Biochem. J. (1980) **188**, 619–632
Printed in Great Britain

Glutamate, Glutamine, Aspartate, Asparagine, Glucose and Ketone-Body Metabolism in Chick Intestinal Brush-Border Cells

John W. PORTEOUS
Department of Biochemistry, University of Aberdeen, Marischal College, Aberdeen AB9 1AS, Scotland, U.K.

(*Received 28 September 1979*)

1. Suspensions of isolated chick jejunal columnar absorptive (brush-border) cells respired on endogenous substrates at a rate 40% higher than that shown by rat brush-border cells. 2. Added D-glucose (5 or 10mM), L-glutamine (2.5mM) and L-glutamate (2.5mM) were the only individual substrates which stimulated respiration by chick cells; L-aspartate (2.5 or 6.7mM), glutamate (6.7mM), glutamine (6.7mM), L-alanine (1 or 10mM), pyruvate (1 or 2mM), L-lactate (5 or 10mM), butyrate (10mM) and oleate (1mM) did not stimulate chick cell respiration; L-asparagine (6.7mM) inhibited slightly; glucose (5mM) stimulated more than did 10mM-glucose. 3. Acetoacetate (10mM) and D-3-hydroxybutyrate (10mM) were rapidly consumed but, in contrast to rat brush-border cells, did not stimulate respiration. 4. Glucose (10mM) was consumed more slowly than 5mM-glucose; the dominant product of glucose metabolism during vigorous respiration was lactate; the proportion of glucose converted to lactate was greater with 10mM- than with 5mM-glucose. 5. Glutamate and aspartate consumption rates decreased, and alanine and glutamine consumption rates increased when their initial concentrations were raised from 2.5 to 6.7 or 10mM. 6. The metabolic fate of glucose was little affected by concomitant metabolism of any one of aspartate, glutamate or glutamine except for an increased production of alanine; the glucose-stimulated respiration rate was unaffected by concomitant metabolism of these individual amino acids. 7. Chick cells produced very little alanine from aspartate and, in contrast to rat cells, likewise produced very little alanine from glutamate or glutamine; in chick cells alanine appeared to be predominantly a product of transamination of pyruvate derived from glucose metabolism. 8. In chick cells, glutamate and glutamine were formed from aspartate (2.5 or 6.7mM); aspartate and glutamine were formed from glutamate (2.5mM) but only aspartate from 6.7mM-glutamate; glutamate was the dominant product formed from glutamine (6.7mM) but aspartate only was formed from 2.5mM-glutamine. 9. Chick brush-border cells can thus both catabolize and synthesize glutamine; glutamine synthesis is always diminished by concomitant metabolism of glucose, presumably by allosteric inhibition of glutamine synthetase by alanine. 10. Proline was formed from glutamine (2.5mM) but not from glutamine (2.5mM) + glucose (5mM) and not from 2.5mM-glutamate; ornithine was formed from glutamine (2.5mM) + glucose (5.0mM) but not from glutamine alone; serine was formed from glutamine (2.5mM) + glucose (5mM) and from these two substrates plus aspartate (2.5mM). 11. Total intracellular adenine nucleotides (22μmol/g dry wt.) remained unchanged during incubation of chick cells with glucose. 12. Intracellular glutathione (0.7–0.8mM) was depleted by 40% during incubation of respiring chick cells without added substrates for 75min at 37°C; partial restoration of the lost glutathione was achieved by incubating cells with L-glutamate + L-cysteine + glycine.

The work described here was carried out in The Metabolic Research Laboratory, Radcliffe Infirmary, Oxford, England, U.K. during leave of absence from the University of Aberdeen.

Recent observations on glucose, ketone body, glutamine, glutamate and aspartate metabolism in the vascularly and luminally perfused small intestine

0306-3283/80/060619-14$01.50/1 © 1980 The Biochemical Society

in vivo and *in vitro* have been reviewed (Porteous, 1978, 1979). One of several advantages of the tissue perfusion technique is that it permits the study of metabolism of substrates supplied alternatively from each side of the intestine. A possible shortcoming of the technique is that the cellular site of metabolism is not identified; it is generally assumed that the intestinal columnar absorptive epithelial cells (brush-border cells) are responsible for the observed metabolism of substrates supplied by the vascular or luminal route. One purpose of the present investigation was to ascertain the capacity of isolated intact brush-border cells to metabolize alanine, pyruvate, lactate, oleate, butyrate, acetoacetate, 3-hydroxybutyrate, glutamine, glutamate, asparagine and aspartate. Glucose metabolism has also been studied because this substrate is the dominant monomeric component of animal diets and the dominant free monomer in the circulation; because glucose was apparently converted to lactate only to a very small degree during its translocation from the lumen to the vascular circuit of perfused rat small intestine (Windmueller & Spaeth, 1978) whereas a substantial conversion of glucose to lactate was observed in similar preparations (Hanson & Parsons, 1976, 1977, 1978), in everted sacs (Pritchard & Porteous, 1977) and in isolated rat jejunal brush-border cells (Towler *et al.*, 1978); and because of the expected interplay between amino acid and glucose metabolism. Observations on glutathione in isolated brush border cells are included because of the postulated role of this peptide in amino acid translocation (Meister & Tate, 1976). The prerequisite assurance that such isolated brush border cells are intact, representative of the absorptive epithelium *in vivo* and essentially free of other tissue components was provided by earlier studies on cells isolated from rat jejunum (Towler *et al.*, 1978). In the present study, analogous cells have been isolated from chick small intestine partly in order to provide comparisons with parallel investigations on cells isolated from rat small intestine in this study and by Watford *et al.* (1979) and partly because isolated chick intestinal brush-border cells survive longer under incubation conditions *in vitro* than do the corresponding rat cells.

Materials and Methods

Reagents

Bovine plasma albumin (fraction V) and ovine testicular hyaluronidase (type 36-231-2) were from Miles Laboratories, Stoke Poges, Bucks. SL2 4LY, U.K.; the albumin was dialysed as described by Krebs *et al.* (1974). NAD^+, NADH, $NADP^+$, NADPH, the sodium salts of pyruvic acid, 2-oxoglutaric acid and oxaloacetic acid, and all enzyme preparations were from Boehringer Corp., Lewes, East Sussex BN7 1LG, U.K. L-Carnitine hydrochloride was from International Enzymes, Windsor, Berks. SL4 5NJ, U.K. Inorganic chemicals glucose, ethanol and glycerol were analytical reagent grade. Sodium L-glutamate, L-glutamine, L-aspartic acid, L-asparagine, L-alanine, acetoacetic acid, D-3-hydroxybutyric acid, sodium butyrate, sodium acetate, sodium citrate, sodium succinate and oleic acid were the best commerically available materials. L-Lactic acid was prepared by Mr. R. Hems. Decanoyl-L-carnitine, 4-pentenoic acid and 4-bromo-octanoic acid were gifts from Dr. D. H. Williamson.

Animals

Male Rhode Island Red × Light Sussex chicks (3–5 weeks old) were supplied by Orchard Farms, Great Missenden, Bucks., U.K., fed on Chick Starter Crumbs ACS (BOCM Silcock, Basingstoke, Hants., U.K.) with free access to water, and were killed by decapitation when 4–7 weeks old. Incubation of isolated intestinal brush border cells was started within the next 40 min.

Preparation of chick intestinal brush-border cells

The whole of the small intestine below the gizzard and above the junction of the caeca with the large intestine (Bolton, 1969; Hill, 1976) was excised and the bilobal pancreas was removed. The lumen of the intestine was washed out at room temperature (about 20°C) with 50 ml of bicarbonate saline (Krebs & Henseleit, 1932) supplemented with dithiothreitol (1.0 mM) and albumin (2.5 mg/ml), and equilibrated with O_2/CO_2 (95:5), final pH 7.4. The washed intestine was slit longitudinally, cut into segments (about 3 cm) and washed rapidly by suspension in 2 × 50 ml of the same medium at room temperature, followed by filtration on a domestic nylon sieve. Washed segments of intestine were incubated at 37°C for 5 min with gentle shaking in 100 ml of the same medium now also supplemented with hyaluronidase (1.5 mg/ml) in a stoppered plastic vessel which was continuously gassed with O_2/CO_2 (95:5). These segments of intestine were filtered as before, washed twice in 50 ml of fresh medium without hyaluronidase, then agitated vigorously with a plastic rod for 1 min at room temperature in a further 50 ml of medium without hyaluronidase to release brush-border cells from the underlying tissue. The cell suspension was harvested into a polypropylene centrifuge tube by filtration of the segments on nylon mesh as before. The residual segments were suspended and agitated again three times in the same way and filtered to fill a further three centrifuge tubes with cell suspension. Cells were sedimented by centrifuging (about 1000 ***g*** sustained for 15 s at room temperature), and supernatant suspensions were discarded and the loosely sedimented cells were gently resuspended in fresh

medium, then sedimented once more. The final combined cell sediments (3–5g wet wt. from each intestine) were resuspended to a concentration of 5–10mg of cell dry wt./ml in fresh medium of the same composition as before except that the albumin concentration was now 25mg/ml.

Preparation of rat jejunal brush-border cells

The cell isolation procedure was that described by Towler *et al.* (1978) except that (i) the cell isolation, washing and incubation medium was the single medium described above, and (ii) apart from the incubation with hyaluronidase at 37°C, all other preparative stages were carried out at room temperature (about 20°C). This procedure differs in a few details from that described by Watford *et al.* (1979). The cells were finally suspended as described for chick cells.

Incubation conditions

Freshly isolated cells (10–20mg dry wt.) were incubated in Krebs-Henseleit bicarbonate saline supplemented with dithiothreitol (1.0mM), EGTA (0.1mM) and albumin (25mg/ml), with and without added substrates as stipulated in the Results section; the total volume of the incubation system was 3ml. The incubation system was contained in the main well of Gevers flasks (Krebs *et al.*, 1974) shaken continuously (100 oscillations/min) at 37°C; the gas phase was O_2/CO_2 (95:5). With very few exceptions, respiration measurements and analyses of the metabolism of endogenous or added substrates were thus performed on the same cell suspension; exceptionally, cells and medium were incubated in stoppered conical flasks with the same gas phase as before. Reactions were terminated by acidification with ice-cold $HClO_4$ (0.5M); extraction of cells was continued with occasional vortex-mixing during 1h at 0°C. Residual cell material was sedimented (3000*g* for 5min at 0°C), the supernatant extract was retained and neutralized with KOH and $KHCO_3$. In some experiments, cells were separated rapidly from the incubation medium by sedimentation into 0.5M-$HClO_4$ using a modification of the special centrifuge tubes described by Hems *et al.* (1975); cell extract and medium were then separately processed and analysed.

Analyses

Oxygen consumption was measured by Warburg respirometry at constant CO_2 concentration (Krebs *et al.*, 1974). Other substrates and products were determined spectrophotometrically (in neutralized extracts of cells plus medium, of cells alone, or in the medium alone) as described in Bergmeyer (1974) by the following authors: glucose (Bergmeyer, H. U., Bernt, E., Schmidt, F. & Stork, H., pp. 1196–1201); pyruvate (Czok, R. & Lamprecht, W., pp. 1446–1451); lactate (Gutmann, I. & Wahlefeld, A. W., pp. 1464–1468); 2-oxoglutarate (Bergmeyer, H. U. & Bernt, E., pp. 1577–1580); oxaloacetate (Wahlefeld, A. W., pp. 1604–1608); alanine (Williamson, D. H., pp. 1679–1685); aspartate and asparagine (Bergmeyer, H. U., Bernt, E., Möllering, H. & Pfleiderer, G., pp. 1696–1700); glutamate (Bernt, E. & Bergmeyer, H. U., pp. 1704–1708); glutamine (Lund, P., pp. 1719–1722); urea (Gutmann, I. & Bergmeyer, H. U., pp. 1794–1798); NH_3 (Kun, E. & Kearney, E. B., pp. 1802–1806); D-3-hydroxybutyrate (Williamson, D. H. & Mellanby, J., pp. 1836–1839); acetoacetate (Mellanby, J. & Williamson, D. H., pp. 1836–1839); ATP (Lamprecht, W. & Trautschold, I., pp. 2101–2110); ADP and AMP (Jaworek, D., Gruber, W. & Bergmeyer, H. U., pp. 2127–2131). Amino acids other than those listed above were determined by automated column chromatography. Reduced and oxidized glutathione were determined as described by Viña *et al.* (1978).

Conduct of experiments and presentation of results

To avoid circadian variations in metabolic activities, all animals were killed between 10.00h and 11.00h. The amounts of each substrate and product present in (*a*) the incubation medium alone and (*b*) the medium plus cells, was always determined at zero time and again (*c*) in the medium plus cells at the end of each incubation period. Zero-time determinations on the medium alone served to check the initial substrate concentrations, those on medium plus cells indicated any initial contribution of substrate or product by the cells to the incubation system. The difference between determinations (*b*) and (*c*) gave the gross consumption or production of a given solute (i) in the absence and (ii) in the presence of any added substrate; results were expressed as μmol/min per g dry wt. of cells. Gross rates of consumption of O_2 by rat brush-border cells are shown in Table 1. Gross rates of consumption or production of a number of solutes by chick cells incubated in the absence of added substrates are shown in Table 2. As all experiments were conducted in the paired manner described above, the change in consumption or production of each solute could be calculated in each experiment as the algebraic difference between results (i) and (ii) described above; in Tables 3 and 4, the means of a number of such determinations and calculations are given, together with standard errors and the significance of the differences between values of (i) and (ii) as determined by the paired *t* test.

All experiments on rat brush-border cells were carried out during January and February 1977. Seasonal variations in metabolic activities of whole ileal tissue have been reported (Neptune, 1965). Most of the results reported here are concerned with

the metabolism, by suspensions of chick brush-border cells, of glucose and amino acids at relatively low (L) initial concentrations (Table 3) or at relatively high (H) initial concentrations (Table 4); these experiments were conducted in the sequence H, L, H, L over the 8-month period February–September 1977. Four sets of results were thus available from cells incubated in the absence of added substrates, and two pairs of sets of results were likewise available from cells incubated with added substrates at different times during the 8 month period. No statistically significant differences could be discerned amongst any of the four sets of results obtained in the absence of added substrates; these results have therefore been collated as a single set (Table 2). Rates of consumption or production of solutes incubated in the presence of low initial concentrations of substrates have likewise been treated as a single set since no statistically significant seasonal variations could be detected (Table 3). Results obtained after incubating cells with relatively high concentrations of substrates have been treated as a single set (Table 4); in this particular instance, the numbers of experiments in the two subsets were too small to permit testing for seasonal variations.

Results

General characteristics of the isolated cells

The microscopic appearance of both rat and chick cells matched that described by Towler *et al.* (1978) but the ratio wet wt./dry wt. of the cells was lower than that previously observed (about 9 for rat cells and about 10 for chick cells); the decrease in this ratio [from a value of 13 reported by Towler *et al.* (1978)] was probably caused by the inclusion of dithiothreitol in the isolation and incubation medium (Watford *et al.*, 1979). Respiration by the rat cells on endogenous substrates or on added glucose (10mM) declined gradually with time so that the rate at 30min was about 80% of that observed during the first 10min. Under the same conditions, chick cells respired at a steady rate for 60min at 37°C; experiments with rat and chick cells were conducted under conditions in which oxygen, glucose and individual amino acids were consumed linearly with time of incubation (Tables 1, 3 and 4).

Rat and chick brush-border cells compared; rates of respiration and substrate consumption

The rates of respiration by rat brush-border cells in the absence of added substrates and in the presence of added glucose (10mM) were indistinguishable (Table 1) from those reported by Watford *et al.* (1979); glutamate (10mM) failed to stimulate rat cell respiration in contrast to the marked stimulation observed with 5mM-glutamate by Watford *et al.* (1979), while glutamine (10mM) stimulated respiration (Table 1) more markedly than did 5mM-glutamine (Watford *et al.*, 1979). These variations in respiration rate in response to different concentrations of these two amino acids do not seem to have been noted before; similar concentration-dependent variations will be noted below in the rates of respiration on added substrates and in the rates of consumption of four amino acids by chick brush-border cells. The remaining observations on respiration by rat brush-border cells (Table 1) are consistent with those reported by Watford *et al.* (1979) except that a marked stimulation of rat cell respiration by added succinate was observed; this latter observation was also made and commented upon by Towler *et al.* (1978).

The rate of respiration on endogenous substrates in chick cells (Table 2) was about 40% greater than in rat cells (Table 1). Of the substrates tested with chick cells, only D-glucose (5mM), L-glutamate (2.5mM) and L-glutamine (2.5mM) raised respiration rates (Table 3) above the already high rate of respiration on endogenous substrates (Table 2). Higher initial concentrations of glucose stimulated respiration less than did 5.0mM-glucose, and higher initial concentrations of glutamate or glutamine failed to stimulate chick cell respiration (Tables 3 and 4). L-Asparagine depressed the respiration rate observed on endogenous substrates (Table 4);

Table 1. *Rat jejunal brush-border-cell respiration rates*
Brush-border cells were isolated from rat jejunum as described by Towler *et al.* (1978) except that the isolation and incubation media were those described in this paper. Results are shown for the rate of respiration in the absence of added substrates and for the gross rates of respiration in the presence of named substrates. Values shown are means ± S.E.M. for the number of experiments indicated in brackets. Values for respiration rates on added substrates were tested by the unpaired *t* test against values for respiration in the absence of added substrates: ***$P<0.001$; *$P<0.05$. All substrates were added at 10mM except for citrate (5mM).

Substrate added	Respiration rate (μmol O_2/min per g dry wt.)	
None	10.2 ± 0.9	(22)
D-Glucose	20.6 ± 1.3***	(25)
L-Glutamate	10.3	(2)
L-Glutamine	19.5 ± 1.1***	(14)
L-Aspartate	11.8 ± 0.9	(9)
Acetate	10.6 ± 1.8	(7)
Succinate	17.4 ± 1.0***	(21)
Citrate	13.4 ± 1.6*	(6)
Ethanol	9.2	(2)
Glycerol	12.4	(2)

Table 2. *Consumption and production (or release) of solutes by chick brush-border cells incubated in the absence of any added substrates*

Cells were isolated from chick jejunum, then incubated, in the absence of any added substrates, as indicated in the Materials and Methods section. Values shown are means ± S.E.M. for the number of experiments shown in brackets. The significances of the differences between mean values and zero are given as $*P<0.05$; $**P<0.01$; $***P<0.001$.

Solute	Rates of consumption (−) and production or release (+) (μmol/min per g dry wt.)	
Oxygen	−14.1 ± 0.69***	(14)
Glucose	+0.03 ± 0.03	(8)
Pyruvate	+0.05 ± 0.03	(13)
Lactate	−0.19 ± 0.14	(12)
Alanine	+0.21 ± 0.05**	(14)
Serine	+0.46 ± 0.04	(3)
Proline	+0.57 ± 0.08	(3)
Ornithine	+0.05 ± 0.03	(3)
Ammonia	+0.35 ± 0.31	(12)
Glutamate	+0.18 ± 0.06**	(16)
2-Oxoglutarate	+0.02 ± 0.006*	(13)
Glutamine	+0.80 ± 0.08***	(10)
Aspartate	+0.11 ± 0.03**	(13)
Asparagine	+0.09 ± 0.02	(4)
Urea	−0.03 ± 0.01	(3)

L-aspartate failed to stimulate chick cell respiration (Tables 3 and 4) and it was notable that aspartate also failed to stimulate rat brush-border cell respiration (Table 1). Pyruvate (1 or 2 mM), L-lactate (5 or 10 mM), L-alanine (1 or 10 mM), butyrate (10 mM) and oleate (1 mM) also failed to stimulate chick brush-border cell respiration. The scope for quantitative comparison of respiration rates by chick and rat cells is limited (Tables 1, 3 and 4; Watford *et al.*, 1979); both species of intestinal brush-border cell appear to use glucose and to a lesser extent, glutamine and glutamate, as respiratory substrates.

Glucose was more rapidly consumed by chick brush-border cells than was any other substrate tested but there was a significant decrease in the rate of glucose consumption when the initial concentration was raised from 5 to 10 mM; decreases in rates of consumption were also observed when the initial concentrations of glutamate and aspartate were raised from 2.5 to 6.7 mM (Tables 3 and 4). In contrast, rates of consumption of glutamine by chick cells increased as the initial concentration was increased (Tables 3 and 4); similarly, the rate of consumption of L-alanine increased from 1.7 to 6.4 μmol/min per g dry wt. when the initial amino acid concentration was raised from 1 to 10 mM (results not tabulated). The rates of consumption of acetoacetate (10 mM) and D-3-hydroxybutyrate (10 mM) were 4.2 and 3.5 μmol/min g dry wt. of cells (results not tabulated) and these rates of substrate consumption were exceeded only by the rates of glucose and alanine consumption; 20% of the acetoacetate consumed appeared as D-3-hydroxybutyrate, whereas 40% of the hydroxybutyrate consumed appeared as acetoacetate. Neither of these ketone bodies stimulated chick-cell respiration, in contrast to the stimulation of rat-cell respiration noted by Watford *et al.* (1979). The rates of glucose consumption by chick cells (Tables 3 and 4) were comparable with that observed by Watford *et al.* (1979) for rat brush-border cells. The rate of consumption of 2.5 mM-glutamate by chick cells (Table 3) was the same as that reported for 5.0 mM-glutamate by rat cells (Watford *et al.*, 1979) but this rate was markedly diminished in chick cells when the initial glutamate concentration was raised (Table 4). Glutamine consumption by chick cells (Tables 3 and 4) proceeded at only 20% of the rate reported for rat cells (Watford *et al.*, 1979).

The metabolic fate of glucose and of selected amino acids in respiring chick brush-border cells

Table 2 collates results obtained in the absence of deliberately added substrates. The high rate of O_2 consumption by chick cells has already been noted. The only other measurements that could be shown to be significantly different from zero were the mean rates of production or release of 2-oxoglutarate, alanine, glutamate, glutamine and aspartate. With the exception of glutamine and ornithine, the mean rate of release of amino acids lay between 0.2 and 0.6 μmol/min per g dry wt. of cells (of the amino acids determined solely by automated column chromatography, only serine, proline and ornithine are shown as representative examples). This slow release of amino acids could signify metabolic production within the cells or extracellular proteolysis by the cells of the albumin in the incubation medium; the present experiments do not distinguish between these possibilities but the rate of release or production of any given amino acid was quite constant. Ammonia production and lactate consumption were more variable from day to day than the production or consumption of other solutes in the absence of added substrates.

Glucose metabolism. The rapid consumption of glucose (Table 3) was accompanied by an increment in O_2 consumption, by increased rates of pyruvate, lactate and alanine production, and by small increases in glutamate and oxoglutarate production. On the ammonia consumed, 80% was accounted for by the increased alanine and glutamate formation though the extreme variability of the values for ammonia consumption leave the precision of this last conclusion in some doubt.

Table 3. *The change in consumption or production of solutes by chick brush-border cells when incubated in the presence of added substrates*

Cells were isolated from chick jejunum, then incubated, in the absence and presence of added substrates, as indicated in the Materials and Methods section. The initial concentrations of D-glucose and of individual L-amino acids were 5 and 2.5 mM respectively. The change in consumption or production of any given solute was calculated as stated in the Materials and Methods section; the values shown are means ± S.E.M. for the number of experiments shown in brackets; the significances of these differences of means were calculated by the paired *t* test and are indicated by †$P<0.1$, *$P<0.05$, **$P<0.01$, ***$P<0.001$. N.D., not determined.

		Changes in consumption (−) and production (+) of solutes in the presence of added amino acids and/or glucose (μmol/min per g dry wt. of cells)							
Solute consumed or produced	Addition ...	Glucose	Glutamate	Glutamate + glucose	Glutamine	Glutamine + glucose	Glutamine + aspartate + glucose	Aspartate	Aspartate + glucose
Oxygen		−4.65*** ± 0.56 (8)	−2.14* ± 0.64 (8)	−4.53*** ± 0.58 (8)	−1.70† ± 0.70 (5)	−3.72** ± 0.78 (5)	−3.68** ± 0.77 (5)	(+0.60) ± 0.78 (5)	−4.42** ± 0.96 (5)
Glucose		−13.1*** ± 1.04 (8)	−0.02 ± 0.10 (3)	−13.9*** ± 1.16 (8)	−0.11 ± 0.11 (3)	−15.2*** ± 0.90 (6)	−15.7*** ± 1.05 (6)	−0.02 ± 0.08 (3)	−14.7*** ± 1.03 (6)
Pyruvate		+1.22** ± 0.24 (8)	−0.04 ± 0.04 (9)	+1.11*** ± 0.17 (9)	+0.01 ± 0.01 (5)	+1.70*** ± 0.19 (6)	+1.95*** ± 0.14 (6)	0	+1.78*** ± 0.16 (5)
Lactate		+16.6*** ± 1.50 (9)	+0.09 ± 0.06 (5)	+16.1*** ± 1.76 (9)	+0.05 ± 0.07 (6)	+19.9*** ± 2.45 (6)	+19.6*** ± 2.02 (6)	−0.13† ± 0.06 (6)	+19.0*** ± 2.04 (6)
Alanine		+0.58*** ± 0.08 (8)	+0.13 ± 0.09 (8)	+1.29*** ± 0.23 (8)	+0.05 ± 0.11 (5)	+1.07** ± 0.13 (5)	+1.54*** ± 0.14 (5)	+0.19** ± 0.04 (5)	+1.37*** ± 0.14 (5)
Serine		−0.01 ± 0.02 (3)	0 (3)	N.D.	+0.02 ± 0.03 (3)	+0.28 ± 0.15 (3)	+0.64 ± 0.30 (3)	0 (3)	N.D.
Proline		−0.12 ± 0.08 (3)	−0.17 ± 0.10 (3)	N.D.	+0.60 ± 0.20 (3)	−0.20 ± 0.17 (3)	−0.23 ± 0.20 (3)	N.D.	N.D.
Ammonia		−0.87 ± 0.56 (8)	−0.14 ± 0.66 (8)	N.D.	+0.32 ± 0.40 (5)	−0.90 ± 0.54 (5)	−0.83 ± 0.45 (5)	−0.16 ± 0.57 (5)	−1.31† ± 0.49 (5)
Glutamate		+0.11* ± 0.03 (8)	−2.68** ± 0.63 (11)	−2.37** ± 0.64 (10)	−0.004 ± 0.04 (10)	+0.20† ± 0.10 (6)	+1.38*** ± 0.13 (7)	+0.96*** ± 0.07 (7)	+1.39*** ± 0.13 (7)
2-Oxoglutarate		+0.05* ± 0.02 (8)	+0.01 ± 0.01 (8)	+0.37*** ± 0.04 (9)	+0.01 ± 0.01 (5)	+0.07* ± 0.03 (6)	+0.05† ± 0.02 (4)	+0.01 (2)	+0.08** ± 0.01 (5)
Glutamine		+0.04 ± 0.09 (9)	+1.61† ± 0.79 (8)	+1.22 ± 1.10 (11)	−1.75* ± 0.75 (10)	−2.19† ± 1.14 (7)	+0.21 ± 0.20 (7)	+0.16† ± 0.07 (7)	−0.06 ± 0.10 (8)
Aspartate		−0.04 ± 0.03 (9)	+0.37*** ± 0.06 (9)	+0.28** ± 0.06 (9)	+0.14** ± 0.02 (6)	−0.02 ± 0.05 (6)	−2.02** ± 0.47 (7)	−2.42** ± 0.41 (6)	−2.60** ± 0.53 (7)
Urea		+0.26 (2)	N.D.	N.D.	−0.15 (2)	−0.11 (2)	+0.06 (2)	+0.15 (2)	−0.05 (2)

Table 4. *Changes in the consumption or production of solutes by chick brush-border cells incubated in the presence of added substrates*

Conditions of experimentation were those given in Table 3 except that the initial concentrations of added glucose and of individual amino acids were 10 and 6.7 mM respectively. Statistical evaluation of results are given as stated in Table 3.

Solute consumed or produced	Addition ...	Change in consumption (−) or production (+) of solutes in the presence of added amino acids and/or glucose (μmol/min per g dry wt. of cells)								
		Glucose	Glutamate	Glutamate + glucose	Glutamine	Glutamine + glucose	Glutamine + aspartate + glucose	Aspartate	Aspartate + glucose	Asparagine
Oxygen		−1.37** ±0.31 (7)	−0.92 ±0.49 (5)	−2.90* ±0.58 (4)	−0.34 ±0.91 (7)	−1.84** ±0.35 (7)	−0.25 (2)	(+0.96) ±0.30 (5)	−1.56 ±0.65 (5)	(+1.95) (2)
Glucose		−10.34** ±0.87 (4)	0 (2)	−10.1* ±0.25 (3)	0 (2)	−9.85** ±0.78 (4)	−11.5* (2)	0 (2)	−12.6* (2)	N.D.
Pyruvate		+1.51** ±0.33 (7)	+0.02 ±0.01 (5)	+1.14† ±0.30 (3)	+0.03 ±0.02 (7)	+1.38** ±0.24 (7)	+2.18 (2)	+0.05 ±0.01 (5)	+1.76*** ±0.29 (5)	+0.04 (2)
Lactate		+19.2*** ±1.83 (7)	+0.23 ±0.14 (5)	+17.7* ±2.54 (3)	+0.13* ±0.04 (6)	+19.3*** ±1.76 (7)	+19.0*** ±1.54 (3)	−0.28 ±0.12 (4)	+18.7*** ±0.77 (5)	−0.17 (2)
Alanine		+0.66** ±0.14 (7)	+0.18* ±0.06 (5)	+1.07 ±0.29 (3)	+0.20* ±0.06 (7)	+1.12** ±0.28 (7)	+1.30 (2)	+0.19* ±0.07 (5)	+1.31 ±0.24 (5)	+0.12 (2)
Ammonia		−0.04 ±0.15 (5)	−0.19† ±0.08 (5)	−0.16 ±0.17 (3)	+1.99 ±1.02 (5)	+1.36 ±0.86 (5)	+0.54 (2)	+0.50** ±0.02 (3)	−0.47 ±0.14 (3)	+1.02 (2)
Glutamate		+0.25* ±0.09 (7)	−0.39 ±0.44 (4)	−0.92* ±0.21 (3)	+0.86** ±0.16 (6)	+0.90* ±0.32 (7)	+1.40 (2)	+0.78** ±0.13 (5)	+1.16*** ±0.11 (5)	+0.31 (2)
α-Oxoglutarate		+0.07 ±0.04 (7)	+0.08* ±0.02 (5)	+0.70* ±0.16 (3)	−0.02 ±0.01 (7)	+0.06 ±0.03 (7)	+0.08 (2)	−0.01 ±0.01 (5)	+0.004 ±0.02 (5)	−0.02 (2)
Glutamine		−0.11† ±0.04 (4)	−0.28 ±0.19 (3)	−0.45 ±0.31 (3)	−2.33† ±1.08 (5)	−0.59 ±0.24 (3)	−1.16 (2)	+0.39 (2)	+0.19 (2)	−0.39 (2)
Aspartate		−0.04 ±0.04 (5)	+0.34* ±0.12 (5)	+0.27 ±0.11 (3)	+0.14 ±0.08 (5)	+0.01 ±0.04 (5)	+0.28 (2)	−1.17 (2)	−2.68 (2)	+0.25 (2)
Urea		+0.03 (2)	0 (2)	+0.23 (2)	0 (2)	0 (2)	N.D.	+0.07 (2)	+0.13 (2)	N.D.

Glutamate metabolism. Glutamate was consumed at only 20% of the rate of glucose consumption (Table 3) and gave only 45% of the stimulation of O_2 consumption; the only notable products of glutamate metabolism observed were aspartate and glutamine, the two together accounting for 74% of the glutamate consumed, but it will be noted that ammonia consumption was insufficient to account for the glutamine produced. The rate of consumption of glutamate was not significantly altered by the concomitant presence of glucose (Table 3) and the rate of production of aspartate was not significantly altered under these circumstances but there was a significant rise in the rate of production of oxoglutarate; the respiration rate, and the rates of glucose consumption and of pyruvate and lactate production were not distinguishable from the rates observed when glucose was added alone but the rate of alanine production was greater than the sum of the rates in the presence of the individual substrates; the mean rate of glutamine production decreased when glucose was also being metabolized and the individual values obtained were more variable. This last point will be taken up again later.

Glutamine metabolism. Glutamine was consumed at 65% of the rate of glutamate consumption, an observation that is quantitatively consistent with observations by Windmueller & Spaeth (1975, 1976) on the different fates of these two amino acids when absorbed from the intestinal lumen; the only notable products detected were aspartate and proline and these together accounted for less than half of the glutamine consumed (Table 3). Respiration was stimulated to an extent that was indistinguishable from that observed with added glutamate. When glucose was added along with glutamine, the rate of glutamine consumption became more variable; about 10% of the consumption was accounted for by a rise in glutamate production. The rates of respiration, glucose consumption, pyruvate and lactate production were restored to values closely similar to those seen in other columns (Table 3) whenever glucose was added to the cells. There was a notable increase in alanine production to balance the change from production of NH_3 when glutamine alone was metabolized to a net consumption of ammonia when glutamine and glucose were metabolized simultaneously; a small production of ornithine (0.2 μmol/min per g dry wt.); and a small serine formation (which was noted in this and only one other circumstance). When aspartate, glutamine and glucose were added together to the chick cells (Table 3), aspartate was consumed at approximately the rate previously observed for glutamine consumption from glutamine and glucose, but consumption of glutamine was now obliterated, glutamate was formed (presumably as a product of aspartate and glucose metabolism) and there was an enhanced production of alanine; otherwise the results of glucose metabolism were quantitatively closely similar to those seen before. A small production of serine was again evident.

Aspartate metabolism. Aspartate when added alone to the chick cells failed to stimulate respiration (Table 3). The only notable products detected were glutamate and a much smaller but significant amount of alanine and some glutamine; together these products accounted for about 55% of the aspartate consumed. Concomitant addition of glucose produced the familiar pattern of increased respiration rate and of glucose metabolism; the rate of consumption of aspartate was not affected but glutamine was no longer formed while the rates of alanine and glutamate formation increased, and were accompanied by a consumption of ammonia.

Serine, proline, ornithine and urea formation. Of the amino acids determined solely by automated column chromatography, only serine, proline and ornithine showed any increased rates of synthesis; the increases were small and occurred only in the circumstances noted (Table 3 and text above). Direct spectrophotometric determinations of glutamate, aspartate and alanine gave results in quantitative accord with those obtained for these same amino acids by column chromatography of the same samples. Thus, though results from only three column chromatographic analyses are reported, results on serine, proline and ornithine production are probably reliable (Tables 2 and 3). The formation of small quantities of ornithine from glutamine and glucose noted in the text above is of interest in the light of the observation by Windmueller & Spaeth (1974) of incorporation of radioactivity from [^{14}C]glutamine into citrulline by perfused rat intestine. This matter is discussed further by Watford *et al.* (1979).

Results shown in Table 3 for urea consumption or formation are too small in number to draw final conclusions but it is perhaps significant that only when glucose or aspartate were added to chick cells was any measurable production of urea noted; analogous observations were made when glutamate + glucose or aspartate + glucose were substrates added at relatively high concentration (Table 4). Presumably only under these four circumstances is the intracellular accumulation of arginine sufficient to elicit the activity of an endogenous arginase.

Summary. The broad picture that emerges from Table 3 is that the rate of respiration on glucose and the rate of consumption of glucose is little affected by the concomitant presence of any of the amino acids listed; production of pyruvate and lactate is also little affected by the presence of these amino

acids but alanine production invariably increases under these circumstances. This last observation will be discussed later. On the other hand, the metabolic fate of the individual amino acids is affected quantitatively as detailed above, by the concomitant metabolism of glucose.

The effects of increasing the initial concentrations of substrates

This broad picture of glucose, glutamate, glutamine and aspartate metabolism in chick intestinal brush-border cells was generally confirmed when cells were incubated with higher initial concentrations of substrates. But, as already noted, the mean rates of consumption of glutamate and aspartate were markedly decreased and the mean rate of glutamine consumption was increased at higher initial substrate concentrations. The rates of alanine formation were not significantly changed by altering the substrate concentrations (Tables 3 and 4); the most notable quantitative differences in metabolic patterns observed with different initial substrate concentrations are described below.

Changes in the metabolism of amino acids. No net formation of glutamine from glutamate (6.7 mM) was observed (Table 4; compare Table 3); the addition of both glucose and glutamate at higher initial concentrations (Table 4) stimulated glutamate consumption (which it did not at lower substrate concentrations; Table 3) and increased the production of oxoglutarate even more markedly (compare Tables 3 and 4).

The net conversion of glutamine to glutamate and NH_3 was absent at low substrate concentrations but prominent at higher initial glutamine concentrations (Table 4; compare Table 3). The concomitant addition of glucose and glutamine, at the higher concentrations, diminished glutamine consumption without affecting significantly the rates of ammonia and glutamate production observed from glutamine alone; these observations contrast with those made when the same substrates were added singly and together at lower concentrations (Table 3).

Whereas aspartate was consumed from a mixture of glutamine, aspartate and glucose at low initial concentrations (Table 3) with no significant net glutamine consumption or production, it was glutamine that was consumed when the initial concentrations of these three mixed substrates were relatively high (Table 4); glutamate was formed rapidly and aspartate was formed slowly under these conditions.

At the higher initial aspartate concentration (Table 4) production of glutamate plus glutamine appeared to account for aspartate consumption but there was an inexplicable net production of NH_3; these events contrasted with those observed when a lower concentration of aspartate was supplied to the cells (Table 3). Aspartate consumption was markedly stimulated by the concomitant addition of glucose at the higher substrate concentrations (Table 4) but not at the lower concentrations (Table 3). Whether glucose was present or absent at the higher initial aspartate concentration (Table 4), a substantial fraction of the aspartate consumed could be accounted for as glutamate and a lesser part as glutamine. This was true only in the absence of glucose at lower aspartate concentrations; no glutamine was formed if glucose was present (Table 3). It was notable that the only circumstance in which glucose failed to stimulate respiration was when it was added together with glutamine and aspartate at the higher initial concentrations (Table 4).

Asparagine was tested only at an initial concentration of 6.7 mM (Table 4). Although it was quite rapidly consumed (1.1 μmol/min per g dry wt. of cells) with formation of an equimolar amount of NH_3, net aspartate and glutamate formation together accounted for only half of the asparagine consumed. Like aspartate, asparagine depressed the rate of respiration observed on endogenous substrates (Table 4).

Changes in the metabolism of glucose. It has already been remarked that the presence or absence of selected amino acids had little effect on the metabolic fate of glucose other than to increase alanine formation (Tables 3 and 4). On the other hand, changes in the initial concentration of glucose did affect markedly some aspects of glucose metabolism. Thus, the glucose-stimulated respiration rate in the presence of 10 mM-glucose was only 30% of the glucose-stimulated rate when glucose was added at an initial concentration of 5 mM (Tables 3 and 4). The mean gross respiration rate in the presence of 10 mM-glucose (15.3 ± 0.96 μmol O_2/min per g dry wt. of cells in seven determinations) was 82% of that in the presence of 5 mM-glucose (18.7 ± 0.68 μmol O_2/min per g dry wt. of cells in nine determinations) the two results being significantly different by the unpaired t test ($P < 0.05$). The reason for this diminution in respiration rate in the presence of a higher concentration of glucose are not clear but the phenomenon could be of considerable significance for whole-body metabolism; chick plasma contains about 15 mM-glucose. The ratio lactic acid produced/pyruvic acid produced in the presence of added glucose in all experiments shown in Tables 3 and 4 was 12.2 ± 0.69 (mean $\pm$ S.E.M for ten sets of results); assuming that the lactate dehydrogenase-catalysed reaction was close to equilibrium in each instance, the small variation about the mean value of this ratio suggests that the cytosolic NAD^+/NADH ratio was held constant whether the initial glucose concentration was 5 or 10 mM. On the other hand, the proportion of glucose metabolized to lactic acid depended

markedly on the initial glucose concentration. When glucose (5 mM) was added to respiring cells, 62.7 ± 1.3% of the glucose consumed was converted to lactic acid (Table 3); when glucose (10 mM) was added, 86.8 ± 4.2% of the diminished amount of glucose metabolized appeared as lactic acid (Table 4). Wide variations have been reported in the proportion of glucose metabolized to lactic acid by different and by analogous preparations of intestine (see Porteous, 1978, 1979). Postulates have been made concerning the regulation of the conversion of glucose to lactic acid (Porteous, 1977) and it may be deduced (Porteous, 1978, 1979) that the proportion of glucose converted to lactic acid could vary with the direction of entry of glucose into the highly polarized brush-border cell. The primary mechanisms determining the pronounced increase in the proportion of glucose metabolized to lactic acid when the initial glucose concentration is increased are not clear from the present investigations, but an increased rate of glucose consumption is not associated with the increased conversion of glucose to lactic acid (Tables 3 and 4).

Adenine nucleotide concentrations in chick brush-border cells

Chick brush-border cells were incubated (*a*) without added substrates, (*b*) in the presence of 5 mM-glucose, (*c*) in the presence of glucose (5 mM) + glutamate (2.5 mM) + glycine (2.5 mM) + cysteine (0.2 mM) for 0, 15, 30 and 45 min under the conditions detailed in the Materials and Methods section. Cells were then separated from the incubation medium and centrifuged into 0.5 M-$HClO_4$ using slight modifications of the procedure described by Hems *et al.* (1975). The results from a typical experiment are shown in Table 5. A decrease in the ATP content of each of three sets of cells was noted between 30 and 45 min of incubation, but there was a slight overall rise in ATP content of cells incubated with rather than without substrates. The ADP content of cells rose during the first 15 min or 30 min of incubation but the AMP content remained almost constant with time in the presence or absence of added substrates. Setting aside these relatively small variations, the total mean adenine nucleotide content of the cells was 22 μmol/g dry wt. and the proportions ATP:ADP:AMP were 0.7:1.0:0.2. The incubation medium was devoid of ATP and AMP but contained ADP equal to about half the ADP content of cells incubated without substrates, and equal to about 30% of the ADP content of cells incubated with the named substrates; the ADP in the medium did not increase during a 45-min incubation.

Glutathione in chick intestinal brush-border cells

Typical results from one of three experiments are shown in Fig. 1. Oxidized glutathione was not found in either the cells or the incubation medium at any time interval. Of the total reduced glutathione present in the incubation system at any time interval, 90% was associated with the cells. Assuming even distribution of the glutathione through cell water, the initial intracellular glutathione concentration was typically 0.7–0.8 mM. Rapid depletion of cell glutathione occurred during incubation without added substrates (Fig. 1); similar losses occurred in isolated hepatocytes (Viña *et al.*, 1978) but could be

Table 5. *The adenine nucleotide content of brush-border cells*

Chick jejunal brush-border cells were isolated and incubated in stoppered flasks, then separated from the incubation medium as described in the Materials and Methods section. The results shown apply to the cells only; further details are given in the text. The results shown are from a single experiment with cells isolated from one intestine; two other batches of isolated cells gave similar results.

Added substrates	Incubation period (min)	Intracellular [adenine nucleotides] (μmol/g dry wt.) ATP	ADP	AMP
None	0	7.3	8.0	2.9
	15	7.8	12.2	2.4
	30	7.3	13.1	2.6
	45	6.2	13.6	2.8
Glucose (10 mM)	0	8.0	8.3	2.9
	15	8.4	11.6	2.4
	30	8.0	12.4	2.4
	45	7.3	11.7	3.9
Glucose (10 mM) + glutamate (1 mM) + glycine (1 mM) + cysteine (0.2 mM)	0	8.7	10.6	2.3
	15	9.0	10.5	2.1
	30	8.8	14.9	2.3
	45	6.8	13.3	2.3

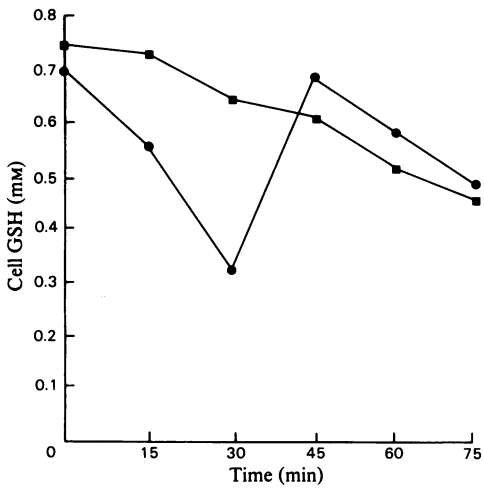

Fig. 1. *Reduced-glutathione content of chick brush-border cells*

Chick jejunal brush-border cells were isolated and incubated in stoppered flasks, then separated from the incubation medium as described in the Materials and Methods section. The results shown apply to the cells only; further details are given in the text. ■, Cells incubated without added substrates; ●, cells incubated with L-glutamate (1 mM) + glycine (1 mM) + L-cysteine (0.2 mM). Two further experiments gave essentially similar results.

prevented by including 0.2 mM-methionine in the hepatocyte incubation medium. This manoeuvre did not prevent depletion of the glutathione content of isolated chick brush-border cells (results not shown). Attempts to sustain the initial intracellular glutathione by incubating chick cells with the direct precursors of glutathione synthesis (Fig. 1) exacerbated the initial depletion of glutathione but later restored the intracellular glutathione concentration to about the same (depleted) level found in cells incubated without added substrates. This phenomenon has not been investigated further. No obvious changes in the adenine nucleotide content of the cells occurred in these experiments (Table 5).

Possible endogenous substrates

Chick intestinal brush-border cells exhibited a high rate of respiration on endogenous substrates; this respiration was not readily enhanced by addition of any one of several substrates other than low concentrations of glucose, glutamate, glutamine (Tables 2, 3, 4 and accompanying text). The endogenous substrates supporting this high rate of respiration have not been identified with certainty; they could include fatty acids. Known inhibitors of fatty acid catabolism gave equivocal results. Decanoyl-L-carnitine (1.0 mM) did not inhibit endogenous respiration, 4-pentenoic acid (0.8 mM) gave variable results and 4-bromo-octanoic acid (0.8 mM) inhibited endogenous respiration by 20%.

Discussion

For reasons that have been reviewed elsewhere (Porteous, 1978, 1979), preparations of intestine that are perfused *in vivo* or *in vitro* by both the luminal and vascular routes are best suited to studies of translocation of solutes in either direction through whole intestinal tissue and to elucidation of the concomitant overall metabolic fate of such solutes. Suspensions of isolated cells, as employed here, cannot distinguish between the possible different metabolic fate of substrates entering the cells across their functionally distinct brush-border and basolateral membranes. The use of such cell suspensions does, however, identify positively the particular intestinal cell component responsible for the observed metabolic events, permits direct and continuous measurements of oxygen consumption under physiologically appropriate conditions and allows more detailed studies of intermediary metabolism than is possible with the perfusion technique. The isolated cell suspensions are thus ideal for rapid quantitative surveys of the metabolic potential of the intestinal epithelium as illustrated by results reported here and elsewhere (Towler *et al.*, 1978; Watford *et al.*, 1979; Porteous *et al.*, 1979; Porteous, 1978, 1979; Morrison & Porteous, 1980); indications of the operation of putative regulatory mechanisms are also quickly revealed.

Glutamine synthesis in chick brush-border cells

Glutamine catabolism in small intestine is well established (Hanson & Parsons, 1977, 1978; Windmueller & Spaeth, 1974, 1975, 1976, 1978; Watford *et al.*, 1979; this paper, Tables 1 and 4). Windmueller & Spaeth (1974) suggested that mammalian intestine may also synthesize glutamine, and Pinkus & Windmueller (1978) reported a positive arteriovenous difference in the glutamine concentration of blood perfusing chick and guinea-pig small intestine. Results shown in Table 3 appear to be the first to demonstrate the capacity of intestinal brush-border cells to synthesize glutamine and to support the suggestions and observations from Windmueller's laboratory. The apparent deficiency in NH_3 consumption during glutamine synthesis cannot be taken as evidence against glutamine synthesis; it is possible that other reactions not measured in this study were producing more NH_3 when added glutamate was the sole known substrate (Table 3). The positive evidence for a net synthesis of glutamine when glutamate (2.5 mM) was the sole added substrate seems secure (Table 3); the mean

glutamine formed accounted for 60% of the mean glutamate consumed. The variability about the mean value for the rate of glutamine formation was such that, for the number of experiments performed, $P < 0.1$. Statistically this is insufficient to provide clear-cut proof of conversion of glutamate to glutamine, but marked variability in the rate of synthesis of glutamine from glutamate is not unexpected. It is consistent with the susceptibility of mammalian glutamine synthetase from several sources to allosteric inhibition of its activity by glycine, serine or alanine in the presence of Mn^{2+}, or of Mg^{2+} and trace concentrations of Mn^{2+} or of Mg^{2+} and orthophosphate (Meister, 1974); see Tables 2 and 3. The slower mean rate of synthesis of glutamine from glutamate in the presence of glucose (Table 3) could then be caused by partial allosteric inhibition of glutamine synthetase by the increased intracellular concentration of alanine that might be expected to arise from the 10-fold increase in the rate of formation of alanine under these circumstances (Table 3). The increased variability in the rate of synthesis of glutamine in the presence of glucose (Table 3) could be a reflection of quite small variations from one experiment to another in the exact intracellular concentrations of allosteric effectors, including alanine, during incubation of cells with both glutamate and glucose. The partially inhibited enzyme would presumably exhibit a sigmoid response of reaction velocity to changes in substrate concentration and would thus also respond most markedly to any small variations in the intracellular concentrations of substrates close to the K_m values; Meister (1974) reports $K_m = 0.18$, 2.3 and 2.5 mM for NH_3, ATP and L-glutamate respectively during assay of the uninhibited enzyme *in vitro*. This last value is identical with the extracellular glutamate concentration used in the present experiments (Table 3). It is notable that a slow rate of glutamine synthesis was also observed from aspartate, presumably via the glutamate that was formed slowly from low or high initial concentrations of aspartate (Tables 3 and 4); again, concomitant metabolism of glucose diminished or eliminated glutamine synthesis, presumably because of inhibition of glutamine synthetase by the alanine which was then formed.

Net glutamine formation was not observed when either glutamate, or glutamate + glucose, were presented at the higher initial concentrations to chick brush-border cells (Table 4). The precise mechanisms that prevent glutamine synthesis from higher concentrations of glutamate alone are not obvious but could be important in defining the role of intestine in whole-body metabolism of glutamate and glutamine. It may be significant that γ-glutamylphosphate is a known intermediate in glutamine synthesis, and that glutamate is known to inhibit ($K_i = 4.5$ mM) other transphosphorylations catalysed by highly purified glutamine synthetases (Meister, 1974).

Differences between rat and chick intestinal brush-border cells

The observation that 10 mM-glutamate did not stimulate rat-cell respiration (Table 1) whereas 5 mM-glutamate did (Watford *et al.*, 1979); and that 10 mM-glutamine stimulated rat-cell respiration (Table 1) more than did 5 mM-glutamine (Watford *et al.*, 1979) requires further investigation. The slow production of alanine from 6.7 mM-glutamate or glutamine by chick cells (Table 4) contrasts with the rapid production of alanine from 5 mM-glutamate or glutamine by rat cells (Watford *et al.*, 1979). Chick cells consumed glutamine (Tables 3 and 4) much more slowly than did rat cells (Watford *et al.*, 1979); this alone could, but does not necessarily, account for much of the diminished rate of alanine formation from glutamine by chick cells as compared with rat cells. The rates of consumption of 5.0 mM-glutamate by rat cells (Watford *et al.*, 1979) and of 2.5 mM-glutamate by chick cells (Table 3) were comparable, but raising the initial glutamate concentration to 6.7 mM diminished markedly the rate of glutamate consumption by chick cells (Table 4). At comparable rates of glutamate consumption, chick cells produced alanine (Table 3) at only 10% of the rate of production by rat cells (Watford *et al.*, 1979). Since aspartate alone gave rise to a slow but significant rate of alanine formation at rates of aspartate consumption comparable with glutamate consumption (Table 3), the enzymes thought to catalyse the conversion of glutamine or glutamate to alanine via malate or oxaloacetate and pyruvate in rat cells (Watford *et al.*, 1979) are presumably present in chick cells; but the enzyme (or enzymes) must then be present at much lower activity in chick cells, or be strongly inhibited under the conditions of incubation used (Table 3). It has then yet to be decided by experiment whether the marked elevation of the rate of alanine production that was observed whenever glucose was a substrate in addition to glutamate, glutamine, aspartate or glutamine and aspartate (Tables 3 and 4) was due to alleviation of the possible inhibition of the enzymes catalysing the conversion of malate or oxaloacetate to pyruvate, or whether the carbon skeleton of alanine arose predominantly from glucose catabolism in the chick cells. Watford *et al.* (1979) observed a 1.5–2.5-fold increase in the ratios alanine produced/glutamate consumed and alanine produced/glutamine consumed when glucose was also present in the incubation medium. In the present experiments the ratio alanine produced/amino acid consumed rose 5- to 15-fold (Table 3) or 2- to 20-fold (Table 4) when glucose was added to an

incubation medium containing one of glutamate, glutamine or aspartate. Such increases were not apparent in experiments on glutamine and glucose metabolism by vascularly perfused rat intestine (Tables 1 and 5 of Hanson & Parsons, 1977). The circumstantial evidence is thus that most of the alanine produced by chick cells (Tables 3 and 4) arose from transamination of pyruvate produced by glucose metabolism. If rat intestinal brush-border cells do produce substantial concentrations of alanine from glutamate (Watford *et al.*, 1979) then glutamine synthesis from glutamate via any glutamine synthetase that was present could well be inhibited by the alanine produced. In contrast, chick brush-border cells produce alanine only very slowly from glutamate or aspartate (Tables 3 and 4); glutamine synthesis from glutamate or aspartate is then possible, as already indicated (Table 3).

Values for changes in NH_3 production or consumption (Tables 2, 3 and 4) were (with three exceptions) statistically insignificant. But it is noticeable that the enhanced production of alanine that always resulted from the incubation of chick cells with glucose or with glucose + glutamate, glutamine, aspartate or glutamine and aspartate (Tables 3 and 4) was also always associated with a small mean consumption of ammonia (Table 3) in contrast to the small mean production of NH_3 when cells were incubated without substrates (Table 2) or with glutamine alone (Table 3). Exceptions to this general rule were seen only when cells were incubated with higher concentrations of glutamine + glucose or glutamine + aspartate + glucose (Table 4) because, as already pointed out, glutamine at the higher concentration was predominantly catabolized to glutamate and NH_3. Rat brush-border cells always showed a small mean production of NH_3 when incubated without substrates or with glucose, and a substantial production when incubated with glutamate or glutamine, or with these amino acids plus glucose (Watford *et al.*, 1979).

Other observations on chick brush-border-cell metabolism

Amongst the other observations made in the present study, the most interesting concern the high rate of chick cell respiration on endogenous substrates; the outstanding role of glucose in stimulating respiration (except when both glutamine and aspartate were also present); the predominant conversion of glucose to lactic acid (even when gross respiration rates were high) and the partial dependence of the extent of this metabolic conversion upon the initial glucose concentration; the variation in the metabolic fates of individual amino acids when their initial concentrations were varied or when glucose was also being metabolized. Each of these observations, like those on glutamine synthesis and degradation, could be of considerable significance for an understanding of the role of small intestine in whole body metabolism. The observed quantitative variations in metabolism just referred to might reflect no more than the response of integrated intracellular metabolic fluxes to the imposed changes in extracellular substrate concentrations; equally, true regulatory (allosteric) mechanisms controlling both vectorial and scalar catalytic events in cell metabolism may be involved and require elucidation. Care has been taken to establish the functional integrity of isolated rat brush-border cells (Towler *et al.*, 1978) and results on the adenine nucleotide contents of chick brush-border cells (Table 5) provide further evidence of functional integrity. But the results on the persistent diminution of the intracellular glutathione content of the chick cells (Fig. 1) suggest that further work on stabilization of the cells and their components is required.

I thank the Medical Research Council for a grant to defray laboratory expenses involved in the work reported here. I thank Professor Sir Hans Krebs for the generous hospitality and support that he provided in The Metabolic Research Laboratory, Radcliffe Infirmary, Oxford. My warm thanks go also to Pat Lund, Dermot Williamson, Marion Stubbs, Reg Hems and David Wiggins for making me welcome; and to Madge Barber, Morag Stuart and Rose Farrell for their willing help on many occasions. I am indebted to Professor D. Kerridge, University of Aberdeen, for the statistical analysis of results presented here.

References

Bergmeyer, H. U. (1974) *Methods of Enzymatic Analysis*, 2nd edn., Verlag Chemie, Weinheim and Academic Press, New York and London

Bolton, W. (1969) *Nutr. Anim. Agric. Importance* **1**, 183–240

Hanson, P. J. & Parsons, D. S. (1976) *J. Physiol. (London)* **255**, 775–795

Hanson, P. J. & Parsons, D. S. (1977) *Biochem. J.* **166**, 509–519

Hanson, P. J. & Parsons, D. S. (1978) *J. Physiol. (London)* **278**, 55–67

Hems, R., Lund, P. & Krebs, H. A. (1975) *Biochem. J.* **150**, 47–50

Hill, K. J. (1976) in *Digestion in the Fowl* (Boorman, K. N. & Freeman, B. M., eds.), British Poultry Science and T. and A. Constable, Edinburgh

Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seylers Z. Physiol. Chem.* **210**, 33–66

Krebs, H. A., Cornell, N. W., Lund, P. & Hems, R. (1974) *Regul. Hepatic Metab. Proc. Alfred Benzon Symp. 6th* 726–750

Meister, A. (1974) *Enzymes, 3rd Ed.* **10**, 699–754

Meister, A. & Tate, S. S. (1976) *Annu. Rev. Biochem.* **45**, 559–604

Morrison, A. & Porteous, J. W. (1980) *Biochem. J.* **188**, 609–618
Neptune, E. M. (1965) *Am. J. Physiol.* **209**, 329–332
Pinkus, L. M. & Windmueller, H. G. (1978) *Arch. Biochem. Biophys.* **182**, 506–517
Porteous, J. W. (1977) *Intest. Permeation, Proc. Workshop Conf. Hoechst, 4th* 240–261
Porteous, J. W. (1978) *Biochem. Soc. Trans.* **6**, 534–539
Porteous, J. W. (1979) *Environ. Health Perspect.* **33** in the press
Porteous, J. W., Furneaux, H. M., Pearson, C. K. & Lake, C. M. (1979) *Biochem. J.* **180**, 455–463
Pritchard, P. J. & Porteous, J. W. (1977) *Biochem. J.* **164**, 1–14
Towler, C. M., Pugh-Humphreys, G. P. & Porteous, J. W. (1978) *J. Cell Sci.* **29**, 53–75
Viña, J., Hems, R. & Krebs, H. A. (1978) *Biochem. J.* **170**, 627–630
Watford, M., Lund, P. & Krebs, H. A. (1979) *Biochem. J.* **178**, 589–596
Windmueller, H. G. & Spaeth, A. E. (1974) *J. Biol. Chem.* **249**, 5070–5079
Windmueller, H. G. & Spaeth, A. E. (1975) *Arch. Biochem. Biophys.* **171**, 662–672
Windmueller, H. G. & Spaeth, A. E. (1976) *Arch. Biochem. Biophys.* **175**, 670–676
Windmueller, H. G. & Spaeth, A. E. (1978) *J. Biol. Chem.* **253**, 69–76