Leading Article

The diarrhoea of famine and severe malnutrition – is glucagon the major culprit?

Intractable diarrhoea is usually the terminal condition of victims of famine and severe malnutrition. Despite many studies on the effects of starvation and malnutrition on the absorptive functions of the small intestine in animals,[1-4] and a few in man,[5-7] no satisfactory explanation exists for this diarrhoea. While it has often been attributed to bacterial infection, extensive bacteriological studies failed to confirm this and it is estimated that only approximately 15% of cases have a diarrhoea of infectious origin.[8-11] In the most detailed analysis of the effects of extreme malnutrition on Danish subjects (who had been incarcerated in a concentration camp), Helweg-Larsen, Hoffmeyer, Kieler *et al*[8] identified three types of diarrhoea. The most prevalent was an endemic, progressive, afebrile 'hunger diarrhoea' while 'infectious diarrhoea' was infrequent. Sudden realimentation caused an 'alimentary diarrhoea'. All were potentially lethal in the severely weakened state of the subjects. According to Roediger,[12] the terminal diarrhoea of kwashiorkor and marasmus (corresponding to hunger diarrhoea) is the result of lack of luminal nutrition of enterocytes, and especially of colonocytes, which severely reduces their absorptive function. In his explanation, the diarrhoea is mainly caused by the loss of the reabsorptive (salvage?) capacity of the colon.

Ethical problems prevent controlled studies on the secretory behaviour of the intestine in starved and severely malnourished individuals but their lack in an experimental animal model is surprising. In order to investigate the effects of starvation and undernutrition on the secretory function of the small and large intestine, the rat has been used as an animal model. Starvation for 24 hours did not significantly alter small intestinal secretory function but between 24 and 72 hours of starvation the duodenum,[13] jejunum,[14] and ileum[15] all showed hypersecretory activity compaired with fed intestine when activated to secrete by neurotransmitters and secretagogues. Moreover, in the case of the ileum, even the fed basal absorptive tone was converted to a secretory one after 24 hours of food withdrawal and its magnitude increased on continued food deprivation.[15] Further studies showed that the proximal colon,[16] and even the rectum,[17] from rats starved for three days responded to cholinergic agonists by electrogenic Cl⁻ hypersecretion. Analysis of the types of secretory responses elicited by various neurotransmitters, bacterial toxins, and secretagogues showed clearly that in the case of the small intestine, all of the agents caused a hypersecretion in the starved rat.[14 15] In the proximal colon, however, only those agents that activate secretion by increasing intracellular Ca^{++} (cholinergic agents, 5-hydroxytryptamine (5HT), A 23187) elicited the hypersecretory response,[16] those acting through increases in intracellular cyclic adenosine monophosphate (AMP) (theophylline, dibutyryl cyclic adenosine monophosphate, forskolin, and prostaglandin₂) or in mid colon through cyclic guanosine monophosphate[18] (*Escherichia coli* STa) did not cause electrogenic Cl⁻ hypersecretion. In fact, they often elicited less secretion compared with the fed state.[19] This lack of effect of cyclic AMP mediated secretagogues to induce hypersecretion has been confirmed in the starved mouse colon.[20] The hypersecretion is not caused by any signifi-

cant change in the enteric neural innervation of the small intestine as it could be obtained even in the presence of the neural toxin tetrodotoxin,[14 15] nor is there any significant change in the enterocyte's receptor sites for the neural transmitters as the hypersecretion could be obtained with agents that bypassed these sites.[14 15] However, cyclic AMP concentrations were higher in enterocytes from starved intestine, both in the basal state and when stimulated by secretagogues.[21] The reversal of the basal absorptive tone in the fed ileum to a secretory one correlated well with the increase in cyclic AMP found in this area on starvation.[21] Confirmation of the hypersecretory action of starvation on small intestinal secretory function in vitro has been reported in the jejunum of starved piglets.[22]

The obvious question that arises from the rat studies is, what causes the hypersecretory behaviour of the enterocytes and colonocytes? Before we can answer this, it is necessary to describe briefly the current model[23] for the secretory behaviour of enterocytes (and colonocytes). Chloride is transported into the cells against its electrochemical gradient by the Na^+-K^+-$2Cl^-$ contransporter in the basolateral membrane. The intracellular Na^+ ion concentration is kept low by the action of the Na^+-K^+-adenosine triphosphatase pump, also in the basolateral membrane, pumping out 3 Na^+ ion in exchange for 2 K^+ ions. The membrane is also equipped with K^+ channels that when opened allow the K^+ to leave the cell under the influence of the K^+ gradient. At the luminal brush border of the cell, Cl⁻ channels which are normally closed can be opened by intracellular second messengers (possible cyclic AMP or Ca^{++}) allowing the intracellular Cl⁻ ions to be driven out of the cell by their concentration difference and the transluminal membrane potential difference (pd, cell interior negative to the lumen) as electrogenic Cl⁻ secretion. This outward movement of Cl⁻ depolarises the luminal membrane potential, which reduces the driving force and attenuates Cl⁻ secretion. Secretagogues, however, activate the basolateral K^+ channels (probably by the raised intracellular Ca^{++}), and the passive outflow of intracellular K^+ hyperpolarises the membrane. Electrical coupling via the intercellular pathway maintains the luminal membrane pd which preserves the electrical potential drive for the Cl⁻ movement. The activation of the enterocyte's luminal Cl⁻ and basolateral K^+ channels is normally via the cholinergic and vipergic neurotransmitters acetylcholine and vasoactive intestinal polypeptide (mediated through intracellular second messengers) but other agencies such as prostaglandins, 5HT, bacterial toxins, and various hormones can also directly or indirectly activate secretion.

Starvation in the rat reduces the mitotic activity in the duodenal/jejunal crypts, they decrease in size and the supply of new enterocytes onto the villus is slowed.[3] As old enterocytes are still sloughed off at the villi tip exfoliation zones, villus height, and cell population decrease. At first it is tempting to explain the increased secretion of the starved small intestine as being the result of this decreased cell turnover and villus size on the basis of the simplistic model that villi absorb and crypts secrete. If the decreased enterocyte population on the villi had a reduced absorption while the crypts secreted even normally, then the imbalance could cause an apparent hypersecretion. We have previously

discussed this as being unlikely for a number of reasons.[14] Firstly, in the starved state the enterocytes are on the villus longer and this ageing seems to increase their absorptive functions[3][14]; secondly, the crypt cell population also decreases yet secretion increases; thirdly, the enterocytes on the villus are now known to be able to secrete Cl⁻ electrogenically[24]; and finally, there is controversy as to whether human villi do decrease in size in conditions known to reduce luminal nutrition.[25] Thus explanations other than the changed balance between crypt and villous enterocytes must be sought.

The pd across the luminal membranes of the starved enterocytes in both the jejunum and the ileum becomes hyperpolarised after only 24 hours of fasting and is maintained for at least 72 hours of starvation.[26] This membrane hyperpolarisation creates an increased driving force on the intracellular Cl⁻ ions when the Cl⁻ channels of the luminal membrane are opened by neurotransmitters or secretagogues. Thus the hyperpolarisation of the starved enterocyte's luminal membrane can account for the hypersecretory activity shown by the small intestine in the food deprived state. However, as described previously, the loss of Cl⁻ via the channels will decrease the transluminal membrane pd and reduce secretion so the K⁺ channels of the enterocyte have also to be included in the mechanism to maintain the luminal membrane pd by their activation and subsequent electrical coupling with this membrane. The increased transluminal membrane pd in the starved enterocytes also affects the charged Na⁺-glucose or amino acid linked cotransporter of the luminal membrane creating an enhanced electrogenic nutrient transfer.[3][27]

One intriguing point that needs comment is that measurements of the enterocyte's transluminal membrane pd after 24 hours of fasting show an obvious increase[26] but assessments of their fluid and electrogenic secretion only become significant after 24–48 hours of starvation.[14][15] A possible explanation for this apparent inconsistency may simply be the different sensitivities of the two techniques. As microelectrode pd measurements are on individual enterocytes, it is possible to assess changes in a small population at or near the top of the villus. The fluid and electrogenic secretory responses are, however, macro measurements needing a large number of enterocytes to be affected before significant differences can be distinguished. It is thus possible that the first changes after 24 hours of starvation are expressed in the most mature cells on the upper part of the villus. The enterocytes lower down the villus follow, but some 24–48 hours later.

The hyperpolarisation of the luminal membrane is caused by a reduction in its passive Na⁺ permeability as the membrane pd in starved enterocytes did not show the linear-log relation with changes in Na⁺ concentration observed in fed enterocytes.[26] The changes seemed specific for Na⁺, as the K⁺ permeability of the membrane in fed and starved intestine was identical. Murer, Hopfer, and Kinne,[28] working with brush border membrane vesicles from fed rat enterocytes, showed that cyclic AMP reduces the passive permeability of the luminal membrane to Na⁺. Presumably, the increased concentrations of cyclic AMP in the starved enterocytes[21] will also influence membrane Na⁺ permeability creating the hyperpolarisation of the luminal membrane.

We can dissect the mechanism of the hypersecretion even further. In starvation, the plasma glucagon in both rat[29] and man[30] is increased; in man it plateaus at a new, higher than fed, value.[30] The raised glucagon stimulates the intestinal uptake of amino acids and glucose as antiglucagon, when injected in starved rats, prevents the enhancement.[29] Even in fed rats, repeated glucagon injections for three days increase the ileal absorption of glucose.[31] The hyperglucagonaemia increases the transluminal membrane pd of jejunal enterocytes[32] in fed rats (from a mean value of −48 mV to −54 mV)

and stimulates the uptake of Na⁺-dependent glucose and amino acids. It is highly likely that the chronic increase in plasma glucagon brought about by starvation induces changes in the enterocytes (perhaps by raising cyclic AMP levels?) creating the hyperpolarisation of the luminal membrane which can then give rise to the various enhanced transport properties of the starved intestine. Interestingly, even when added directly to small intestine in vitro, glucagon has been shown to cause Cl⁻ secretion across mouse jejunum,[33] and when acutely injected causes jejunal secretion in human subjects in vivo.[34]

At present all the experimental evidence linking glucagon with the hypersecretion of starvation is only attributable to the small intestine. It is not known whether glucagon is also involved in the hypersecretion induced in the colonocytes. Even if further experimental studies show that glucagon is implicated in colonic hypersecretion an explanation has to be sought for the finding that starved colonocytes respond to Ca-mediated secretagogues by hypersecretion but not to those mediated by cyclic AMP.

One final problem remains, namely, how is intestinal secretion activated in the starved animal or human? In both humans and rats in the fasted state, a regular propagated wave of electrical and smooth muscle activity called the migrating myoelectric complex (MMC) passes down the small intestine.[35] Food disrupts the regularity of the activity. The MMC activates secretion in the stomach and pancreas when it passes down the tract[36] and increases transiently the transmural electrical pd across the small intestine.[37][38] This increase was interpreted as being the result of neural activation of electrogenic Cl⁻ secretion.[37][38] Recordings obtained across the small intestine of cystic fibrosis patients have confirmed this interpretation, because the subjects do not show such electrogenic secretory activity as their intestines cannot secrete Cl⁻ electrogenically.[39] Thus the MMC continually passes down the starved intestine (both in rats and in humans) stimulating the enteric plexii to activate the cellular hypersecretion. Other possible luminal activators of secretion are bacterial toxins[15][40] and even intestinal viral agents.[41] The net result of all these agencies is to make the starved intestine secrete excessive amounts of fluid and thus create diarrhoea by overloading the depressed absorptive function of the colon.[12]

Preliminary studies in rats have shown that the simple drinking of glucose during the three day starvation, while greatly ameliorating the hypersecretion in the duodenum, jejunum, and ileum, leaves the colon's hypersecretory activity unaffected.[42][43] The rat model also offers the possibility of investigating dietary regimens that will not only ameliorate both small and large intestinal hypersecretory activity of starvation but will also act as stimulants for their absorptive functions.[44]

R J LEVIN

Department of Biomedical Science,
University of Sheffield,
Sheffield S10 2TN

1 Debnam ES, Levin RJ. Effects of fasting and semi starvation on the kinetics of active and passive sugar absorption across the small intestine in vivo. *J Physiol Lond* 1975; 252: 681–700.

2 Levin RJ, Mitchell MA. Intestinal adaptations to fasting – use of corrected kinetic parameters to assess responses of jejunal and ileal absorption in vivo: In: Robinson JW, Dowling RH, Riecken E-O, eds. *Mechanisms of intestinal adaptation. Falk Symposium No 30.* Lancaster: MTP Press Limited, 1982: 103–10.

3 Levin RJ. Intestinal adaptation to dietary change as exemplified by dietary restriction studies. In: Batt RM, Lawrence TJ, eds. *Function and dysfunction of the small intestine.* Liverpool: Liverpool University Press, 1984; 77–93.

4 Levin RJ. The intestinal absorption of some essential and non-essential amino acids in fed and fasting rats. *Life Sci* 1970; 9: 61–8.

5 Steiner M, Farrish GCM, Gray SJ. Intestinal uptake of valine in calorie and protein deprivation. *Am J Clin Nutr* 1969; 22: 871–7.

6 Adibi SA, Allen ER. Impaired jejunal absorption rates of essential amino acids induced by either dietary caloric or protein deprivation in man. *Gastroenterology* 1970; 59: 404–13.

7 Vasquez JA, Morse EL, Adibi SA. Effect of starvation on amino acids and peptide transport and peptide hydrolysis in humans. *Am J Physiol* 1985; 249: 563–6.

8 Helweg-Larsen P, Hoffmeyer H, Kieler J, Thaysen EH, Thaysen JH, Thygesen P, et al. Famine disease in German concentration camps: complications and sequels. Acta Med Scand 1952; 274 (suppl): 1–460.

9 Medical Research Council. Studies of undernutrition: Wuppertal 1946–9. Medical Research Council Special Report Sciences nos 275. London: HMSO, 1951: 61–3.

10 Winnick M. Hunger disease – studies by Jewish physicians in the Warsaw Ghetto. New York: John Wiley, 1979.

11 James WPT. Effects of protein-calorie malnutrition on intestinal absorption. Ann NY Acad Sci 1971; 176: 244–61.

12 Roediger WEW. Metabolic basis for starvation diarrhoea: implications for treatment. Lancet 1986; i: 1082–4.

13 Levin RJ, Young A. Nutritional level and rat proximal duodenal secretion in vitro and in vivo. J Physiol Lond 1988; 403: 47P.

14 Young A, Levin RJ. Diarrhoea of famine and malnutrition: investigations using a rat model. i. Jejunal hypersecretion induced by starvation. Gut 1990; 31: 43–53.

15 Young A, Levin RJ. Diarrhoea of famine and malnutrition: investigations using a rat model ii. Ileal hypersecretion induced by starvation. Gut 1990; 31: 162–9.

16 Levin RJ, Nzegwu HC, Young A. Proximal colon secretion in fed and fasted rats. J Physiol Lond 1988; 396: 33P.

17 Levin RJ, Parker A. Rectal electrogenic secretion – is it a putative indicator of intestinal secretory status induced by nutritional deprivation in the rat? Exp Physiol 1990; 75: 609–11.

18 Levin RJ, Nzegwu H, Pereira M, Young A. Malnourishment enhances the in vitro electrogenic secretory response to E. Coli STa enterotoxin in rat small and large intestine. J Physiol Lond 1988; 406: 56P.

19 Nzegwu H, Levin RJ. Dietary reduction increases secretion in rat distal colon induced by secretagogues acting via Ca'' but not cyclic AMP. Proc Nutr Soc 1990; 49: 178A.

20 Sagmanligil V, Levin RJ. Starvation enhances mouse colonic electrogenic secretion evoked by cholinergic but not cyclic AMP mediation in vitro. J Physiol Lond 1990; 426: 75P.

21 Young A, Levin RJ. Cyclic AMP levels stimulated by secretagogues are greater in ileal enterocytes isolated from starved compared to fed rats. J Physiol Lond 1990; 424: 8P.

22 Carey HV, Tucker KE. Intestinal secretion is altered by the absence of luminal contents in piglets. Gastroenterology 1991; 100 (part 2): A681.

23 Hardcastle J, Hardcastle PT. Membrane permeability changes in intestinal secretion. Med Sci Res 1987; 15: 471–3.

24 Stewart CP, Turnberg LA. A microelectrode study of responses to secretagogues by epithelial cells on the villus and crypt of rat small intestine. Am J Physiol 1989; 257: G334–43.

25 Levin RJ. Dietary carbohydrate and the kinetics of intestinal functions in relation to hexose absorption. In: Dobbing J, ed. Dietary starches and sugars in man: a comparison. Human Nutrition Reviews, International Life Sciences Institute. London: Springer-Verlag, 1989: 89–117.

26 Debnam ES, Thompson CS. The effect of fasting on the potential difference across the brush border membrane of enterocytes in rat small intestine. J Physiol Lond 1985; 355: 449–56.

27 Thompson CS, Debnam ES. Starvation-induced changes in the autoradiographic localisation of valine uptake by rat small intestine. Experientia 1986; 42: 945–8.

28 Murer H, Hopfer U, Kinne R. Adenylate cyclase system in the enterocyte: cellular localization and possible relation to transepithelial transport. In: Bonfils S, Fromageot P, Rosselin G, eds. First International Symposium on Hormonal Receptors in Digestive Tract Physiology. INSERM symposium No 3. Amsterdam: Elsevier/North-Holland Biomedical Press, 1977: 425–34.

29 Rudo ND, Lawrence A, Rosenberg TH. Treatment with glucagon-binding antibodies alters intestinal response to starvation in the rat. Gastroenterology 1975; 69: 1265–8.

30 Marliss EB, Aoki TT, Unger RH, Soeldner S, Cahill PG. Glucagon levels and metabolic effects in fasting man. J Clin Invest 1970; 49: 2256–70.

31 Debnam ES. Effects of fasting and glucagon on the kinetics of active hexose absorption across the rat distal ileum in vivo. Q J Exp Physiol 1982; 67: 587–94.

32 Thompson CS, Debnam ES. Hyperglucagonaemia: effects on active nutrient uptake by the rat jejunum. J Endocrinol 1986; 111: 37–42.

33 Kaufman ME, Dinno MA, Huang KC. Effect of glucagon on ion transport in mouse intestine. Am J Physiol 1980; 238: G491–4.

34 Hicks T, Turnberg LA. Influence of glucagon on the human jejunum. Gastroenterology 1974; 67: 1114–8.

35 Ruckebush M, Fioramonti J. Electrical spiking activity and propulsion in small intestine in fed and fasted rats. Gastroenterology 1975; 68: 1500–8.

36 Vantrappen GR, Peeters TL, Janssens J. The secretory component of the interdigestive migrating motor complex in man. Scand J Gastroenterol 1979; 14: 663–7.

37 Read NW, Smallwood RH, Levin RJ, Holdsworth CD, Brown BH. The relationship between changes in intraluminal pressure and transluminal potential difference in the human and canine jejunum in vivo. Gut 1977; 18: 141–51.

38 Read NW. The migrating motor complex and spontaneous fluctuation of transmural potential difference in the human small intestine. In: Christensen J, ed. Gastrointestinal motility. New York: Raven Press, 1980; 299–306.

39 Baxter PS, Read NW, Hardcastle PT, Hardcastle J, Wilson AJ, Taylor CJ. Abnormal jejunal potential difference in cystic fibrosis. Lancet 1989; i: 464–6.

40 Evered D, Whelan J, eds. Microbial toxins and diarrhoeal disease. Ciba Foundation Symposium No 112. London: Pitman Publishing Ltd, 1985.

41 Stephen J. Functional abnormalities in the intestine. In: Farthing MJ, ed. Viruses and the gut. Proceedings 9th BSG-SKF International Workshop 1988. Welwyn Garden City: Smith Kline and French Ltd, 1989: 41–4.

42 Young A, Levin RJ. The effects of glucose feeding during starvation on rat jejunal secretion. Proc Nutr Soc 1987; 46: 24A.

43 Young A, Nzegwu H, Levin RJ. Glucose feeding during starvation and its effects on secretion in the rat ileum and colon. Proc Nutr Soc 1988; 47: 144A.

44 Lifschitz CH, Shulman RJ. Nutritional therapy for infants with diarrhoea. Nutr Rev 1990; 48: 329–38.