The turnover of L-type pyruvate kinase in cultured rat hepatocytes

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Hepatocytes were isolated by collagenase perfusion of livers from rats that had been allowed access to a carbohydrate-rich diet or laboratory chow or had been deprived of food 48 h before use. By incubation with L-[4,5-³H]leucine and precipitation with anti-(L-type pyruvate kinase) sera the rates of synthesis and degradation of L-type pyruvate kinase were measured in freshly prepared cells and hepatocytes maintained in monolayer culture for up to 5 days. Hepatocytes from carbohydrate-rich-diet-fed rats synthesized more L-type pyruvate kinase than did cells from chow-fed animals, which in turn synthesized more than cells from 48 h-starved rats. Hepatocytes maintained in culture for up to 5 days synthesized L-type pyruvate kinase at similar rates to freshly prepared cells. The degradation of [³H]leucine-labelled L-type pyruvate kinase was shown to be biphasic. A phase with $t_{1/2}$ (half-time) 4.9 h and a duration of 8–10 h was followed by a phase with $t_{1/2}$ 79.2 h. Cells from chow-fed and carbohydrate-rich-diet-fed rats showed similar patterns of degradation of L-type pyruvate kinase. The addition of 2 mM-fructose and 0.1 μ M-insulin to the culture medium increased the $t_{1/2}$ of the rapid phase to 12 h in cells isolated from carbohydrate-rich-diet-fed rats, but not in cells from chow-fed rats. The secondary, slower, phase of degradation remained unaffected. The degradation of fructose 1,6-bisphosphatase and total cell protein followed first-order kinetics. The half-life of fructose 1,6-bisphosphatase was 41.0 h in cells from chow-fed animals and 48.5 h in cells from carbohydrate-rich-diet-fed donors. Fructose and insulin did not affect the rate of enzyme degradation. We propose that there is a role for protein catabolism in the short-term and long-term control of L-type pyruvate kinase concentration.

The isoenzyme of pyruvate kinase (ATP:pyruvate 2-O-phosphotransferase, EC 2.7.1.40) present in the parenchymal cells of the liver, the L-type isoenzyme, occupies a key position in the control of hepatic metabolism, and is subject to a complex pattern of regulation. The enzyme shows co-operative binding of phosphoenolpyruvate (Rozengurt *et al.*, 1969) and is allosterically activated by fructose 1,6-bisphosphate (Taylor & Bailey, 1967; Ekman *et al.*, 1976) and by 6-phosphogluconate (Smith & Freedland, 1979). L-type pyruvate kinase is inhibited by alanine (Seubert *et al.*, 1968) or glucagon-stimulated phosphorylation (Ishibashi & Cottam, 1978; Riou *et al.*, 1978).

The concentration of L-type pyruvate kinase is under dietary control (Krebs & Eggleston, 1965). An increase in enzyme activity is observed on feeding (Hopkirk & Bloxham, 1979) or weaning (Weber *et al.*, 1965; Taylor *et al.*, 1967; Vernon & Walker, 1968a,b; Hopkirk & Bloxham, 1980) and a decrease in activity on starvation (Van Berkel *et al.*,

1977, 1978). Increased activity is associated with increased rates of enzyme synthesis *de novo* (Cladaras & Cottam, 1980a; Hopkirk & Bloxham, 1980), and an increase in the concentration of mRNA for L-type pyruvate kinase (Cladaras & Cottam, 1980b). The half-life of L-type pyruvate kinase is between 47 and 59 h in adult rats (Hopkirk & Bloxham, 1979; Cladaras & Cottam, 1980a) and 76 h in young rats weaned on to a carbohydrate-rich diet (Hopkirk & Bloxham, 1980). In meal-trained animals the total enzyme activity undergoes a rapid diurnal variation (Hopkirk & Bloxham, 1979). The observed increases may be explained by an increased rate of enzyme synthesis; the decreases in activity, however, cannot be adequately accounted for by a degradation process with a half-life of greater than 50 h. In the light of these findings it was decided to re-examine the synthesis and degradation of L-type pyruvate kinase *in vitro*. A system which permits such an examination of hepatic protein metabolism in the absence of complicating factors

which apply to experiments performed *in vivo* or to perfusion is that of isolated hepatocytes maintained in primary culture (Spence & Pitot, 1979; Hopgood *et al.*, 1980, 1981; Grinde & Seglen, 1981; Schwarze & Seglen, 1981).

This work involves the use of isolated rat hepatocytes maintained in short-term suspension culture and long-term monolayer culture to examine the synthesis and degradation of L-type pyruvate kinase *in vitro* by the quantitative immunoprecipitation of L-type pyruvate kinase that has been labelled with [^3H]leucine.

Materials and methods

Male Wistar Albino rats (125–200 g) were used for all experiments. Animals were fed on either standard laboratory diet (chow) or high-carbohydrate diet (Bloxham *et al.*, 1977), or were deprived of food 48 h before use.

The experimental procedures for the preparation of rabbit anti-(L-type pyruvate kinase) and rabbit anti-(fructose 1,6-bisphosphatase) sera and the assay of hepatic pyruvate kinase and fructose 1,6-bisphosphatase have been described previously (Hopkirk & Bloxham, 1980). Lactate dehydrogenase was assayed by the method of Berg *et al.* (1972). One unit of enzyme activity catalysed the formation of 1 μmol of product/min at 37°C. The sources of chemicals were as previously described (Poole *et al.*, 1982).

Cell culture conditions

The details for the culture of hepatocytes, the sequential immunoprecipitation of fructose 1,6-bisphosphatase and L-type pyruvate kinase, and the measurement of synthesis of L-type pyruvate kinase and fructose 1,6-bisphosphatase have been reported previously (Poole *et al.*, 1982). The final concentration of L-leucine was 0.38 mM; an incorporation of 1000 d.p.m. of L-[4,5- ^3H]leucine (58 Ci/mmol) represents the synthesis of protein containing 52 pmol of L-leucine.

Measurement of fatty acid synthesis

The incorporation of [U- ^{14}C]lactate into hepatocyte lipid was measured as previously described (Hopkirk & Bloxham, 1979). Routinely cells were incubated in suspension or monolayer culture with 3 mM-[U- ^{14}C]lactate (0.1 $\mu\text{Ci}/\text{mmol}$) at 37°C for periods of up to 1 h.

Expression of results

All results are expressed as the means $\pm$ S.E.M. of *n* independent observations. All cell weights represent dry weights of material.

Results

Synthesis of L-type pyruvate kinase by isolated rat hepatocytes

Hepatocytes isolated from rats fed on a diet rich in carbohydrate and low in fat possessed a higher activity of pyruvate kinase (61 ± 5 units/g dry wt. of cells; 3650 units/g of DNA; *n* = 3) than did hepatocytes isolated from chow-fed (30 ± 3 units/g of cells; 1410 units/g of DNA; *n* = 4) or 48 h-starved animals (18 ± 2 units/g of cells; 630 $\pm$ 7 units/g of DNA; *n* = 3). This is in agreement with previous reports *in vivo* (Kohl & Cottam, 1976, 1977; Cladaras & Cottam, 1980a; Hopkirk & Bloxham, 1980) and *in vitro* (Smith & Freedland, 1981). It has been suggested that the increased concentrations are due to an increase in the rate of synthesis of L-type pyruvate kinase. The rate of synthesis of L-type pyruvate kinase *in vitro* was measured in freshly prepared hepatocytes maintained in suspension culture. Cells (5–10 mg/ml final concn.) were incubated in a final volume of 3 ml with 10 μCi of L-[4,5- ^3H]leucine (40–60 Ci/mmol) at 37°C for up to 3 h. Fructose 1,6-bisphosphatase and L-type pyruvate kinase were sequentially precipitated as described in the Materials and methods section. Freshly prepared hepatocytes incorporated [^3H]leucine into L-type pyruvate kinase, fructose 1,6-bisphosphatase and total hepatocyte protein linearly with time for at least 3 h. Incorporation of label into L-type pyruvate kinase was greatest in cells isolated from rats fed on a carbohydrate-rich diet. Cells from animals fed on chow incorporated more label into L-type pyruvate kinase than did cells from 48 h-starved rats (Table 1). The increased rates of synthesis of L-type pyruvate kinase were not due to an overall increase in the rate of total protein synthesis; there was, instead, a significant increase in the percentage of the total labelled protein that was precipitated as L-type pyruvate kinase from hepatocytes isolated from carbohydrate-rich-diet-fed rats (0.43%) compared with chow-fed (0.17%) and 48 h-starved animals (0.02%).

The concentration of fructose 1,6-bisphosphatase showed little change with the dietary status of the animal (carbohydrate-rich-diet-fed; 6.9 ± 0.2 units/g dry wt. of cells; 413 ± 6 units/g of DNA; *n* = 3; chow-fed: 7.4 ± 0.1 units/g of cells; 348 ± 5 units/g of DNA; *n* = 4; 48 h-starved: 8.6 ± 0.2 units/g of cells; 300 ± 7 units/g of DNA; *n* = 3). Incorporation of [^3H]leucine into fructose 1,6-bisphosphatase occurred at similar rates in hepatocytes isolated under the three conditions studied (Table 1).

Maintenance of hepatocytes in primary monolayer culture

An alternative hypothesis for the rapid changes in concentrations of L-type pyruvate kinase that occur

Table 1. Incorporation of [^3H]leucine into hepatocyte protein

Hepatocytes were established in suspension or monolayer culture, labelled with L-[4,5- ^3H]leucine for 3 h and the incorporation of radioactivity into specific liver proteins and total protein was measured as described in the Materials and methods section. Values are the means $\pm$ S.E.M. for n independent observations.

		[^3H]Leucine incorporated (d.p.m./3 h per mg of cells)							
		L-type pyruvate kinase		Fructose 1,6-bisphosphatase		Total protein ($\times 10^{-3}$)			
Culture	Donor animal	n	Control	+cycloheximide	Control	+cycloheximide	Control	+cycloheximide	
Fresh suspension	Carbohydrate-fed	3	2550 $\pm$ 140	100 $\pm$ 10	2450 $\pm$ 30	110 $\pm$ 10	590 $\pm$ 20	22.8 $\pm$ 0.4	
	Chow-fed	4	920 $\pm$ 40	30	2650 $\pm$ 40	150 $\pm$ 40	539 $\pm$ 10	21.2 $\pm$ 0.2	
	48 h-starved	3	100 $\pm$ 20	10	2110 $\pm$ 20	190 $\pm$ 10	501 $\pm$ 5	12.1 $\pm$ 0.4	
5-day monolayer	Carbohydrate-fed	3	3010 $\pm$ 40	90 $\pm$ 10	2810 $\pm$ 30	220 $\pm$ 20	562 $\pm$ 20	34.5 $\pm$ 0.2	
	Chow-fed	4	1040 $\pm$ 20	20	2730 $\pm$ 60	100 $\pm$ 10	545 $\pm$ 20	24.1 $\pm$ 0.7	
	48 h-starved	3	120 $\pm$ 10	10	2760 $\pm$ 100	160 $\pm$ 10	563 $\pm$ 70	30.1 $\pm$ 0.3	

with changing dietary status *in vivo* may be an alteration in the rate at which the enzyme is degraded. To study this phenomenon *in vitro* it is necessary to maintain cells in culture for up to 5 days. Fig. 1 outlines a typical protocol observed in such experiments. Culture dishes were inoculated with cells suspended in supplemented Waymouth's medium containing 5% foetal-calf serum and 0.1 μM -insulin. The inclusion of insulin in the inoculation medium facilitated the adhesion of the cells to the dishes (Poole *et al.*, 1981). After 1–2 h incubation the cells began to adhere to the plates, the maximum adhesion was reached after 4 h at 37°C. After adhering, the cells spread to form a confluent monolayer of cells. After 4 h the cells that had not adhered were removed and the medium was replaced with one containing no foetal-calf serum. It was at this stage that the different experimental media were added. During the next 12 h of culture, those cells that were only loosely adhering to the plates detached. Cell numbers did not change significantly over the next 4–5 days in culture. Cell number was determined from the amount of DNA present per plate. Cells from carbohydrate-rich-diet-fed, chow-fed and 48 h-starved rats contained respectively 16.71 $\pm$ 0.12, 21.27 $\pm$ 0.14 and 28.64 $\pm$ 0.23 μg of DNA/mg dry wt. of cells.

Hepatocytes isolated from the three experimental groups of animals incorporated [^3H]leucine into L-type pyruvate kinase, fructose 1,6-bisphosphatase and total cell protein linearly with time for at least 3 h and at similar rates to those freshly prepared cells in suspension culture. The differences in the rates of synthesis of L-type pyruvate kinase observed with freshly prepared cells in the three dietary states was maintained after 5 days in culture. Protein synthesis was inhibited by 100 μg of cycloheximide/ml (Table

Fig. 1. Maintenance of adult rat hepatocytes in primary monolayer culture

Culture dishes that had been pretreated for 1 h with supplemented Waymouth's medium containing 0.1 μM -insulin and 5% foetal-calf serum were inoculated with hepatocytes (A). After 4 h the medium containing unattached cells was replaced with differentiated experimental media containing no foetal-calf serum (B). After a further 12 h culture the medium containing cells which had detached during this period was removed and the experiment typically commenced after 16 h (C). Medium was then changed every 12 h. Cell number was determined as described in the Materials and methods section. The data are taken from a single representative cell preparation (S.E.M. < 5%).

1), but not by 100 μg of chloramphenicol/ml (results not shown). Such a finding is consistent with there being no appreciable contamination of the culture with prokaryotic organisms. Inhibition by cycloheximide shows the protein to come from a eukaryotic ribosomal site of synthesis.

The metabolic integrity of hepatocytes maintained in primary monolayer culture was assessed by their ability to synthesize specific liver protein (L-type pyruvate kinase) and their response to glucagon with respect to its inhibition of fatty acid synthesis. Hepatocytes isolated from chow-fed rats maintained in primary monolayer culture for 5 days incorporated $8.6 \pm 0.2 \mu\text{mol}$ of $[\text{U-}^{14}\text{C}]\text{lactate/h}$ per g of cells ($404 \pm 9 \mu\text{mol/h}$ per g of DNA) into fatty acids ($n=3$), compared with a rate of $9.4 \pm 0.3 \mu\text{mol/h}$ per g of cells ($442 \pm 14 \mu\text{mol/h}$ per g of DNA; $n=3$) for freshly prepared cells. Glucagon ($0.1 \mu\text{M}$) inhibited fatty acid synthesis by about 90% in both cases ($1.6 \pm 0.1 \mu\text{mol/h}$ per g of cells; $75 \pm 5 \mu\text{mol/h}$ per g of DNA; $n=3$ for fresh cells; $1.2 \pm 0.1 \mu\text{mol/h}$ per g of cells; $56 \pm 5 \mu\text{mol/h}$ per g of DNA; $n=3$ for cultured cells).

Estimation of the lifetime of L-type pyruvate kinase in vitro

Monolayer cultures of hepatocytes were labelled after 16 h at 37°C with $10 \mu\text{Ci}$ of $\text{L-[4,5-}^3\text{H]leucine}$ for 3 h. The medium was removed and replaced with medium containing no radioactively labelled amino acid. The amounts of radioactivity incorporated into L-type pyruvate kinase and fructose 1,6-bisphosphatase were determined at various times after the removal of the $[\text{}^3\text{H}]\text{leucine}$ as described above. Cells isolated from carbohydrate-rich-diet-fed rats incorporated significantly more $[\text{}^3\text{H}]\text{leucine}$ into L-type pyruvate kinase ($2550 \pm 140 \text{ d.p.m./3 h}$ per mg of cells; $15260 \pm 840 \text{ d.p.m./3 h}$ per $100 \mu\text{g}$ of DNA; $n=6$) than did cells from chow-fed animals ($920 \pm 40 \text{ d.p.m./3 h}$ per mg of cells; $4330 \pm 190 \text{ d.p.m./3 h}$ per $100 \mu\text{g}$ of DNA; $n=6$). Cells from 48 h-starved animals did not incorporate sufficient label into L-type pyruvate kinase to permit the determination of the half-life of the enzyme under these conditions.

The degradation of L-type pyruvate kinase was biphasic (Figs. 2 and 3). A similar pattern of disappearance of $[\text{}^3\text{H}]\text{leucine}$ -labelled enzyme was observed in cells, isolated from both carbohydrate-rich-diet-fed and chow-fed rats, which were cultured

Fig. 2. *Lifetime of L-type pyruvate kinase in cultured rat hepatocytes isolated from chow-fed animals*

Hepatocytes were established in monolayer culture in supplemented Waymouth's medium (O) or supplemented Waymouth's medium containing 2 mM-fructose and $0.1 \mu\text{M}$ -insulin (●). After incubation for 3 h with $\text{L-[4,5-}^3\text{H]leucine}$, the medium was replaced with medium containing no radioactivity. At time points after this replacement, the amount of label in L-type pyruvate kinase was determined as described in the Materials and methods section. The half-time for each phase was calculated from the first-order rate equation $t_{1/2} = 0.693/k_d$, where k_d is the apparent rate constant for loss of ^3H from L-type pyruvate kinase. Results are shown as means $\pm$ S.E.M. ($n=4$).

Table 2. *Lifetimes of $[\text{}^3\text{H}]\text{leucine}$ -labelled protein in cultured rat hepatocytes*

The half-lives of L-type pyruvate kinase, fructose 1,6-bisphosphatase and total trichloroacetic acid-precipitable material were determined as described in the Materials and methods section. Results are the means $\pm$ S.E.M for n independent observations. The significance of the differences in half-life in the presence or absence of fructose and insulin were measured by Student's t test: * $P < 0.05$.

Condition	Culture medium	Half-life (h)			
		L-type pyruvate kinase (1)	L-type pyruvate kinase (2)	Fructose 1,6-bisphosphatase	Total protein
Chow-fed	Waymouth's	4.9 ± 0.1 (4)	79.2 ± 1.3 (4)	41.0 ± 0.6 (4)	19.8 ± 0.2 (4)
	Waymouth's + 2 mM-fructose + $0.1 \mu\text{M}$ -insulin	4.9 ± 0.1 (3)	79.2 ± 1.7 (3)	41.0 ± 0.7 (3)	22.9 ± 0.3 (3)
Carbohydrate-rich-diet-fed	Waymouth's	5.8 ± 0.2 (4)	85.0 ± 2.4 (4)	48.5 ± 1.4 (4)	20.7 ± 0.1 (4)
	Waymouth's + 2 mM-fructose + $0.1 \mu\text{M}$ -insulin	11.9 ± 0.2 (3)*	78.4 ± 1.1 (3)	49.4 ± 0.3 (3)	23.9 ± 0.3 (3)

Fig. 3. Lifetime of L-type pyruvate kinase in cultured rat hepatocytes isolated from carbohydrate-rich-diet-fed rats

Hepatocytes were established in monolayer culture in supplemented Waymouth's medium (○) or supplemented Waymouth's medium containing 2 mM-fructose and 0.1 μ M-insulin (●). The experimental conditions were as described in Fig. 2.

in supplemented Waymouth's medium. A rapid phase, during which 60–70% of the labelled enzyme was degraded and lasting for 8–10 h, was followed by a more gradual decrease in the amount of labelled enzyme. The initial rapid phase had a half-time of about 5 h and the secondary slower phase a half-time of about 80 h (Table 2). However, addition of 2 mM-fructose and 0.1 μ M-insulin to the culture medium significantly increased the half-life of the initial rapid phase of L-type pyruvate kinase degradation by cells from carbohydrate-rich-diet-fed rats, but not by cells from chow-fed animals. The duration of the initial phase was increased from 10 to 20 h; the extent by which the enzyme was degraded during this time was, however, unaltered (Fig. 3). The secondary, slower, phase of degradation remained unaffected in both cases.

Hepatocytes isolated from carbohydrate-rich-diet-fed rats incorporated similar amounts of radioactivity into fructose 1,6-bisphosphatase as did cells isolated from chow-fed animals (Table 1). The loss of ^3H from immunoprecipitates of fructose 1,6-bisphosphatase with time followed first-order kinetics (Fig. 4), in contrast with the degradation of L-type pyruvate kinase. The apparent rate constant for degradation was similar for both cells types, yielding values of 48.5 ± 1.4 h and 41.0 ± 0.6 h for the half-lives of fructose 1,6-bisphosphatase in cells from carbohydrate-rich-diet-fed and chow-fed rats respectively (Table 2). These values compared favour-

Fig. 4. Lifetime of fructose 1,6-bisphosphatase in cultured rat hepatocytes

Hepatocytes were isolated from chow-fed (●) and carbohydrate-rich-diet-fed rats (○) were established in monolayer culture in supplemented Waymouth's medium. The experimental conditions were as described in Fig. 2.

ably with that of 45 h reported for the enzyme *in vivo* (Zalitis & Pitot, 1979). The inclusion of fructose and insulin in the culture medium did not affect the rate of enzyme degradation (Table 2).

The half-life of the total cellular protein was determined from the rate of disappearance of trichloroacetic acid-precipitable radioactivity from the cells; both cell types degraded total protein at a similar rate, by a process following first-order kinetics. Additions to the culture medium did not extend the lifetime of the total labelled protein in hepatocytes from either type of animal (Table 2).

Discussion

Hepatocytes maintained in monolayer culture may be used to determine the half-life of a specific radioactively labelled liver protein *in vitro*. Using hepatocytes isolated from rats under a variety of nutritional conditions and maintained in primary monolayer culture, we have shown that the rate of synthesis of L-type pyruvate by isolated rat hepatocytes is dependent on the nutritional status of the donor animal; significantly, the different cells types maintained their different rates of synthesis of L-type pyruvate kinase for at least 5 days when cultured in supplemented Waymouth's medium. This suggests that there may be some form of programming within the hepatocytes which maintains their prescribed rate of synthesis of L-type pyruvate kinase. The degradation of [^3H]leucine-labelled L-type pyruvate kinase by cultured rat hepatocytes did not follow first-order kinetics with a half-time of 56 h as described *in vivo* (Hopkirk & Bloxham, 1979; Cladaras & Cottam, 1980a); instead a rapid phase

with a half-time of 5 h was followed by a slower phase of enzyme degradation with a half-time of 80 h. A similar pattern of degradation was observed in cells from rats fed on either chow or a diet rich in carbohydrate. Previous studies on the degradation of L-type pyruvate kinase (Leffert *et al.*, 1978) and of glucokinase (Spence & Pitot, 1979) by cultured rat hepatocytes reported a rapid phase of enzyme degradation that was attributed to a loss of enzyme activity by leakage from damaged freshly prepared cells. We have found that culturing cells for 12–16 h before use overcomes this problem; damaged cells detached from the culture dishes during this time and could be removed by aspiration. Fructose 1,6-bisphosphatase is fairly constitutive in the liver under the dietary conditions studied, and may be employed as an internal marker of the efficiency of the culture system. The degradation of fructose 1,6-bisphosphatase followed first-order kinetics, with a half-time of 41 h compared with a reported value of 45 h *in vivo* (Zalitis & Pitot, 1979). The biphasic degradation of L-type pyruvate kinase is therefore a real phenomenon, providing both a short-term and a long-term regulation of enzyme concentration. Previous experiments to determine the half-life of the enzyme have involved measurements of total enzyme activity (Hopkirk & Bloxham, 1979), which does not allow for enzyme synthesis *de novo*, or the disappearance of radioactivity from immunoprecipitable L-type pyruvate kinase (Cladaras & Cottam, 1980a; Hopkirk & Bloxham, 1980), after injection *in vivo*. The latter approach is hampered by the necessity to allow 6–10 h after injection for amino acid equilibration within the animal and for sufficient label to be incorporated into L-type pyruvate kinase. A delay of 10 h before the zero-time point would involve missing completely the initial rapid phase of degradation of L-type pyruvate kinase that is observed by using a shorter pulse label *in vitro*.

Feeding rats on a diet rich in carbohydrate (sucrose) and low in fat is characterized by increased portal-venous concentrations of glucose, fructose, lactate and insulin. The inclusion of fructose and insulin in the culture medium induces an increase in synthesis of L-type pyruvate kinase by hepatocytes isolated from 48 h-starved rats (Poole *et al.*, 1982). In cells from carbohydrate-rich-diet-fed rats it was possible to retard the initial rapid phase of degradation of L-type pyruvate kinase by the inclusion of fructose and insulin together in the culture medium. Neither fructose nor insulin alone, nor the inclusion of lactate in the medium, significantly retarded the rapid phase of enzyme breakdown. The inclusion of fructose and insulin in the medium did not alter the rate of enzyme degradation in the secondary slower phase or change the extent to which the enzyme was degraded in the rapid phase. Neither phase of

degradation was affected in cells isolated from chow-fed animals. The rates of total protein degradation and fructose 1,6-bisphosphatase degradation also remained unaltered. The retardation of the rapid phase of enzyme degradation in cells from carbohydrate-rich-diet-fed rats by pseudo-carbohydrate diet *in vitro* may be due to a net dephosphorylation of the enzyme in the presence of insulin and fructose. L-type pyruvate kinase from rats fed on a carbohydrate-rich diet exists almost completely in the dephosphorylated form (Van Berkel *et al.*, 1977). Phosphorylation of purified L-type pyruvate kinase makes the enzyme more susceptible to proteolysis by subtilisin *in vitro* (Bergström *et al.*, 1978) and by unspecified proteinases *in vivo* (Hall *et al.*, 1979).

It has been postulated that hepatocytes contain two major pools of proteolytic enzyme activity: one intralysosomal and one cytoplasmic (Seglen *et al.*, 1979). Intralysosomal protein degradation is characterized by its inhibition by weak bases such as ammonia, methylamine and chloroquine. Preliminary results from this laboratory (G. P. Poole, unpublished work) have shown that the initial rapid phase of degradation of L-type pyruvate kinase is inhibited by chloroquine, whereas the slower secondary phase is unaffected, possibly indicating separate intracellular sites of enzyme hydrolysis for the two phases; chloroquine did not inhibit fructose 1,6-bisphosphatase degradation by hepatocytes, suggesting that the two enzymes may have different sites of degradation within the liver cell. We therefore conclude that the alterations in concentration of L-type pyruvate kinase in isolated rat hepatocytes are controlled by changes in the rates of both enzyme synthesis and enzyme degradation, although the mechanism by which the enzyme is degraded remains as yet unknown.

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References

- Berg, T., Bowman, D. & Seglen, P. O. (1972) *Exp. Cell Res.* **72**, 571–573
- Bergström, G., Ekman, P., Humble, E. & Engström, L. (1978) *Biochim. Biophys. Acta* **532**, 259–267
- Bloxham, D. P., Fitzsimons, J. T. R. & York, D. A. (1977) *Horm. Metab. Res.* **9**, 304–309
- Cladaras, C. & Cottam, G. L. (1980a) *Arch. Biochem. Biophys.* **200**, 426–433
- Cladaras, C. & Cottam, G. L. (1980b) *J. Biol. Chem.* **255**, 11499–11503
- Ekman, P., Dahlquist, U., Humble, P. & Engström, L. (1976) *Biochim. Biophys. Acta* **429**, 374–382
- Grinde, B. & Seglen, P. O. (1981) *Hoppe-Seyler's Z. Physiol. Chem.* **362**, 549–556

- Hall, E. R., McCully, V. & Cottam, G. L. (1979) *Arch. Biochem. Biophys.* **195**, 315–324
- Hopgood, M. F., Clark, M. G. & Ballard, F. J. (1980) *Biochem. J.* **186**, 71–79
- Hopgood, M. F., Clark, M. G. & Ballard, F. J. (1981) *Biochem. J.* **196**, 33–40
- Hopkirk, T. J. & Bloxham, D. P. (1979) *Biochem. J.* **182**, 383–397
- Hopkirk, T. J. & Bloxham, D. P. (1980) *Biochem. J.* **192**, 507–516
- Ishibashi, H. & Cottam, G. L. (1978) *J. Biol. Chem.* **253**, 8767–8771
- Kohl, E. A. & Cottam, G. L. (1976) *Arch. Biochem. Biophys.* **176**, 671–682
- Kohl, E. A. & Cottam, G. L. (1977) *Biochim. Biophys. Acta* **484**, 49–58
- Krebs, H. A. & Eggleston, L. V. (1965) *Biochem. J.* **94**, 3c–4c
- Leffert, H., Moran, T., Sell, S., Skelly, H., Ibsen, K., Mueller, M. & Arias, I. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 1834–1838
- Poole, G. P., Postle, A. D. & Bloxham, D. P. (1981) *Biochem. Soc. Trans.* **9**, 399
- Poole, G. P., Postle, A. D. & Bloxham, D. P. (1982) *Biochem. J.* **204**, 81–87
- Riou, J. P., Claus, T. H. & Pilgis, S. J. (1978) *J. Biol. Chem.* **253**, 656–659
- Rozengurt, E., Jimenez de Asua, L. & Carminatti, H. (1969) *J. Biol. Chem.* **244**, 3142–3147
- Schwarze, P. E. & Seglen, P. O. (1981) *In Vitro* **17**, 71–76
- Seglen, P. O., Grinde, B. & Solheim, A. E. (1979) *Eur. J. Biochem.* **95**, 215–225
- Seubert, W., Henning, H. V., Schoner, W. & L'Age, M. (1968) *Adv. Enzyme Regul.* **6**, 153–187
- Smith, S. B. & Freedland, R. A. (1979) *J. Biol. Chem.* **254**, 10644–10648
- Smith, S. B. & Freedland, R. A. (1981) *Am. J. Physiol.* **240**, E279–E285
- Spence, J. T. & Pitot, H. C. (1979) *J. Biol. Chem.* **254**, 12331–12336
- Taylor, C. B. & Bailey, E. (1967) *Biochem. J.* **102**, 32c–33c
- Taylor, C. B., Bailey, E. & Bartley, W. (1967) *Biochem. J.* **105**, 717–722
- Van Berkel, T. J. C., Kruijt, J. K. & Koster, J. F. (1977) *Eur. J. Biochem.* **81**, 423–433
- Van Berkel, T. J. C., Kruijt, J. K., Van den Berg, G. B. & Koster, J. F. (1978) *Eur. J. Biochem.* **92**, 553–561
- Vernon, R. G. & Walker, D. G. (1968a) *Biochem. J.* **106**, 321–329
- Vernon, R. G. & Walker, D. G. (1968b) *Biochem. J.* **106**, 331–338
- Weber, G., Singhal, R. L., Stamm, N. B., Lea, M. A. & Fisher, E. A. (1965) *Adv. Enzyme Regul.* **4**, 59–81
- Zalitis, J. G. & Pitot, H. C. (1979) *Arch. Biochem. Biophys.* **194**, 620–631