Biochemical Pharmacology, Vol. 25, pp. 527–532. Pergamon Press, 1976. Printed in Great Britain.

EFFECT OF 6-MERCAPTOPURINE ON INOSINIC ACID DEHYDROGENASE IN CULTURED HUMAN FIBROBLASTS*

ALBERT LEYVA, EDWARD W. HOLMES, JR. and WILLIAM N. KELLEY

Departments of Medicine and Biochemistry, Duke University Medical Center, Durham, N.C. U.S.A., 27710

(*Received* 5 *August* 1974; *accepted* 16 *May* 1975)

Abstract—6-Mercaptopurine, but not other purine analogs, produced an increase in IMP dehydrogenase specific activity in cultured human skin fibroblasts. The conversion of 6-mercaptopurine to its ribonucleotide form, 6-thioinosine 5′-monophosphate, was required for this effect, as evidenced by the lack of an effect of 6-mercaptopurine in cells deficient in hypoxanthine-guanine phosphoribosyltransferase activity. This effect of 6-mercaptopurine on IMP dehydrogenase was blocked by inhibition of RNA or protein synthesis. Furthermore, stabilization of IMP dehydrogenase activity by 6-mercaptopurine or its metabolic products to heat or trypsin inactivation could not be demonstrated *in vitro*. These results suggest that 6-thioinosine 5′-monophosphate or one of its nucleotide derivatives leads to an increase in the synthesis of IMP dehydrogenase at the transcriptional level.

6-Mercaptopurine (6-MP) is an analog of hypoxanthine which interferes with purine nucleotide biosynthesis at a number of different sites (see review by Elion [1]). 6-MP competes with hypoxanthine and guanine for conversion to ribonucleotide derivatives by the salvage pathway enzyme, hypoxanthine-guanine phosphoribosyltransferase (HGPRT). More importantly, the ribonucleotide form of 6-MP, 6-thioinosine 5′-monophosphate (6-thioIMP), is an inhibitor of several different steps of purine nucleotide biosynthesis *de novo*, including the synthesis of phosphoribosylamine, the first reaction committed to this pathway, and the conversions of IMP to adenylosuccinic acid, adenylosuccinic acid to AMP and IMP to XMP. The latter reaction is the first step unique to the pathway for the synthesis of GMP *de novo* and is catalyzed by inosinic acid (IMP) dehydrogenase [2] (IMP:NAD^+ oxidoreductase, EC 1.2.1.14).

Atkinson *et al.* [3] demonstrated that IMP dehydrogenase from Ehrlich ascites tumor cells was inhibited by 6-thioIMP in a manner which was competitive with respect to IMP. A similar type of inhibition of IMP dehydrogenase from sarcoma 180 cells by 6-chloroinosine 5′-monophosphate was noted by Anderson and Sartorelli [4]. Holmes *et al.* [5] have also observed inhibition of IMP dehydrogenase from human placenta by 6-thioIMP. Hampton and Nomura [6] proposed from their studies of the enzyme from *Aerobacter aerogenes* that the nucleotide derivatives of 6-MP, 6-thioguanine (6-TG), and 6-chloropurine (6-ClP) react with a sulfhydryl group in the proximity of the IMP binding site to form either a stable disulfide or thioether bond.

In the present study we have demonstrated that 6-MP causes an increase in the specific activity of IMP dehydrogenase in cultured human fibroblasts. The data suggest that this effect is mediated by a nucleotide derivative of 6-MP and may be dependent on RNA and protein synthesis.

* This research supported by USPHS Research Grant No. AM-14362, USPHS Training Grant No. GM-00233 and USPHS Training Grant No. AM-05620.

MATERIALS AND METHODS

Hypoxanthine[8-^{14}C] (2·48 mCi/m-mole), L-leucine[4,5-^{3}H] (64 Ci/m-mole) and uridine[2-^{14}C] (56·7 mCi/m-mole) were purchased from New England Nuclear Corp. Nicotinamide adenine dinucleotide, sodium phosphoribosylpyrophosphate, 6-MP, 6-mercaptopurine ribonucleoside (6-MPR), 6-thioIMP (barium salt), 6-TG, 6-ClP, XMP, actinomycin D, trypsin and trypsin inhibitor were obtained from Sigma Chemical Co. Dithiothreitol, cycloheximide and puromycin were purchased from Calbiochem Co. All other chemicals were commercial products of highest available purity.

Cell culture. Cultures of human skin fibroblasts were initiated from punch skin biopsies of normal individuals and patients with the Lesch Nyhan syndrome as described previously [7]. Fibroblasts were cultured in monolayer in 100-mm plastic sterile disposable Petri dishes (Falcon Plastics). The cells were grown in Eagle's minimum essential medium (GIBCO), supplemented with 10% fetal calf serum (dialyzed or undialyzed, GIBCO), nonessential amino acids, penicillin (50 U/ml), and streptomycin (50 μg/ml) and incubated at 37° in a humidified 5% CO_2–95% air atmosphere. Cultures were serially propagated by splitting 1 to 4 after reaching confluency. In each experiment, replicate cultures were prepared by pooling cells harvested from several cultures before transferring 10-ml aliquots of cell suspension to new Petri dishes. Only confluent cultures in the fifth to fifteenth passage were used and the culture medium (supplemented with undialyzed serum except where indicated otherwise) was changed every 2 days. Under these conditions there was little variation in IMP dehydrogenase specific activity in any particular experiment. As noted later, however, the basal level of IMP dehydrogenase activity was lower when dialyzed rather than undialyzed serum was used as a component of the cell culture medium.

Cells (1–3 × 10^6) were harvested from culture dishes by rapid trypsinization. The culture medium was removed by aspiration and the cell sheets were

washed with phosphate-buffered saline (PBS). Cells were incubated with 1 ml of 0·1% trypsin (9300 BAEE units/mg; Worthington Biochemical) with agitation at room temperature for 1–5 min. The detached cells were suspended in cold fresh medium, centrifuged at 600 *g* and washed with PBS. Cell pellets were stored at −70° until time of extraction. IMP dehydrogenase activity was found to be stable under these conditions for at least 2 weeks. Cells were suspended in 0·2 ml of ice-cold 50 mM potassium phosphate buffer, pH 7·4, with 1 mM dithiotreitol and lysed by rapidly freezing and thawing twice. Lysates were centrifuged at 10,000 *g* for 20 min, and the supernatant was dialyzed against the same buffer for 6–8 hr at 4°.

Enzyme assays. IMP dehydrogenase activity was determined by a radiochemical method similar to that described by Pehlke *et al.* [8]. The reaction mixture contained 0·0083 μmole IMP[8-^{14}C],* 0·1 μmole NAD^+, 10 μmoles KCl, 0·1 μmole EDTA, 0·05 μmole dithiothreitol, 5 μmoles potassium phosphate buffer, pH 7·4, and 25–100 μg fibroblast protein (50 μl dialyzed cell extract) in a final volume of 100 μl. The reaction was allowed to proceed for 1 hr at 37° and stopped by the addition of an equal volume of cold 95% ethanol. Precipitated protein was removed by centrifugation at 10,000 *g* and 50 μl of the supernatant, along with 20 μg carrier XMP, was spotted on Whatman DE81 paper and subjected to descending chromatography in 0·2 M ammonium formate buffer, pH 5·0 (R_f: XMP, 0·32; IMP, 0·72). The XMP spot was located with u.v. light, cut out and counted at 63 per cent efficiency in a Packard Tri-Carb liquid scintillation counter. The assay was linear with respect to time up to 90 min and with respect to amount of protein up to 125 μg. Under these assay conditions endogenous 5′-nucleotidase activity had no significant effect on the measurement of IMP dehydrogenase activity. In the presence of EDTA less than 10 per cent of the substrate, IMP, and less than 5 per cent of the product, XMP, were found to be dephosphorylated. In addition, potential difficulty arising from the metabolism of NAD^+ in the extract was avoided by providing NAD^+ in excess. Reducing the concentration of NAD^+ by 50% yielded the same level of enzyme activity. A higher concentration of NAD^+ had an inhibitory effect. Essentially the same results were obtained using extracts prepared from cells grown in the presence of 6-MP.

HGPRT activity was measured by a radiochemical method similar to that described by Kelley and Meade [9]. Thymidine triphosphate was not used to inhibit the breakdown of the nucleotide product to the nucleoside derivative by endogenous 5′-nucleotidase. However, comparable results were obtained by using the sum of the radioactivity in IMP and inosine to quantitate enzyme activity.

Protein was measured by the method of Lowry *et al.* [10] using bovine serum albumin as a standard.

RNA and protein synthesis. Rates of RNA and protein synthesis were estimated in confluent cultures which had attained a cell density of $3·4 \times 10^4$ cells/cm^2. These cultures were incubated for 2 hr with 5 ml of medium containing 0·1 μCi uridine[2-^{14}C] and 1 μCi L-leucine[4,5-^{3}H]. Subsequent steps were carried out at 0–4°. After the labeling period the cells were washed three times with 5-ml portions of PBS and lysed with 4 ml of 0·5% sodium dodecyl sulfate in 0·01 M Tris·HCl, pH 7·4. An equal volume of 10% trichloroacetic acid was added to the lysates to precipitate nucleic acids and protein. The precipitates were collected on glass fiber filters (Reeve Angel, No. 934-Ah, 2·4-cm diameter) and washed four times with 5 ml of 5% trichloroacetic acid and twice with 5 ml of 95% ethanol. The filters were dried and counted in a toluene-based scintillation fluid. Tritium counts were corrected for 8·3 per cent spillover of ^{14}C counts. Tritium was counted at 14·5 per cent efficiency and ^{14}C was counted at 36·5 per cent efficiency. The labeling conditions described here gave linear incorporation rates for both isotopes up to 8 hr.

Stability of IMP dehydrogenase in vitro. Fibroblast extract, in 100-μl aliquots, was incubated at 70° for up to 8 min and then chilled rapidly at 0–4°. Residual IMP dehydrogenase activity was assayed and expressed as a per cent of the initial activity of the unheated control. Fibroblast extract, in 100-μl aliquots, was also incubated at 25° for up to 10 min with 10 μl trypsin (1 mg/ml). Proteolysis was terminated by rapid chilling at 0–4° and the addition of 10 μl trypsin inhibitor (2 mg/ml). Residual IMP dehydrogenase activity was expressed as a per cent of the activity of the control, in which trypsin inhibitor was added immediately after trypsin.

RESULTS

The change in specific activity of IMP dehydrogenase 3 days after the addition of 6-MP to fibroblasts cultured in medium supplemented with undialyzed or dialyzed serum is illustrated in Table 1. With undialyzed serum, enzyme specific activity varied between 8·9 and 15·6 nmoles/mg of protein/hr in control cultures and increased as much as 67 per cent in 6-MP-treated cells. With dialyzed serum, enzyme specific activity varied between 5·3 and 9·7 nmoles/mg of protein/hr and was increased by as much as 150 per cent in 6-MP-treated cells. The maximum IMP dehydrogenase activity in extracts of 6-MP-treated cells was 20–22 nmoles/mg of protein/hr using either type of medium. A maximum effect was exerted by 10^{-3} M 6-MP in experiments using medium supplemented with undialyzed serum, whereas 10^{-4} M 6-MP was most effective in cells grown in medium supplemented with dialyzed serum. This observation suggests that substances which interfere with the action of 6-MP may be present in undialyzed serum. The addition of purine bases to either medium, however, had no effect on IMP dehydrogenase activity levels. Also, the activity of at least one other enzyme involved in purine metabolism, phosphoribosylpyrophosphate synthetase, was found to respond similarly to undialyzed and dialyzed serum (unpublished data).

The kinetics of the increase in IMP dehydrogenase specific activity after the addition of 6-MP is demonstrated in Fig. 1. A maximum increase was observed within 3 days after the addition of 6-MP and the enzyme activity level remained stable for at least 2 additional days. The specific activity of another en-

* IMP[8-^{14}C] was enzymatically synthesized from hypoxanthine[8-^{14}C] using partially purified human HGPRT according to Pehlke *et al.* [8].

Table 1. Effect of 6-MP on IMP dehydrogenase specific activity*

Culture medium	No. of experiments	Concn of 6-MP (M)	Per cent of control
Undialyzed serum supplemented		0	100
	4	10^{-5}	122 (111–150)
	5	10^{-4}	152 (139–171)
	3	10^{-3}	167 (133–193)
Dialyzed serum supplemented		0	100
	3	10^{-5}	190 (169–226)
	5	10^{-4}	250 (155–362)
	3	10^{-3}	172 (158–183)

* 6-MP was added to cultures at concentrations ranging from 10^{-5} to 10^{-3} M. Cells were collected 3 days later and IMP dehydrogenase specific activity was measured. For each individual experiment the per cent of control was determined for the enzyme activity observed in the presence of 6-MP. Listed are the mean and range of the per cent values from three to five separate experiments. The IMP dehydrogenase specific activity in the controls ranged from 8·9 to 15·6 nmoles/mg of protein/hr for cells grown in the presence of undialyzed serum and from 5·3 to 9·7 nmoles/mg of protein/hr for cells grown in presence of dialyzed serum.

zyme of purine metabolism, HGPRT, which was also constant during confluency, was not altered by 6-MP at concentrations ranging from 10^{-5} to 10^{-3} M for up to 5 days (Fig. 2). There was no apparent change in cell morphology or cell adhesion as observed with light microscopy during the course of the experiments under these conditions.

The effect of 6-MP on the rate of incorporation of labeled precursors of RNA and protein into acid-insoluble material is shown in Table 2. 6-MP had virtually no effect on the incorporation of labeled leucine into acid-insoluble material. Labeled uridine incorporation was reduced at all concentrations of 6-MP examined. Maximum inhibition of approximately 60 per cent was noted at concentrations of 6-MP in the range of 10^{-3}–10^{-4} M.

The increase in IMP dehydrogenase specific activity in response to 6-MP did not occur in cells with reduced HGPRT activity. As illustrated in Table 3, the specific activity of IMP dehydrogenase in cell strain 230, with less than 2 per cent of the HGPRT activity present in normal cells, decreased slightly

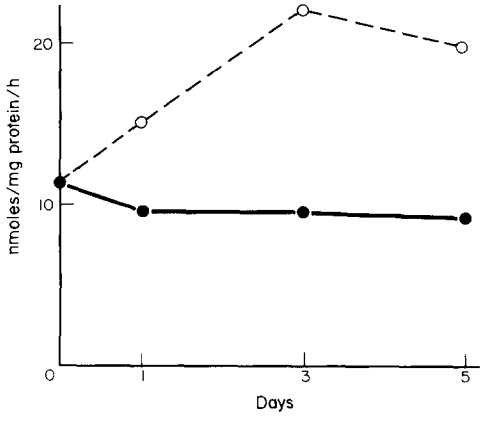

Fig. 1. Time course of 6-MP-mediated increase of IMP dehydrogenase specific activity. 6-MP (10^{-5} M) was added to confluent cultures in dialyzed serum-supplemented medium, and IMP dehydrogenase activity was assayed 1, 3 and 5 days later. ●—●, Control, no 6-MP added; ○–○, 6-MP added.

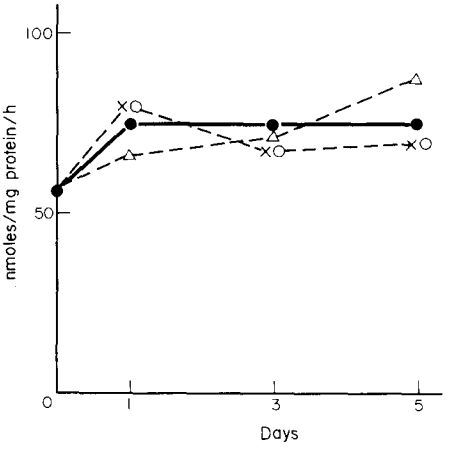

Fig. 2. Effect of 6-MP on HGPRT specific activity in cell culture. Three groups of replicate confluent cultures were incubated in the presence of 6-MP (10^{-5}, 10^{-4} and 10^{-3} M). A control group was not treated with 6-MP. Cells were collected 1, 3 and 5 days after the initiation of the experiment and assayed for HGPRT activity. ●—●, Control, no 6-MP added; ○–○, 10^{-5} M 6-MP added; △–△, 10^{-4} M 6-MP added; ×–×, 10^{-3} M 6-MP added.

Table 2. Effect of 6-MP on the incorporation of [^{3}H]leucine and [^{14}C]uridine into acid-insoluble material*

Concn of 6-MP (M)	Per cent of control	
	[^{3}H]leucine incorporation	[^{14}C]uridine incorporation
0	100†	100‡
10^{-5}	114	68
10^{-4}	95	42
10^{-3}	98	40

* Replicate cultures were incubated for 12 hr in the presence of 6-MP before determining incorporation of labeled precursor into acid-insoluble material as described under Materials and Methods.

† 485 cpm.

‡ 421 cpm.

Table 3. Effect of 6-MP on IMP dehydrogenase specific activity in normal and Lesch Nyhan cells*

Cell strain	HGPRT (nmoles mg protein/hr)	Concn of 6-MP (M)	IMP dehydrogenase (nmoles mg protein/hr)
233 (Normal)	75		
		0	11·8
		10^{-4}	16·4
		10^{-3}	20·4
230 (Lesch Nyhan)	1·0		
		0	14·4
		10^{-4}	13·4
		10^{-3}	13·4

* Normal and Lesch Nyhan cells in the ninth and twelfth passage, respectively, were incubated in the presence of 6-MP, (10^{-4} or 10^{-3} M) for 3 days, harvested and assayed for IMP dehydrogenase activity. HGPRT activity was measured before the start of the experiment.

when the cells were incubated with 6-MP at concentrations of 10^{-4} or 10^{-3} M. These concentrations of 6-MP produced a 40 and 70 per cent increase, respectively, in the specific activity of IMP dehydrogenase in normal fibroblasts.

The effects of 6-MP and other related compounds on the specific activity of IMP dehydrogenase are compared in Table 4. In this experiment enzyme activity in extracts of control cells was low (5·5 nmoles/mg of protein/hr) and the stimulatory effect of 6-MP was over 3-fold. 6-MPR at an equimolar concentration caused only a 2·4-fold increase in IMP dehydrogenase specific activity. Conversely, 6-MMPR, 6-TG and 6-ClP at equimolar concentration led only to a decline in IMP dehydrogenase activity.

The 6-MP-mediated increase in IMP dehydrogenase activity was examined for dependence on RNA and protein synthesis. RNA synthesis was blocked by actinomycin D (0·1 μg/ml), which inhibited the incorporation of labeled uridine into the acid-insoluble material to 5 per cent of control values. Protein synthesis was blocked by cycloheximide (5 μg/ml) or puromycin (3 μM), which inhibited the incorporation of labeled leucine into the acid-insoluble material to 10 and 40 per cent of control values respectively. These inhibitors at the doses used had only a minor effect on the morphological appearance of confluent cultures. The effect of these inhibitors on IMP dehydrogenase activity was tested on cells cultured in the presence and absence of 6-MP at a concentration of 10^{-4} M (Fig. 3). In control cultures IMP dehydrogenase activity fluctuated between 5·5 and 6·6 nmoles/mg of protein/hr, whereas in 6-MP-treated cultures enzyme activity levels increased gradually to 18·2 nmoles/mg of protein/hr by the third day. Actinomycin D added at the beginning of the experiment to either control or 6-MP-treated cultures resulted in a decrease in enzyme activity after 2 days. Actinomycin D, added 1 day after 6-MP, blocked the further increase in IMP dehydrogenase activity but did not cause a decline of the elevated enzyme activity levels. In control cultures cycloheximide did not affect IMP dehydrogenase levels over a 2-day period, whereas puromycin added during the same time period caused a decline in enzyme specific activity. Both cycloheximide and puromycin effectively prevented the increase of enzyme activity in 6-MP-treated cultures when added at the beginning of the experiment. These inhibitors also blocked any further increase of the elevated IMP dehydrogenase activity levels when added to cultures 1 day after 6-MP treatment.

Experiments were performed *in vitro* utilizing heat and trypsin inactivation in order to determine if the effect of 6-MP in IMP dehydrogenase could also be attributed in part to enzyme stabilization. The results are illustrated in Figs. 4 and 5. No difference in enzyme stability to heat inactivation or trypsin digestion was observed for extracts of cells grown in 10^{-4} M

Table 4. Effect of various purine analogs on IMP dehydrogenase specific activity in cell culture*

Compound added to culture medium	IMP dehydrogenase (nmoles/mg protein/hr)	Per cent of control
None	5·5	100
6-Mercaptopurine	18·2	331
6-Mercaptopurine ribonucleoside	13·2	240
6-Methylmercaptopurine ribonucleoside	3·7	67
6-Thioguanine	2·5	45
6-Chloropurine	3·2	58

* Replicate cultures in dialyzed serum-supplemented medium were grown for 3 days in the presence of the different compounds listed above, each at a concentration of 10^{-4} M. The results are the average of duplicate samples expressed as specific activity and as a per cent of the activity in untreated cells.

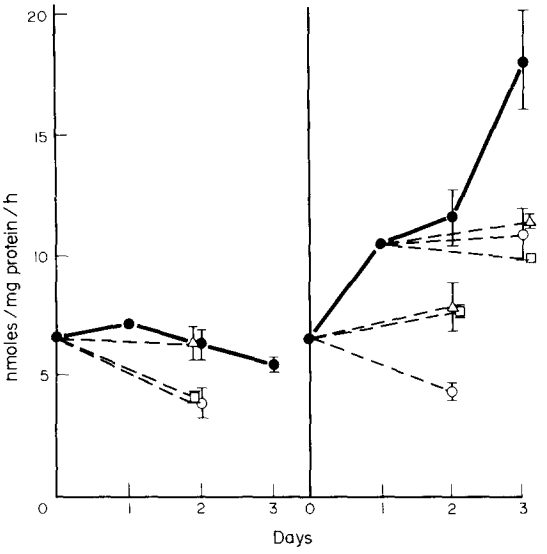

Fig. 3. Effect of inhibitors of RNA and protein synthesis on the increase of IMP dehydrogenase specific activity by 6-MP. Replicate cultures grown to confluency in dialyzed serum-supplemented medium were incubated for 3 days in the absence (left panel) and presence (right panel) of 6-MP (10^{-4} M). Actinomycin D, 0·1 μg/ml, cycloheximide, 5 μg/ml, or Puromycin, 3 μM, was added to some 6-MP-treated cultures at the beginning of the experiment (day 0) or 1 day later. These drugs were added to untreated cultures only on day 0. Cells were harvested from duplicate cultures at the indicated times and IMP dehydrogenase activity was assayed. Left panel and right panel: ●–●, control; ○–○, actinomycin D added; △–△, cycloheximide added, and □–□, puromycin added. Vertical bars indicate range of activity.

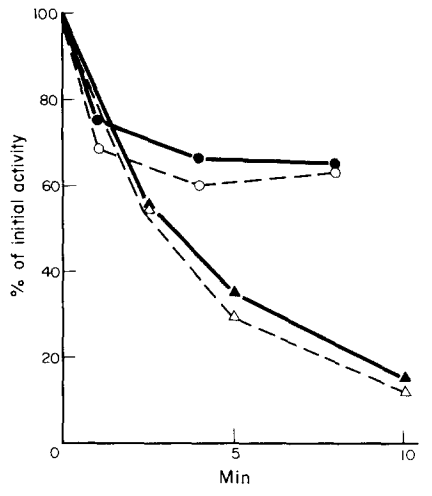

Fig. 4. Stability of IMP dehydrogenase activity in extracts of 6-MP-treated cells. Fibroblasts were cultured in the presence of 10^{-4} M 6-MP for 3 days, during which IMP dehydrogenase activity increased nearly 2-fold. These cells and untreated cells were then harvested and extracted in 0·05 M potassium phosphate, pH 7·4. The undialyzed extracts were adjusted with buffer to the same protein concentration, 3 mg/ml, and subjected to heat or trypsin inactivation as described under Materials and Methods. The levels of IMP dehydrogenase activity in extracts of control cells and 6-MP-treated cells were 28 and 51 nmoles/hr respectively. Enzyme activity is expressed as per cent of initial activity. ●——●, Control, heat inactivation; ○–○, cells grown in presence of 6-MP, heat inactivation; ▲——▲, control, trypsin inactivation; △–△, cells grown in presence of 6-MP, trypsin inactivation.

6-MP when compared to control cultures (Fig. 4). 6-ThioIMP was also examined for a stabilizing effect on IMP dehydrogenase activity in fibroblast extract (Fig. 5). This nucleotide analog was added to dialyzed lysate at a final concentration of 10^{-4} M. Only a slight decrease in heat stability and little or no effect on trypsin inactivation resulted; however, inhibition of enzyme activity by over 80 per cent indicated an interaction between 6-thioIMP and IMP dehydrogenase. A comparison of the stability of IMP dehydrogenase activity in undialyzed and dialyzed control extracts (Figs. 4 and 5) suggests that certain cellular metabolites or ions removed by dialysis may stabilize enzyme activity.

DISCUSSION

In this study it has been demonstrated that the specific activity of IMP dehydrogenase in cultured human fibroblasts is increased as much as 2- to 3-fold by the addition of the purine analog, 6-MP. This effect of 6-MP is not a general one on enzyme activity, since the specific activity of hypoxanthine-guanine phosphoribosyltransferase, another soluble enzyme, was not altered. The absence of an effect by 6-MP on cells deficient in hypoxanthine-guanine phosphoribosyltransferase activity indicated a requirement for conversion of the drug to its ribonucleotide form, 6-thioIMP. Increased IMP dehydrogenase specific activity was also observed with 6-mercaptopurine ri-

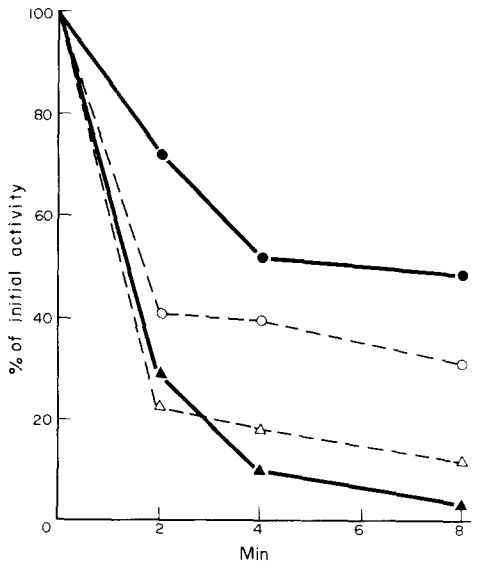

Fig. 5. Effect of 6-thioIMP on the stability of IMP dehydrogenase. Fibroblast extract, dialyzed against 0·05 M potassium phosphate, pH 7·4, and containing 3 mg/ml of protein and 30 nmoles/hr of IMP dehydrogenase activity, was incubated for 15 min at 25° in the presence and absence of 10^{-4} M 6-thioIMP and subjected to heat inactivation or trypsin inactivation as described under Materials and Methods. Enzyme activity is expressed as per cent of initial activity. ●——●, Control, heat inactivation; ○–○, 6-thioIMP added, heat inactivation; ▲——▲, control, trypsin inactivation; △–△, 6-thioIMP added, trypsin inactivation.

bonucleoside (6-MPR), but not with other purine analogs, including 6-methylmercaptopurine ribonucleoside, 6-thioguanine and 6-chloropurine. Since human fibroblasts do not appear to have the enzymatic capacity to phosphorylate 6-MPR, the effect of this compound on IMP dehydrogenase activity is probably due to its conversion to 6-MP in a reaction catalyzed by purine nucleoside phosphorylase.

It cannot be ascertained from these experiments whether 6-thioIMP or one of its metabolites is the active agent. Other known nucleotide metabolites of 6-MP include 6-methylmercaptopurine ribonucleoside 5′-monophosphate [11], 6-thioguanosine 5′-monophosphate [12] and 6-thioxanthosine 5′-monophosphate [13]. The first two derivatives are not likely to be responsible for this effect of 6-MP, since 6-methylmercaptopurine ribonucleoside and 6-thioguanine, which could be converted to their respective nucleotide forms, did not lead to an increase of IMP dehydrogenase specific activity in cell culture.

An attempt was made to determine the molecular mechanism responsible for the increase of IMP dehydrogenase specific activity produced by 6-MP. Inhibition of RNA synthesis by actinomycin D and inhibition of protein synthesis by cycloheximide or puromycin prevented the effect of 6-MP, suggesting that both RNA and protein synthesis may be required. This approach to the study of enzyme regulation, however, is compromised by the possibility that nonspecific metabolic toxicity of actinomycin D, cycloheximide or puromycin may influence the results. Thus, interpretation of these experiments is limited and more supporting or conclusive evidence is needed. Nonetheless, one interpretation of these data is that 6-MP, via a nucleotide derivative, in some manner stimulates the synthesis of IMP dehydrogenase at the transcriptional level.

In a recent study by Tidd and Paterson [14] using mouse lymphoma cells, 6-MP was found to cause a significant reduction in the guanine ribonucleotide pool size with only a slight decrease in the adenine ribonucleotide pool size. In addition, 6-thioguanine was shown to have little effect on either ribonucleotide pool, whereas 6-methylmercaptopurine ribonucleoside was noted to decrease the pools of both adenine and guanine ribonucleotides. Based on this study, 6-MP appeared to be the only one of the three purine analogs to have a specific effect on the cellular content of guanine ribonucleotides. Thus, the increase in IMP dehydrogenase specific activity mediated by 6-MP could possibly reflect derepression of the enzyme due to a decrease in the levels of guanine ribonucleotides. IMP dehydrogenase in bacterial cells has been shown to be controlled by end-product repression through guanine nucleotides [15]. Our results are consistent with this model.

Since IMP dehydrogenase from bacterial [6] or mammalian [4] cells forms a stable complex with 6-thioIMP through a disulfide linkage, stabilization of the human enzyme by the purine nucleotide analog was also considered as a possible explanation for the 6-MP-mediated increase in IMP dehydrogenase activity. The increased IMP dehydrogenase activity in lysates of 6-MP-treated cells, however, was not stabilized to inactivation by thermal denaturation or proteolysis. Furthermore, the addition of 6-thioIMP to fibroblast extract also failed to stabilize the enzyme to trypsin inactivation and may actually have decreased its stability to heat inactivation. Although, more precise methods of studying specific enzyme turnover will be required to exclude the possibility of enzyme stabilization, it is noteworthy that protection of dihydrofolate reductase activity from proteolytic or heat inactivation by amethopterin correlated well with the later observation that the intracellular degradation of dihydrofolate reductase is inhibited as a result of the tight binding of amethopterin to the enzyme molecule [16, 17].

6-MP has been used clinically as an immunosuppressive agent and an antileukemic drug. Several studies of the inhibition of cell proliferation by 6-MP have described a "delayed" cytotoxicity. The more recent studies by Tidd *et al.* [18] on the delayed response of cultured mouse lymphoma cells to 6-MP have led to subsequent biochemical work relating drug lethality to the degree of incorporation of 6-MP into DNA rather than to the inhibitory action on purine nucleotide biosynthesis [14, 19]. Since only confluent cell cultures characterized by a very low growth rate were used in the present studies, they presumably deal primarily with the latter metabolic effects of 6-MP and not with its effect on cell replication.

REFERENCES

1. G. B. Elion, *Fedn Proc.* **26,** 898 (1967).
2. B. Magasanik, H. S. Moyed and L. B. Gehring, *J. biol. Chem.* **226,** 339 (1957).
3. M. R. Atkinson, R. K. Morton and A. W. Murray, *Biochem. J.* **89,** 167 (1963).
4. J. H. Anderson and A. C. Sartorelli, *Biochem. Pharmac.* **18,** 2737 (1969).
5. E. W. Holmes, D. M. Pehlke and W. N. Kelley, *Biochim. biophys. Acta* **364,** 209 (1974).
6. A. Hampton and A. Nomura, *Biochemistry, N.Y.* **6,** 1679 (1967).
7. W. N. Kelley and J. B. Wyngaarden, *J. clin. Invest.* **49,** 602 (1970).
8. D. M. Pehlke, J. A. McDonald, E. W. Holmes and W. N. Kelley, *J. clin. Invest.* **51,** 1398 (1972).
9. W. N. Kelley and J. C. Meade, *J. biol. Chem.* **246,** 2953 (1971).
10. O. H. Lowry, W. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193,** 265 (1951).
11. P. W. Allan, H. P. Schnebli and L. L. Bennett, *Biochim. biophys. Acta* **114,** 647 (1966).
12. D. J. Nelson, C. J. L. Bugge, H. C. Krasny and T. P. Zimmerman, *J. Chromatog.* **77,** 181 (1973).
13. M. R. Atkinson, G. Eckermann and J. Stephenson, *Biochim. biophys. Acta* **108,** 320 (1965).
14. D. M. Tidd and A. R. P. Paterson, *Cancer Res.* **34,** 733 (1974).
15. H. J. J. Nijkamp and P. G. Dehaan, *Biochim. biophys. Acta* **145,** 31 (1967).
16. J. R. Bertino and R. L. Hillcoat, *Adv. Enzyme Regulat.* **6,** 335 (1968).
17. R. C. Jackson and F. M. Huennekens, *Archs Biochem. Biophys.* **154,** 192 (1973).
18. D. M. Tidd, S. C. Kim, K. Horakova, A. Moriwaki and A. R. P. Paterson, *Cancer Res.* **32,** 317 (1972).
19. D. M. Tidd and A. R. P. Paterson, *Cancer Res.* **34,** 738 (1974).