Nucleotide Contents of Ascites Hepatoma Cells and Their Changes Induced by D-Galactosamine

Dietrich O. R. Keppler and David F. Smith

Department of Chemistry, Florida State University, Tallahassee, Florida 32306

SUMMARY

AS-30D and Novikoff rat ascites hepatoma cells were incubated in a medium selected according to the following criteria: (a) low leakage of lactate dehydrogenase; (b) low uptake of trypan blue; (c) high adenosine phosphate content; (d) high adenosine triphosphate/adenosine diphosphate ratio and adenylate energy charge. A procedure for defined cooling, freezing, and deproteinization of the incubated cells has been developed. This technique allows enzymatic analyses of nucleotide contents in both cell lines.

The content of total acid-soluble adenine, guanine, cytosine, and uracil 5'-nucleotides amounts to 3.74, 0.78, 0.67, and 1.51 μmoles/g of cell wet weight, respectively, in AS-30D cells and to 3.44, 0.58, 0.41, and 1.38 μmoles/g, respectively, in Novikoff cells. Measurements of adenosine mono-, di-, and triphosphates and of uridine mono-, di-, and triphosphates revealed a strong predominance of the nucleoside triphosphates. The adenylate energy charge amounts to 0.96 and 0.94 in AS-30D and Novikoff cells, respectively; the energy charge of the uridylate system is 0.94 and 0.90, respectively. The relative ratios of adenosine phosphates determined by direct enzymatic analysis are significantly higher than those obtained by chromatographic separation of the nucleotides after labeling with adenosine-8-14C.

The amount of uridine triphosphate in both cell lines is 2.6-fold higher than in liver. The analyses of the UDP-sugar pattern revealed a selective deficiency of UDP-glucose and of UDP-galactose. UDP-glucuronate and UDP-N-acetylhexosamines are in the same range as in rat liver. The extremely low UDP-glucose levels in both hepatoma cell lines may explain the virtual absence of glycogen.

SUMMARY

the controls when AS-30D cells are incubated in the presence of 1 and 2 mM galactosamine, respectively.

INTRODUCTION

Many chemical agents with antitumor activity interfere with the metabolism and concentration of nucleotides of the malignant cell. This has been demonstrated for purine and pyrimidine analogs (13, 17, 40) and for the amino sugar D-glucosamine (6, 7, 9, 36). Quantitative analyses of the nucleotide contents are required for an understanding of the mode of action of several antitumor agents. Furthermore, a comparison of the nucleotide pattern of ascites hepatoma cells reported in this paper with that determined previously in rat liver (20, 25–27) revealed significant differences, particularly in the levels of UDP-sugars.

Nucleotide contents reflecting the in vivo situation have been determined in various tissues (18, 25, 27, 49), including solid hepatomas (48) by application of the freeze-clamp method (51). The lability of nucleotides requires an equivalent technique for determinations in tumor cells growing in suspension. It was an objective of this investigation to establish conditions required for incubation and rapid inactivation of Novikoff (32) and AS-30D (43) ascites hepatoma cells allowing direct enzymatic analyses of their absolute nucleotide contents. Nucleotide pools and the relative distribution of nucleoside phosphates have been studied in detail by Plagemann (33–35) using radioactive labeling of cultured Novikoff rat hepatoma cells (subline N151-67) followed by paper chromatography and electrophoresis.

High concentrations of D-galactosamine have been shown to inhibit viability, transplantability, and incorporation of labeled precursors into macromolecules of Sarcoma 180 and Ehrlich ascites carcinoma cells (4, 5, 44). Furthermore, D-galactosamine is the most efficient sugar analog inducing a selective deficiency of UTP and UDP-hexoses in liver (11, 25, 26), followed by an inhibition of RNA and protein synthesis (2, 11, 25, 39), and leading to hepatocellular necrosis (11, 24). We have therefore used this amino sugar to interfere with the metabolism and concentration of nucleotides in ascites hepatoma cells.

MATERIALS AND METHODS

Chemicals, Enzymes, and Isotopes. Powdered Swim's S-77 medium was purchased from Grand Island Biological
Company, Grand Island, N. Y.; Pluronic F68 (46) was obtained from BASF Wyandotte Corp., Wyandotte, Mich.; D-galactosamine HCl was from C. Roth, Karlsruhe, Germany. UDP-galactose 4-epimerase, lactate dehydrogenase, glucose 6-phosphate dehydrogenase, yeast hexokinase, and all coenzymes required for the enzymatic analyses were purchased from Sigma Chemical Co., St. Louis, Mo., in the highest purity available; all other enzymes used in this investigation were from Boehringer Mannheim Corp., New York, N. Y. Adenosine-8-14C, 52 μCi/μmole, was from Schwarz/Mann, Orangeburg, N. Y.; and UDP-(uniformly labeled glucuronic acid-14C) acid, 275 μCi/μmole, was from International Chemical and Nuclear Corp., Irvine, Calif.

Ascites Hepatoma Cells. The transplantable rat ascites hepatomas AS-30D (43) and Novikoff (32) were obtained from Dr. E. F. Walborg, Jr., University of Texas at Houston, M. D. Anderson Hospital and Tumor Institute, Houston, Texas. Both tumor lines were carried in 7- to 9-week-old female Sprague-Dawley rats (Cherokee Laboratory Supply Company, Atlanta, Ga.). The tumor cells were transplanted at 7-day intervals by i.p. injection of 0.5 ml of ascitic fluid collected under sterile conditions. The AS-30D line (43) was studied from transplant generations 226 to 258.

Standard Medium and Incubation of Cells. Swim’s powdered S-77 medium (9 g/liter) containing amino acids, salts, vitamins, streptomycin, and glucose at a concentration of 5.6 mM, was supplemented as follows: Pluronic F68 (46), 1 g/liter; L-glutamine, 2 mM; L-cystine, 0.06 mM; Na3HPO4, 25 mM (to give a final concentration of 26 mM); NaHCO3, 10 mM. The medium was filtered before addition of NaHCO3, and the pH was adjusted at 37° to 7.40.

Cells were removed from the opened peritoneal cavity under sterile conditions at 6 to 7 days after inoculation and suspended in standard medium. Both cell lines were washed 3 times in 10 volumes of medium by centrifugation at 20 χ g for 8 min at room temperature. The cells were incubated under air in closed Erlenmeyer flasks at 37° on a metabolic shaker. Novikoff cells were suspended at a concentration of 1.4 (± 0.3) χ 106/ml; the AS-30D cell concentration was 2.2 (± 0.4) χ 106/ml. Cell wet weights were determined in triplicate with a variation of less than 2% by weighing the pellet from 10-ml aliquots of the suspension after centrifugation at 750 χ g for 10 min at 4°C. One g of wet cells corresponded to 2.0 χ 106 Novikoff and to 3.0 χ 106 AS-30D cells.

Cooling, Freezing, and Deproteinization of Cells in Suspension. Aliquots of 25 ml of the cell suspension were pipetted into 50-ml polypropylene centrifuge tubes (Ivan Sorvall, Inc., Norwalk, Conn.) while the latter were immersed for 10 sec with their outside into liquid nitrogen. The tubes (preferably 2 at a time) were centrifuged immediately at −20° for 1 min at 750 χ g. The temperature of the supernatant was at 7 ± 1°C when the centrifuge had stopped. The supernatants were poured off without delay, and remaining fluid on the inner wall of the tubes was wiped off. The pellets were frozen by immersion of the tubes into liquid nitrogen; simultaneously, 1.0 ml of 0.8 M perchloric acid was pipetted and frozen on top of the pellet. The tubes were kept at −20° until the homogenization was performed at 4°C with a motor-driven Teflon pestle fitting into the polypropylene tubes. The acidic supernatant after centrifugation at ca. 15,000 χ g for 15 min at 4°C was poured off and neutralized with solid KHCO3.

Enzymatic Analyses of Nucleotides. 14CAMP, 14CGMP, 14SUMP, and 14CMP were measured after a quantitative hydrolysis of the nucleotides by means of snake venom phosphodiesterase yielding 5'-nucleoside monophosphates (19, 27). AMP (1), GMP (15, 29), CMP (19), and UMP (27) were assayed spectrophotometrically. These assays do not discriminate between ribonucleotides and the small percentage of deoxyribonucleotides in cell or tissue extracts. ATP was determined with yeast hexokinase and glucose 6-phosphate dehydrogenase (28). Pyruvate kinase and adenylate kinase were used for the measurements of ADP and AMP, respectively (1). UDP-glucose, UDP-galactose, UTP, UDP, and UMP were assayed with high specificity as described previously (27); UDP-galactose 4-epimerase was used for the analysis of UDP-galactose; an additional assay was performed for the separate determination of UTP and UDP, respectively (22, 23). UDP-glucuronate and UDP-N-acetylhexosamines were measured by means of isotope dilution combined with chromatographic separation and enzymatic assay of the UMP moiety (26).

14C Labeling and Chromatographic Separation of Adenosine Phosphates. Cells were incubated for 60 min in standard medium supplemented with adenosine-8-14C (2 μCi/ml). Aliquots of 1.5 ml were pipetted slowly into 50-ml plastic tubes immersed into liquid nitrogen; thereby, the cell suspension was frozen immediately upon contact with the tube. Perchloric acid (3 ml, 0.5 ml) was frozen on top of the cells. Aliquots of the neutralized supernatant after homogenization and centrifugation were chromatographed on Whatman No. 3MM paper. Adenosine phosphates were separated by ascending chromatography at 4°C for 18 hr in a solvent composed of isobutyric acid/water/concentrated NH4OH, pH 3.6 (66/33/1, by volume). The relative radioactivities of ATP, ADP, and AMP were quantitated on a Berthold-Varian radiochromatogram scanner.

Criteria for Cell Viability. The following criteria for intact cell function and viability under various conditions were used. (a) Leakage of intracellular macromolecules was determined by assaying lactate dehydrogenase activities (8) in the supernatant of cell suspensions centrifuged at 500 χ g for 5 min at 4°C. This activity was compared to the activity of a sonicate prepared from the same cell suspension. (b) Uptake or exclusion of an extracellular dye was determined by the use of trypan blue (5) followed by examination of the cells by phase-contrast microscopy. (c) The energy state of the cells was analyzed by the measurement of contents and relative ratios of ATP, ADP, and AMP, as described in this paper.

The abbreviations used are: 14CAMP, sum of all acid-soluble adenine 5'-nucleotides (including ribonucleotides and deoxyribonucleotides); 14CGMP, sum of acid-soluble guanine 5'-nucleotides; 14SUMP, sum of acid-soluble uracil 5'-nucleotides; 14CMP, sum of acid-soluble cytosine 5'-nucleotides; UDP-N-acetylhexosamines, sum of UDP-N-acetylgalactosamine and UDP-N-acetylgalactosamine.
RESULTS

Selection of Buffer and Incubation Medium. Media described previously for incubation (10) and culture (37) of Novikoff rat hepatoma cells were unsatisfactory for the cell lines used in this study. Incubation in Krebs-Ringer-P, buffer containing 10 mM potassium phosphate (pH 7.5) (10) was associated with a pH drop to values below 7.0 within 50 min. The medium BM 42 (37) consistently lead to an aggregation, particularly of Novikoff cells, caused by calcium ions at a concentration of 2 mM. The phosphate-bicarbonate buffer of our standard medium was replaced by the following buffers: N-2-hydroxyethylpiperazine-N-2'-ethanesulfonic acid (35 mM); Tris (35 mM); sodium bicarbonate (26 mM); sodium phosphate (35 mM); triethanolamine (35 mM). At 60 min of incubation all buffers maintained the pH between 7.35 and 7.50, and both Novikoff and AS-30D cells showed a trypan blue uptake by less than 4% of the cells. However, the ATP content and the ATP/ADP ratio were markedly lower in both cell lines using the nonphysiological buffers. This difference was most pronounced for Novikoff cells where the adenosine phosphate pools and the ATP/ADP ratios were lower than 55 and 25%, respectively, as compared to the values in standard medium (Table 1). The buffer of the standard medium was also superior when it was replaced by either phosphate or bicarbonate, if adenosine phosphate contents and ratios were used as criteria. We were unable, however, to differentiate between these 3 buffers during the initial 2 hr of incubation by trypan blue uptake or by lactate dehydrogenase leakage. Among the 3 viability criteria used under various conditions, trypan blue had the lowest and adenosine phosphate contents and ratios had the highest sensitivity to detect both reversible and irreversible cell damage.

Adenine Nucleotide Contents and Ratios. ΣAMP was measured specifically as 5'-AMP after venom phosphodiesterase hydrolysis and amounted to 3.74 and 3.44 μmoles/g of wet cells in AS-30D and Novikoff hepatoma cells, respectively (Table 2). A similar value, 3.90, has been measured for ΣAMP in rat liver (27). In contrast to liver, however, the pattern of adenine nucleotides was different in ascites hepatoma cells and characterized by a pronounced predominance of ATP. This was in accordance with the remarkably high ATP/ADP ratios and adenylate energy charges in both tumor lines (Table 1) as compared to liver where ATP/ADP amounts to 3.36 (20) and the energy charge to 0.81 (20). These high ratios in ascites hepatoma cells were also observed when the phosphate concentration of the medium was lowered to 6 mM. The time course of nucleotide contents during incubation is shown on Chart 1.

The adenosine phosphate pattern found by direct enzymatic analysis (Table 1) was compared with the pattern obtained by labeling of AS-30D cells for 60 min with adenosine-8-14C as described under "Materials and Methods." The chromatogram was developed at 4° since consist
ently lower ATP/ADP ratios were observed when the chromatography was performed at 25° in the same solvent. The following mean values for relative radioactivities ± S.D. were measured in 6 extracts: ATP, 4.7 ± 0.65; ADP, 1.0 ± 0.4; AMP, 0.5 ± 0.07; the adenylate energy charge was 0.84. The low pH required for the chromatographic separation of adenosine phosphates should be considered as a factor contributing to a degradation of ATP even at 4°.

The commonly used labeling and chromatography of adenine phosphates indicated significantly lower ATP/ADP ratios and adenylate energy charges when compared to those obtained by direct enzymatic analysis (Table 1; Chart 1).

Guanine and Cytosine 5'-Nucleotides. The sum of all acid-soluble guanine and cytosine 5'-nucleotides, respectively (Table 2), was in the same range as the contents measured in liver (19, 25, 27). Higher nucleotide contents were observed in AS-30D cells as compared to Novikoff cells (Table 2). The sum of acid-soluble adenine, guanine, cytosine, and uracil 5'-nucleotides was 6.70 and 5.81 μmoles/g of cell, wet weight, in AS-30D and Novikoff hepatoma cells, respectively, as compared to a value of 5.86 μmoles/g of liver (19).

Uracil Nucleotide Contents. The virtually complete pattern of uridine phosphates and UDP-sugars has been determined for the 1st time in AS-30D and Novikoff ascites hepatoma cells (Table 3). Contents and ratios of uracil nucleotides were similar in both cell lines. However, the comparison with rat liver revealed highly significant differences (Table 3). The UTP levels were 2.6 times higher than in liver and also higher than in any other mammalian tissue studied thus far (27). High UTP/UDP ratios and high energy charges of the uridylicate system were observed in both hepatoma cell lines (Table 3). This corresponded to the adenosine phosphate pattern given in Table 1.

Analyses of the UDP-sugar levels revealed a selective deficiency of UDP-glucose and UDP-galactose in both cell lines. As shown in Table 3, UDP-glucose was depressed to 16 and 13% as compared to liver in AS-30D and Novikoff ascites hepatoma cells, respectively. By contrast, UDP-glucuronate and UDP-N-acetylhexosamine contents were in the same range as determined in liver. Only the latter observation established the selectivity of the UDP-hexose deficiency.

Effect of Uridine. AS-30D cells were incubated for 2 hr in the presence of 1 mM uridine (Chart 2). The content of each of the uracil nucleotides was almost doubled. The experiment failed, however, both in AS-30D and in Novikoff cells to elevate the UDP-glucose or UDP-galactose level to a range comparable to the hepatonic one. No significant changes in the relative distribution of uracil nucleotides were observed, although the ∑UMP increased from 1.5 to 2.9 μmoles/g of AS-30D cells during 2 hr. The pronounced absolute increase of UTP, UDP-N-acetylhexosamines, and UDP-glucuronate was associated with a minor decrease of adenosine phosphates by less than 10%.

Galactosamine-induced Changes of Nucleotide Contents. Galactosamine was metabolized by both hepatoma cell lines causing a concentration-dependent depletion of the uridine phosphate pools. The same depression of uridine phosphates, however, was observed at an at least 2-fold lower galactosamine concentration in AS-30D cells as compared to Novikoff cells. The effect of the amino sugar on AS-30D cells was therefore studied more extensively. As shown in Chart 3, there was only a minimal depression in the amount of ∑UMP. However, the UTP content was depressed to 30 and 15% of the controls in the presence of 1 and 2 mM galactosamine, respectively (Chart 3). The changes of the nucleotide pattern induced by galactosamine occurred rapidly, and no major differences were observed whether the nucleotide contents were analyzed at 35 (Chart 3) or at 65 min of incubation. The depletion of UTP was associated with a depression of UDP-glucose and UDP-galactose to about 60% as compared to the controls in the presence of 1 and 2 mM galactosamine.

In contrast to rat liver, in which galactosamine interferes very selectively with uracil nucleotide metabolism (21, 23), a concomitant depression of adenosine and guanine nucleotides was observed in the ascites hepatoma cells. The ∑AMP and

<table>
<thead>
<tr>
<th>Uracil nucleotide measured</th>
<th>AS-30D cells</th>
<th>Novikoff cells</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>UTP</td>
<td>0.66 ± 0.05a (12)a</td>
<td>0.68 ± 0.06 (9)</td>
<td>0.26 ± 0.04 (18)</td>
</tr>
<tr>
<td>UDP</td>
<td>0.07 ± 0.01 (12)</td>
<td>0.08 ± 0.03 (8)</td>
<td>0.06 ± 0.01 (18)</td>
</tr>
<tr>
<td>UMP</td>
<td>0.023 ± 0.008 (8)</td>
<td>0.04 ± 0.01 (12)</td>
<td>0.03 ± 0.01 (18)</td>
</tr>
<tr>
<td>UTP/UDP</td>
<td>9.65 ± 1.26 (8)</td>
<td>9.4 ± 3.3 (8)</td>
<td>4.4 ± 0.5 (18)</td>
</tr>
<tr>
<td>E.C.,</td>
<td>0.940 ± 0.007 (8)</td>
<td>0.904 ± 0.015 (8)</td>
<td>0.830 ± 0.015 (18)</td>
</tr>
<tr>
<td>UDP-glucuronate</td>
<td>0.30 ± 0.08 (4)</td>
<td>0.26 ± 0.08 (3)</td>
<td>0.28 ± 0.06 (7)</td>
</tr>
<tr>
<td>UDP-N-acetylhexosamines</td>
<td>0.39 ± 0.11 (4)</td>
<td>0.34 ± 0.07 (6)</td>
<td>0.32 ± 0.12 (6)</td>
</tr>
<tr>
<td>UDP-glucose</td>
<td>0.052 ± 0.010 (20)</td>
<td>0.041 ± 0.005 (12)</td>
<td>0.32 ± 0.04 (65)</td>
</tr>
<tr>
<td>UDP-galactose</td>
<td>0.017 ± 0.002 (16)</td>
<td>0.016 ± 0.001 (10)</td>
<td>0.09 ± 0.01 (19)</td>
</tr>
</tbody>
</table>

a Mean ± S.D.

a Numbers in parentheses, number of cell extracts analyzed.

E.C., energy charge (3) of the uridylicate system.

The nucleotide contents of freeze-clamped rat livers are data from previous work (25-27).
UDP-glucose 0 30 60 90 120
INCUBATION TIME (min)

Chart 2. Effect of uridine on UTP and UDP-glucose contents of AS-30D cells; 2.5 x 10^6 cells/ml were incubated in standard medium at 37°C without additions (■, △) or in the presence of 1 mM uridine (□, ▽). Duplicate samples from each suspension were removed for analysis at the times indicated; the nucleotide contents of duplicate samples differed by less than 10%.

ΣGMP decreased to 72% of the control values given in Table 2, when AS-30D cells were incubated for 65 min in the presence of 1 mM galactosamine. The concentration-dependent loss of purine nucleotides was associated with a drop of the ATP/ADP ratio (Chart 3). Accordingly, the adenylate energy charge decreased from 0.96 to 0.72 when the cells were incubated in the presence of 5 mM galactosamine as described in the legend to Chart 3. This lowering of the phosphorylation potential, however, is only a minor contributing factor to the induction of UTP deficiency, since the sum of UTP + UDP + UMP was strongly depleted as well (Chart 3).

DISCUSSION

The procedure for defined cooling, freezing, and deproteinization described in this study for ascites hepatoma cells can be applied to a variety of tumor cells in suspension. This technique allows in addition a direct spectrophotometric analysis of nucleotides. High ATP/ADP ratios and energy charges in the same range as described for AS-30D and Novikoff ascites cells were also observed in Ehrlich ascites tumor cells (D. Keppler, unpublished studies). Skeletal muscle, to our knowledge, is the only nonmalignant tissue with a comparable predominance of ATP; an ATP/ADP ratio of 12 and an adenylate energy charge of 0.96 can be calculated for this tissue (16). The high energy charge of the adenylate system in the hepatoma cells corresponds to a high energy charge of uridine phosphates (Table 3). The ratios and energy charges of adenosine and uridine phosphates are not identical (Tables 1 and 3). This may be related to the existence of different sizes and ratios of ribonucleoside phosphate pools in ascites hepatoma cells. Studies by Plagemann (34, 35) indicated independent nuclear and cytoplasmic ribonucleoside phosphate pools in cultured Novikoff rat hepatoma cells. Such a compartmentalization of nucleotides complicates a calculation of cellular nucleotide concentrations; we therefore prefer the term nucleotide content (18).

The distinct alteration of the sugar nucleotide pattern in AS-30D and in Novikoff ascites hepatoma cells, as compared to liver, is the 1st indication of a possible new mechanism responsible for changes in the composition and structure of carbohydrate-containing macromolecules of these tumor cells. Winzler (50) has discussed the hypothesis that the concentrations of sugar nucleotides, in addition to the relative activities of sugar transferases, are a determining factor for the structure of oligosaccharides in mucins and glycoproteins. This hypothesis may be further investigated by a comparison of the carbohydrate composition and structure of cell surface glycopeptides (41, 42, 47) and the sugar nucleotide pattern in various ascites hepatoma cell lines.

Most experimental hepatomas, including Novikoff ascites hepatoma cells, have an extremely low content of glycogen (30–32, 38, 45). The deficiency of UDP-glucose demonstrated in this study (Table 3; Chart 2) may well serve as a possible explanation. As a proof, however, the UDP-glucose content in glycogen-rich hepatomas (38) must be determined. A loss of rat liver glycogen to less than 5% of the controls has been observed when the UDP-glucose content was decreased to 0.05 μmole/g of liver by means of galactosamine (20).

Galactosamine induced a marked change in the distribu-
tion of uracil nucleotides in AS-30D and Novikoff ascites hepatoma cells. The extent and time course of the depletion of UTP was very similar when AS-30D cells were incubated for 35 min in the presence of 2 mM galactosamine (Chart 3) or when isolated rat livers were perfused for the same time with the same concentration of this galactose analog (21). The trapping of uridine phosphate has been shown in liver as a consequence of the rapid accumulation of UDP-amino sugars derived from galactosamine (12, 25, 26). These UDP-amino sugars comprise the major portion of the UDP under condition of galactosamine-induced UTP deficiency (25, 26).

The high selectivity of hepatic UTP deficiency (25) was not observed in hepatoma cells (Chart 3). The extent and time course of the depletion of total uracil nucleotides when AS-30D cells were incubated in the presence of galactosamine (Chart 3) was very similar when AS-30D cells were incubated with the same concentration of this galactose analog (21).

A rapid phosphorylation of galactosamine by hepatoma cells apparently leads to a metabolic response pattern that has been described in liver as a result of an excess trapping of phosphate and is caused by the rapid phosphorylization of, e.g., fructose or 2-deoxy-D-glucose (for review see Ref. 14). A rapid phosphorylization of galactosamine by hepatoma cells apparently leads to a similar response pattern. However, there was no significant loss of total uracil nucleotides when AS-30D cells were incubated in the presence of galactosamine (Chart 3). This indicated that the deficiency of UTP results almost exclusively from uridine phosphate trapping. This is in contrast to the effects of glucosamine on cultured Novikoff hepatoma cells, where a rapid degradation of both uracil and adenine nucleotides has been observed (36).

Analogous to liver (11, 25, 39), galactosamine may be useful as an efficient inhibitor of UTP-dependent macromolecular syntheses in ascites hepatoma cells.

REFERENCES


29. Meich, R. P., and Parks, R. E., Jr. Adenosine Triphosphate: Guanosine Monophosphate Phosphotransferase. Partial Purification and...
Nucleotide Contents of Ascites Hepatoma Cells


Nucleotide Contents of Ascites Hepatoma Cells and Their Changes Induced by d-Galactosamine

Dietrich O. R. Keppler and David F. Smith

Cancer Res 1974;34:705-711.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/34/4/705

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.