15 °C. In contrast to the effect of temperature, changing the K^+ concentration of the bathing medium had no significant effect on the ouabain-sensitivity of fluid secretion, despite the fact that the rate of fluid secretion, itself, increased with increasing K^+ concentration. The mean rates of fluid secretion (in the absence of ouabain) were 3.4 ± 0.9 nl/min ($n=22$) in the presence of 10 mM K^+, 4.4 ± 0.7 nl/min ($n=25$) in the presence of 20 mM K^+ and 7.3 ± 1.0 nl/min ($n=21$) in the presence of 40 mM K^+. At all concentrations of K^+, the level of ouabain-inhibition of fluid secretion was about 57%.

The results presented in the table show that the inhibition of Na^+-K^+-activated ATPase activity by ouabain decreases as the temperature is lowered. At 30 °C, enzyme activity was inhibited by about 50% whereas at 20 and 15 °C the levels of inhibition were about 32% and 15% respectively.

Discussion. As mentioned briefly in the introduction, a number of researchers have failed to demonstrate an inhibitory effect of ouabain on the Malpighian tubules of *Calliphora*[12], *Rhodnius*[13], *Carausius*[14], *Glossina morsitans*[15], *Locusta*[5] and *Zonocerus*[5]. In contrast, other workers have shown that Malpighian tubule function is ouabain-sensitive in *Locusta*[1,3], *Drosophila hydei*[16] and *Glossina morsitans*[17]. In addition, Farquharson[18] has shown that fluid secretion by the Malpighian tubules of the pill millipede, *Glomeris marginata,* is sensitive to ouabain at concentrations as low as 5 µM. One of the main differences in methodology in the literature concerns the temperature at which fluid secretion by the Malpighian tubules has been studied. Rafaeli-Bernstein and Mordue[5], who report no effect of ouabain on fluid secretion in *Locusta,* carried out their experiments at 24–25 °C. Other workers[12–15] have performed their experiments at room temperature (19–22 °C). The inhibition of Na^+-K^+-activated ATPase by ouabain has been shown to be substantially affected by temperature in the present study; confirming the results of other workers[6,19]. In addition, fluid secretion by *Locusta* Malpighian tubules was far less sensitive to ouabain at 15 and 20 °C than at 30 °C (the temperature used by Anstee and Bell[1] and Anstee et al.[3]). Temperature is clearly an important factor in determining the extent to which ouabain inhibits Malpighian tubule function in *Locusta.* This may be expected if a Na^+-K^+-activated ATPase is involved in the mechanism of fluid secretion and one must conclude that failure to demonstrate inhibition of fluid secretion at room temperature, by ouabain, should not be taken as evidence against the involvement of this enzyme in the secretory mechanism.

In the present study, varying the K^+ concentration from 10 to 40 mM failed to affect the ouabain-sensitivity of the fluid secretory process. It would seem, therefore, that the K^+ concentration of the bathing medium may vary quite substantially without effecting a significant reduction in ouabain-inhibition.

1 J.H. Anstee and D.M. Bell, J. Insect Physiol. *21,* 1779 (1975).
2 J.H. Anstee and D.M. Bell, Insect Biochem. *8,* 3 (1978).
3 J.H. Anstee, D.M. Bell and H. Fathpour, J. Insect Physiol. *25,* 373 (1979).
4 J.H. Anstee and K. Bowler, Comp. Biochem. Physiol. *62A,* 763 (1979).
5 A. Rafaeli-Bernstein and W. Mordue, Physiol. Ent. *3,* 59 (1978).
6 J.S. Charnock, A.F. Almeida and R. To, Archs Biochem. Biophys. *167,* 480 (1975).
7 C.R. Kinsolving, R.L. Post and D.L. Beaver, J. cell. comp. Physiol. *62,* 85 (1963).
8 J.D. Judah and K. Ahmed, J. cell. comp. Physiol. *64,* 355 (1964).
9 H. Matsui and A. Schwartz, Biochem. biophys. Res. Comm. *25,* 147 (1966).
10 T. Akera, Biochim. biophys. Acta *249,* 53 (1971).
11 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193,* 265 (1951).
12 M.J. Berridge, J. exp. Biol. *48,* 159 (1968).
13 S.H.P. Maddrell, J. exp. Biol. *51,* 71 (1969).
14 D.E.M. Pilcher, J. exp. Biol. *53,* 465 (1970).
15 J.L. Gee, J. exp. Biol. *65,* 323 (1976).
16 U. Atzbacher, F. Hevert, E. Weber-Von Grotthuss and A. Wessing, J. Insect Physiol. *20,* 1989 (1974).
17 R.H. Gooding, Experientia *31,* 938 (1975).
18 P.A. Farquharson, J. exp. Biol. *60,* 29 (1974).
19 A.J. Peacock, K. Bowler and J.H. Anstee, Insect Biochem. *6,* 281 (1976).

Physical exercise stimulates marked concomitant release of β-endorphin and adrenocorticotropic hormone (ACTH) in peripheral blood in man

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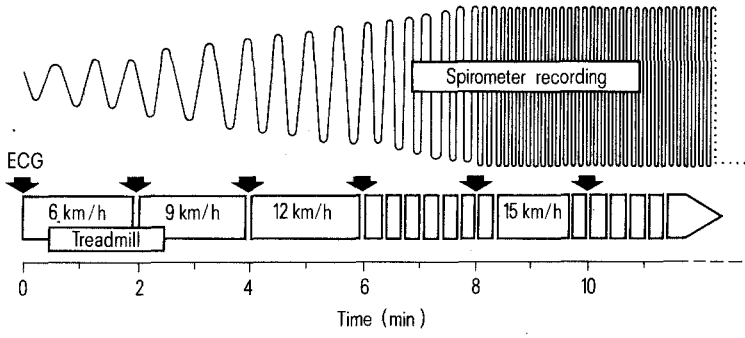
Summary. ACTH and β-endorphin have been evaluated by means of a specific and sensitive radioimmunoassay in athletes reaching a status of physical stress. A concomitant marked increase of these 2 peptides has been recorded. The implications of this finding lead to the conclusion that stress stimulates the synthesis of the common precursor (31 K) in the pituitary.

Endorphins possess an analgesic action and probably produce other opiate-like effects as well, but the real physiological significance of these substances has not yet been elucidated. The fact that 2 different compartmentalized endorphin pools exist, one in peripheral blood coming from the pituitary[1,2], the other directly synthesized by the peptidergic neurons in the brain[3,4], seems to indicate different biological actions of these substances, one with a direct effect on the neurons in the CNS, another with a hormonal effect at peripheral level. So far only an analgesic effect, and no other physiological significance, has been demonstrated. Whether or not the peripheral effects, subsequent to pituitary release, are mediated by a target organ remains to be elucidated. Furthermore, it is not yet known whether a specific release mechanism exists in the brain or pituitary.

Stress may play a role in releasing these peptides, thus indicating a common biosynthetic pathway[5], at least in the pituitary, with that of ACTH.

Rossier et al.[6] have shown that foot-shock stress in rats leads to an increase in opioids at the peripheral level, but not in the brain, indicating a specific release mechanism for



Graphic representation of physical exercise on the treadmill in 8 male athletes aged 21 ± 2 years. Arrows indicate ECG recording. Blood samples were collected in basal conditions, upon reaching maximum aerobic capacity and then 15 and 30 min after stopping exercise.

these substances during this condition. Whether this phenomenon is present in man has not yet been shown and results obtained in animals cannot always be extrapolated to man. The aim of the present investigation was to ascertain whether a specific release mechanism for endorphin exists during stress in man and if this release is concomitant with that of ACTH. 8 professional male athletes aged 21 ± 2 years, all following the same training programme, were investigated. A catheter was introduced into the forearm vein and maintained in situ throughout the experiment. Physical stress was produced by means of a treadmill, and monitored by a spirometer for aerobic capacity. Athletes ran on the treadmill (figure 1) for 2 min at 6 km/h, 2 min at 9 km/h and 2 min at 12 km/h (warming-up phase), after which the treadmill was automatically set at 15 km/h till the athletes reached maximum aerobic capacity monitored by the continuous spirometer recording. It should be pointed out that a large number of athletes were investigated, but only these 8 reached the status described and were therefore eligible for the study.

Particular care was taken to ensure a stress status which was reproducible and could be quantitatively expressed. Blood was collected by means of a peristaltic pump under basal conditions, upon reaching maximum aerobic capacity, and then 15 and 30 min after stopping. Upon collection the test tubes containing blood samples were placed on ice and plasma was immediately separated and frozen by means of an acetone-dry-ice mixture.

Radioimmunoassay of ACTH was performed by a previously described method[7]. The antiserum used was specific and sensitive and did not cross react with opiates (β-endorphin and β-lipotropin). An antiserum kindly supplied by Dr Candace Pert, NIH, Bethesda Md, was used for the β-endorphin radioimmunoassay (table 1). While this antiserum did not cross-react with ACTH, it showed a complete cross-reactivity with human β-lipotropin. Plasma β-endor-

Table 1. RIA of β-endorphin** specificity of the antiserum

Peptides and hormones	Origin	Molar ratio × 100*
β-endorphin	Immunonuclear Co, (Stillwater, MI, USA)	100
Leu-enkephalin		0.02
Met-enkephalin		0.02
Substance P		0.08
Somatostatin	Serono (Rome, Italy)	0.02
LHRH		0.02
Ovine β-lipotropin	Dr. C.H. Li	100
ACTH 1-39	CIBA (Basel, Switzerland)	0.05
B-MSH		0.04
Human chorionic somatomammotropin	Sclavo (Siena, Italy)	0.02
Human chorionic gonadotropin	Serono (Rome, Italy)	0.02

* Molar ratio × 100 of each compound when compared to β-endorphin for 50% competition of ^{125}I β-endorphin binding to the antibody. ** RIA was performed using Chloramine T, ^{125}I-labelled synthetic β-endorphin. 0.1 ml of labelled hormone, 0.1 ml of antiserum diluted 1/3000 and standard or sample solution, all diluted in phosphate buffer 0.05 M, pH 7.4, BSA 0.1%, were incubated for 24 h at 4 °C before adding 0.1 ml of goat antirabbit gamma-globulin serum. After a second 6 h incubation at 4 °C, 2 ml of buffer were added and all tubes were centrifuged for 30 min at 3000 × g at 4 °C. The supernatant was discarded and the pellets counted. Results were calculated by means of the logit-log RIA programme[8] using a MK vs BKG computer system (Honeywell). Intra-assay variation was 9% as c.v. while interassay variation did not exceed 14% as c.v. Sensitivity, expressed as the minimum detectable dose, was less than 5 pg/tube. It should be pointed out that serum was assayed after chromatography on Sephadex G75 in 0.3 M acetic acid as described in the text. In this experimental condition ^{125}I β-endorphin and cold β-endorphin used as marker, were eluted between 80 and 110 ml. These 1-ml fractions were pooled, lyophilised and then assayed. The recovery by this procedure, calculated on cold synthetic β-endorphin, was about 65%.

Table 2. Values of β-endorphin and ACTH before, during and after physical exercise. Results are expressed as mean values ± SD (n = 8). The condition of maximal effort was reached by all subjects between 8 and 15 min

Condition	Basal conditions	100% maximal effort	15 min after stopping	30 min after stopping
ACTH (pg/ml)	80 ± 15	850 ± 25	320 ± 45	95 ± 30
β-Endorphin (pg/ml)	320 ± 70	1620 ± 250	1080 ± 120	420 ± 65

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

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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