Vitamin A deficiency and small intestinal secretory function in the rat

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Abstract
The influence of vitamin A on the functions of the small intestine was examined in rats made vitamin A deficient for 40 days by feeding a special diet after weaning and in pair fed vitamin A deficient rats that were given supplementary vitamin A (240 IU/day) in their drinking water. The basal and stimulated electrogenic secretory and absorptive functions of the jejunum and proximal and distal ileum removed from these rats were examined in vitro using the short circuit current as the index of transport activity. The basal short circuit current in the jejunum and proximal ileum was not significantly different but that of the distal ileum was lower. Electrogenic glucose transfer was not significantly affected by the vitamin deficiency. Cholinergic stimulation using the M1/M2 agonist betahanechol showed a greatly enhanced electrogenic secretion in the jejunum of the deficient rats while secretion stimulated by dibutyryl cyclic adenosine monophosphate was significantly greater in their distal ilea compared with the supplemented group. The vitamin deficiency also disrupted the normal higher/lower hierarchical pattern of transport activity between the proximal and distal ileum. The enhanced secretory activity of the vitamin A deficient small intestine offers a putative explanation for the well known relation between vitamin A deficiency and diarrhoea found in humans.

Vitamin A deficiency has been claimed to rank worldwide among the most common of dietary deficiencies. In humans it is usually associated with diarrhoea, but despite many observational, clinical, interventional, and field studies it is still unclear whether the deficiency is the direct cause of the diarrhoea or merely a consequence of poor nutritional health. While there have been a few animal studies on the effects of vitamin A deficiency on the small intestine, these have focused either on changes in the population dynamics of the enterocytes, such as the decrease in number of goblet cells, an increased cell cycle time coupled with an impaired migration rate out of the jejunal crypts, or on biochemical changes in nuclear RNA and protein synthesis and the enzymes alkaline phosphatase and Na–K adenosine triphosphatase. Apart from a brief note by Kancha and Anasuya that vitamin A deficiency enhanced in vitro the duodenal transfer of calcium and the jejunal transfer of oxalate, there have been no investigations on the absorptive or secretory functions of the small and large intestine in vitamin A deficiency. The present study was devised to establish whether vitamin A deficiency by itself influenced the secretory mechanisms of the intestine in rats. Because vitamin A deficiency reduces food intake the use of pair fed controls is essential as a reduction in food intake is now known to induce hypersecretory activity in both small and large intestine and rectum in the rat. This paper describes the secretory behaviour of the rat small intestine in vitamin A deficiency. A preliminary report has been published.

Methods
ANIMALS AND DIET
Weaning 3 week old male albino rats (weight approximately 80 g) of the Sheffield strain were divided into five groups. Group 1 was fed a commercially supplied vitamin A deficient diet (diet ssDii from SDS Diets, England) ad libitum and had free access to water. The diet contained casein (15%), rice starch (35%), safflower oil (10%), solkafloc (a bulking agent) (10%), icing sugar (25%), and a mixture of vitamins (excluding vitamin A) and essential minerals (5%) and was quoted as having a retinol concentration of 0 mg/kg diet by analysis. The diet was fed for 40 days and the food intake of the rats was measured daily. Group 2 was pair fed the vitamin A deficient diet using the food intake values of group 1 but had a soluble preparation of vitamin A dissolved in their drinking water (Rovimix A 500W, Roche Products) such that their intake approximated to 80 IU vitamin A/day. Similarly, group 3 was also pair fed on the vitamin A deficient diet according to the intake of group 1 but had an intake of approximately 240 IU vitamin A per day in their drinking water. Group 4 was pair fed the standard CRM pelleted diet used in all our previous studies (Diet CRM Labsure, London, containing 1500 IU vitamin A/kg diet) while group 5 comprised age matched controls fed the standard CRM pelleted diet ad libitum. All the rats were housed in plastic cages and were kept at 20(1)°C and 72% humidity with the lights on from 5 30 am until 6 30 pm. The food intakes of the vitamin A deficient group are shown in Table I for days 1, 10, 20, 30 and 40.

IN VITRO PREPARATION
On the day of use (after 40 days on the diets) the rats were anaesthetised with intraperitoneal pentobarbitone (May & Baker; 60 mg/kg body weight). On achieving surgical anaesthesia, a midline incision was made and the mid-jejunum, some 40–45 cm from the ligament of Treitz, identified. A 5 cm length of this area was removed, cut open, and mounted as a flat sheet
between two identical chambers as described previously. Similarly mounted was a 5 cm segment of ileum (15–20 cm aboral from the ileocaecal junction) and a 5 cm segment of distal ileum (removed aboral from the ileocaecal junction). The segments were incubated at 38°C in bicarbonate saline gassed with 95% O₂ 5% CO₂. The tissue potential difference (in mV), short circuit current (μA/cm² serosal area), and the tissue resistance (ohms/cm² serosal area) were all obtained by a previously published standard technique. Secretory function was assessed in the basal state without the addition of secretagogues and as the maximum secretory current minus the basal secretory current after the serosal addition (1 mmol/l) of the stable muscarinic (M₁/M₂) agonist Bethanechol or dibutyryl cyclic adenosine monophosphate (AMP). The absorptive function of the segments was monitored by the mucosal addition of 28 mmol/l glucose (balanced osmotically by the serosal addition of 28 mmol/l mannitol) which generated, by Na⁺– coupled electrogenic glucose transfer, a transfer short circuit current.

PLASMA VITAMIN A ESTIMATION
The concentration of vitamin A in the plasma of the various experimental groups was estimated by a modification of Robinson's of the techniques of Grys and Bayfield and Cole.

MATERIALS
All the chemicals were purchased from Sigma Chemical, Poole, England. The caged vitamin A deficient diet (ssDi) was purchased from SDS Diets, England, and the normal control diet CRM from Labsure, London. The soluble vitamin A used in the drinking water (Rovamix, A Type 500W) was a gift from Roche Products, Welwyn Garden City, England.

STATISTICAL ANALYSIS
All the results are shown as the mean (SE). For statistical comparisons the unpaired Student's t-test with 0.05 as the level of significance was used. When multiple comparisons were needed the Kruskal–Wallis analysis of variance was used followed by Conover's multiple r test to delineate specific differences.

Results
FOOD INTAKE
Table I gives the food intake of group 1 for days 1, 10, 20, 30, and 40 on the vitamin A deficient diet. There was an increase of 1:2-fold from day 20 to day 40. For comparison the food intake of the rats fed the normal control diet (CRM, Labsure) is shown for day 40, it being approximately 2:7 times greater than that of the vitamin A deficient group. It should be noted that for groups 4 and 5, who were fed the normal CRM diet, group 4 represents a chronically under-nourished group compared with group 5 (as on day 40, for example, group 4 rats received only 9 g CRM diet/day while those in group 5 averaged 24 g/day). Group 4 rats on 9 g/day would have an average daily vitamin A intake of 135 IU vitamin A while those on 24 g (group 5) would have an intake of 360 IU/day.

VITAMIN A PLASMA LEVELS
The plasma concentrations of vitamin A for the five groups are given in Table II. The rats on the vitamin deficient diet (group 1) had the lowest concentrations. The average daily requirement for vitamin A in adult rats has been estimated as 40–80 IU. The vitamin A deficient rats fed 80 IU vitamin A/day (group 2) showed an increased plasma vitamin A concentration, but this was not significantly different from group 1 (p>0.05), while those fed 240 IU vitamin A/day (group 3) had a vitamin A concentration approximately 3:5 times greater (p<0.001) than group 1. Moreover, the concentration for this group was not significantly different from that in rats pair fed the normal CRM diet (group 4) and the age matched rats fed the normal CRM diet ad libitum (group 5). Thus feeding 80 IU vitamin A/day in the drinking water does not satisfactorily restore the plasma vitamin A state of the vitamin deficient rats to normal, but giving 240 IU vitamin A/day does.

INTESTINAL PHYSICAL AND BIOELECTRIC PARAMETERS
Several basic physical and bioelectric parameters were measured in the intestines of the vitamin deficient rats (group 1) and the vitamin deficient rats supplemented with 240 IU vitamin A/day (group 3). Because there was no significant difference between the groups for total intestinal length or weight of jejunal, proximal ileal, and terminal ileal segments per cm² serosal area and...
for basal potential difference, these have not been shown. The data for the basal current are given in Table III. While no significant differences were found between the vitamin deficient (group 1) and the vitamin deficient pair fed groups (groups 2 and 3), a significant rise in the jejunal basal current was evident in group 4 (+49%, p<0.001) and group 5 (+46%, p<0.001) compared with group 1. In the ileum no significant differences were found between the vitamin deficient rats (group 1) and other control groups 2 and 3, but the pair fed rats in group 4 showed a significant increase (+42%, p<0.005). The tissue resistance for the jejunum and distal ileum was not significantly different between groups 1 and 3 but that of the proximal ileum was significantly lower by approximately 22% (p<0.001) in the vitamin deficient group (group 1, n=19 mean (SE) 57 (5) ohms/cm² serosal area compared with group 3, n=14, 73 (5) ohms/cm² serosal area).

SECRETORY CURRENTS INDUCED BY BETHANECHOL

The secretory currents induced in the jejunum, proximal ileum, and distal ileum by serosal bethanechol in the five groups are given in Table IV. The time course of the response for the jejunum is shown in Figure 1. Comparing the vitamin A deficient (group 1) with the deficient rats supplemented with 240 IU vitamin A (group 3) the maximum minus basal secretory current induced in the jejunum was 86% greater (p<0.001), that in the proximal ileum was 37% greater (but not significant), while that in the distal ileum was 33% greater (p<0.05). Even in the vitamin A deficient animals supplemented with 80 IU vitamin A (group 2) the maximum minus basal secretory current of the jejunum (+118.5%, p<0.001) and proximal ileum (40%, p<0.02) was significantly greater than that in group 3. The fed rats (group 4) showed enhancement of bethanechol secretion brought about by chronically reduced food intake when compared to the maximum minus basal secretory current induced in the rats fed ad libitum on the CRM diet (group 5). The secretory current of the jejunum in group 4 rats was 203% greater (p<0.001), in the proximal ileum 76% greater (p<0.001), and in the distal ileum 117% greater (p<0.001) than that in group 5. That in group 3 rats was not significantly enhanced in the jejunum and proximal ileum, but in the distal ileum it was significantly greater (44% p<0.001) than in group 5.

SECRETORY CURRENTS INDUCED BY DIBUTYRYL CYCLIC AMP

The secretory currents induced by dibutyryl cyclic AMP in the jejunum, proximal ileum, and distal ileum in the five groups are shown in Table V. The time course of response for the distal ileum is shown in Figure 2. An increase in the maximum minus basal secretory current was observed only in the terminal ileum (+40%, p<0.01) when the vitamin deficient rats (group 1) were compared with the vitamin deficient supplemented with 240 IU vitamin A/day (group 3); the secretory currents of the jejunum and proximal ileum were not significantly different. The currents of the CRM pair fed rats (group 4) showed increases in the jejunum (+33%, p<0.05) when compared with those in the rats fed ad libitum with the CRM diet (group 5). The currents for group 3 rats were not significantly different from those of group 4, indicating that feeding 240 IU vitamin A/day restored the intestinal function to the level of the rats with normal vitamin A concentrations but on a reduced food intake.
ELECTROGENIC GLUCOSE TRANSFER

The transfer currents induced by the addition of 28 mmol/l glucose to the mucosal fluids of the jejunal, proximal ileum, and terminal ileum from the various nutritional groups are shown in Table VI. No significant difference was observed for the maximum minus basal transfer currents between the vitamin A deficient rats (group 1) and the vitamin deficient rats supplemented with 240 IU vitamin A/day (group 3). In group 4 the values for the proximal ileum (+163%, p<0.01) and the terminal ileum (+39%, p<0.05) were significantly greater than the values for group 5 fed ad libitum.

Discussion

The results show that vitamin A deficiency has complex effects on the secretory functions of the small intestine when assessed in vitro. While the basal intestinal current, which is known to be mainly electrically Cl secretion,14-16 seems to be unaltered, the maximum minus basal current induced by bethanechol, a stable muscarinic M1/M2 agonist, was significantly greater in jejunum and so was that induced by dibutyryl cyclic AMP in the distal ileum of the vitamin A deficient rat. Bethanechol induced secretion is mainly electrogeneric Cl in the jejunum,14 but there is some electrogeneric bicarbonate secretion in the ileum.15 Previous studies on the distal ileum15 have shown that this area has significantly less secretory and absorptive capacity than the proximal ileum and did not adapt to starvation.16 The former finding16 is confirmed in the present study when the data of the distal ileum are compared with that of the proximal ileum in group 5 rats, those fed the normal control diet (CRM, Labsure) ad libitum. The basal current and the bethanechol stimulated, dibutyryl cyclic AMP stimulated, and glucose stimulated maximum minus basal currents of the distal ileum were clearly less than those of the proximal ileum. This pattern is completely disrupted in the vitamin A deficient group (group 1) where the basal current and the bethanechol induced and glucose induced maximum minus basal currents of the distal ileum are not lower than those of the proximal ileum. Even in the case of the dibutyryl cyclic AMP induced current, that of the distal ileum in the vitamin deficient group is actually greater than in the proximal ileum. Vitamin A deficiency thus alters dramatically the balance between the distal and proximal ileum.

In a previous study we presented evidence to suggest that one property of the distal ileum may be its 'chemosensing' function.15 Whether vitamin A deficiency also interferes with this putative distal ileal function is a question to be answered in further studies.

It is interesting that the alterations in intestinal function brought about by vitamin A deficiency in the rat seem to be selective for the secretory function, for no change could be found in the absorptive electrogeneric transfer of glucose. The only other data on the absorptive function of the vitamin A deficient rat is a brief note by Kancha and Anasuya,17 who reported that the in vitro duodenal transfer of calcium and the jejunal transfer of oxalate were enhanced compared with those of control intestine from pair fed rats. Their rats, however, were made vitamin A deficient over 84 days compared with the 40 days in our study. It is not known whether this increase in duration of the deficient state is an important difference between the studies. Only two other investigations have examined secretory function in the gastrointestinal tract of vitamin A deficient rats. The basal transmural gastric potential difference was not altered significantly in vitamin A deficient rats,18 but unfortunately no measurements were made in the rats stimulated to secrete acid, which is probably the more important measurement. In the case of the salivary gland19 impaired secretion of saliva was found during the induction of rapid, synchronous vitamin A deficiency.

Vitamin A deficiency in the rat seems to induce an increase in jejunal electrogeneric secretion (chloride) when activated by bethanechol and in the distal ileum when activated by dibutyryl cyclic AMP. Feeding vitamin A in those fed the normal diet to vitamin A deficient rats prevented the changes. This is the first clear cut demonstration that vitamin A deficiency alone can enhance the secretory function of the small intestine. This enhanced
secretory activity observed in the rat offers an explanation for the well known relation between vitamin A deficiency and diarrhoea found in humans.

Acknowledgement is given to the Medical Research Council for financial support.

2 Feachem RG. Vitamin A deficiency and diarrhoea – a review of interrelationships and their implications for the control of xerophthalmia and diarrhoea. Trop Dis Bull 1987; 84: R1-16.

16 Levin RJ, Nazgawi C, Young A. Proximal colon secretion in fed and fasted rats. J Physiol (Lond) 1987; 396: 33P.
18 Levin RJ, Parker AJ. Rectal electrogenic secretion – is it a putative indicator of intestinal secretory status induced by nutritional deprivation in the rat? Exper Physiol 1990; 75: 609-11.
19 Levin RJ, Nazgawi C. Vitamin A deficiency and its effects on in vitro jejunal and ileal electrogenic secretion in the rat. J Physiol (Lond) 1990; 422: 66P.
21 Robinson ME. Retinoids in organ culture of the cheek pouch and in other tissues of the Syrian hamster (mesocricetus auratus). M Sc Guelph, Ontario: University of Guelph. (thesis.)
25 Young A, Levin RJ. The rat distal ileum has a reduced absorptive and secretory capacity compared with proximal ileum – is it to facilitate its chemo sensing function? J Exp Physiol 1989; 74: 563-3.
26 Levin RJ, Young A. Proximal but not distal rat ileum shows adaptive responses to starvation. J Physiol (Lond) 1990; 420: 143P.