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Effects of differentiation-inducing agents on purine nucleotide metabolism in an ovarian cancer cell line

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Abstract. The effects of the differentiation-inducing agents sodium butyrate (NaOBt), dimethylsulfoxide (DMSO) and mycophenolic acid (MA), on purine nucleotide metabolism, was studied in an ovarian carcinoma cell line (GZL-8). Exposure to these agents inhibited cell proliferation, but did not affect cell viability. Three hours following exposure, NaOBt and DMSO moderately decelerated purine synthesis de novo, but MA accelerated it three-fold, this being associated with a two-fold increase in the excretion of hypoxanthine and xanthine into the incubation medium. NaOBt and DMSO did not affect the cellular nucleotide content, but MA caused a 73% decrease in GTP content and about a 50% increase in the cellular content of UTP. The following alterations in cellular enzyme activity were observed 72 h following exposure: NaOBt decreased the activity of hypoxanthine–guanine phosphoribosyltransferase and increased the activity of IMP and of AMP 5′-nucleotidases, DMSO increased the activity of IMP 5′-nucleotidase, and MA increased the activity of the two nucleotidases. The results suggest that, in the carcinoma cell line studied, the differentiation process induced by NaOBt and DMSO may be associated with a general shift in the direction of purine metabolism from anabolism to catabolism, whereas that induced by MA is associated with a specific decrease in the production of GTP.

Key words: De novo purine synthesis – Differentiation-inducing agents – Dimethylsulfoxide – Guanine nucleotides – IMP dehydrogenase – Mycophenolic acid – Purine metabolism – Sodium butyrate

Abbreviations: DMSO, dimethylsulfoxide; MA, mycophenolic acid; *P*Rib*PP*, 5′-phosphoribosyl 1-pyrophosphate; NaOBt, sodium butyrate, HGPRT, hypoxanthine-guanine phosphoribosyltransferase;

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Introduction

Malignant transformation of multiple cell types is associated with programmed changes in "housekeeping" metabolic pathways, which are essential to the new machinery of the transformed cell. One of the main metabolic pathways that changes dramatically during malignant transformation is that of purine nucleotide metabolism. These pathways were studied extensively in hepatic cells transformed by carcinogens (Weber et al. 1976; Weber 1983). In general, malignant transformation is associated with a shift in the anabolic direction, which in purine metabolism is marked by dramatic increases in the activities of IMP dehydrogenase (IMP:NAD^+ oxidoreductase; EC 1.2.1.14) ribonucleotide reductase, 5′-phosphoribosyl-1-pyrophosphate (*P*Rib*PP*) amidotransferase (5-phosphoribosylamine: pyrophosphate phosphoribosyltransferase; EC 2.4.2.14) and succinyl-AMP synthetase (IMP:L-aspartate ligase; EC 4.3.2.2). Salvage enzyme activities are changed less markedly (Weber 1983; Natsumeda et al. 1984; Reem and Friend 1967; Prajda et al. 1979).

Only a few reports have been published concerning the effects of chemical inducers of differentiation (differentiation-inducing agents) on purine metabolism (Reem and Friend 1976; Dexter et al. 1981; Cass et al. 1977; Lucas et al. 1983; Sokoloski et al. 1991). Sodium butyrate (NaOBt), dimethylsulfoxide (DMSO) and mycophenolic acid (MA) are established differentiation-inducing agents in hematopoietic and solid-tumor cell lines (Leder and Leder 1975; Prasad 1980; Nordenberg et al. 1986; Sidi et al. 1988; Yu et al. 1989; Kim et al. 1980; Kiguchi et al. 1990a, b; Langdon et al. 1988). It has been suggested that differentiation-inducing agents might potentiate the action of other therapeutic modalities, such as irradiation and chemotherapy (Arundel et al. 1985; Pommier et al. 1988; Leith 1988; Waxman et al. 1992). We were interested in studying the effects of differentiation-inducing agents in ovarian cancer cell lines, since a higher grade of tumor

differentiation correlates with improved survival (Young and Devita 1975; Baak et al. 1986). An ovarian cancer cell line GZL-8, established in our laboratory from ascitic fluid of an ovarian cancer patient, was shown to exhibit differentiated features following treatment with NaOBt (Wasserman et al. 1989, 1992). The purpose of the present study was to delineate the changes in purine metabolism induced by chemical inducers of differentiation in this cell line. We have chosen to study the effects of NaOBt and DMSO, differentiation inducers that are not known to be directly involved with purine metabolism, in comparison to MA, which is known to cause differentiated phenotypic alterations, probably through inhibition of IMP dehydrogenase (Lucas et al. 1983). Such data may be helpful in the design of therapeutic combinations of differentiation-inducing agents and other medications targeted at purine metabolic pathways.

Materials and methods

[8-^{14}C]Hypoxanthine, (50 mCi/mmol), [8-^{3}H]adenine (28.6 Ci/mmol), [^{14}C]formic acid (sodium salt; 55 mCi/mmol), [8-^{14}C]adenosine 5′-monophosphate (50–60 mCi/mmol) and [8-^{14}C]inosine 5′-monophosphate (52.6 mCi/mmol) were purchased from Amersham (Buckinghamshire, UK). Purine nucleotides, nucleosides and bases, microcrystalline cellulose (Sigmacell type 20) and all other chemicals were purchased from Sigma (St. Louis, Mo., USA). RPMI-1640 medium, glutamine, antibiotics and fetal calf serum were obtained from Biological Industries (Kibbutz Beth Haemek, Israel). Culture dishes and flasks were the product of Nunc (Roskilde, Denmark).

The cell line GZL-8, derived in our laboratory from ascitic fluid of an ovarian carcinoma patient (Wasserman et al. 1992), was used. Cells were grown in RPMI-1640 medium supplemented with fetal calf serum and antibiotics. For cell growth experiments 10^5 cells were incubated in the absence or presence of the differentiation-inducing agents in 1 ml growth medium. Cell growth was assessed by counting the cells with a Coulter counter following their detachment with EDTA (1 mM in phosphate-buffered saline).

The rate of de novo purine nucleotide synthesis was studied in the intact cells by measuring the rate of incorporation of [^{14}C]formate into total cellular purines and into the total purines excreted into the incubation medium (Zoref et al. 1975). For the study of incorporation of [^{14}C]formate into purines, monolayer cultures in 250-ml culture flasks were incubated for 2 h at 37°C with 10 μCi sodium [^{14}C]formate. After incubation, the culture media were collected and handled separately. The cells, following trypsinization, were washed twice with ice-cold saline and collected by centrifugation at 4°C. Samples of 1 ml 1 M perchloric acid and 0.2 μCi [8-^{3}H]adenine, as a recovery marker, were added, and the tubes were thoroughly mixed. The purine nucleotides and nucleosides were hydrolyzed at 100°C for 1 h. The hydrolysate was adjusted with water to a volume of 1.5 ml, chilled and 0.7 mg adenine was added to each tube as carrier. The purine bases were then precipitated as a purine-silver complex, washed, extracted in 0.1 M HCl at 100°C for 1 h, and counted. The purines in the total volume of the growth medium (excreted purines) were subjected to hydrolysis in 1 M perchloric acid, as described above, precipitated and counted. The results were corrected for incomplete recovery of purines and are related to total cell protein (Lowry et al. 1951). Labeling of purine nucleotides and of purines excreted into the medium was linear with time for at least 4 h, and for a fixed period of incubation it was also proportional to the number of cells incubated. For the determination of distribution of label among total intracellular and total extracellular purines, the purines extracted from the purine-silver complex were separated by chromatography on microcrystalline cellulose, using *n*-butanol/methanol/H_2O/NH_4OH 25% (60:20:20:1, by vol.) as solvent (Zoref Shani et al. 1988).

The activity of the various enzymes was determined in cell extracts. Washed trypsinized cells were suspended in 0.5 ml H_2O and subjected to freezing and thawing three times. The cell lysates were subjected to centrifugation at 17 000 rpm at 4°C for 20 min, and the supernatant was dialyzed for 2 h at 4°C against 1 mM TRIS/HCl buffer, pH 7.4. The enzymes were assayed radiochemically (Brosh et al. 1990) in a total volume of 200 μl incubation mixture and the reaction was arrested by addition of 40 μl 15% perchloric acid. Products were separated from substrates by TLC on microcrystalline cellulose, using appropriate solvents as specified. For the assay of hypoxanthine–guanine phosphoribosyltransferase (IMP:pyrophosphate phosphoribosyltransferase; EC 2.4.2.8; HGPRT), the reaction mixture contained 55 mM TRIS/HCl buffer (pH 7.4), 5 mM $MgCl_2$, 1 mM *P*Rib*PP*, 0.1 mM [8-^{14}C]hypoxanthine and up to 100 μg cell extract protein. Incubation was carried out at 37°C for 10 min and 20 min. IMP plus inosine were separated from the respective substrate base, using NH_4HCO_3 solution (16 g/100 ml H_2O) as solvent. The incubation mixture for the assay of 5′-nucleotidases (EC 3.1.3.5) contained 55 mM TRIS/HCl buffer (pH 7.4), 5 mM $MgCl_2$, and 0.5 mM [8-^{14}C]IMP for IMP 5′-nucleotidase determination, or 0.5 mM [8-^{14}C]AMP for AMP 5′-nucleotidase determination, and 20–100 μg cell extract protein. Incubation was carried out at 37°C for 15 min and 30 min. Nucleosides plus base products were separated from the substrate using *n*-butanol/methanol/H_2O/NH_4OH 25% (60:20:20:1, by vol.). In all cases, enzyme activities were linear with time and protein concentration (Lowry et al. 1951).

Purine nucleotides, nucleosides and bases, in extracts from cells and culture media, were determined employing HPLC methodology. The growth medium was separated, and the cells extracted immediately in 1 ml cold 1 M perchloric acid. The cell extract was neutralized with 0.7 ml 0.5 M $KHCO_3$ in 1.5 M KOH 2 min following the acid extraction and was then subjected to centrifugation (40 000 *g*, 15 min at 4°C). The growth medium was acidified and neutralized as described above. The extracted purines were kept at −70°C until analyzed. The purine nucleotides in the cell extracts were quantified on a SAX-10 Partisil column (Whatman). The column was equilibrated with 7 mM KH_2PO_4, pH 4.0. The injection of the nucleotide extract was followed by pumping of 7 mM KH_2PO_4, pH 4.0, for 15 min at 1.5 ml/min, after which a linear gradient was initiated to 0.25 M KH_2PO_4 in 0.5 M KCl, pH 4.5, at 1.5 ml/min over a period of 45 min. The high-molarity eluent was continued at the same flow rate for an additional 30 min (Hartwick and Brown 1975).

The purine nucleosides and bases in the culture media were separated on LiChrosorb RP-18 columns (Merck). The injection of the samples was followed by pumping of 20 mM KH_2PO_4, pH 4.5, for 6 min at 1.5 ml/min, after which a linear gradient was initiated to 40% 20 mM KH_2PO_4, pH 4.5, and 60% methanol (60% in water), over a period of 24 min at 1.5 ml/min (Osborne et al. 1983). The HPLC system employed was a Varian 9010 solvent-delivery system, with a Varian 9050 variable-wavelength detector set at 254 nm and with a computerized integrator.

Results

Treatment of GZL-8 cells with NaOBt, DMSO or MA inhibited their proliferation (Table 1), which was not associated with decreased cell viability.

The inhibition of cell growth was associated with significant changes in the rate of de novo purine synthesis (Table 2). After 3 h exposure, NaOBt and DMSO decelerated purine synthesis moderately, but MA accelerated it more than threefold. The acceleration effect of MA was associated with a marked, more than sevenfold, increase in the rate of excretion of labeled purines into the culture medium. The proportion of excreted purines following exposure to MA rose to 72%, in comparison to 8% in the control cells. The largest part of the label was found in hypoxanthine derivatives (83%).

Analysis of the distribution of label from [^{14}C]formate among the intracellular purines revealed that in the control cultures most of the label (44%) was found in adenine derivatives (adenine nucleotides and their degradation

Table 1. Proliferative activity of GZL-8 cells exposed to differentiation-inducing agents for 72 h in vitro

Differentiating agent	$10^{-4} \times$ Cell number
None	30.3 ± 0.8
Sodium butyrate	14.0 ± 0.8
Mycophenolic acid	15.3 ± 2.4
Dimethylsulfoxide	16.4 ± 2.7

Initial number of cells plated was 10^5

product adenosine), somewhat less (36%) in guanine derivatives (guanine nucleotides and their degradation products guanosine and guanine) and markedly less (13%) in hypoxanthine derivatives (IMP, and its degradation products inosine and hypoxanthine) and in xanthine (7%). Exposure of the cultures to DMSO and NaOBt did not affect the distribution, but exposure to MA was associated with a 50% decrease of the label in guanine derivatives (to 16% of the total intracellular labeling) (Table 3).

Analyis (by HPLC) of the purines excreted from the cells into the incubation medium revealed two peaks, identified as hypoxanthine and xanthine. The excretion of xanthine had increased twofold and that of hypoxanthine about threefold 3 h following exposure to MA. The other differentiation-inducing agents did not affect the excretion of purines (Table 4).

The AMP content of the cells could not be assayed accurately. Therefore, the energy charge was calculated as $(\frac{1}{2}\,\mathrm{ADP} + \mathrm{ATP})/(\mathrm{ADP} + \mathrm{ATP})$. According to this calculation, the energy charge of the cell line was 0.93. Exposure of the cultures to the differentiation-inducing agents did not affect this value, nor did it affect the nucleotide content, except for MA, which caused a very marked (73%) decrease in the cellular GTP content and about a 50% increase in the cellular content of UTP (Table 5).

Some of the above-mentioned parameters of purine nucleotide metabolism were also assessed 72 h following exposure of the cultures to the differentiation-inducing agents. After this period, the cultures exposed to NaOBt and to DMSO still exhibited a decelerated rate of purine synthesis, but the cultures exposed to MA exhibited a rate similar to that in the unexposed cells (Table 2). The decreased rate of purine synthesis following exposure to NaOBt and DMSO did not affect the distribution of label among the intracellular purines or the rate of excretion of labeled purines into the incubation medium. However, exposure to MA resulted in decreased labeling of guanine derivatives and increased labeling of adenine derivatives (Table 3). At 72 h following exposure, the cultures exposed to MA exhibited principally the same alterations in cellular nucleotide content as found at 3 h following the addition of this agent (a decreased content of GTP and increased content of pyrimidine nucleotides). These alterations did not affect the energy charge of the cells. The

Table 2. Rate of de novo purine nucleotide synthesis of GZL-8 cells exposed to differentiation-inducing agents in vitro

Differentiating agent	3 h after exposure ($n = 8$)[a]		72 h after exposure ($n = 5$)[a]	
	Rate of [^{14}C]formate incorporation into total purines [cpm (mg protein)$^{-1}$ h^{-1}]	Labeled purines excreted into medium (% of total)	Rate of [^{14}C]formate incorporation into total purines [cpm (mg protein)$^{-1}$ h^{-1}]	Labeled purines excreted into medium (% of total)
None	4300 ± 500	8 ± 11	7440 ± 910	16 ± 4
Sodium butyrate	3170 ± 400*	10 ± 13	2960 ± 730**	$14 + 8$
Mycophenolic acid	13213 ± 2500*	72 ± 5***	7620 ± 1860	19 ± 7
Dimethylsulfoxide	3488 ± 540*	10 ± 10	3960 ± 430**	12 ± 7

*,**,*** Significance of difference from control group: $*P < 0.02$; $**P < 0.01$; $***P < 0.001$

[a] The assay (incorporation of [^{14}C]formate into purines) started at this time and was carried out for 2 h; n, number of experiments. Results are expressed as means $\pm$ SD

Table 3. Distribution of label from [^{14}C]formate among the various intracellular purine derivatives in GZL-8 cells exposed to differentiation-inducing agents in vitro

Differentiating agent	Distribution of label among the various purine derivatives (% of total incorporation)							
	Adenine		Hypoxanthine		Guanine		Xanthine	
	3 h	72 h	3 h	72 h	3 h	72 h	3 h	72 h
None	44 ± 3	47 ± 6	13 ± 6	10 ± 3	36 ± 8	35 ± 3	7 ± 5	8 ± 2
Sodium butyrate	35 ± 4	41 ± 8	22 ± 6	18 ± 7	37 ± 11	30 ± 9	6 ± 5	11 ± 4
Mycophenolic acid	56 ± 3	35 ± 4*	17 ± 6	6 ± 3	16 ± 8*	48 ± 8*	11 ± 7	11 ± 4
Dimethylsulfoxide	36 ± 8	44 ± 7	11 ± 4	15 ± 3	43 ± 8	32 ± 7	10 ± 5	9 ± 2

Results are expressed as means $\pm$ SD of values from four experiments

* Significance of difference from control group: $P < 0.001$

[a] Incubation with [^{14}C]formate was carried for 2 h following exposure for 3 h or 72 h

Table 4. Rate of excretion of oxypurines in GZL-8 cells exposed to differentiation-inducing agents for 3 h in vitro

Differentiating agent	n	Oxypurine excretion [nmol (mg protein)$^{-1}$ (3 h)$^{-1}$]	
		Xanthine	Hypoxanthine
None	3	93.4 ± 5.6	16.6 ± 0.1
Mycophenolic acid	5	174.0 ± 55*	46.0 ± 16.7**
Sodium butyrate	3	87.9 ± 1.3	18.7
Dimethylsulfoxide	3	97.8 ± 1.8	17.9 ± 0.6

Results are expressed as means ± SD
*,** Significance of difference from control group: *$P < 0.05$; **$P < 0.01$

cultures exposed to NaOBt exhibited a lower ATP and a higher GDP content, with a somewhat decreased energy charge. The cultures exposed to DMSO exhibited a normal nucleotide content and normal energy charge (Table 5).

The effect of exposure of the cultures to the differentiation-inducing agents on the activity of enzymes participating in purine nucleotide synthesis (HGPRT) and degradation (IMP and AMP 5′-nucleotidases) was determined in cell extracts from cultures 72 h following exposure to the differentiation-inducing agents. Cultures exposed to NaOBt exhibited decreased activity of HGPRT and increased activity of the 5′-nucleotidases. Cultures exposed to DMSO exhibited increased activity of IMP 5′-nucleotidase. Cultures exposed to MA exhibited increased activity of the 5′-nucleotidases (Table 6).

Discussion

Exposure of the carcinoma GZL-8 cell line to differentiation-inducing agents resulted in alterations in purine metabolism. NaOBt and DMSO are not known to affect purine metabolism directly; nevertheless, 3 h following exposure to either agent, the cell cultures exhibited a decreased rate of de novo purine synthesis. This relatively fast effect could reflect an as yet unknown direct influence of these agents on purine metabolism, or an effect secondary to the onset of the differentiation process. The effect of DMSO on *PRibPP* amidotransferase, catalyzing the first-committed, rate-limiting step in the pathway of purine synthesis, has previously been studied in murine erythroleukemic cells undergoing differentiation by DMSO in vitro. It was found that the activity of this enzyme decreased markedly only after 72 h of exposure (Reem and Friend 1976). Our results imply that DMSO and NaOBt cause much earlier changes in the regulation of the metabolic flux through the pathway of de novo purine synthesis. The deceleration of the purine synthesis was also observed 72 h following exposure of the cells to these differentiation-inducing agents, this time probably reflecting the stage of cell differentiation.

Table 5. Nucleotide contents of GZL-8 cells exposed to differentiation-inducing agents in vitro

Nucleotide	Nucleotide content (nmol/mg protein) with different differentiating agents							
	None		Mycophenolic acid		Sodium butyrate		Dimethylsulfoxide	
	3 h ($n = 7$)	72 h ($n = 4$)	3 h ($n = 3$)	72 h ($n = 4$)	3 h ($n = 4$)	72 h ($n = 4$)	3 h ($n = 4$)	72 h ($n = 5$)
ADP	4.30 ± 1.7	4.9 ± 0.5	4.40 ± 1.28	3.6 ± 1.9	5.56 ± 4.6	6.8 ± 1.8	3.35 ± 1.54	3.6 ± 1.2
GDP	0.97 ± 0.31	0.9 ± 0.1	0.39 ± 0.15	0.4**	1.54 ± 0.75	1.3 ± 0.2*	0.97 ± 0.40	0.8 ± 0.2
UTP	7.60 ± 0.95	5.6 ± 0.7	11.60 ± 1.44***	12.6 ± 2.9**	7.86 ± 2.32	5.5 ± 0.2	10.70 ± 1.15	5.9 ± 3.1
CTP	2.85 ± 0.43	2.0 ± 0.5	3.55 ± 0.58	4.4 ± 0.6***	2.88 ± 1.00	2.3 ± 0.3	3.70 ± 1.20	2.6 ± 0.8
ATP	30.0 ± 3.9	34.8 ± 5.6	23.7 ± 5.9	33.9 ± 4.1	29.9 ± 7.4	25.9 ± 0.6*	29.8 ± 8.6	24.6 ± 0.4
GTP	7.18 ± 0.23	7.1 ± 1.8	1.95 ± 54***	2.0 ± 0.4***	6.40 ± 2.50	5.5 ± 0.4	7.37 ± 2.50	5.1 ± 2.1
EC	0.93	0.93	0.92	0.95	0.92	0.89	0.94	0.93

Results are expressed as means ± SD. n, number of experiments for each exposure time. EC, energy charge
*,**,*** Significance of difference from control group: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

Table 6. Enzyme activities of GZL-8 cells exposed to differentiation-inducing agents for 72 h in vitro

Differentiating agent	Enzyme activity [nmol (mg protein)$^{-1}$ h^{-1}]					
	HGPRT		IMP 5′-nucleotidase		AMP 5′-nucleotidase	
	Control	PE	Control	PE	Control	PE
Sodium butyrate	836 ± 110	495 ± 51*	90 ± 5	149 ± 6*	115 ± 51	174 ± 16*
Mycophenolic acid	717 ± 110	888 ± 116	89 ± 7	149 ± 11*	127 ± 8	155 ± 7*
Dimethylsulfoxide	891 ± 128	661 ± 103	70 ± 1	111 ± 12*	211 ± 46	236 ± 31

Results are expressed as means ± SD (four experiments). HGPRT, hypoxanthine–guanine phosphoribosyltransferase; PE, post exposure
* Significance of difference from control group: $P < 0.05$

The cultured GZL-8 cell line exhibited a lesser need for purine nucleotides 3 days following exposure to NaOBt and DMSO. This is inferred from the observed decrease in the capacity of the cells to produce nucleotides (decrease in the rate of de novo production of nucleotides and in the activity of HGPRT, catalyzing salvage nucleotide synthesis), and from the increased activity of the nucleotide degradation enzymes, IMP and AMP 5′-nucleotidases. These alterations reflect a programmed change in the direction of purine nucleotide homeostasis from an anabolic to a catabolic state. Increased activity of salvage enzymes, such as HGPRT, in tumor cells, has been considered to underly the resistance of the cells to purine anti-metabolites (Natsumeda et al. 1984). Diversion of purine nucleotide homeostasis from biosynthesis towards degradation, induced by differentiation-inducing agents, may be utilized in designing combination therapy (Weber et al. 1992). Such combinations may include differentiation-inducing agents and purine anti-metabolites. Differentiation-inducing agents may limit the cellular ability to salvage purines and thereby increase cellular susceptibility to purine anti-metabolites.

Of the three differentiation-inducing agents studied, only MA is known to exert a direct effect on purine metabolism. MA is a potent inhibitor of IMP dehydrogenase, causing depletion of cellular guanine nucleotides. This effect has been shown in several tissues to be associated with enhancement of de novo purine nucleotide synthesis. Guanine nucleotides are potent inhibitors of *P*Rib*PP* synthetase (ribosephosphate pyrophosphokinase; EC 2.7.6.1) (Zoref et al. 1975) and *P*Rib*PP* amidotransferase (Wood and Seegmiller 1973; Holmes et al. 1973). A decreased cellular content of guanine nucleotides results in activation of these enzymes, enhancing purine synthesis. Activation of *P*Rib*PP* synthetase has a major impact in this respect, since *P*Rib*PP* is a limiting and regulatory substrate for the *P*Rib*PP* amidotransferase, catalyzing the first-committed, rate-limiting step in the pathway of purine synthesis (Wood and Seegmiller 1973; Holmes et al. 1973). Indeed, exposure to MA for 3 h resulted in a decreased flux from IMP to GMP, manifested in a decreased cellular GTP content and enhanced rate of de novo purine synthesis. In addition, inhibition of IMP dehydrogenase and the accelerated rate of de novo IMP synthesis resulted in a marked increase in the degradation of the excessive IMP produced, as reflected by the increased excretion of its degradation products hypoxanthine and xanthine into the culture medium (Tables 2–5).

Depletion of guanine nucleotides caused by MA has been suggested to be the primary trigger for the differentiation induced by this compound. Indeed, cell proliferation, whether normal (such as liver regeneration), or due to malignant transformation, is associated with increased activity of IMP dehydrogenase (Weber et al. 1976). On the other hand, inhibition of this enzyme has been shown to induce differentiation of transformed cells (Sidi et al. 1988, 1989; Yu et al. 1989; Kiguchi et al. 1990a, b; Collart and Huberman 1990). It has been suggested (Lucas et al. 1983) that MA, as well as other differentiation-inducing agents, induces differentiation of HL60 cells through guanine nucleotide depletion. However, no such depletion was observed in our cells following exposure to NaOBt or DMSO.

In addition to its lowering effect on cellular GTP content, MA also increased the cellular content of UTP. MA-induced elevation of cellular pyrimidine nucleotides has been reported before, and attributed to enhanced synthesis of *P*Rib*PP*, which is a limiting substrate for UMP synthesis from orotic acid (Cass et al. 1977). The MA-induced increase in *P*Rib*PP* generation is attributed to decreased feedback inhibition of *P*Rib*PP* synthetase, on the basis of the lowered cellular content of guanine nucleotides, as discussed above.

The effect of MA on purine metabolism was also assessed 72 h following exposure of the cells to this agent. In such cells, the GTP content was low and pyrimidine nucleotides were elevated. The rate of incorporation of labeled formate into total purines (de novo synthesis) was similar to that in untreated cells, but more than double that in to cells exposed to NaOBt and DMSO. The increase in the flux from IMP to GMP, as observed in these cells 72 h following the exposure to MA, is not compatible with the other results. This finding probably reflects increased IMP dehydrogenase activity, compensating for the cellular deficit in guanine nucleotides. However, as evident from the low GTP content at the time of measurement, this increase did not fully replenish the guanine nucleotide pool at that time. An increase in IMP dehydrogenase, compensating for the guanine nucleotide deficit, was reported previously in HGPRT-deficient erythrocytes (Pehlke et al. 1972; Sweetman and Nyhan 1972), and may reflect the importance of this enzyme in the regulation of intracellular guanine nucleotide content.

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