, consistent with an increase in electrogenic Cl− secretion, although the difference in JCl net between the tissues did not reach statistical significance.

Fasting animals for 72 h resulted in a rise in Isc in both groups, producing a significantly higher Isc in E− than in E+ animals (E+, 91 ± 7 μA/cm²; E−, 122 ± 6 μA/cm²; n=8; P<0.05). This response to fasting involves a modulation of adrenergic neural tone in the rat small intestine [9], and the results of a brief pharmacological study of aminergic secretion and absorption are presented below.

**Table 2. Steady-state unidirectional and net ion fluxes in stripped jejuna from 6-month old E+ and E− rats. Abbreviations:** lsc, mucosal to serosal flux; lnet, serosal to mucosal flux; jr, short-circuit current; jrnet, residual ion flux; Rn, electrical resistance; +, net absorption; −, net secretion; NS, not significant; IBMX, 200 μmol/l serosal and mucosal IBMX. Values are means ± SEM (n=6 paired tissues for each group).

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**Fig. 1.** Cumulative concentration-response curves for serosal acetylcholine in jejuna pretreated with 1.25 × 10⁻⁶ mol/l serosal TTX and 10⁻⁴ mol/l serosal neostigmine. O, E+, △, E−. Values are means ± SEM (n=8 in both groups).

**Electrogenic secretory responses in the presence of serosal TTX**

Cumulative concentration-response curves for acetylcholine (10⁻⁹ − 10⁻⁴ mol/l) after serosal pretreatment with TTX and neostigmine produced a greater ΔIsc max in E− jejuna (Fig. 1), yet there was no significant difference in EC₅₀ [ΔIsc max]: E+, 112 ± 10 μA/cm²; n=8; E−, 167 ± 21 μA/cm²; n=8; P<0.05; EC₅₀: E+, 5.0 × 10⁻⁷ mol/l (95% confidence interval 2.9–8.2 mol/l); E−, 4.4 × 10⁻⁷ mol/l (95% confidence interval 3.1–6.2 mol/l)]. The secretory response to 350 μmol/l dbcAMP was significantly higher in E− jejuna (ΔIsc: E+, 33 ± 4 μA/cm²; E−, 48 ± 4 μA/cm²; n=8; P<0.05). The ΔIsc max for the electrogenic secretory response to the specific phosphodiesterase inhibitor IBMX was also higher in E− jejuna (ΔIsc max: E+, 189 ± 9 μA/cm²; E−, 211 ± 10 μA/cm²; n=8; P<0.05). The EC₅₀ for responses to IBMX were comparable [EC₅₀: E+, 8.1 × 10⁻⁶ mol/l (95% confidence interval 4.4–15.0 × 10⁻⁶ mol/l); E−, 1.1 × 10⁻⁵ mol/l (95% confidence interval 0.75–1.9 × 10⁻⁵ mol/l); n=8; not significant]. E. coli STa (35 mouse units/ml) produced a similar increment in Isc in both groups (E+, 31 ± 4 μA/cm²; E−, 28 ± 4 μA/cm²; n=7; not significant).
Electrogenic responses to amines (5-HT and noradrenaline)

Cumulative concentration–response curves for the electrogenic response to 5-HT (Fig. 2) revealed a greater $\delta I_{\text{se}}/\text{max}$ in E+ animals ($E^+, 48 \pm 8 \mu A/cm^2$; $E^-, 21 \pm 3 \mu A/cm^2$; $n=7$, $P<0.01$), and a lower $EC_{50}$ in E+ jejuna ($E^+, 1.6 \times 10^{-6}\text{mol/l}$ (95% confidence interval 1.0–2.5 $\times 10^{-6}\text{mol/l}$), $E^-, 4.9 \times 10^{-6}\text{mol/l}$ (95% confidence interval 2.3–10.4 $\times 10^{-6}\text{mol/l}$); $n=7$; $P<0.05$). Pretreatment of the serosal surface with the 5-HT$_3$ antagonist ondansetron (1$\mu$mol/l) resulted in a shift of the concentration–response curve to the right in E+ jejuna with no change in $\delta I_{\text{se}}/\text{max}$, yet a 10-fold increase in $EC_{50}$ ($P<0.05$, Fig. 2). In E- jejuna $\delta I_{\text{se}}/\text{max}$ remained unchanged and the increment in $EC_{50}$ (as compared with E- tissues not pretreated with ondansetron) failed to reach statistical significance (i.e. $P>0.05$) [$\delta I_{\text{se}}/\text{max}$: $E^+, 41 \pm 6 \mu A/cm^2$; $E^-, 20 \pm 3 \mu A/cm^2$; $EC_{50}^+$: $E^+, 1.02 \times 10^{-5}\text{mol/l}$ (95% confidence interval 0.6–1.5 $\times 10^{-5}\text{mol/l}$); $EC_{50}^-$: $E^-, 0.93 \times 10^{-5}\text{mol/l}$ (95% confidence interval 10.62–1.39 $\times 10^{-5}\text{mol/l}$); $n=8$]. The $ED_{50}$ values were comparable in E+ and E- animals after ondansetron pretreatment.

Noradrenaline exerts (mainly) an $\alpha_2$-adrenergic proabsorptive effect in the small intestine associated with a fall in $I_{\text{se}}$. Therefore, to study this effect, tissues were maximally stimulated with 200$\mu$mol/l IBMX and then sequentially exposed to serosal pargyline (a monoamine oxidase inhibitor), tyramine (a false neurotransmitter which releases endogenous noradrenaline stores), noradrenaline and yohimbine (a selective $\alpha_2$ adrenergic antagonist). Yohimbine was used to verify that the response to noradrena-

line was mediated through $\alpha_2$-adrenoceptors, and in all cases it brought about a $\geq 90\%$ reversal of the noradrenaline effect on $I_{\text{se}}$. Serosal pargyline (250$\mu$mol/l) produced a greater decrement in $I_{\text{se}}$ in E+ than in E- jejuna ($E^+, 47 \pm 3 \mu A/cm^2$; $E^-, 28 \pm 3 \mu A/cm^2$; $n=6$; $P<0.01$). Tyramine (100$\mu$mol/l) did not change $I_{\text{se}}$ in either group. Subsequent addition of 100$\mu$mol/l serosal noradrenaline again brought about a greater decrement in $I_{\text{se}}$ in E+ jejuna ($E^+, 28 \pm 7 \mu A/cm^2$; $E^-, 11 \pm 4 \mu A/cm^2$; $n=6$; $P<0.01$).

**DISCUSSION**

This study demonstrates a number of changes in ion secretion and absorption and their control in rat jejunum in chronic vitamin E deficiency.

Ion flux studies demonstrated net secretion of Na$^+$ and Cl$^-$ in E- jejuna in the basal (unstimulated) state as opposed to net Na$^+$ and Cl$^-$ absorption in E+ jejuna. The electrogenic secretory responses to dbcAMP, to phosphodiesterase inhibition and to non-neuronal cholinergic (muscarinic M$_3$ [8]) stimulation are also enhanced in E- jejuna. These changes, together with the poor response of E- jejuna to the proabsorptive $\alpha_2$-adrenergic influence of noradrenaline, would be maladaptive in individuals with PDD and might serve to enhance secretion. These observations are in keeping with our hypothesis that a deficiency of vitamin E could promote diarrhoea and predispose to the perpetuation of PDD.

The origins of the increase in intestinal secretion in association with vitamin E deficiency have not been defined in the present study. Vitamin E is both an important structural element and an antioxidant in biological membranes. Depletion of vitamin E will therefore render the lipids and proteins of the intestinal mucosa susceptible to damage by oxidative stimuli and this might modify intestinal secretion by a number of mechanisms. Peroxidation of membrane lipids, which we have demonstrated in the intestine in vitamin E deficiency [3], has an effect on the biophysical characteristics of the enterocyte apical membrane [10]. This alteration in membrane lipid dynamics might give rise to a change in the functioning of membrane transporter proteins, perhaps as a result of altered lipid–protein interactions. Vitamin E may also protect membrane proteins from direct oxidative damage [11], the consequences of which could also modulate epithelial function. Other ways in which oxidative stress might modulate intestinal secretion include the release of prostaglandins which act on enterocytes and enteric nerves to cause secretion [12], an increase in cytosolic calcium [13], an increase in paracellular permeability [14] and activation of guanylate cyclase [15]. We have made observations pertinent to some of these phenomena in our animal model. Addition of either TTX or indomethacin (10$^{-6}\text{mol/l}$) (prostaglandin synthase inhibition) to
the serosal surface of E+ and E− jejuna had no effect on basal I_{se} (data not shown). We have not, however, studied the effects of these agents on basal fluxes of Na⁺ and Cl⁻. The isotopic flux data suggest, if anything, a reduction in paracellular permeability in E− jejuna. Previous studies in this same animal model have failed to demonstrate any differences in unstimulated cycle nucleotide (cyclic AMP and cyclic GMP) levels in the small intestine in vitamin E deficiency [16].

The change in jejunal morphology in the E− jejuna (an increase in villus length) may have a bearing on the secretory response if the recent observation that the muscarinic M₃ receptor, the final common pathway of cholinergic secretion, is confined to villus enterocytes is substantiated [17]. The poor response of E− jejuna to 5-HT is of interest. 5-HT has a role in modulating intestinal secretion through its influences within the submucosal nerve plexi. The action of exogenous 5-HT involves both cholinergic and non-cholinergic neurons [18]. Chronic vitamin E deficiency causes a characteristic neuropathy in both humans and the rat [19], which may also affect the gastrointestinal tract [20]. An enteric neuropathy, if present, might explain (at least in part) the reduction in the electrogenic secretory response to 5-HT in E− animals. Cyclic AMP-mediated secretion is enhanced in vitamin E deficiency but not the cyclic GMP-mediated electrogenic secretory response to E. coli STa. The secretory response to E. coli STa is mediated in part via the serotonergic 5HT₃ receptor [21]. It is of interest, therefore, that the 5HT₃ receptor antagonist ondansetron had little effect on the 5-HT concentration–response curve in E− jejuna, and yet demonstrated competitive antagonism in E+ jejuna. These findings support the notion that the 5HT₃ receptor may be affected by vitamin E deficiency. Further clarification of this point is, however, needed.

Fasting animals for 72 h is known to increase small-intestinal I_{se}, although the origins of this change remain ill understood. Part of the increase arises from a reduced release of noradrenaline from sympathetic afferent fibres within the enteric nervous system [9]. It is interesting to note that in this same animal model, after a period of 12 months of vitamin E deficiency, the release of [³H]noradrenaline from nerve terminals within the caecum muscularis is decreased and that this is associated with an increase in the noradrenaline content of caecal muscle (C. Hoyle, personal communication). Our observations of reduced responsiveness to noradrenaline and of a larger increment in I_{se} on fasting in E− jejuna may therefore be different manifestations of an alteration in catecholamine turnover within the enteric nervous system in vitamin E deficiency. The smaller response to pargyline in E− jejuna is difficult to interpret as this may reflect in part a reduced responsiveness of these tissues to noradrenaline.

This study provides evidence that vitamin E deficiency is associated with abnormalities of intestinal secretion and absorption and that muscarinic M₃ [22], serotonergic 5HT₃, and cyclic AMP-mediated secretory mechanisms and a₂-adrenergic proabsorptive mechanisms are affected. Whatever the origins of the vitamin E deficiency in infants and children with PDD, a strong case can be made for early and aggressive replacement therapy with vitamin E and other vitamins/trace metals which help provide the individual with competent defences against oxidative stress.

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