Pattern of methionine adenosyltransferase isoenzyme expression during rat liver regeneration after partial hepatectomy

Laura M. Frago[a], América Giménez[b], Elías Nelson Rodriguez[a], Isabel Varela-Nieto[a,*]

[a] *Instituto de Investigaciones Biomédicas, Consejo Superior de Investigaciones Científicas (CSIC), Arturo Duperier 4, 28029 Madrid, Spain*
[b] *Liver Unit, Fundacio Clinic, Universitat de Barcelona, 08036 Barcelona, Spain*

Received 11 March 1998

Abstract The present report investigates the pattern of expression of liver-specific and extrahepatic methionine adenosyltransferase (MAT) isoenzymes in regenerating rat liver after partial hepatectomy. The results show that there is a switch in the expression of these isoenzymes that is coincident with maximal cell proliferation in the remaining liver lobes. Extrahepatic MAT levels increase about three times 4 h after hepatectomy, reaching a maximum 36 h later. This is accompanied by a rapid and transient increase in total MAT activity and levels of related metabolites *S*-adenosyl-L-methionine and *S*-adenosyl-L-homocysteine. Liver-specific MAT levels are reversibly down-regulated within 24–48 h post surgery. Our results indicate that MAT isoenzyme expression is tightly regulated during liver regeneration after two-third hepatectomy. The implications of these observations for evaluation of the degree of liver regeneration are briefly discussed.

Key words: Methionine adenosyltransferase; Partial hepatectomy; Proliferative cell nuclear antigen; *S*-Adenosyl-L-methionine; Liver regeneration

1. Introduction

The liver is one of few adult quiescent tissues that retains the capacity to regenerate. Adult liver cells can replicate rapidly, thus allowing the liver to recover from toxic-induced damage and to regenerate in a few days after surgical procedures that remove most of its mass [1]. A considerable effort has been devoted to the search for molecular markers of this process to survey liver damage after chronic liver dysfunction caused by cirrhosis and to evaluate the degree of liver regeneration in patients after partial hepatectomy for liver cancer (for recent reviews see [1–3]).

S-Adenosyl-L-methionine (AdoMet) is the major donor of methyl groups in transmethylation reactions and a pivotal metabolite for methionine breakdown and re-synthesis, a key metabolic crossroad of the liver [4]. Methionine adenosyltransferase (MAT) is the enzyme that catalyses AdoMet synthesis from methionine and ATP [5]. AdoMet can be then metabolised to *S*-adenosyl-L-homocysteine (AdoHcy) which will be finally reconverted into methionine. This set of metabolic reactions is known as the methylation cycle. Alterations in the ratio AdoMet:AdoHcy have been associated with a number of liver pathologies (see for example [4,6]). Indeed, AdoMet has been used in the therapy of cholestatic chronic liver disease [7,8], since AdoMet treatment prevents hepatotoxicity and liver necrosis caused by thioacetamide, acetaminophen or carbon tetrachloride among others in animal models [4,9–12].

Mammalian MAT exists as two isoenzymes, the liver-specific and the extrahepatic type, designated MAT I/III and MAT II respectively [13]. MAT isoenzymes exhibit a differential expression pattern during rat liver development [14]. Liver-specific MAT I/III is upregulated rapidly after birth whilst extrahepatic MAT II is highly expressed during foetal life and is downregulated postnatally, its expression being almost negligible in the adult liver. This distinct expression pattern is typical of liver-specific proteins such as albumin, α-fetoprotein or glucokinase versus hexokinase [1,15,16]. Therefore, MAT I/III has been proposed to be a marker for liver differentiation [14]. On the other hand, MAT II has been reported to be expressed in rat and human hepatoma cells [17]. Hence, the suggestion was that MAT II expression should be upregulated in highly proliferative hepatic cell types and during liver growth.

In the present work, we have studied the pattern of expression of both MAT isoenzymes and measured total MAT activity and levels of AdoMet and in the regenerating liver. We found that extrahepatic MAT is rapidly and highly induced within 3 h after partial hepatectomy. Concomitantly there is an increase in total MAT activity and levels of AdoMet and AdoHcy. Liver-specific MAT mRNA and protein levels decreased coinciding with the peak of cell proliferation. Our data indicate that there is a switch in MAT isoenzyme expression during liver regeneration that may provide a means of measuring the degree of the regenerative response in clinical situations.

2. Materials and methods

2.1. Animals and partial hepatectomies

Male Sprague-Dawley rats weighing 200–250 g were used for all experiments. The animals were treated according to the European Community laws for animal care. Rats were maintained on a 12 h light/12 h darkness schedule. Food and water were provided ad libitum, but the rats were fasted overnight before experiments. Partial hepatectomy was carried out according to the procedure of Higgins and Anderson [18], by which the median and left lateral lobes of the liver (two-thirds of liver mass) were removed. Sham-operated rats were subjected to midventral laparotomy with minimal trauma to the liver. Surgery was carried out with the rats under ether anaesthesia. Animals were allowed to recover and at the times indicated the remaining liver lobes were surgically removed, immediately frozen in liquid nitrogen and stored at −70°C until used.

2.2. Immunoblotting

For Western blotting, rat liver samples of approximately 100 mg were homogenised in homogenisation buffer: 10 mM Tris-HCl pH 7.5, 0.3 M sucrose, 0.1 mM EGTA pH 7.5 with protease inhibitors.

*Corresponding author. Fax: (34) (1) 585-4587.
E-mail: ivarela@iib.uam.es

Abbreviations: MAT, methionine adenosyltransferase; AdoMet, *S*-adenosyl-L-methionine; AdoHcy, *S*-adenosyl-L-homocysteine; PCNA, proliferative cell nuclear antigen

PII S0014-5793(98)00360-3

Afterwards the samples were centrifuged and the supernatants were collected to measure protein concentration. 10 μg of protein was resolved using 10% SDS-PAGE before being electrophoretically transferred onto PVDF membranes (Dupont-NEN, Boston, MA, USA). Filters were blocked with Tris-buffered saline containing 5% (w/v) non-fat dried milk, and incubated with the primary specific antibody. Filters were subsequently washed and incubated with the corresponding secondary antibody conjugated with peroxidase. Bound peroxidase activity was visualised by chemiluminescence (Dupont-NEN, Boston, MA, USA) and quantified by densitometry.

Rabbit polyclonal antibody to rat MAT I/III was a generous gift from Dr. Maria A. Pajares (IIB-CSIC, Madrid, Spain) (1:10 000 dilution), which specifically recognises the liver-specific isoenzyme without cross-reactivity with the extrahepatic MAT [14]. The proliferative cell nuclear antigen (PCNA) is a nuclear protein of 36 kDa associated with the cell cycle and is essential for DNA synthesis [19]. Mouse monoclonal anti-PCNA was from ATOM (ATOM, Barcelona, Spain, 1:2000 dilution). Secondary antibodies conjugated with peroxidase were purchased from Bio-Rad (Richmond, CA, USA). Salts, organic solvents and other reagents were of analytic grade.

2.3. RNA extraction and Northern analysis

RNA from hepatectomised or sham-operated rat liver samples was isolated by acid guanidinium thiocyanate-phenol-chloroform extraction [20]. Total RNA (20 μg per sample) was separated by 1.2% agarose electrophoresis under denaturing conditions (1.1 M formaldehyde and MOPS buffer) and transferred to nitrocellulose filters (Schleicher and Schuell, Keene, NH, USA). Prehybridisation and hybridisation of the membranes were carried out under moderate stringency conditions (50% formamide at 42°C) for 5 and 24 h respectively. Filters were hybridised with an *Eco*RI fragment of plasmid pSSRL, containing 2.2 kbp cDNA for rat MAT I/III [21], or 1.1 kbp for rat MAT II [14] and a cDNA for 18S RNA as loading control. MAT and 18S probes were a generous gift from Drs. Luis Alvarez (IIB-CSIC, Madrid, Spain) and Isabel Fabregat (Department of Biochemistry, Facultad de Farmacia, Universidad Complutense, 28040 Madrid, Spain) respectively. Probes were labelled with [α-^{32}P]dCTP by the random priming method (specific activity 5–10×10^8 cpm/mg DNA) according to the manufacturer's instructions (Amersham International, Amersham, UK). Blots hybridised with the MAT I/III probe were exposed overnight at −70°C, whereas for MAT II detection blot hybridisation exposure was 5 days at −70°C. Hyperfilm MP films (Amersham) were used in both cases. For quantification we analysed scanned images of the obtained Northern blots using the program NIH Image 1.59 on Macintosh computers. The relative intensities of the corresponding bands were then compared.

2.4. Determination of MAT activity

MAT activity was determinate as described [22]. Briefly, frozen liver portions of approximately 100 mg were homogenised in cold 10 mM Tris-HCl pH 7.5, containing 0.3 M sucrose, 0.1% β-mercaptoethanol, 1 mM benzamidine, 0.1 mM PMSF, 10 μg/ml soybean trypsin inhibitor. The homogenate was centrifuged ($10\,000\times g$, 20 min) and the resulting supernatant was centrifuged again ($100\,000\times g$, 60 min). Samples of 160 μl were incubated with 90 μl of a reaction mixture containing 75 mM Tris-HCl (pH 8.0), 250 mM KCl, 9 mM $MgCl_2$, 10 mM dithiothreitol, 5 mM methionine, and 5 mM [2-^{3}H]ATP (4 Ci/mol). The incubation was carried out for 30 min at 37°C and was stopped by the addition of 4–5 ml distilled water. The samples were immediately loaded on 1 ml cation exchanger AG 50W-X4 columns (Bio-Rad, Richmond, CA, USA) equilibrated in water. Columns were washed with water (20 ml), and [^{3}H]AdoMet was then eluted with 4 ml of 3 M ammonium hydroxide. Radioactivity was determined in the presence of 1 ml glacial acetic acid and 10 ml scintillation liquid (Optiphase Hisafe 3, Pharmacia, Uppsala, Sweden).

2.5. Determination of AdoMet, AdoHcy and ATP

AdoMet and AdoHcy levels were measured as in [11]. ATP concentration was determined by the method of Lowry and Passonneau [23]. Total protein was measured using Bradford reagent (Bio-Rad, Richmond, CA, USA).

2.6. Statistical analysis

When indicated, data are shown as mean ± S.E.M. of at least four experiments. Statistical significance was estimated by ANOVA for multiple matched unpaired groups. A P value <0.05 was considered significant.

3. Results and discussion

Liver regeneration could be considered a paradigm of controlled tissue growth in contrast to carcinogenesis. In this study we approached the question of which mechanisms allow the liver to grow while maintaining differentiated functions by studying MAT isoenzyme expression and activity after partial hepatectomy. Extrahepatic MAT II mRNA expression increase is already visible 3 h after partial hepatectomy (2.4 ± 0.5-fold increase, $n=4$), with the maximum level of expression at time intervals of 12–36 h (3 ± 0.3-fold increase, $n=4$) (Fig. 1, filled symbols). MAT II expression started to decrease after 36 h and equalled 0 h levels 72 h later, then its expression was maintained for the following 14 days, the latest time studied (data not shown). The observed values of MAT II levels in the sham-operated groups at the different times were similar to those observed at time 0 h (Fig. 1, open symbols). MAT II expression in the adult normal liver is almost negligible [14]. Hence, MAT II expression was gradually increased in the beginning, remained highly expressed, and presented a plateau at the expected time of maximal liver regeneration activity. The time kinetics of DNA synthesis during liver regeneration have been reported [1,24] and MAT II pattern of expression seemed to correlate well with cellular proliferation. To assess hepatocyte proliferation we used Western blot analysis of PCNA levels (Fig. 2). There is a good correlation between the levels of the nuclear protein PCNA and the rates of cellular proliferation and DNA synthesis [25]. PCNA immunostaining of liver sections has been used as a cell proliferation index in the evaluation of the rate of liver regeneration [24,26]. PCNA protein levels increased up to 8.8 ± 3.2-fold ($n=4$) 24 h after two-third hepatectomy as

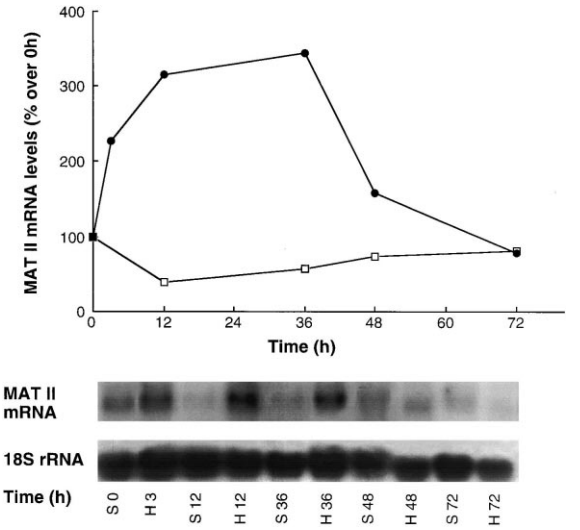

Fig. 1. MAT II mRNA expression in the regenerating liver after partial hepatectomy. Northern blot analysis was performed at different times post operation. Filter was hybridised sequentially to a ^{32}P-labelled extrahepatic MAT II cDNA and an 18S ribosomal RNA probe. Corrected densitometric quantitation is shown. Values are expressed as percent of value at time 0. Filled circles represent values at the indicated times post hepatectomy and open squares the values after sham operations. A representative experiment of four is shown. S indicates sham-operated and H hepatectomy.

compared with the sham-operated rat groups which exhibited no changes in the profile of PCNA levels with respect to post-operation time. Therefore, MAT II mRNA increase precedes and accompanies the peak of cellular proliferation.

Liver-specific MAT mRNA levels are shown in Fig. 3. An abrupt decrease was observed at the time interval 24–36 h (2.6 ± 0.1-fold decrease, $n = 4$). Then MAT I/III mRNA levels recovered and reached normal levels 48–72 h post hepatectomy (Fig. 3, filled symbols). Basal levels maintained for the following 14 days, the latest time point tested (data not shown). Sham-operated rats exhibited a sustained high expression of the liver-specific MAT at all the times studied (Fig. 3, open symbols). Liver-specific MAT immunoreactivity was detected by Western blot analysis in cytosolic protein extracts prepared from aliquots of the same liver samples that have been used for Northern blotting (Fig. 3). MAT I/III levels remained constant in sham-operated animals whilst there was a clear decrease in the regenerating liver. Representative time points are shown in the lower panel of Fig. 3. Taken altogether our data indicate that coinciding with maximal cell proliferation there is a switch in the MAT isoform expressed in the regenerating liver. Concerning the mechanisms that may operate to induce MAT isoenzyme switch during liver regeneration, it has been reported that the balance between cell proliferation and differentiation is strictly regulated in a time-dependent process by a pool of cytokines, growth factors and hormones [1,27]. In this context, it is worth noting that MAT I/III expression is modulated by several of these extracelluar factors [14,28–30], and within the promoter of the liver-specific gene there are cytokine-responsive elements [31].

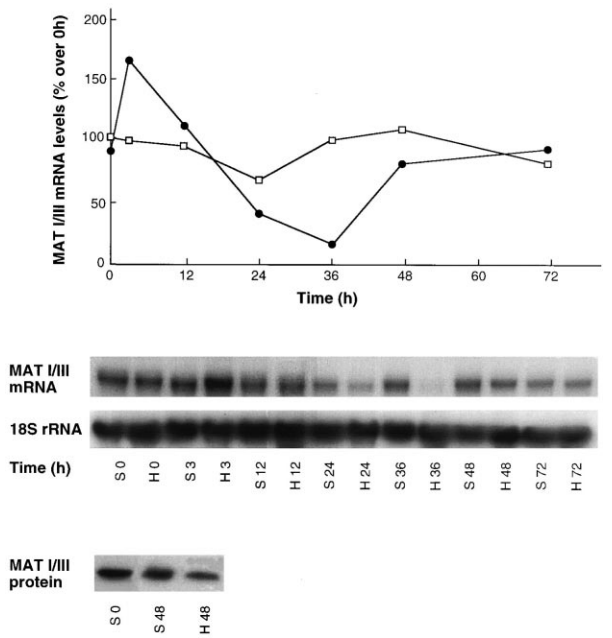

Fig. 3. MAT I/III mRNA and protein levels in the regenerating liver after partial hepatectomy. Northern blot analysis was performed at different times post operation. Filter was hybridised to a ^{32}P-labelled hepatic MAT I/III cDNA or to an 18S ribosomal RNA probe. A corrected densitometric quantitation is shown in the upper panel. Values are expressed as percent of value at time 0. Filled symbols represent values at the indicated times post hepatectomy and empty squares represent values obtained in sham-operated liver samples. The lower row shows a Western blot analysis of MAT I/III levels. S indicates sham operation and H partial hepatectomy. A representative experiment of four is shown.

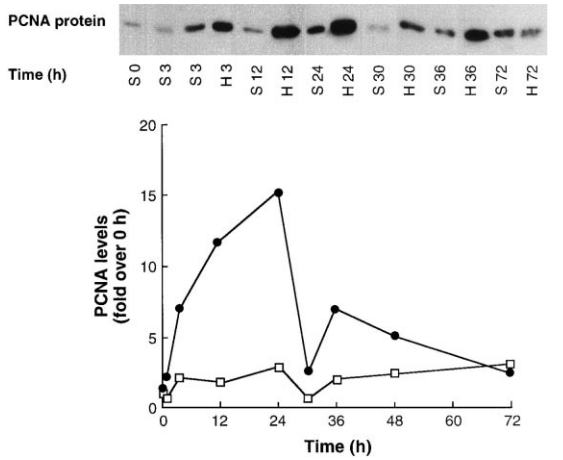

Fig. 2. PCNA protein levels in the regenerating liver after partial hepatectomy. Western blot analysis of PCNA levels. S indicates sham operation and H partial hepatectomy. A corrected densitometric quantitation is shown in the lower panel. Values are expressed as fold over time 0. Filled circles represent values at indicated times post hepatectomy and empty squares the values after sham operations. A representative experiment of four is shown.

Measurement of total MAT activity indicates that there is a stimulation (from 157 ± 9.6 to 366 ± 20 pmol/min/mg protein, $P < 0.05$) 3 h post hepatectomy followed by a slow decay to equal basal levels 72 h later (Fig. 4A). Basal levels of activity are maintained up to 14 days later, when the regeneration process has been completed. These results correlate well with the observed pattern of MAT isoenzyme expression (Figs. 1 and 3). At shorter times MAT II expression has already increased and still MAT I/III levels are maintained. Therefore, total MAT activity is increased as an earlier response to liver trauma. The next step was to determine AdoMet and AdoHcy levels. As can be observed in Fig. 4B,C, the levels of both metabolites are increased with the same time-dependent evolution as observed for MAT activity. AdoHcy levels increased from 0.07 ± 0.01 to 0.15 ± 0.05 ng/mg protein, $P < 0.05$, and AdoMet levels increased from 0.20 ± 0.05 to 0.37 ± 0.02 ng/mg protein, $P < 0.05$, 3 h post hepatectomy. The levels of ATP, a AdoMet precursor, were measured in parallel and no significant variation could be observed (data not shown), ruling out the possibility that changes in AdoMet levels were a consequence of ATP availability. Despite the changes observed in both metabolites the ratio AdoMet:AdoHcy, the methylation ratio, did not change and was around a value of 3 in all the groups studied, whether hepatectomised or sham-operated. This suggests that during liver regeneration following partial hepatectomy no impairment in the availability of the methyl groups required for lipid, DNA and protein methylation should be expected.

Our results indicate that the regulation of the pattern of expression and activity of MAT isoenzymes is strictly regulated during liver regeneration. The capacity of adult liver cells to proliferate after hepatectomy is singular in that the hepatocytes at the same time support the essential liver functions with minimal disturbances [1]. Our data indicate that the regulation of AdoMet metabolism and MAT expression fulfils this goal, since they suffer profound changes but the essential parameter for a normal homeostasis, the methylation ratio, is kept constant along the process. A switch from adult liver-type isoforms to other isoform patterns corresponding to a

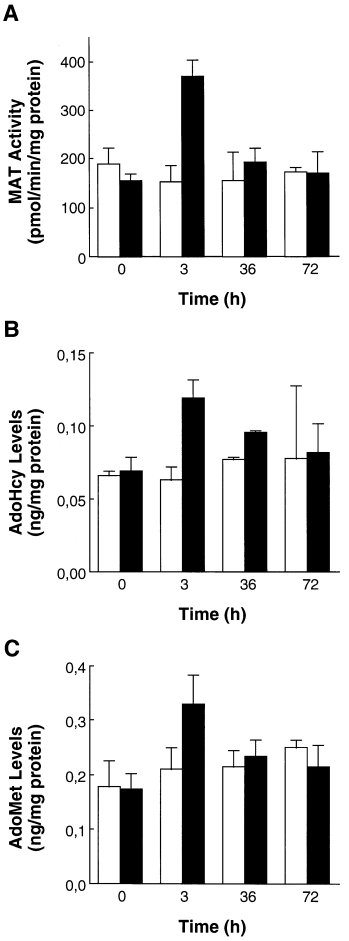

Fig. 4. MAT activity and levels of AdoMet and AdoHcy after partial hepatectomy. A: MAT activity was assayed at saturating concentrations of the substrates 5 mM methionine and 5 mM [^{3}H]ATP. A representative experiment of four is shown. Values are expressed as the mean ± S.E.M. of three different samples measured in each group. B, C: AdoMet and AdoHcy levels were analysed by HPLC. Values are expressed as the mean ± S.E.M. of four different experiments. Open columns indicate sham-operated rat samples and black columns regenerating rat liver samples after partial hepatectomy.

less differentiated stage has been described during hepatic regeneration after hepatectomy. Extrahepatic MAT expression is almost absent in adult normal liver, whilst it is upregulated in states of activated cellular proliferation including foetal liver [14], liver regeneration (this work) and hepatoma cells [17]. On the other hand, liver-specific MAT expression is characteristic of adult liver and it is downregulated in proliferative situations. Our data confirm that MAT I/III is a molecular marker of liver differentiation. In conclusion, relative levels of MAT isoenzymes could be taken as a functional index to evaluate the degree of regeneration in the rat liver after partial hepatectomy.

Acknowledgements: The authors are grateful to Estefanía Fernández (Finnova, Comunidad de Madrid) for technical assistance. Laura M. Frago is a fellow of Europharma (Boehringer Ingelheim group), América Giménez and Nelson Rodriguez were supported by research contracts from the Ministerio de Educación and the Ministerio de Asuntos Exteriores, Spain, respectively. We are also grateful to Drs. Pablo Ortiz and Luis Alvarez for the critical reading of the manuscript and helpful discussion. We also thank J.M. Mato's laboratory for the facilities provided. This study was supported by grants from the Dirección General de Investigación, Ciencia y Tecnología (PM96-0075) and Europharma (Boehringer Ingelheim group).

References

[1] Michaelopoulos, G.K. and DeFrances, M.C. (1997) Science 276, 60–66.
[2] Steer, C.J. (1995) FASEB J. 9, 1396–1400.
[3] Fausto, N. and Webber, E.M. (1994) in The Liver: Biology and Pathobiology (Arias, I.M., Boyer, J.L., Fausto, N., Jakoby, W.B., Schachter, D.A. and Shafritz, D.A., Eds.), pp. 1059–1084, Raven Press, New York.
[4] Mato, J.M., Alvarez, L., Corrales, F.J. and Pajares, M.A. (1994) in The Liver: Biology and Pathobiology (Arias, I.M., Boyer, J.L., Fausto, N., Jakoby, W.B., Schachter, D.A. and Shafritz, D.A., Eds.), pp. 461–470, Raven Press, New York.
[5] Cantoni, G.L. (1975) Annu. Rev. Biochem. 44, 435–441.
[6] Garcea, R., Daino, L., Pascale, R., Simile, M.M., Puddu, M., Ruggiu, M.E., Seddaiu, M.A., Satta, G., Sequenza, J. and Feo, F. (1989) Carcinogenesis 10, 1183–1192.
[7] Cacciatore, L., Varriale, A., Cozzolino, G., Dionisio, M., LeGrazie, C. and Bortolini, M. (1989) Acta Ther. 15, 363–371.
[8] Frezza, M., Surrenti, C., Manzillo, G., Fiaccadori, F., Bortolini, M. and Di Padova, C. (1990) Gastroenterology 99, 211–215.
[9] Mesa, M.L., Carrizosa, R., Martínez-Honduvilla, C., Benito, M. and Fabregat, I. (1996) Hepatology 23, 600–606.
[10] Lieber, C.S., Casini, A., De Carli, L.M., Iowe, N., Sasaki, R. and Leo, M.A. (1990) Hepatology 11, 165–172.
[11] Varela-Moreiras, G., Alonso-Aperte, E., Rubio, M., Gassó, M., Deolofeau, R., Alvarez, L., Caballería, J., Rodés, J. and Mato, J.M. (1995) Hepatology 22, 1310–1315.
[12] Friedel, H.A., Goa, K.L. and Benfield, P. (1989) Drugs 38, 389–416.
[13] Kotb, M., Mudd, S.H., Mato, J.M., Geller, A.M., Kredich, N.M., Chou, J.Y. and Cantoni, G.L. (1997) Trends Genet. 13, 51–52.
[14] Gil, B., Casado, M., Pajares, M.A., Boscá, L., Mato, J.M., Martín-Sanz, P. and Alvarez, L. (1996) Hepatology 24, 876–881.
[15] Bonney, R.J., Hopkins, H.A., Walker, P.R. and Potter, V.R. (1973) Biochem. J. 136, 115–124.
[16] Tsanev, R. (1975) in: Results and Problems in Cell Differentiation (Reiner, J. and Holzer, H., Eds.), p. 197, Springer-Verlag, Berlin.
[17] Cai, J., Sun, W.M., Hwang, J.J., Stain, S.C. and Lu, S.C. (1996) Hepatology 24, 1090–1097.
[18] Higgins, G.M. and Anderson, R.M. (1931) Arch. Pathol. 12, 186–201.
[19] Bravo, R. and MacDonald-Bravo, H. (1987) J. Cell Biol. 105, 1549–1554.
[20] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[21] Alvarez, L., Asunción, M., Corrales, F., Pajares, M.A. and Mato, J.M. (1991) FEBS Lett. 290, 142–146.
[22] Martin-Duce, A., Ortiz, P., Cabrero, C. and Mato, J.M. (1988) Hepatology 8, 65–68.
[23] Lowry, O.H. and Passonneau, J.V. (1972) in: A Flexible System of Enzymatic Analysis (Lowry, O.H. and Passonneau, J.V., Eds.), pp. 151–157, Academic Press, New York.
[24] Theocharis, S.T., Skopelitou, A.S., Margeli, A., Pavlaki, K.J. and Kittas, C. (1994) Dig. Dis. Sci. 39, 245–252.
[25] Celis, J.E. and Celis, A. (1985) Proc. Natl. Acad. Sci. USA 82, 3262–3266.
[26] Wolf, H.K. and Michalopoulos, G.K. (1992) Hepatology 15, 707–713.
[27] Cressman, D.E., Greenbaum, L.E., DeAngelis, R.A., Ciliberto, G., Furth, E.E., Poli, V. and Taub, R. (1996) Science 274, 1379–1383.
[28] Gil, B., Pajares, M.A., Mato, J.M. and Alvarez, L. (1997) Endocrinology 138, 1251–1258.
[29] Vara, E., Arias-Díaz, J., Villa, N., Hernández, J., García, C., Ortiz, P. and Balibrea, J.L. (1995) Cyobiology 32, 422–427.
[30] Arias-Díaz, J., Vara, E., García, C., Villa, N., Rodríguez, J.M., Ortiz, P. and Balibrea, J.L. (1996) J. Surg. Res. 62, 79–84.
[31] Alvarez, L., Sánchez-Góngora, E., Mingorance, J., Pajares, M.A. and Mato, J.M. (1997) J. Biol. Chem. 272, 22875–22883.