and guinea pigs (n = 5). Bile was collected every 10 min during a 2–3 h experimental period. Blood was withdrawn from the carotid aretery (1.0 ml) during spontaneous secretion, and during and after choleresis or cholestasis. Total phosphorus was measured as described by Bartlett[3], whereas inorganic phosphate was quantitated enzymatically as reported by Hwang and Cha[7]. Choline-containing phospholipids (from now on referred to as phospholipids) were determined enzymatically[4]. The accuracy and reproducibility of these methods were validated in our laboratory.

Results and discussion. Inorganic phosphate, total phosphorus, and phospholipid concentrations in plasma were virtually constant throughout the bile collection period, regardless of whether bile secretion was stimulated of inhibited. Their plasma levels in rats and guinea pigs were, respectively: inorganic phosphate = 0.88 ± 12 (SD) and 0.97 ± 0.15 mmoles/l; total phosphorus = 2.33 ± 0.35 and 1.67 ± 0.27 mmoles/l, phospholipids = 1.68 ± 0.19 and 0.56 ± 0.09 mmoles/l. The biliary concentrations are reported in the table. Two features of these results are of importance. First, inorganic phosphate concentration in bile during spontaneous secretion accounted for approximately 12% of total biliary phosphorus in the rat, but as much as 60% in the guinea pig. Unlike the rat, in fact, the guinea pig's bile contained very low levels of phospholipids. Second, and more importantly, inorganic phosphate concentrations increased significantly in the post-choleretic period, and during and after cholestasis. In either species, the increment in inorganic phosphate levels accounted almost entirely for that seen in total biliary phosphorus.

At present, it is not clear why biliary inorganic phosphate concentration changes following alterations in bile secretion·rate produced by choleretic or cholestatic bile acids. The mechanism by which inorganic phosphate enters bile is not known, so that interpretation of the present results is not possible. Similarly, it remains to be demonstrated whether the changes observed in the rat and guinea pig occur in other laboratory animals and man.

To our knowledge, detailed studies of inorganic phosphate excretion into bile of laboratory animals have not been conducted, and available data are limited to clinical observations. Interestingly, however, Wiegard and Murphy[8] and Sutor and Wilkie[6] have observed a wide variation in inorganic phosphate levels in hepatic biles and duodenal aspirates of patients who underwent cholecystectomy. In some cases, inorganic phosphate accounted for up to 50% of total phosphorus[8]. It is thus possible that in man and other animal species as well, changes in inorganic phosphate concentrations occur, at least in part, secondarily to changes in bile secretory function.

In conclusion, these findings stress the importance of determining inorganic phosphate in bile when total biliary phosphorus, measured in whole bile samples, is taken as an estimate of phospholipid content. Our studies indicate that correction for inorganic phosphate is essential 1) in animal species, e.g. the guinea pig, in which the latter accounts for a major fraction of total biliary phosphorus; and 2) under conditions associated with changes in bile secretion rate.

1 This work was supported by grant HD-17556 from the National Institute of Child Health and Human Development.

2 Fiske, C.H., and Subbarow, Y., J. biol. Chem. *66* (1925) 375.
3 Bartlett, G.R., J. biol. Chem. *234* (1959) 466.
4 Gurantz, D., Laker, M.F., and Hofmann, A.F., J. Lipid Res. *22* (1981) 373.
5 Qureshi, M.Y., Murphy, G.M., and Dowling, R.H., Clinica chim. Acta *105* (1980) 407.
6 Sutor, D.J., and Wilkie, L.I., Clinica chim. Acta *77* (1977) 31.
7 Hwang, W.I., and Cha, S., Analyt. Biochem. *55* (1973) 379.
8 Wiegard, J., and Murphy, G.H., Clinica chim. Acta *89* (1978) 169.

0014-4754/85/091160-02$1.50 + 0.20/0

Urogastrone-epidermal growth factor is trophic to the intestinal epithelium of parenterally fed rats

R.A. Goodlad, T.J.G. Wilson, W. Lenton, H. Gregory*, K.G. McCullough** and N.A. Wright

Department of Histopathology, Royal Postgraduate Medical School, Hammersmith Hospital, Ducane Road, London (England), 6 September 1984

Summary. The weight of the stomach, small intestine and colon and the mucosal crypt cell production rate of these tissues were significantly decreased after 10 days on an isocaloric TPN diet when compared to orally fed controls. Continuous infusion of recombinant beta urogastrone, at a dose below that needed to inhibit gastric acid secretion, largely prevented this decrease in crypt cell production rate and gastrointestinal tissue weights.
Key words. Urogastrone; epidermal growth factor; gastrointestinal tract; trophic; total parenteral nutrition.

Despite the considerable interest shown in the in vitro actions of urogastrone-epidermal growth factor (Uro-EGF) a definitive physiological role for the polypeptide has yet to be confirmed. However the location of the main sites of production of Uro-EGF in the salivary glands and Brunners glands of the duodenum of man[1] and the rat[2] implies that Uro-EGF may have a role in the maintenance of gastrointestinal homeostasis.

β-urogastrone (human epidermal growth factor-hEGF) is a natural human polypeptide which has similar chemical, physical and physiological properties to rat and mouse EGF[3,4]. While the growth promoting actions of Uro-EGF in vitro are well characterized[5], its role in vivo is uncertain: in the fetus and newborn animal, Uro-EGF stimulates the proliferation and differentiation of the epidermis, maturation of the pulmonary epithelium and accelerates the healing of corneal epithelium[5]. Uro-EGF also stimulates the proliferation and maturation of the neonatal intestine[6–8], where it also increases the activity of intestinal ornithine decarboxylase[9], an enzyme associated with the initiation of rapid cell proliferation. The presence of Uro-EGF in a variety of body fluids, including saliva, plasma[5] and milk[10], its production by the salivary and Brunners glands[1,2], the trophic action of saliva on the intestine[11], the demonstration of Uro-EGF receptors in intestinal epithelial cells[12] and the reported cytoprotective effects on the duodenal mucosa[13] suggest that it has a role in the control of gastrointestinal homeostasis other than its ability to inhibit gastric acid secretion[18].

Although Uro-EGF would appear to stimulate intestinal epithelial cell proliferation in the neonate, its role in the adult intestine is not clear. Injection of Uro-EGF in rodents has been reported to increase the incorporation of tritiated thymidine into

Experientia 41 (1985), Birkhäuser Verlag, CH-4010 Basel/Switzerland

DNA at all sites in the gastrointestinal tract[14,15], or alternatively only in the stomach[16] or only in starved animals[17,18]. The use of the gross incorporation of tritiated thymidine as the sole criterion of proliferative rate has recently been extensively critisized[19], because the incorporation of tritiated thymidine into DNA by the salvage enzyme thymidine kinase depends on the activity of many factors other than the proportion of cells in DNA synthesis. A study of the short-term effects of Uro-EGF administration using the more robust[19] stathmokinetic method[20], showed a trophic effect which varied from site to site in the intestine and depended on the time after injection; however this study used starved rodents which are considerably stressed and no longer in a steady state of cell proliferation.

A more suitable model of the hypoplastic intestine exists in the rat maintained by isocaloric total parenteral nutrition (TPN)[21], which is generally agreed to be the pertinent system for the study of effects of humoral factors on the intestine[22]; since the TPN rat is in a steady state, the direct and indirect effects of food (luminal nutrition) are abolished, and the effects of endogenous secretions are considerably reduced.

Methods. Male 200 g Wistar rats were housed individually in wire bottomed cages and were fed either orally, or were maintained parenterally for 10 days, with or without β-urogastrone. The urogastrone was recombinant polypeptide (supplied by ICI and GD Searle), derived from the expression of a synthetic gene in *E. coli*[23] and purified to > 97% as judged by amino acid analysis. The biological activity of the cloned urogastrone was identical to that of natural urogastrone purified from human urine. 250 ng/ml of β-urogastrone were added to the test TPN diet. The diet was infused at a rate of 60 ml/rat/day giving a daily intake of 15 µg/rat/day.

The right jugular vein of the TPN rats was cannulated with a silastic catheter which was brought round to the back of the neck and then connected via a skin button and tether to a minature fluid swivel joint (Harvard Apparatus Ltd, Fircroft Way, Edenbridge, Kent). The TPN diet was infused at 60 ml/rat/day which gave 1.8 g N, 6.0 g triglyceride and 250 kcal per kg of rat. 1 l of the TPN diet contained 715 ml of Vamin glucose, 94 ml intralipid 20% (Kabivitrum Ltd, Riverside Way, Uxbridge), 140 ml dextrose 50%, 10 ml Vitlipid infant (Kabivitrum), 1 vial Solvito (Kabivitrum), 20 ml Addiphos (Kabivitrum), 12.8 ml 10% calcium gluconate, 3.4 ml 50% magnesium sulphate and 8.7 ml 23.4% sodium chloride.

Crypt cell production rate. After 10 days on their diets, 1 mg/kg of vincristine sulphate (Eli Lilly, Basingstoke) was injected via the jugular catheter. The rats were then killed at timed intervals and the gut was removed and fixed in Carnoys fluid. The tissue was later stained with the Feulgen reaction and the antral pits, intestinal crypts and colonic crypts were microdissected; the number of arrested metaphases in 10 crypts was then counted and the mean values plotted against time after injection. The slope of the line, fitted by least squares linear regression, gave the crypt cell production rate (CCPR)[19].

Statistics. CCPR's are shown +/− SE and weights are given +/− SE. Differences between groups were tested by a two-sided Student's t-test.

Results. 10 days of TPN significantly decreased the weights of all sections of the intestine (table 1), and whilst 15 µg/rat/day/ of Uro-EGF significantly increased the relative weight of the stomach, small intestine and colon in comparison with the relevant TPN control groups, only the stomach and colon weights were restored to the fed level. No significant changes in the weight of the other major body organs were observed. 10 days of TPN caused a reproducible reduction in the CCPR at all sites in the gastrointestinal tract studied (table 2) and this was statistically significant at the three sites in the small intestine studied in both experiments and in the stomach in the second experiment. The infusion of 15 µg/rat/day Uro-EGF along with the TPN diet increased the CCPR at all sites in the gastrointestinal tract when compared with relevant groups on TPN alone. This was significantly so for all the sites in the second experiment and in two of the small bowel sites in the first experiment.

Discussion. The results of these studies would indicate that one of the in vivo actions of Uro-EGF is the maintenance of gastrointestinal epithelial cell proliferation and growth. There is strong evidence for a humoral basis for the marked adaptation of the intestine to a variety of stimuli, especially intestinal resection[24], and it is possible that Uro-EGF may also have a role in these hyperplastic responses.

Table 1. The effects of oral feeding, total parenteral nutrition (TPN) and TPN plus 15 µg/rat/day urogastrone on the relative b.wts of the gastrointestinal tract (expressed as a percentage of total b.wt)

Group	Orally Fed	TPN1	TPN2	TPN + UG 1	TPN + UG 2
n =	10	8	7	6	10
Weight stomach					
+/−	0.553	0.533	0.476	0.656	0.597
	0.011	0.022	0.031[a]	0.023[c]	0.019[c]
Weight small intestine					
+/−	2.327	1.490	1.334	1.856	1.859
	0.046	0.029[b]	0.030[b]	0.027[b,d]	0.063[b,d]
Weight caecum					
+/−	0.458	0.321	0.287	0.355	0.340
	0.024	0.020[b]	0.023[b]	0.016[b]	0.015[b]
Weight colon					
+/−	0.530	0.364	0.330	0.488	0.507
	0.014	0.012[b]	0.011[b]	0.018[d]	0.018[d]

TPN1, rats maintained by total parenteral nutrition in the first experiment; TPN2, rats maintained by total parenteral nutrition in the second experiment; TPN + UG1, rats maintained by total parenteral nutrition with 15 µg/rat/day of urogastrone in the first experiment; TPN + UG2, rats maintained by total parenteral nutrition with 15 µg/rat/day of urogastrone in the second experiment. [a] significantly lower than the orally fed group $p < 0.05$; [b] significantly lower than the orally fed group $p < 0.01$; [c] significantly higher than the respective TPN group $p < 0.01$; [d] significantly higher than the respective TPN group $p < 0.001$.

Table 2. The effects of oral feeding, TPN or TPN plus 15 µg/rat/day urogastrone on crypt cell production rates

	Orally Fed	TPN1	TPN2	TPN + UG 1	TPN + UG 2
n =	10	8	7	6	10
The antrum of the stomach					
+/−	2.47	0.89	0	2.11	2.00
	0.80	0.35	0.24[b]	0.51	0.57[e]
10% of the length of the small intestine					
+/−	17.96	9.25	8.28	9.92	14.80
	1.72	0.14[c]	1.16[c]	2.05[a]	1.95[d]
50% of the length of the small intestine					
+/−	24.52	7.35	7.54	15.37	20.14
	2.86	0.92[c]	1.14[c]	2.31[a,e]	2.73[f]
90% of the length of the small intestine					
+/−	27.71	8.37	5.44	14.10	12.19
	3.32	1.43[c]	1.55[c]	1.77[b,d]	2.11[b,d]
50% of the length of the colon					
+/−	9.64	4.51	4.22	6.44	11.45
	2.12	1.76	1.61	1.88	2.47[d]

TPN1, rats maintained by total parenteral nutrition in the first investigation; TPN2, rats maintained by total parenteral nutrition in the second investigation; TPN + UG1, rats maintained by total parenteral nutrition with 15 µg/rat/day of urogastrone in the first investigation; TPN + UG2, rats maintained by total parenteral nutrition with 15 µg/rat/day of urogastrone in the second investigation. [a] significantly lower than the orally fed group $p < 0.05$; [b] significantly lower than the orally fed group $p < 0.01$; [c] significantly lower than the orally fed group $p < 0.001$; [d] significantly higher than the respective TPN control group $p < 0.05$; [e] significantly higher than the respective TPN control group $p < 0.01$; [f] significantly higher than the respective TPN control group $p < 0.001$.

Acknowledgment. We thank the Cancer Research Campaign for financial assistance, and Kabivitrum Ltd for the gift of the Vamin glucose and Intralipid.

* ICI, Alderley Park, Macclesfield.

** High Wycombe, Bucks.

1 Heitz, P.U., Kaspar, M., Van Norden, S., Polak, J.M., Gregory, H., and Pearse, A.G., Gut *19* (1978) 408.
2 Olsen, P.S., and Nexo, E., Scand. J. Gastroent. *18* (1983) 771.
3 Gregory, H., Nature *257* (1975) 325.
4 Carpenter, G., and Cohen, S., A. Rev. Biochem. *48* (1979) 193.
5 Carpenter, G., Epidermal growth factor, in: Tissue growth factors, p. 89. Ed. R. Baserga. Springer-Verlag, Berlin 1981.
6 Malo, C., and Menard, D., Gastroenterology *83* (1982) 28.
7 Oka, Y., Ghrisan, F.K., Greene, H.L., and Orth, D.N., Endocrinology *112* (1983) 940.
8 Calvert, R., Beaulieu, J.F., and Menard, D., Experientia *38* (1982) 1096.
9 Feldman, E.J., Aures, D., and Grossman, M.I., Proc. Soc. exp. Biol. Med. *159* (1978) 400.
10 Carpenter, G., Science *210* (1980) 198.
11 Li, A.K., Schattenkerk, M.E., Huffman, R.G., Ross, J.S., and Malt, R.A., Gastroenterology *84* (1983) 949.
12 Forgue-Lafitte, M.E., FEBS Lett. *114* (1980) 243.
13 Kirkegaard, P., Olsen, P.S., Poulsen, S.S., and Nexo, E., Gastroenterology *85* (1983) 1277.
14 Schieving, L.A., Yeh, Y.C., Tsai, T.H., and Schieving, L.E., Endocrinology *105* (1979) 1475.
15 Schieving, L.A., Yeh, Y.C., Tsai, T.H., and Schieving, L.E., Endocrinology *106* (1980) 1498.
16 Johnson, L.R., and Guthrie, P.D., Am. J. Physiol. *238* (1980) G45–G49.
17 Chabot, J.G., Paget, N., and Hugon, J.S., Comp. Biochem. Physiol. pt A *74* (1983) 247.
18 Dembinski, A., Gregory, H., Konturek, S.J., and Polanski, M., J. Physiol., Lond. *325* (1982) 35.
19 Goodlad, R.A., and Wright, N.A., Quantitative studies on epithelial replacement in the gut, in: Techniques in the Life Sciences. Techniques in digestive physiology, vol. P2, p. 212. Ed. T.A. Titchen. Elsevier Biochemical Press, Ireland 1982.
20 Al-Nafussi, A., and Wright, N.A., Virchows Arch. (cell path.) *40* (1982) 63.
21 Hughes, C.A., and Dowling, R.H., Clin. Sci. 59 (1980) 317.
22 Robinson, J.W.L., Dowling, R.H., and Reichen, E.O., (eds), Intestinal Adaptation II. MTP Press, Lancaster 1982.
23 Smith, J., Cook, E., Fotheringham, I., Phelby, S., Derbyshire, R., Eaton, M.A.W., Doel, M., Lilley, D.M.J., Pardon, J.F., Patel, T., Lewis, H., and Bell, L.D., Nucleic Acid. Res. *10* (1982) 4467.
24 Sagor, G.R., Ghatei, M.A., Al-Mukhtar, M.Y.T., Wright, N.A., and Bloom, S.R., Br. J. Surg. *69* (1982) 14.

0014-4754/85/091161-03$1.50 + 0.20/0

Antidiuretic hormone involvement in the release of α-melanocyte-stimulating hormone by hyperosmotic stimuli

A. Howe and K.S. Ray

Department of Physiology, Chelsea College, University of London, London (England), 23 November 1984

Summary. In the normal Wistar rat, the plasma α-MSH level was raised by hypertonic saline injection (as compared with control rats injected with isotonic saline). No such rise in α-MSH followed hypertonic saline administration in the Brattleboro (hereditary diabetes insipidus) animal (compared to isotonic saline injected controls). It is suggested that, in the rat, endogenous antidiuretic hormone is involved in the secretory response of the pars intermedia to osmotic stimuli.
Key words. Antidiuretic hormone; pars intermedia; α-MSH secretion.

A functional relationship between the pars intermedia of the pituitary gland and the adjacent neurohypophysis has been postulated[1]. This view is based essentially upon the observation that the pars intermedia is activated to release melanocyte-stimulating hormone (MSH) by various forms of osmotic stimuli, such as the injection of hypertonic saline, ingestion of sodium chloride, or deprivation of water[2–5], stresses well known to represent potent stimuli for the release of the neurohypophysial hormones, antidiuretic hormone (ADH) and oxytocin[3–8]. Such stimuli represent useful tools for the experimental investigation of pars intermedia function, but neither the physiological significance of this response nor the mode of activation of the pars intermedia under these conditions is clear.

Whether or not the release of MSH by osmotic stress is dependent on neurohypophysial hormones could be tested by examining the response (MSH secretion) of the pars intermedia to an osmotic stimulus in an animal lacking a functional neurohypophysial system. Experimentally, this poses technical problems; for example it is virtually impossible to destroy either the ADH or the oxytocin system selectively by lesion techniques. However, such a model is available in the form of the genetically abnormal Brattleboro rat, an animal with hereditary diabetes insipidus (D.I.), which is deficient in ADH while possessing a normally functional oxytocin system[8,9]. Experiments were, therefore, carried out to see if the Brattleboro rat would release MSH in response to an osmotic stimulus (hypertonic saline), which was effective in causing the release of MSH in the normal laboratory (Wistar) rat.

Materials and methods. A total of 14 adult male Wistar rats (b.wt 160–210 g) and 11 homozygous adult rats (both male and female) of the Brattleboro strain (b.wt 175–240 g and exhibiting marked diabetes insipidus), were used and were maintained under standard conditions of lighting and temperature. The animals were anesthetized with urethane (15% w/v in distilled water, at a dose of 1.5 g/kg b.wt, i.p.). Both the Wistar and

The effect of i.v. injection of hypertonic (2%) saline on the plasma α-MSH level in anesthetized Wistar and Brattleboro rats

Animal	Injection (i.v.)	Number of animals	Plasma α-MSH (pg/ml) 12 min after NaCl Mean	± SEM	Significance
Wistar rats	Hypertonic (2%) NaCl	9	4598	294	Significant ($p < 0.01$)
	Control-isotonic (0.9%) NaCl	5	1053	111	
Battleboro rats	Hypertonic (2%) NaCl	5	922	81	Not significant
	Control-isotonic (0.9%) NaCl	6	1265	271	