phin was therefore concentrated from 8 ml of whole plasma and extracted on glass powder[7] and then separated by means of a 1.8×60 cm Sephadex G75 column in 0.3 M acetic acid. The fractions in the area of the ^{125}I β-endorphin, which eluted experimentally in the same area as cold B-endorphin used as marker were pooled, lyophylised and then assayed.

Results are shown in table 2: a large increase in ACTH and β-endorphin is recorded in correspondence with maximal effort.

What is the biological significance of this large increase? It may be related to a pain control mechanism. In fact, during physical exercise various catabolic compounds are accumulated in the human muscles and blood[9,10] and this should be pain-producing, but the feeling of pain is no longer present above certain levels of physical stress. Pain appears at high levels of muscle tiredness, but training raises the painful stress threshold. This increase is, however, at peripheral level, while perception of pain is central and therefore, taking into consideration the above mentioned finding of Rossier et al.[6], it is hard to speculate on an analgesic action of this peripheral increase.

This peripheral increase therefore remains to be elucidated. The marked increase found in our experimental conditions however clearly indicates that the stress mechanism in man stimulates the synthesis and release of the pituitary β-endorphins and ACTH probably through a common biosynthetic pathway (31 K).

The biological significance of this phenomenon is open to speculation.

1 H. Teschmacher, K.E. Opheim, B.M. Cox and A. Goldstein, Life Sci. *16*, 1771 (1975).
2 B.M. Cox, K.E. Opheim, H. Teschmacher and A. Goldstein, Life Sci. *16*, 1777 (1975).
3 J. Hughes, Brain Res. *88*, 295 (1975).
4 L. Terenius and A. Wahlstrom, Life Sci. *16*, 1759 (1975).
5 R. Mains, B.A. Eipper and N. Ling, Proc. natl Acad. Sci. USA *74*, 3014 (1977).
6 J. Rossier, E.D. Franch, C. Rivier, N. Ling, R. Guillemin and F.E. Bloom, Nature *270*, 618 (1977).
7 A.R. Genazzani, F. Fraioli, C. Conti and P. Fioretti, J. nucl. Biol. Med. *18*, 67 (1974).
8 V. Faden and D. Rodbard, Rep. N.T.I.S. No P.B. 246224, vol. 1. 3rd edn, 1975.
9 C.A. Keele and D. Armstrong, in: Substances producing pain and itch, p.247. E. Arnold, London 1964.
10 L. Vecchiet, V. Dolce and F. Fini, Sports Med. *13*, 3 (1973).

Effect of splanchnectomy and vagotomy on the pepsinogen response to intragastric acid

D.E. Webber[1] and Shelagh M. Morrissey

Department of Physiology, Queen Elizabeth College, London W8 7AH (England), 10 December 1979

Summary. Perfusion of the stomach in the anaesthetized rat with saline acidified to pH 2.5 with hydrochloric acid induced a small but significant release of pepsinogen into the perfusate. This stumulus to secretion was unaffected by splanchnectomy but was abolished by vagotomy. It is concluded that to a modest degree acid secretion in the rat may stimulate pepsinogen secretion by a vagal pathway.

Instillation of acid into the canine Heidenhain pouch[2] and into the neurally intact human stomach[3] was reported to induce substantial and significant increases in pepsinogen output. These observations suggest that pepsinogen secretion may be physiologically dependent upon an initial increase in acid output. Stimuli to pepsinogen secretion might therefore be suspected of operating, at least in part, through increases in acid secretion.

Dependence of pepsinogen secretion upon lowered pH would be unsurprising, as optimally active proteolysis of most substrates occurs at pH 1.5–2.5[4]. However, a recent study in neurally intact rats found no stimulation of pepsinogen output by intragastric acid[5]. The present work was designed to resolve this situation, and to examine the involvement of the autonomic nervous system.

Materials and methods. Fasted male Wistar rats, weighing 200–300 g, were anaesthetised with urethane (150 mg/100 g b.wt, i.p.) and tracheotomized. 2 perspex cannulae were placed in the stomach after the method of Ghosh and Schild[6], as described by Webber and Morrissey[7]. Untied ligatures were placed under both splanchnic nerves and the vagi in the abdomen, permitting subsequent denervation without mechanical stimulation of the stomach[8]. Continuous perfusion of the stomach with 0.9% sodium chloride solution, pH 5.0–5.5 (osmolality=275 mosmole/kg), was then commenced. After establishment of basal acid secretory values, saline acidified to pH 2.5 with hydrochloric acid (osmolality=280 mosmole/kg) was substituted as the perfusate for 20–30 min. This level of acidity represents maximal acid output to insulin after dilution in saline with the flow rate in use (approximately 1.1 ml/min)[7]. The perfusate was collected over 2.3-min periods for pepsin assay. Pepsin activities were estimated before, during and after acid perfusion using the method of Anson[9] as described by Bell and Webber[10]. Acidified saline perfusion was repeated using the same animal after splanchnectomy and then after vagotomy. Results from 9 animals were analyzed conjointly by means of Student's t-test for unpaired data, $p < 0.05$ being taken as level of significance.

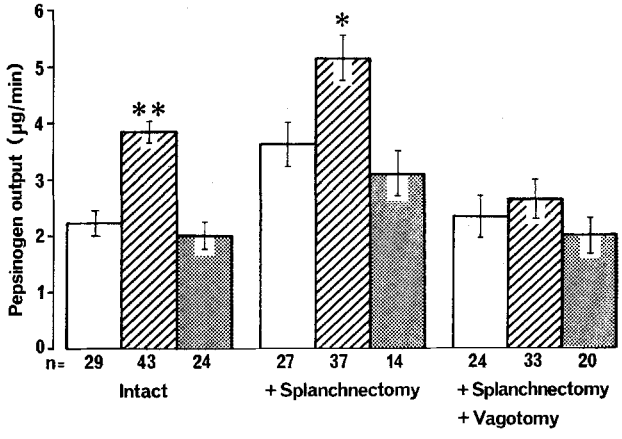
Results. Perfusion of the stomach with acidified saline in neurally intact rats resulted in a small but significant increase in gastric pepsinogen secretion (figure). The elevated pepsinogen levels generally returned to control, basal values immediately upon cessation of the stimulus.

Following splanchnectomy, pepsinogen output was slightly raised in the period before and after perfusion with acidifed saline. Pepsinogen output rose further upon perfusion of the stomach with acidified saline; a significant increase, similar to the neurally intact animals being recorded (figure). Following vagotomy in the same animals no increase in pepsinogen output was observed upon perfusion of the stomach with acidified saline (figure).

Control experiments showed that the increases in pepsin activity could not be duplicated merely by acidification of the gastric juice. Repeated acidified saline perfusion in neurally intact rat gave reproducible increases in activity.

Discussion. The present work shows that significant, but very slight, increases in pepsin output can be elicited upon perfusion of an acidic solution through the rat stomach. Division of the splanchnic nerves to the stomach did not

alter this response. This was despite the slightly raised basal values following sympathectomy, as has been previously observed[11]. The sympathetic nervous supply is recognized as having inhibitory actions on gastric secretion[12]. However, the similarity between the pepsin increases before and after splanchnectomy suggests that the splanchnic nerves play no part in the regulation of the response to acid. Bilateral vagotomy virtually abolished the response, suggesting that a vagal pathway is in operation. It is worth noting that pepsin secretion, unlike acid, is not totally abolished by vagotomy[13,14].



Gastric pepsin output induced by acidified saline perfusion of the intact, splanchnectomized and vagotomized (totally denervated) stomach in anaesthetized rats. Mean values (± SEM) before (□), during (▨) and after (▩) perfusion with acidified saline. n = Number of estimations of pepsin activity in 9 rats. Significant difference between pepsinogen secretion during acidified saline perfusion, and pepsinogen secretion into normal saline is shown by * ($p < 0.01$) and ** ($p < 0.001$).

The substantial pepsin secretion in response to acid instillation previously reported by Johnson[2] and Bynum and Johnson[3] could not be duplicated using the present preparation. The instillate pH employed by these authors (pH 1-2) was lower than ours, and may not represent physiological norms. Although these pH values may be obtained by stimulation of the parietal cells in the fasted animal, it is unlikely that such large amounts of acid occur normally in the empty stomach. Furthermore, these authors used a phosphate buffer, pH 7.4, for control instillations. Pepsin is readily inactivated by weak alkali and at pH 7.4 only a fraction of the true pepsin concentration would be detected[15]. This could lead to exaggeration of any true increase that occurred when the pH was lowered. It is clear, therefore, that normal pepsinogen secretion in the rat stomach is not simply the result of initial acid secretion, and stimuli of pepsin and acid secretion can be considered to act on each cell type independently.

1 Present address: Department of Medicine, Royal Veterinary College, North Mymms, Hatfield, Herts. AL9 7TA (England).
2 L.R. Johnson, Am. J. Physiol. *223*, 847 (1972).
3 T.E. Bynum and L.R. Johnson, Am. J. digest. Dis. *20*, 607 (1975).
4 D.F. Magee, Med. Clins, N. Am *58*, 1277 (1974).
5 J. Puurunen, Eur. J. Pharmac. *55*, 207 (1979).
6 M.N. Ghosh and H.O. Schild, Br. J. Pharmac. *13*, 54 (1958).
7 D.E. Webber and S.M. Morrissey, Can. J. Physiol. Pharmac. *57*, 1283 (1979).
8 R. Lambert, Surgery of the digestive system in the rat. C.C. Thomas, Springfield, Illinois, 1965.
9 M.L. Anson, J. gen. Physiol. *22*, 79 (1938).
10 F.R. Bell and D.E. Webber, J. Physiol. *291*, 413 (1979).
11 P.W. Shafer and C.F. Kittle, Surgery *29*, 1 (1951).
12 D.L. Sanders, Agents Actions *6*, 385 (1976).
13 W.H. Bachrach, Am. J. digest. Dis. *7*, 1071 (1962).
14 W.W. Duke, B.I. Hirschowitz and S.G. Sachs, Lancet *2*, 871 (1965).
15 D.W. Piper and B.H. Fenton, Gut *6*, 506 (1965).

Potassium transfer from brain to blood during sustained hyponatraemia in the newborn calf[1]

R.M. Gardiner and A.G. Wilkinson[2]

Physiological Laboratory, University of Cambridge, Cambridge CB2 3EG (England), 2 November 1979

Summary. The effect of dilutional hyponatraemia on cerebral blood flow and oxygen consumption, and net transfer of K^+ to the circulation from brain tissue drained by the sagittal sinus was investigated in anaesthetized calves. Cerebral blood flow decreased, and net transfer of K^+ to the circulation increased during hyponatraemia.

The K^+ content of brain tissue is reduced after prolonged hyponatraemia and it has been suggested that this loss of intracellular electrolyte represents the means by which brain swelling is mitigated in hyposmolar conditions[3]. However, the route, mechanism and time course of brain K^+ loss during hyponatraemia remain uncertain. These experiments were undertaken to quantify net transfer of K^+ to the circulation from brain tissue drained by the sagittal sinus during normonatraemia and during sustained hyposmolar hyponatramia.

The animals were anaesthetized with i.v. sodium pentobarbitone (30 mg/kg b.wt), paralyzed with gallamine triethiodide and ventilated via a tracheostomy. Cerebral blood flow was measured using an intra-arterial injection technique, in which the clearance of H_2 from cerebral venous blood is determined by a platinum anode located in the sagittal dural sinus[4]. Cerebro-spinal fluid (CSF) was sampled and its pressure recorded from a needle placed p.c. in the cisterna magna. Estimations were performed in duplicate on paired samples withdrawn simultaneously from the aorta and sagittal dural sinus at the beginning and end of each flow determination. Plasma and CSF electrolyte concentrations were determined with a Corning 455 Flame Photometer after dilution 1 in 200, and whole blood oxygen content by a polarographic technique[5]. Osmolality was determined by freezing point depression. Cerebral oxygen consumption and net K^+ transfer were calculated from the blood or plasma flow and arterio-cerebral venous concentration difference. Arterial blood gas tensions, pH, blood pressure and EEG were also monitored. The experiments were carried out on 12 pedigree Jersey calves aged between 10 and 46 days. Measurements were made