Plasma enteroglucagon, peptide YY and gastrin in rats deprived of luminal nutrition, and after urogastrone-EGF administration. A proliferative role for PYY in the intestinal epithelium?

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Summary. Intestinal tissue mass was significantly reduced throughout the gastrointestinal tract ($p < 0.001$) of intravenously fed (TPN) rats. Urogastrone-epidermal growth factor, (URO-EGF), reversed these changes. Although plasma enteroglucagon and gastrin levels showed a small increase with URO-EGF, this was far less than the gut tissue weight change, suggesting that it was unlikely that they were involved in modulating the proliferative response of the intestine to URO-EGF. Peptide tyrosine tyrosine (PYY) levels were however significantly increased by URO-EGF, indicating that PYY may possibly have a role in the modulation of intestinal cell proliferation.
Key words. Intravenous (parenteral) nutrition; gastrointestinal tract; epithelium; hormones; glucagon; enteroglucagon; gastrin; PYY; Cell division; cell proliferation; growth control.

EGF[2] can stimulate the proliferation and maturation of the neonatal intestine[3,4], and human EGF or urogastrone (URO-EGF) has been shown to be a potent stimulator of intestinal epithelial cell proliferation in adult animals[5,6] and humans[7].

The effects of URO-EGF were studied on those gut-related peptides, implicated in the control of intestinal epithelial cell proliferation[8]. Adult rats were given a dose of URO-EGF which markedly stimulates cell proliferation throughout the intestine[6].

Methods. Three groups of 12 male 230-g Wistar rats (Olac Ltd., Blackthorn, Oxon) were maintained on the following treatments for eight days. The first group was fed a standard pelleted diet (Labshure PRD, Poole Dorset) the second was maintained on an intravenous (TPN) diet[5,6], the third group was on TPN plus 60 µg/rat/day of URO-EGF. The URO-EGF (ICI, Alderley Park, Macclesfield) was recombinant urogastrone derived from the expression of a synthetic gene in *E. coli* and had the same amino acid sequence and biological activity as natural URO-EGF[9]. The animals were anaesthetised with pentobarbitone and killed by exsanguination. The gastrointestinal tract was removed, rinsed with sterile ice-cold saline, blotted and weighed. Gut hormones were measured by previously described radioimmunoassays for pancreatic glucagon[10], enteroglucagon[11], PYY[12] and gastrin[13]. Enteroglucagon was calculated by subtracting specifically measured pancreatic glucagon (C-terminal immunoreactivity measured with the antiserum RCS5 from total N-terminal glucagon immunoreactivity (measured with antiserum R59)). The assays were capable of detecting, pancreatic glucagon 2 pmol/l, enteroglucagon 5 pmol/l PYY 2.5 pmol/l and gastrin 2 pmol/l with 95% confidence. All results are presented as the mean ± SEM. Data was tested by a two-sided *t*-test.

Results. There was no difference in the end body weight of the TPN or the TPN + URO-EGF groups (223.8 ± 2.5 and 218.8 ± 4.3, respectively). The orally fed rats were however, significantly heavier 288.8 ± 4.0 ($p < 0.001$). The weight of the stomach, small intestine, caecum and colon were significantly reduced ($p < 0.001$) after intravenous feeding (table 1). Urogastrone (URO-EGF) significantly increased the wet weight of all these regions of the gastrointestinal tract and restored the weight of the stomach, caecum and colon to orally fed levels (table 2). The small intestine was however still significantly lighter, but this difference was removed if the results were expressed as a percentage of body weight, and the relative weight of the other regions of the gastrointestinal tract was then significantly greater than that of the orally fed rats ($p < 0.001$).

All hormone levels measured were significantly reduced in the small intestine of the intravenously fed animals when compared to the orally fed group ($p < 0.001$). The reduction in pancreatic glucagon, enteroglucagon and gastrin was however greater than that in tissue weights (table 2), while the decrease in PYY was similar to the decrease in intestinal tissue weight.

URO-EGF treatment significantly increased enteroglucagon, ($p < 0.05$) but not pancreatic glucagon (fig.). Enteroglucagon levels were nonetheless still significantly less than those of the orally fed rats ($p < 0.001$) and the increase with URO-EGF only increased plasma levels from 14% to 24% of the oral value (table 2).

Plasma gastrin was very highly significantly increased by URO-EGF ($p < 0.001$), but like enteroglucagon its levels were still very much less than those of the orally fed group ($p < 0.001$) (table 2).

Plasma PYY was also very highly significantly increased by URO-EGF when compared to the TPN group ($p < 0.001$); moreover, it was also significantly elevated when compared to the orally fed group ($p < 0.05$) (table 2).

Discussion. The dramatic proliferative response of the gastrointestinal tract to URO-EGF previously observed[5,6] was substantiated by the large increase in tissue weight seen in

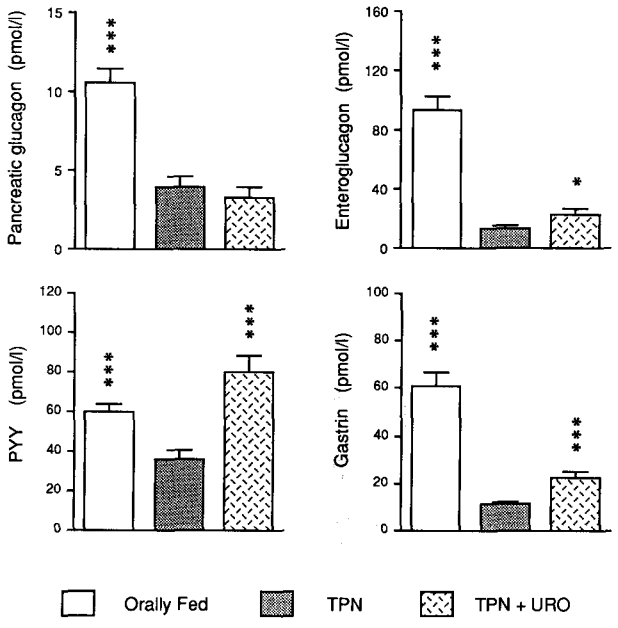
Table 1. The effects of oral feeding, total parenteral nutrition (TPN) and TPN + 60 µg/rat/day of urogastrone-epidermal growth factor on the wet weight (g) of the major regions of the gastrointestinal tract.

	Orally fed	TPN	TPN ± URO-EGF
Stomach	1.730 ± 0.038	1.154 ± 0.028 [+]	1.633 ± 0.030 [*]
Small intestine	10.460 ± 0.330	4.902 ± 0.113 [+]	7.736 ± 0.244 [+ *]
Caecum	1.367 ± 0.058	0.855 ± 0.036 [+]	1.371 ± 0.070 [*]
Colon	1.882 ± 0.060	0.910 ± 0.028 [+]	2.015 ± 0.084 [*]

[+] significantly lower then the orally fed group ($p < 0.001$); [*] significantly greater than the TPN group ($p < 0.001$).

Table 2. The relative effects of oral feeding, total parenteral nutrition (TPN) and TPN + 60 µg/rat/day of urogastrone-epidermal growth factor on the weight of the major regions of the gastrointestinal tract and on the plasma levels of pancreatic glucagon, enteroglucagon, gastrin and peptide YY. The results are expressed as a percentage of the values found in the orally fed group of rats.

	Orally fed	TPN (%)	TPN + URO-EGF (%)
Stomach weight	100	66.7	94.4
Small intestine weight	100	46.9	74.0
Caecum weight	100	62.5	100.3
Colon weight	100	45.4	107.1
Plasma pancreatic glucagon	100	37.5	30.5
Plasma enteroglucagon	100	13.9	24.4
Plasma gastrin	100	18.7	37.2
Plasma PYY	100	61.1	134.0



The effects of oral feeding, intravenous nutrition (TPN) and TPN + URO-EGF on plasma hormone levels. *significantly greater than the TPN group ($p < 0.05$); **significantly greater than the TPN group ($p < 0.01$); ***significantly greater than the TPN group ($p < 0.001$).

this experiment. The proliferative effect of URO-EGF on the small intestine was less pronounced than that of the other regions of the gastrointestinal tract, confirming that the stomach and colon are more susceptible to the effects of URO-EGF which could perhaps be the consequence of their being considerably more EGF receptors in the colon than in the small intestine[14].

The activity of all hormones fell dramatically in the TPN rats where the intestine was deprived of 'luminal nutrition'[15], with the levels of pancreatic glucagon, enteroglucagon and gastrin falling proportionately more than the loss of tissue weight. The drop in PYY was more in line with the fall in tissue mass. URO-EGF increased all the hormones except pancreatic glucagon, but the response of enteroglucagon and gastrin was not of the same order of magnitude as the proliferative changes, suggesting that they do not have a major role in modulating the proliferative response of the intestine to URO-EGF.

Plasma PYY however, increased to values significantly greater than those of the orally fed group in the URO-EGF treated group. The response of this peptide reported here raises the possibility that it might be involved in modulating the proliferative effects of the intestine to EGF. The correlation between PYY and intestinal epithelial cell production in dietary manipulation studies[16] and after intestinal resection[17], leads to the speculation that this effect may be more general.

Changes in plasma enteroglucagon and PYY are usually seen in all models of altered intestinal epithelial cell proliferation, suggesting that they may be major factors in the control of intestinal cell renewal[8]. The different response of enteroglucagon and PYY observed in this study is especially interesting as the two hormones often respond similarly[5,16]. Enteroglucagon and PYY producing cells are believed to be co-localized in the distal intestine and caecum[18], thus it is of interest that they can behave so differently.

PYY producing cells are located distally[19] whilst receptors for PYY have been reported in the proximal intestine[20]; thus the possibility that a feedback loop from the distal gastrointestinal tract to the proximal exists would appear attractive.

The lack of any major response of enteroglucagon to URO-EGF, despite the concomitant increase in proliferation, contradicts the proposal that raised enteroglucagon levels are merely the inevitable consequence of increased intestinal cell division or mass. These results would also suggest that the inferred proliferative effect of enteroglucagon and the directly observed effects of URO-EGF exert their influence through different mechanisms; which is substantiated by the observation that enteroglucagon has rarely been associated with increased intestinal epithelial cell division in the colon where URO-EGF has its greatest effect.

It is very likely that many mechanisms are involved in the control of gastrointestinal epithelial cell renewal, the exact nature of these have yet to be resolved[15], but an important role for PYY in this cannot be excluded.

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