Table 1. Reactions catalysed by enzyme preparations of different purity from *Cl. pasteurianum*

Crude enzyme was prepared by $(\text{NH}_4)_2\text{SO}_4$ precipitation (50–70% saturation) and had a threonine aldolase activity of 20 munit/mg of protein; purified enzyme was prepared by $(\text{NH}_4)_2\text{SO}_4$ precipitation, acetic acid precipitation and DEAE-cellulose chromatography as described by Dainty (1970), and had a threonine aldolase activity of 320 munit/mg of protein. For determination of heat stability enzyme solutions were pretreated for 5 min at elevated temperatures, prior to the assay. Reaction conditions: 50 mM-potassium phosphate buffer, pH 7.0; 10 mM-substrate(s); 0.1–0.4 unit of threonine aldolase/ml; 0.05 mM-pyridoxal phosphate (PLP) and 1 mM-dithiothreitol (both in reactions 2–5); 0.5 mM-tetrahydrofolic acid (THF; in reactions 3 and 5; 2 mM solution freshly prepared in 50 mM-phosphate buffer, pH 7.0, with 0.1% 2-mercaptoethanol). Incubation 10–240 min at 30 °C. Abbreviation: n.d., not determined.

Reaction catalysed	Relative reaction rate (%)		Dependence on exogenous PLP	Molar ratio threonine:glycine at equilibrium	Temperature for 50% inhibition (°C)
	Crude enzyme	Purified enzyme			
1 L-Threonine → glycine + acetaldehyde	100	100	—	1:0.45	48
2 L-Allothreonine → glycine + acetaldehyde	50	3	+	1:6	56
3 L-Serine $\xrightarrow{\text{THF}}$ glycine + formaldehyde	30	n.d.	+	n.d.*	n.d.
4 Glycine + acetaldehyde → (allo)threonine	3 (0.5)†	0.2	+	1:6	56
5 Glycine + formaldehyde $\xrightarrow{\text{THF}}$ serine	25	2	+	1:1 (serine:glycine)	55

* Equilibrium dependent on THF concentration.
† Value in parentheses obtained in the absence of PLP.

organism apparently does not attack any of the substrates (Bell & Turner, 1977). On the other hand, in rat liver cytoplasm an enzyme with specificity similar to that of the clostridial threonine aldolase has been detected (Palekar *et al.*, 1973).

In reactions 2 and 4 of Table 1, which are obviously catalysed by serine hydroxymethyltransferase, a real equilibrium of the reactants is attained from both directions. On the other hand, no significant L-threonine synthesis due to threonine aldolase is observed. Also the pH optimum of the cleavage of threonine by threonine aldolase (pH 7.0) is different from that of reactions 2 and 4 (pH 8.0). These findings are strong evidence that the product obtained in incubations with crude extracts from glycine and acetaldehyde is allothreonine.

Therefore it must be assumed that also Dainty (1970), who had enriched the enzyme to an even higher purity, had obtained at least in part allothreonine from glycine and acetaldehyde catalysed by serine hydroxymethyltransferase. It can therefore be stated that the practical synthesis of L-threonine from glycine and acetaldehyde by means of threonine aldolase from *Clostridium pasteurianum* is not possible.

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Fructose 2,6-bisphosphate in isolated rat enterocytes

A study of the regulation of phosphofructokinase from epithelial cells of rat small intestine was recently published by Kellett and coworkers. Phosphofructokinase from these cells was found to be distinct (Khoja & Kellett, 1983) from other isoenzymes previously described and was designated phosphofructokinase D, following the nomenclature of Tsai & Kemp (1973). After starvation (Jamal & Kellett, 1983a) or streptozotocin-diabetes (Jamal & Kellett, 1983b) the susceptibility of phosphofructokinase to inhibition by ATP was increased when the enzyme was assayed under suboptimal conditions at pH 7. This modification of the regulatory properties could result from a decrease in the concentration of a stimulator such as fructose 2,6-bisphosphate. This interpretation was apparently ruled out since neither

Table 1. Effect of glucose concentration on lactate release and fructose 2,6-bisphosphate concentration in isolated rat enterocytes from fed rats

Isolated rat enterocytes were prepared and incubated according to Watford *et al.* (1979) for 15 min at 37 °C in the presence of the indicated glucose concentrations. Lactate was measured (Hohorst, 1963) in neutralized perchlorate extracts. For the measurement of fructose 2,6-bisphosphate, 1 ml samples of cells were taken after 15 min of incubation, treated with 50 μ l of 1 M-NaOH and heated at 80 °C for 10 min. Values are means $\pm$ S.E.M. for at least six cell preparations.

Concn. of glucose (mM)	Lactate release (μ mol/min per g dry wt.)	Concn. of fructose 2,6-bisphosphate (nmol/g dry wt.)
0	0	5.6 $\pm$ 0.3
0.25	6.8 $\pm$ 0.8	8.0 $\pm$ 0.3
0.5	11.6 $\pm$ 1.5	9.0 $\pm$ 0.7
1.0	15.5 $\pm$ 1.2	10.0 $\pm$ 0.5
2.0	17.1 $\pm$ 1.0	10.2 $\pm$ 0.5
5.0	19.5 $\pm$ 0.8	10.7 $\pm$ 0.8
10.0	20.3 $\pm$ 1.5	11.7 $\pm$ 1.5

fructose 2,6-bisphosphate nor 6-phosphofructo-2-kinase were detected in intestinal mucosa (Jamal *et al.*, 1984). However, mucosal phosphofructokinase, like that of other tissues (Hers *et al.*, 1982), was stimulated by fructose 2,6-bisphosphate (Jamal *et al.*, 1984).

We have reinvestigated this problem by using a very sensitive method of measuring fructose 2,6-bisphosphate (Van Schaftingen *et al.*, 1982; Van Schaftingen, 1984) in isolated rat enterocytes. Isolated rat enterocytes, prepared according to Watford *et al.* (1979), were chosen because the influence of glucose on lactate release is easily demonstrable in this preparation. Indeed, glucose elicited a dose-dependent release of lactate with half-maximal effect at about 0.4 mM-glucose (Table 1). Thus, lactate release was maximal in the physiological range of glucose concentration (5–10 mM) and was not influenced by the substrate concentration in this range. Table 1 also shows that fructose 2,6-bisphosphate was detectable in those cells and that its concentration increased in parallel with glucose concentration and lactate release. Assuming that 1 g cell wet wt. corresponds to 0.1 g dry wt. (Watford *et al.*, 1979) and that the cytosolic space represents 50% of the total volume, one can calculate that the intracellular concentration of fructose 2,6-bisphosphate is between 0.6 and 2.3 μ M (Table 1). This is above the K_a value of phosphofructokinase for the stimulator (0.6 μ M), determined at 0.5 mM-fructose 6-phosphate and 2.5 mM-ATP (Jamal *et al.*, 1984). Therefore, in contrast to the claim of Kellett and coworkers (see above), fructose 2,6-bisphosphate is present in rat enterocytes and might even

exert a regulatory role in these cells. Moreover, in enterocytes prepared from 48 h-starved rats, and incubated with 5 mM-glucose, fructose 2,6-bisphosphate content was 5.2 ± 0.5 ($n = 3$) nmol/g dry wt., i.e. about 50% of that in enterocytes from fed rats. This may explain, at least in part, the change in regulatory properties previously observed (Jamal & Kellett, 1983a). The activity of 6-phosphofructo-2-kinase was also measured (according to Rider & Hue, 1984) and was found to be 3.8 ± 0.7 (mean $\pm$ S.E.M. for five preparations) nmol of fructose 2,6-bisphosphate formed/min per g dry wt. at 30 °C; this activity was decreased by about 40% after 48 h of starvation.

The discrepancy between our data concerning fructose 2,6-bisphosphate and those of Kellett and coworkers can probably be explained by differences in methodology: (i) fructose 2,6-bisphosphate is not stable during the preparation of extracts (more than 50% of fructose 2,6-bisphosphate is lost during a 1 min centrifugation of enterocytes) and immediate quenching of the cells is required (50 mM-NaOH, 80 °C), (ii) the method we have used (Van Schaftingen *et al.*, 1982; Van Schaftingen, 1984) to measure fructose 2,6-bisphosphate is at least 20 times more sensitive than that used by Jamal *et al.* (1984). We therefore believe that the experimental protocol followed by Kellett and coworkers (Jamal *et al.*, 1984) did not allow them to detect fructose 2,6-bisphosphate in epithelial cells from small intestine.

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