Uridylate trapping, induction of UTP deficiency, and stimulation of pyrimidine synthesis *de novo* by D-galactosone

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D-Galactosone (D-lyxo-2-hexosulose) is phosphorylated and metabolized to the uridine diphosphate derivative in AS-30D hepatoma cells and rat liver. These reactions were catalysed *in vitro* by galactokinase and hexose-1-phosphate uridylyltransferase. Nucleotide analyses by high-performance liquid chromatography and enzymic assays revealed that this galactose analogue interferes with cellular pyrimidine nucleotide metabolism leading to a deficiency of UTP. [^{14}C]Uridine labelling of hepatoma cells indicated a division of [^{14}C]uridylate from UTP into UDP-galactosone; the latter was formed at a rate of more than $1.7\text{ mmol} \times \text{h}^{-1} \times (\text{kg AS-30D or liver wet wt.})^{-1}$. As a consequence of UTP deficiency, D-galactosone (1 mmol/l or 1 mmol/kg body wt.) strongly enhanced the rate of pyrimidine synthesis *de novo* as evidenced by incorporation of $^{14}\text{CO}_2$ into uridylate and by an expansion of the uridylate pool. This resulted in a doubling of the total acid-soluble uridylate pool within 70 min in the hepatoma cells and within 110 min in rat liver. Combined treatment of hepatoma cells with D-galactosone and *N*-(phosphonoacetyl)-L-aspartate, an inhibitor of aspartate carbamoyltransferase, prevented the expansion of the uridylate pool and led to a synergistic reduction of UTP to 10% of the content in control cells. Hepatic UTP deficiency was selective with respect to other nucleotide 5'-triphosphates but was associated with reduced contents of UDP-glucose, UDP-glucuronate, and UDP-*N*-acetylhexosamines. Isolation of the UDP derivative of D-galactosone revealed an extremely alkali-labile UDP-sugar, probably an isomerization product of UDP-galactosone, that was degraded by elimination of UDP with a half-life of 45 min at pH 7.5 and 37°C. The instability of UDP-galactosone may contribute *in vivo* to limit the time period of severe uridine phosphate deficiency in addition to the compensatory role of pyrimidine synthesis *de novo*. During the initial time period, however, D-galactosone is effective as a powerful uridylate-trapping sugar analogue.

D-Galactosone (D-lyxo-2-hexosulose) was among the first sugar analogues synthesized (Fischer, 1889). In contrast to D-glucosone, this C-2-modified D-galactose analogue was previously not considered to be toxic (Bayne, 1952; Bayne & Fewster, 1956). Earlier work has established that the toxicity of some sugar analogues, including D-galactosamine, is due to their interference with uracil nucleotide

metabolism (Decker & Keppler, 1974; Keppler, 1975). The uridylate-trapping effect of these sugars comprises the formation and accumulation of UDP-sugar derivatives leading to a decrease in UTP and a subsequent stimulation of pyrimidine synthesis *de novo* (Keppler *et al.*, 1970a; Holstege *et al.*, 1982).

Our present investigations with a D-galactose-metabolizing hepatoma cell line (Keppler, 1977a) and in rat liver have revealed that D-galactosone can act as a most powerful uridylate-trapping agent,

Abbreviation used: h.p.l.c., high-performance liquid chromatography.

inducing a higher rate of pyrimidine synthesis *de novo* than any of the sugar analogues studied previously. D-Galactosone induces severe UTP deficiency in rat liver *in vivo*.

Materials and methods

Chemicals, isotopes and enzymes

D-Galactosone was synthesized according to Bayne (1963). *N*-(Phosphonoacetyl)-L-aspartate, an inhibitor of aspartate carbamoyltransferase (Collins & Stark, 1971), was kindly given by Dr. S. Marinis (Goedecke AG, Freiburg, Germany). [2-¹⁴C]-Uridine (55 Ci/mol) and [¹⁴C]NaHCO₃ (52 Ci/mol) were purchased from The Radiochemical Centre, Amersham. Galactokinase from galactose-adapted yeast (28 units/mg of protein as defined by the supplier) was obtained from Sigma. Hexose-1-phosphate uridylyltransferase from calf liver (1.5 units/mg of protein) as well as further enzymes, nucleotides and cofactors were from Boehringer Mannheim.

Phosphorylation of D-galactosone and measurement of hexose-1-phosphate uridylyltransferase activity

D-Galactosone (4 mmol/l) was phosphorylated by yeast galactokinase (180 mg/l) at 37°C in the presence of ATP (10 mmol/l), magnesium acetate (15 mmol/l), and triethanolamine (100 mmol/l) at pH 7.4. After 2 h the incubation was terminated by addition of HClO₄ (0.6 mol/l). The supernatant after centrifugation was neutralized with KHCO₃. Unreacted D-galactosone and the product D-galactosone 1-phosphate were assayed by the sequential addition of galactose dehydrogenase (Wallenfels & Kurz, 1962) and alkaline phosphatase in the presence of NAD⁺.

Hexose-1-phosphate uridylyltransferase from calf liver (300 units/l) was assayed spectrophotometrically at 25°C and pH 7.4 with varied concentrations of galactosone 1-phosphate or galactose 1-phosphate in the presence of UDP-glucose (0.6 mmol/l), cysteine (14 mmol/l), triethanolamine (70 mmol/l), glucose 1,6-bisphosphate (50 µmol/l), Mg²⁺ (1 mmol/l), NADP⁺ (0.7 mmol/l), phosphoglucomutase and glucose-6-phosphate dehydrogenase (2 k-units/l each).

Incubation and deproteinization of hepatoma cells

AS-30D ascites hepatoma cells (Smith *et al.*, 1970) were obtained and incubated as described earlier (Keppler & Smith, 1974; Keppler, 1977a). Cell suspensions had a concentration of $(1.8-2.4) \times 10^9$ cells/l and were kept on a gyratory shaker at 37°C under CO₂/air (1:20) at a pH between 7.45 and 7.25. The concentrations of phosphate and bicarbonate in the standard medium (Keppler & Smith, 1974) were 2 and 26 mmol/l, except for the [¹⁴C]NaHCO₃-labelling experiments where they

were changed to 5 and 20 mmol/l respectively. Experiments were preceded by a 40 min preincubation period. D-Galactosone was added at an initial concentration of 1 mmol/l of cell suspension. Since D-galactosone is hygroscopic and unstable, particularly at alkaline pH values, its concentration in the freshly prepared solution was based on the enzymic analysis with galactose dehydrogenase and NAD⁺ (Wallenfels & Kurz, 1962) in the presence of Tris/HCl buffer (0.1 mol/l, pH 8.0). Cells were collected by centrifugation and frozen in liquid nitrogen (Keppler & Smith, 1974). Deproteinization was performed in the cold with 5 vol. of HClO₄ (0.6 mol/l); the supernatants after centrifugation were collected and neutralized with KHCO₃ to a pH value of about 6. In order to preserve acid-labile metabolites, a deproteinization with HgCl₂ (0.11 mol/l) adjusted to pH 6.5 was performed where indicated; the supernatant after centrifugation could be directly applied to h.p.l.c.

Experiments in vivo

Female rats of the Wistar strain (Ivanovas, Kisslegg, Germany) weighing 100 ± 5 g had free access to water and a carbohydrate-rich, 20% protein diet. Animals were injected intraperitoneally with a fresh aqueous solution of D-galactosone (1 mmol/kg). Livers were freeze-clamped *in situ* under light pentobarbital anaesthesia followed by homogenization in 5 vol. of HClO₄ (0.6 mol/l).

Metabolite analyses

Enzymic analyses were performed on neutralized HClO₄ extracts. UMP and UDP-glucose were assayed specifically with UDP-glucose dehydrogenase as indicator enzyme (Keppler *et al.*, 1970b). Total acid-soluble uracil, cytosine, guanine, and adenine 5'-nucleotides (ΣUMP, ΣCMP, ΣGMP, ΣAMP) were determined as UMP, CMP, GMP, and AMP, respectively, after snake venom phosphodiesterase hydrolysis of cell and tissue extracts (Keppler *et al.*, 1970b).

Nucleoside 5'-phosphates, NAD⁺ and UDP-sugars, including the UDP-derivative of D-galactosone, were separated by anion-exchange h.p.l.c. (Kontron, Eching, Germany) on a Partisil-10 SAX (Whatman, Clifton, NJ, U.S.A.) column using flow and buffer gradients (Hartwick & Brown, 1975) as described by Holstege *et al.* (1982). Peaks were recorded at 262 nm and integrated automatically (Spectra-Physics, Darmstadt, Germany); nucleotides were quantified by referring to standards of the highest purity that had been analysed enzymically in NAD⁺- or NADP⁺-coupled assays (Bergmeyer, 1974). UDP-glucose served as the standard for the UDP derivative of D-galactosone, since the nucleotide moieties were identical and the retention times differed by less than 20 s. Alternatively, the UDP

derivative could be quantified by measurement of the UDP released at pH 9.

Ion-pair reverse-phase h.p.l.c. (Hoffman & Liao, 1977; Walseth *et al.*, 1980) was used to separate the intermediates of pyrimidine synthesis *de novo* on a 5 μ m-particle ODS-Hypersil RP18 column (Shandon, Frankfurt, Germany). Isocratic elution was performed with an aqueous solution containing tetrabutylammonium hydroxide (5 mmol/l) and KH_2PO_4 (50 mmol/l) and adjusted with phosphoric acid to pH 4.0. Peaks were recorded at 230 nm. Carbamoyl aspartate was measured after its conversion to the hydantoin (Nyc & Mitchell, 1947) during the HClO_4 deproteinization of the frozen cell pellets.

For the separation of ^{14}C -labelled metabolites, fractions were collected every 20 s and counted at an efficiency of 90% in a Triton/toluene scintillation mixture.

Results

Phosphorylation of D-galactosone and conversion to its UDP derivative

Incubation of AS-30D cells in the presence of D-galactosone at a concentration of 1 mmol/l as determined with galactose dehydrogenase led to D-galactosone phosphate contents of 1.5, 1.9, 2.4, and 2.7 mmol/kg after 15, 30, 60 and 120 min, respectively. The identity of this phosphorylated derivative with D-galactosone 1-phosphate was indicated by its reactivity with hexose-1-phosphate uridylyltransferase in the presence of UDP-glucose, yielding similar amounts as in the sequential assay with galactose dehydrogenase and alkaline phosphatase. Mild acid hydrolysis of the cell and tissue supernatants at 95°C and pH 2.0 (adjusted with phosphoric acid) led to a dephosphorylation with a half-life of 18 min. Under the same conditions, D-galactosone 1-phosphate synthesized with yeast galactokinase was dephosphorylated with a half-life of 12 min, whereas D-galactose 1-phosphate and D-galactose 6-phosphate were hydrolysed with half-lives of 5 and 2430 min, respectively.

D-Galactosone 1-phosphate was a substrate for hexose-1-phosphate uridylyltransferase from calf liver with an apparent K_m value of 0.35 mmol/l as compared with 0.65 mmol/l for D-galactose 1-phosphate. The maximal velocity, however, was only 2.7% of that for D-galactose 1-phosphate in the presence of the substrate analogue and under the conditions described under 'Materials and methods'. The $K_m/V_{\max}$ ratio was 0.7 and 14 for D-galactose 1-phosphate and D-galactosone 1-phosphate, respectively. In AS-30D cells the formation and accumulation of the UDP derivative of D-galactosone, amounting to 0.94 mmol/kg after 30 min, became detectable by h.p.l.c. (Fig. 1). This UDP derivative

Fig. 1. Changes in uracil nucleotides induced by D-galactosone

AS-30D hepatoma cells were incubated in the presence of D-galactosone (1 mmol/l). Freeze-pellets were obtained from 25 ml aliquots of the suspension for metabolite analyses as described under 'Materials and methods'. Nucleotide contents are based on cell wet weights determined in the same suspension (Keppler & Smith, 1974). Mean values from four to ten separate experiments, with all the s.d. values below 15% of the mean, are given. Abbreviation: GalO, D-galactosone.

had a retention time on the anion-exchange column hardly distinguishable from that for UDP-galactose and UDP-glucose. Isolation of this material by preparative h.p.l.c. at pH values between 4.1 and 4.5 allowed its partial characterization. Treatment with snake venom phosphodiesterase yielded 5'-UMP that was quantified by specific enzymic analysis (Keppler *et al.*, 1970b) and corresponded to the amount of UDP-derivative determined by chromatography and absorbance at 262 nm. The isolated UDP derivative was extremely alkali-labile; treatment in potassium phosphate buffer at pH 7.50 and 37°C led to an elimination of UDP with a half-life of 45 min. The hydrolysed material did not react with galactose dehydrogenase to form NADH. This indicates that the carbohydrate moiety of the UDP derivative underwent isomerization or degradation upon hydrolysis. The isolated UDP derivative was degraded at pH 2.0 and 95°C with a half-life of 10 min, yielding 55% UDP and 15% UMP after 20 min. Isolation of UDP-glucosone from D-glucosone-treated cells (Holstege *et al.*, 1982) and comparative analysis of its stability indicated that it was 40 times less alkali-labile, eliminating UDP at pH 7.50 and 37°C with a half-life of 1800 min.

Labelling of AS-30D hepatoma cells with [^{14}C]-uridine demonstrated the diversion of labelled uridylate into the UDP derivative of D-galactosone (Fig. 2). While the latter comprised 61% of labelled uracil nucleotides, the relative radioactivity in UTP decreased from 31% in controls to 8% in cells exposed to D-galactosone (Fig. 2). The uptake of [^{14}C]uridine was increased by 30% when AS-30D cells were treated with the uridylate-trapping galactose analogue, as described under Fig. 2.

Changes in nucleoside 5'-triphosphate contents induced by D-galactosone in hepatoma cells suspended in vitro

The most significant and early effect of D-galactosone in AS-30D cells was a drop of UTP levels to 56% of the control level after 15 min

Fig. 2. Diversion of [^{14}C]uridylate into the UDP derivative of D-galactosone

AS-30D cells were incubated for 2 h in the presence of [^{14}C]uridine ($200\ \mu\text{Ci/l}$; $3.6\ \mu\text{mol/l}$) prior to freezing of the cell pellet (320 mg) and homogenization in 1 ml of HgCl_2 ($0.11\ \text{mol/l}$; pH 6.5). D-Galactosone ($1\ \text{mmol/l}$) was added 30 min after [^{14}C]uridine. Cell extracts ($50\ \mu\text{l}$) from control cells (○) and cells exposed for 1.5 h to D-galactosone (●) were separated by anion-exchange h.p.l.c. Shaded peak areas mark the chromatogram from D-galactosone-treated cells. Abbreviations: GalO, D-galactosone; Hex, hexose.

($P < 0.001$; Fig. 3). The depression of UTP in these cells *in vitro* was followed by a lowering of CTP content, which is consistent with their precursor-to-product relation. Severe UTP deficiency was induced when the compensatory formation of UTP by pyrimidine synthesis *de novo* was blocked by *N*-(phosphonoacetyl)-L-aspartate, resulting in a synergism with the uridylate-trapping effect of D-galactosone (Fig. 4).

The interference of D-galactosone in pyrimidine nucleotide metabolism was rather selective, as the changes in ATP, GTP, and other purine nucleotides were only limited (Fig. 3).

The duration of the D-galactosone-induced depression of UTP was studied by following the time course of UTP recovery and D-galactosone 1-phosphate decrease in AS-30D cells that had been incubated for 1 h in the presence of the D-galactose analogue under the conditions described in the legend to Fig. 1, followed by centrifugation, resuspension and further incubation in fresh

Fig. 3. Nucleoside 5'-triphosphate contents of hepatoma cells exposed to D-galactosone

Nucleotide levels were measured in HClO_4 extracts by h.p.l.c. Mean values from four separate experiments are expressed as % of the respective nucleoside triphosphate (NTP) content in control cells. Control levels for GTP, ATP, UTP, and CTP after 3 h amounted to 0.53 ± 0.02 (s.d.), 3.66 ± 0.13 , 1.09 ± 0.06 , and $0.56 \pm 0.03\ \text{mmol/kg}$ of AS-30D cell wet wt., respectively.

Fig. 4. Synergistic depletion of UTP pools in AS-30D cells by uridylate trapping and inhibition of pyrimidine synthesis *de novo*

Selective inhibition of aspartate carbamoyltransferase by *N*-(phosphonoacetyl)-L-aspartate (PALA) (Collins & Stark, 1971) at a concentration of 1 mmol/l intensified the depression of UTP levels by D-galactosone (GalO; 1 mmol/l). UTP was determined in neutralized HClO_4 extracts from AS-30D cells by anion-exchange h.p.l.c. Mean values from four to ten separate experiments are shown, with the s.d. values averaging 13% of the mean.

Fig. 5. Total acid-soluble uracil 5'-nucleotides (Σ UMP) and their changes induced by D-galactosone and *N*-(phosphonoacetyl)-L-aspartate (PALA)

The rise in Σ UMP observed in AS-30D cells exposed to D-galactosone (GalO; 1 mmol/l) was completely suppressed by the simultaneous addition of PALA (1 mmol/l) as inhibitor of the second enzyme in pyrimidine synthesis *de novo*. Each point represents the mean from four to ten separate experiments with the s.d. values averaging 9% of the mean.

medium. As compared with its control level, UTP was at 55, 79, 100, and 105% at 1, 2, 3, and 4 h after resuspension, respectively; in the same experiments D-galactosone 1-phosphate dropped to 30, 15, 11, and 9% of its initial level after resuspension. These changes were accompanied by a decrease in the content of the UDP derivative of D-galactosone and a recovery of UDP-*N*-acetylhexosamines to their control levels.

Stimulation of pyrimidine synthesis *de novo* by D-galactosone

The acid-soluble pool of uridylate (Σ UMP) increased 2.7-fold at a rate of $1.4 \text{ mmol} \times \text{h}^{-1} \times (\text{kg of cells})^{-1}$ during the initial 2 h after addition of D-galactosone to hepatoma cell suspensions (Fig. 5). Within this time period the UTP level dropped to less than 60% of control (Fig. 3). The rate of increase in Σ UMP was markedly smaller, however, when UTP

was near 70% of the control level between 2 and 3 h after addition of the uridylate-trapping sugar analogue (Fig. 1). Selective inhibition of the second enzyme in pyrimidine synthesis *de novo* by *N*-(phosphonoacetyl)-L-aspartate (Swyryd *et al.*, 1974) prevented the expansion of the uridylate pool (Fig. 5).

The enhanced rate of pyrimidine nucleotide synthesis *de novo* was directly measured by incorporation of $^{14}\text{CO}_2$ into the uridylate pool. As compared with untreated control cells, D-galactosone led to a 3.7-fold increase in the labelling of Σ UMP $^{14}\text{CO}_2$ (Fig. 6). Based on the specific activity of $^{14}\text{CO}_2$, this corresponds to rates of pyrimidine synthesis *de novo* over the 3 h incorporation period of 0.31 and $1.17 \text{ mmol} \times \text{h}^{-1} \times (\text{kg of cells})^{-1}$ in control and D-galactosone-treated cells, respectively. During the same time period, the average expansion of the uridylate pool (Σ UMP) in D-galactosone-treated cells amounted to $1.06 \text{ mmol} \times \text{h}^{-1} \times (\text{kg of cells})^{-1}$ (Fig. 5). The enhanced rate of pyrimidine synthesis *de novo*, as determined by the labelling of

Fig. 6. D-Galactosone-induced enhancement of [^{14}C]-uridylylate formation from $^{14}\text{CO}_2$ and labelling of the intermediates of pyrimidine synthesis *de novo*

AS-30D cells had been incubated for 10 min in the presence of D-galactosone (1 mmol/l) when [^{14}C]- NaHCO_3 (150 mCi/l) was added to a final concentration of 20 mmol/l. $^{14}\text{CO}_2$ incorporation lasted for 3 h in a final volume of 1 ml and was discontinued by deproteinization of the cell pellet (7.1 mg) with 250 μl of HClO_4 (0.6 mol/l). The acid treatment was associated with a breakdown of carbamoyl phosphate and formation of the hydantoin from carbamoyl aspartate. Uracil 5'-nucleotides were converted to UMP by snake venom phosphodiesterase hydrolysis and the radioactive intermediates were separated by ion-pair reverse-phase h.p.l.c. as described under 'Materials and methods'. The radioactivity in the metabolite fractions is given in the sequence of pyrimidine synthesis *de novo* as indicated in the scheme below the Figure, not in the sequence of elution upon chromatography. In the scheme the reduction of ubiquinone (Q) to ubiquinol (QH_2) by dihydro-urotate dehydrogenase represents a simplified version (Forman, 1980; Keppler & Holstege, 1982). Mean values from two closely corresponding experiments each with control cells (open bars) and D-galactosone-treated cells (shaded bars) are given. Abbreviations: Cbm, carbamoyl; H_2Oro , dihydro-urotate; Oro, urotate.

ΣUMP from $^{14}\text{CO}_2$, was not associated with major changes in the intermediates of this pathway, which remained at low steady levels of radioactive labelling (Fig. 6).

Table 1. Changes in liver nucleotide contents induced by D-galactosone

Livers from female Wistar rats were freeze-clamped 1 h after an intraperitoneal dose of D-galactosone (1 mmol/kg). Nucleotides were analysed by h.p.l.c., except for UDP-glucose, which was determined enzymically. Mean values are from four to seven rats in each group ($\pm$ S.D.).

Nucleotide	Amount (mmol/kg of liver) in:	
	Control	D-Galactosone treated
UTP	0.31 ± 0.04	0.06 ± 0.03
CTP	0.07 ± 0.01	0.13 ± 0.02
GTP	0.27 ± 0.02	0.34 ± 0.04
ATP	2.60 ± 0.30	2.57 ± 0.25
ADP	0.69 ± 0.06	0.73 ± 0.10
AMP	0.12 ± 0.02	0.15 ± 0.06
NAD $^+$	0.72 ± 0.09	0.80 ± 0.04
UDP-glucose	0.33 ± 0.05	0.07 ± 0.01
UDP-N-acetylhexosamines	0.28 ± 0.09	0.10 ± 0.01
UDP-glucuronate	0.32 ± 0.09	0.11 ± 0.04
UDP-galactosone	—	1.74 ± 0.10
ΣUMP	1.39 ± 0.04	2.16 ± 0.26
ΣCMP	0.22 ± 0.06	0.34 ± 0.06
ΣGMP	0.40 ± 0.03	0.53 ± 0.03
ΣAMP	4.35 ± 0.27	4.42 ± 0.46

Effects of D-galactosone on rat liver nucleotides

A single dose of the galactose analogue resulted in a pronounced change in the distribution of hepatic uracil nucleotides *in vivo* (Table 1). Formation of D-galactosone 1-phosphate (0.7 ± 0.2 mmol/kg of liver) and accumulation of the UDP derivative of D-galactosone led to a fall of the UTP and UDP-glucose contents to about 20% of the controls after 1 h. D-Galactosone markedly reduced the contents of UDP-glucuronate and of UDP-N-acetylhexosamines in the liver (Table 1). The pool of acid-soluble uracil nucleotides (ΣUMP) expanded within 1 h by 0.77 mmol/kg of liver; this amount corresponds to 55% of the pool in normal rat liver. CTP and total cytosine nucleotides (ΣCMP) increased *in vivo*, whereas most purine nucleotide levels remained in the control range (Table 1). The ATP/ADP ratio and the adenylate energy charge were 3.8 and 3.5, and 0.86 and 0.85 in normal and D-galactosone-treated livers, respectively.

Time course studies after a single dose under the conditions described for Table 1 demonstrated UTP contents of 0.08 ± 0.01 and 0.15 ± 0.03 (S.D., $n = 4$) mmol/kg at 2 and 3 h, respectively, and completely normal metabolite contents after 24 h. After 3 h hepatic D-galactosone 1-phosphate was below 0.1 mmol/kg, while its UDP derivative still amount-

ted to 1.36 ± 0.15 mmol/kg. The latter was not detectable any more after 24 h.

Discussion

D-Galactosone acts as a powerful uridylate-trapping sugar analogue in hepatoma cells and liver (Figs. 1 and 2 and Table 1). Phosphorylation of D-galactosone precedes the rapid formation of the UDP derivative, which accumulates at a rate of more than $1.7 \text{ mmol} \times \text{h}^{-1} \times (\text{kg wet wt.})^{-1}$ (Fig. 1, Table 1). This rate of uridylate diversion into the UDP derivative exceeds uridylate synthesis, as reflected by the rise in ΣUMP , by about 25% in AS-30D hepatoma cells and by 125% in rat liver. This imbalance between uridylate synthesis and uridylate trapping results in moderate UTP deficiency in the hepatoma cells (Fig. 3) and a depletion of liver UTP to 20% of control (Table 1). Additional inhibition of pyrimidine synthesis *de novo* by *N*-(phosphonoacetyl)-L-aspartate prevents the compensatory increase in total uridylate (ΣUMP) (Fig. 5) and intensifies the deficiency of UTP in hepatoma cells (Fig. 4). This synergistic action of D-galactosone and *N*-(phosphonoacetyl)-L-aspartate corresponds to the effect of D-galactosamine and *N*-(phosphonoacetyl)-L-aspartate (Keppler, 1977b) or D-galactosamine and 6-azauridine (Keppler, 1975, 1977a).

The first and rate-determining enzyme of pyrimidine synthesis *de novo*, glutamine-dependent carbamoyl-phosphate synthetase, is controlled by UTP and 5-phosphoribose 1-diphosphate as feedback inhibitor and allosteric activator, respectively (Tatibana & Shigesada, 1972). D-Galactosone elicited an approx. two-fold higher rate of uridylate synthesis *de novo* than did D-galactosamine in hepatoma cells (Keppler, 1977a) and liver (Keppler *et al.*, 1970a); however, both galactose analogues lowered the UTP contents to a similar extent. D-Galactosone may have less effect on 5-phosphoribose 1-diphosphate than other D-galactose analogues which have, as a side effect, a significant phosphate-trapping action in addition to their uridylate-trapping property. A lowering of cellular P_i levels directly affects 5-phosphoribose 1-diphosphate synthesis (Roth & Deuel, 1974; Planet & Fox, 1976). The phosphate-trapping effect of 2-deoxy-D-galactose (Smith & Keppler, 1977; Starling & Keppler, 1977) and D-galactosamine (Stermann *et al.*, 1978) is due to the rapid accumulation of the respective D-galactose analogue 1-phosphate; the latter is three to eight times smaller 1 h after D-galactosone administration in AS-30D hepatoma cells as well as liver. Accordingly, D-galactosone did not induce significant changes in the ATP/ADP ratio or in the adenylate energy charge.

The increased incorporation of labelled CO_2 into

total uridylate (Fig. 6) corresponds quantitatively to the expansion of the uridylate pool (Fig. 5) and is in line with the regulation of glutamine-dependent carbamoyl-phosphate synthetase by UTP (Pausch *et al.*, 1975). This site of regulation of pyrimidine synthesis *de novo* is also consistent with the labelling pattern of the intermediates of this pathway, all of which remained at low levels (Fig. 6). The channeling of OMP by UMP synthase (multienzyme pyr 5, 6 according to Jones, 1980) explains the minimal labelling of OMP.

Cellular UTP deficiency leads to a release of feedback inhibition of uridine-cytidine kinase and to an increased uptake of uridine (Keppler & Holstege, 1982) and uridine analogues (Holstege *et al.*, 1978). Accordingly, the uptake of labelled uridine by D-galactosone-treated cells was enhanced (Fig. 2). UTP deficiency induced by D-galactosamine in rat liver *in vivo* is associated with an enhanced uptake of extracellular cytidine (Seifert & Buchar, 1981) and an increased hepatic content of CTP, and ΣCMP (Decker *et al.*, 1971). Corresponding changes have been observed after D-galactosone administration *in vivo* (Table 1). However, in cell suspensions *in vitro*, where extracellular cytidine is lacking, a decrease in UTP contents is accompanied by a drop in CTP levels (Henninger *et al.*, 1979; Fig. 3).

The effect of D-galactosone on uridine phosphate contents is limited in its duration by the strong stimulation of pyrimidine synthesis and by the degradation of UDP-galactosone. The former provides uridine phosphate by synthesis *de novo*, whereas the latter serves as a direct source of UDP. The UTP content had recovered to 48% of control in rat liver 3 h after D-galactosone, whereas UTP levels were still at their minimum 3 h after administration of D-galactosamine (Decker & Keppler, 1974) or 2-deoxy-D-galactose (Starling & Keppler, 1977). At this time, and after equimolar doses of the D-galactose analogues, the content of D-galactosone 1-phosphate was below 0.1 mmol/kg whereas D-galactosamine 1-phosphate and 2-deoxy-D-galactose 1-phosphate were at about 3 and 5 mmol/kg, respectively. This indicates that the substrate for the uridylate-trapping action of hexose-1-phosphate uridylyltransferase is available for a much shorter time period after D-galactosone administration. This is in line with the observation that the UDP derivative of D-galactosone decreased between 1 and 3 h and had disappeared from the liver after 24 h, while the UDP derivatives of D-galactosamine and 2-deoxy-D-galactose increased during at least 5 h and were still present in significant amounts after 24 h (Keppler *et al.*, 1970a; Starling & Keppler, 1977).

The extreme lability of the isolated UDP derivative of D-galactosone at pH 7.50 and 37°C may result from rapid isomerizations of UDP-galacto-

sone, possibly occurring *in vivo* and *in vitro*, and leading via the 2,3-enediol intermediate to UDP-D-lyxo-3-hexosulose and, by elimination of the UDP moiety, to further degradation products of D-galactosone. Analogous degradation pathways have been studied for methyl D-arabino-2-hexosulose (Theander, 1958, 1980) and benzyl 3-pentopyranuloses (Heyns *et al.*, 1962). The completely different stability of UDP-glucosone as compared with the isolated UDP derivative of D-galactosone points to the importance of the position of the 4'-hydroxyl for the isomerization reactions leading to UDP elimination. It is of interest that the isolated UDP derivative of D-galactosone was about ten times more resistant to acid at pH 2 when compared with UDP-glucose (Caputto *et al.*, 1950).

The interference of uracil nucleotide metabolism induced by D-galactosone indicates that this C-2-modified hexose analogue can be highly effective in D-galactose-metabolizing tissues and cells such as liver and hepatoma. The inhibitory and cytotoxic effects depend, however, on the duration and the extent of UTP deficiency (Decker & Keppler, 1974). The structure of D-galactosone and its labile UDP derivative is the one of an agent acting strongly but only for a limited time period. This property may be most useful in experimental chemotherapy where uridylate-trapping hexose analogues serve to induce short-term UTP deficiency, which enables an increased cellular uptake of the analogues such as 5-fluorouridine (Holstege *et al.*, 1978; Anukarahanonta *et al.*, 1980; Keppler & Holstege, 1982).

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