Substrate utilization by rat stomach *in vivo*

Arteriovenous differences for glucose, lactate, ketone bodies, fatty acids and glycerol under control and acid-secreting conditions

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1. Arteriovenous differences for several potential metabolic substrates were measured across the fundic wall of the stomach of rats that had been starved overnight. 2. There was an uptake of glucose and D-3-hydroxybutyrate, but no significant arteriovenous differences for acetoacetate, pyruvate, non-esterified fatty acids and glycerol were apparent. 3. Lactate output represented a substantial fraction of glucose uptake when the arterial lactate concentration was within the resting physiological range, but when the arterial lactate concentration was above 1.3 mM, lactate was taken up by the stomach. 4. Stimulation of acid secretion by pentagastrin did not affect the value of arteriovenous differences. Thus blood flow to the fundic mucosa and substrate metabolism may be similarly enhanced by pentagastrin. 5. It is concluded that metabolism of glucose and D-3-hydroxybutyrate, and to a lesser extent of glutamine and branched-chain amino acids [Anderson & Hanson (1983) *Biochem. J.* 210, 451–455], could supply energy to power acid secretion.

The selection of particular metabolic fuels by the gastric mucosa as sources of energy for acid secretion could arise for a number of reasons. Firstly, a particular metabolic pathway might be intimately associated with the secretory mechanism (Sernka & Harris, 1972; Hersey, 1977). Secondly, acid secretagogues might enhance the entry of particular substrates into the parietal cell, or activate enzymes controlling the flux through particular metabolic pathways; or thirdly, only certain metabolic pathways might be sufficiently active to meet the high energy demands of acid secretion. Experiments *in vitro* have produced conflicting evidence of the relative importance of carbohydrates and fuels derived from lipids as substrates for acid secretion (Davenport & Chavré, 1950; Sernka & Harris, 1972; Hersey, 1977, 1981; Forte *et al.*, 1980). A problem with such experiments *in vitro* is that potential substrates are often presented alone to the tissue, and/or at non-physiological concentrations. The aim of this work was to make a first attempt to establish the pattern of utilization of carbohydrate and lipid-derived fuels by the stomach *in vivo*. An advantage of this approach is that substrates are presented to the tissue in combination and usually at physiological concentrations. This work complements a previous report of amino acid

metabolism by the stomach *in vivo* (Anderson & Hanson, 1983).

Materials and methods

Animals

Male Wistar rats (175–225 g body wt.) were obtained from Banting and Kingman, Hull, U.K. Rats were fed on Heygates breeding diet (modified 41 b diet) supplied by Pilsbury, Edgbaston, Birmingham, U.K. Rats were starved overnight (17 h) before use, unless specifically stated otherwise.

Reagents

Substrates, enzymes and coenzymes were obtained from Boehringer. 1,5-Diphenylcarbazine was obtained from Sigma. Inorganic chemicals, solvents and sodium heparin were purchased from BDH.

Measurement of arteriovenous differences

The technique, which has been described in detail by Anderson & Hanson (1983), involves the simultaneous sampling of blood from the vein draining the dorsal fundic region of the stomach (coronary vein; Greene, 1955) and from the abdominal aorta of an intact anaesthetized animal *in vivo*. Rats were anaesthetized with sodium pento-

barbitone (Sagatal; 60 mg/kg body wt.) and injected intravenously with heparin [500 i.u. in 0.2 ml of saline (0.9% NaCl)] just before sampling. When required, acid secretion was stimulated by subcutaneous injection of pentagastrin (Peptavlon, ICI Pharmaceuticals; 250 mg/kg body wt.); arteriovenous differences were then measured 20 min after injection (see Anderson & Hanson, 1983, where the extent and time course of the acid-secretory response are recorded).

Assay of substrates and metabolites

Non-esterified fatty acids were assayed in plasma by the method of Falholt *et al.* (1973). In all other cases measurements were made on neutralized HClO_4 extracts of whole blood (0.5–0.7 ml of blood added to 4 ml of ice-cold 1 M- HClO_4 , mixed, centrifuged at 4°C for 15 min at 2790 g, and neutralized with 4 M-KOH), and the following assays were performed: glucose (Bergmeyer *et al.*, 1974); lactate (Hohorst, 1963); pyruvate (Czok & Lamprecht, 1974); glycerol (Wieland, 1974); D-3-hydroxybutyrate (Williamson & Mellanby, 1974); and acetoacetate (Mellanby & Williamson, 1974).

Measurement of substrates and metabolites in stomach contents

Rats were starved for 24 h, and then allowed to feed for 1 h, after which food was removed, and 30 min later the animal was killed and the total contents of the stomach were removed and weighed. The contents were then centrifuged at 10000 g for 4 min (Beckman Microfuge), the volume of the supernatant was recorded, and a neutralized HClO_4 extract of supernatant (prepared as above) was used for assay of all except fatty acids, which were extracted from the untreated supernatant.

Results and discussion

Stimulation of acid secretion and the pattern of substrate utilization

Of the seven potential substrates investigated, only glucose and D-3-hydroxybutyrate were taken up by the wall of the fundus when substrate concentrations in arterial blood were in the physiological range (Table 1). Values for mean arteriovenous differences were unaffected by the stimulation of acid secretion with pentagastrin (Table 1). Injection of pentagastrin appeared to lower the concentration of glucose in arterial blood (Table 1), but the comparison between arteriovenous differences in control and pentagastrin-stimulated rats is unlikely to have been affected by this difference, since no relationship between the arteriovenous difference for glucose and the concentration of glucose in arterial blood was found.

If an increased blood flow to the mucosa accompanies stimulation of acid secretion by pentagastrin (Main & Whittle, 1973), then there must be an increase, of similar size, in substrate utilization by the mucosa, for otherwise mean arteriovenous differences would not be the same in control and pentagastrin-stimulated rats. Neither the present nor previous work (Anderson & Hanson, 1983) suggests any change in the pattern of substrate utilization after stimulation of acid secretion by pentagastrin.

Glucose, lactate and pyruvate

The largest negative arteriovenous differences in both control and pentagastrin-injected rats were for glucose (Table 1). Consideration of those experiments in which the arterial blood lactate concentration was within ± 2 s.d. of the value for resting conscious rats (Hawkins *et al.*, 1971b) showed that

Table 1. *Arteriovenous differences for glucose, lactate, pyruvate, ketone bodies, non-esterified fatty acids and glycerol across the stomach of control and pentagastrin-injected rats in vivo*

Results are expressed as means $\pm$ s.e.m., with the numbers of animals in parentheses. Arterial blood was sampled from the abdominal aorta and venous blood from the coronary vein (Greene, 1955). Injection of rats with pentagastrin did not alter the concentration of most substrates in the arterial blood, and values have therefore been pooled, the exception being glucose, where the values for control ($\dagger$) and pentagastrin-injected ($\ddagger$) rats were different ($P < 0.05$) in this series of experiments. The probability, P , of arteriovenous differences being significantly different from zero (paired t test) is denoted as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Abbreviation used: n.d., not determined.

Substrate	Arterial blood concn. (mM)	Venous – arterial concn. (mM)	
		Control	Pentagastrin-injected
Glucose	6.34 $\pm$ 0.31 (9) $\dagger$ 4.97 $\pm$ 0.39 (8) $\ddagger$	–0.149 $\pm$ 0.022 (9)***	–0.109 $\pm$ 0.026 (8)**
Lactate	0.677 $\pm$ 0.036 (19)	0.159 $\pm$ 0.035 (10)**	0.147 $\pm$ 0.033 (9)**
Pyruvate	0.088 $\pm$ 0.009 (6)	0.0005 $\pm$ 0.009 (6)	n.d.
D-3-Hydroxybutyrate	0.456 $\pm$ 0.052 (16)	–0.096 $\pm$ 0.012 (9)***	–0.086 $\pm$ 0.020 (7)**
Acetoacetate	0.198 $\pm$ 0.016 (16)	–0.016 $\pm$ 0.010 (9)	0.005 $\pm$ 0.011 (7)
Non-esterified fatty acids	0.661 $\pm$ 0.033 (21)	–0.001 $\pm$ 0.039 (11)	0.008 $\pm$ 0.023 (10)
Glycerol	0.112 $\pm$ 0.008 (15)	–0.004 $\pm$ 0.009 (7)	0.020 $\pm$ 0.010 (8)

Fig. 1. Relationship between arteriovenous difference for lactate and arterial blood lactate concentration. The lines were drawn by linear regression analysis of the data, the regression equations being: control, $y = 0.30 - 0.23x$ ($n = 13$); pentagastrin-injected rats, $y = 0.31 - 0.23x$ ($n = 16$). Both regression coefficients were significantly different from zero ($P < 0.001$).

there was an output of lactate by the tissue under these circumstances (Table 1). If all values for the arteriovenous differences for lactate were plotted against the lactate concentration in arterial blood, a significant linear relationship between the two measurements was found, which was similar for both control and pentagastrin-stimulated rats (Fig. 1). No effect of the variation in the arteriovenous concentration difference for lactate on the arteriovenous difference for glucose was apparent [see also Windmueller & Spaeth (1978), Table 1, for the jejunum]. Too deep an anaesthesia may have contributed to the substantial elevation of the arterial lactate concentration in some experiments, for anaesthesia induced by sodium pentobarbitone does appear to increase arterial lactate (Hawkins *et al.*, 1971*b*). Either a decreased removal of lactate from the blood by the liver or an increased production of lactate, consequent perhaps on anoxia in some tissue, could have raised the arterial lactate concentration. However, it seems unlikely that the stomach was anoxic under such circumstances, for utilization of blood lactate is a characteristic of only well-oxygenated tissues. An uptake of lactate by the fundic wall occurred when the arterial lactate concentration was greater than 1.3 mM. Thus, although lactate metabolism can support gastric acid secretion *in vitro* (Davenport & Chavré, 1950; Hersey, 1981), it is somewhat unlikely that it does so *in vivo* unless the arterial concentration of lactate is raised above 1.3 mM. There was no significant

arteriovenous difference for pyruvate under control conditions (Table 1).

Ketone bodies

The mean uptake of D-3-hydroxybutyrate was significant, and similar, in both control and pentagastrin-stimulated rats (Table 1), but in neither condition was there a significant uptake of acetoacetate [cf. *in vitro* Davenport & Chavré (1951)]. In contrast with the stomach, the brain (Hawkins *et al.*, 1971*a*) and small intestine (Windmueller & Spaeth, 1978) take up both D-3-hydroxybutyrate and acetoacetate *in vivo*. There was, as for brain (Hawkins *et al.*, 1971*a*), a linear relationship between the uptake of D-3-hydroxybutyrate by the stomach and its concentration in arterial blood (Fig. 2), although this was only found with rats that had been injected with pentagastrin. In control animals it is possible that regulation of D-3-hydroxybutyrate uptake might lie within the tissue, rather than the arterial concentration of D-3-hydroxybutyrate being the determinant of uptake.

In gastric glandular mucosa, activities of 3-oxo acid CoA-transferase (EC 2.8.3.5) (Hanson & Carrington, 1981) and D-3-hydroxybutyrate dehydrogenase (MacGill *et al.*, 1982) were higher than in most other rat tissues, which implied that ketone bodies might be important substrates for the stomach. This suggestion is validated by our finding of a high fractional extraction of D-3-hydroxybutyrate from arterial blood by the fundic wall (18%

Fig. 2. Relationship between arteriovenous difference for D-3-hydroxybutyrate and arterial blood D-3-hydroxybutyrate concentration

The line was drawn by linear regression analysis of the data, the regression equations being: control rats $y = -0.07 - 0.05x$ ($n = 12$); pentagastrin-injected rats, $y = -0.01 - 0.20x$ ($n = 11$). The regression coefficient was only significantly different from zero ($P < 0.01$) when rats had been injected with pentagastrin, and therefore a line has only been drawn in this instance. All rats were starved overnight, except for three control and four pentagastrin-injected rats, which were starved for 48h in an attempt to widen the range of arterial D-3-hydroxybutyrate concentrations.

in control rats and 24% in rats injected with pentagastrin).

Non-esterified fatty acids and glycerol

No significant arteriovenous differences for either non-esterified fatty acids or glycerol were demonstrated (Table 1). The large values for the S.E.M., however, cause the confidence limits of the means to be wide, and the possible involvement in stomach metabolism of either lipolysis, which would probably be associated with generation of glycerol, or the uptake of non-esterified fatty acids from the plasma cannot be completely excluded.

Utilization of substrates from the lumen

In the small intestine, glutamine (Hanson & Parsons, 1977; Windmueller & Spaeth, 1980) is taken up from the lumen and is used as a metabolic substrate, as are short-chain fatty acids by the colon (Roediger, 1982). As a first step in determining whether stomach cells might be able to utilize substrates from the gastric lumen, measurements of the concentrations of potential substrates and some metabolites were performed (Table 2). Glucose and lactate were present at high concentrations, and, given that entry into the epithelial mucous cells can occur, their metabolism by such cells seems to be a possibility. It is less likely that luminal substrates could provide energy for acid secretion by parietal cells, for these cells are located towards the bottom of the gastric glands, most of their cell membrane faces the contraluminal side of the gland, and

Table 2. Concentration of some potential substrates and metabolites in the fluid present in rat stomach after a meal

Rats were starved overnight, allowed access to food for 1h, and then the contents of the stomach were removed 30 min later, and treated as described in the Materials and methods section. Glutamine, glutamate and ammonia were assayed by the methods used by Anderson & Hanson (1983); for other assays see the Materials and methods section. Results are means $\pm$ S.E.M. for four rats in each case. The volume of fluid obtained by centrifugation of the stomach contents was 0.679 ± 0.068 ml (13).

Substance	Concn. in fluid (mM)
Glucose	19.5 ± 1.60
Lactate	5.44 ± 0.41
D-3-Hydroxybutyrate	Undetectable
Acetoacetate	0.16 ± 0.057
Non-esterified fatty acids	0.44 ± 0.062
Glycerol	5.71 ± 0.69
Glutamate	0.80 ± 0.076
Glutamine	0.18 ± 0.041
Ammonia	0.92 ± 0.14

substrates would have to reach the cell against an opposing flow of acid.

General discussion and conclusions

The stomach wall takes up glucose and D-3-hydroxybutyrate (Table 1), and to a lesser extent glutamine and the branched-chain amino acids (Anderson & Hanson, 1983), from blood. The extent

to which such results can be used to draw conclusions about metabolism of parietal cells now requires discussion. Since parietal cells make a considerable contribution to the oxygen consumption, in the presence of histamine, by a crude preparation of cells from dog fundic mucosa (Soll, 1978), and since it is unlikely that the stomach muscle will make a major metabolic contribution (obvious motility was absent), it seems that arterio-venous differences for the whole of the fundic wall may relate to parietal cell metabolism under conditions where acid secretion is stimulated. Therefore parietal cells probably metabolize glucose, D-3-hydroxybutyrate, glutamine and branched-chain amino acids to provide energy for acid secretion. However, to substantiate this suggestion fully the end-products of metabolism of these substrates must be established. The results do not support an association between the utilization of a particular metabolic substrate and the provision of energy to support acid secretion, although clearly the stomach wall exhibits a preference for certain metabolic substrates.

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References

- Anderson, N. G. & Hanson, P. J. (1983) *Biochem. J.* **210**, 451–455
- Bergmeyer, H. U., Bernt, E., Schmidt, F. & Stork, H. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 1196–1201, Verlag Chemie, Weinheim, and Academic Press, New York
- Czok, R. & Lamprecht, W. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 1446–1451, Verlag Chemie, Weinheim, and Academic Press, New York
- Davenport, H. W. & Chavré, V. J. (1950) *Gastroenterology* **15**, 467–480
- Davenport, H. W. & Chavré, V. J. (1951) *Am. J. Physiol.* **166**, 456–461
- Falholt, K., Lund, B. & Falholt, W. (1973) *Clin. Chim. Acta* **46**, 105–111
- Forte, J. G., Black, J. A. & Forte, J. G., Jr. (1980) *Am. J. Physiol.* **238**, G353–G357
- Greene, E. C. (1955) *Anatomy of the Rat*, p. 228, Hafner, New York
- Hanson, P. J. & Carrington, J. M. (1981) *Biochem. J.* **200**, 349–355
- Hanson, P. J. & Parsons, D. S. (1977) *Biochem. J.* **166**, 509–519
- Hawkins, R. A., Williamson, D. H. & Krebs, H. A. (1971a) *Biochem. J.* **122**, 13–18
- Hawkins, R. A., Alberti, K. G. M. M., Houghton, C. R. S., Williamson, D. H. & Krebs, H. A. (1971b) *Biochem. J.* **125**, 541–544
- Hersey, S. J. (1977) *Gastroenterology* **73**, 914–919
- Hersey, S. J. (1981) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **40**, 2511–2518
- Hohorst, H. J. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 266–270, Verlag Chemie, Weinheim, and Academic Press, London
- MacGill, A. K., Trotman, C. N. A. & Carrington, J. M. (1982) *Proc. Univ. Otago Med. Sch.* **60**, 77–78
- Main, I. H. M. & Whittle, B. J. R. (1973) *Br. J. Pharmacol.* **49**, 534–542
- Mellanby, J. & Williamson, D. H. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 1840–1843, Verlag Chemie, Weinheim, and Academic Press, New York
- Röediger, W. E. W. (1982) *Gastroenterology* **83**, 424–429
- Sernka, T. J. & Harris, J. B. (1972) *Am. J. Physiol.* **222**, 25–32
- Soll, A. H. (1978) *J. Clin. Invest.* **61**, 370–380
- Wieland, O. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 1404–1409, Verlag Chemie, Weinheim, and Academic Press, New York
- Williamson, D. H. & Mellanby, J. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 1836–1839, Verlag Chemie, Weinheim, and Academic Press, New York
- Windmueller, H. G. & Spaeth, A. E. (1978) *J. Biol. Chem.* **253**, 69–76
- Windmueller, H. G. & Spaeth, A. E. (1980) *J. Biol. Chem.* **255**, 107–112