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Regulation of Purine Metabolism in Lymphocytes (42117)

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Abstract. Three general questions regarding nucleosides and lymphocytes are discussed: (a) Why are so many measurements being made of adenosine deaminase activity, what do the results mean, and why is there still disagreement about some of the conclusions; (b) what do we understand about nucleosides and lymphocyte death; and (c) to what extent do we really understand nucleoside and nucleotide metabolism in lymphocytes? Experimental studies show that treatment of mice with deoxycoformycin, to produce accumulation of deoxyadenosine, leads to rapid thymus involution, elevated dATP concentrations in thymus and liver, and inhibition of adenosylhomocysteine hydrolase in these tissues. Deoxyguanosine inhibits the growth of mouse lymphoma L5178Y cells, and this toxicity is prevented by deoxycytidine plus adenine. In cells treated with deoxyguanosine, concentrations of both GTP and dGTP are elevated, and this is not affected by deoxycytidine. Adenine, however, reduces GTP concentrations to normal, and prevents most of the elevation in dGTP concentrations. Contrary to previous belief, it has been demonstrated that lymphocytes and nucleated bone marrow cells will synthesize purine nucleotides *de novo* if incubated in an appropriate medium; carbon dioxide is particularly important for this process. © 1985 Society for Experimental Biology and Medicine.

"Nucleosides and lymphocytes" represents an area of scientific investigation that is unique in many areas. It is not just concerned with the biochemical basis of a single disease, or with a single metabolic pathway, or with the metabolism of a single precursor, or really, even with the metabolism of a single tissue or cell type. Instead, this biological system involves a wide range of cell types or subtypes as well as of many functional states, all of which are included under the term "lymphocytes." The nucleoside and nucleotide metabolism of this family of cells varies during normal development from earliest stages right up to the programmed death that is the fate of many lymphocytes. There is also a variety of functional states related to the complexity of the immune response in which lymphocytes play such a central role. Both immunodeficiency diseases and many kinds of leukemia and lymphoma are of concern, together with a variety of therapeutic modalities related to them.

From the point of view of intermediary metabolism, molecular biology, and biochemical pharmacology, several enzymes that were previously thought to have little significance—adenosine deaminase, purine nucleoside phosphorylase and 5′-nucleotidase in particular—or about which little was known, such as adenosylhomocysteine hydrolase, have now come to the fore. Purine catabolism and its regulation is now receiving much needed attention. There is also an increased concern with the extracellular environment in terms of nucleoside and nucleotide concentrations, and with the relationship between extracellular and intracellular metabolism and effects of nucleosides. Biochemical events involved in cell death are now receiving a great deal of attention, and new classes of drugs are being evaluated therapeutically and used investigationally.

The complexity of the field of nucleosides and lymphocytes, however, does mean that individual investigators have to focus on specialized subtopics; it is also difficult to maintain an overall knowledge of developments in the field as a whole, and those who approach the area with biochemical, immunologic, oncologic, or other backgrounds may not have the whole range of methodologic expertise and conceptional knowledge that would ideally be desirable. It is, therefore, an area that invites—even requires—collaboration, as well as regular occasions on which progress and problems in the field as a whole can be addressed, and, finally, a consistently critical approach. This conference offers an opportunity to achieve several of these goals.

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In this paper three questions having to do with the area of nucleosides and lymphocytes will be raised, and data related to each presented. Both the questions and data are by way of example, and clearly do not exhaust the subject.

Materials and Methods. Sources of most of the materials used, and methods for cell culture, cell extraction, measurement of ribonucleotide and deoxyribonucleotide concentrations, cell incubations, and separation of radioactive nucleotides, have been presented in detail (1).

To analyze nucleotides in liver, mice were anesthetized with Penthrane (Abbott Laboratories) and one lobe of the liver was exposed and clamped with tongs cooled in liquid nitrogen. The frozen tissue was ground to a powder under liquid nitrogen and homogenized in 0.4 *M* perchloric acid. To analyze nucleotides in thymus, mice were anesthetized and the thymus was removed quickly, rinsed with 0.154 *M* NaCl, and homogenized in cold 0.4 *M* perchloric acid. Lymphocytes were prepared from heparinized blood by layering on Ficoll–Hypaque; nucleated bone marrow cells and spleen erythroblasts were also prepared using this method.

To assay adenosylhomocysteine hydrolase activity, 50 μl of tissue supernatant (30,000*g*, 30 min) was added to a mixture consisting of 50 μl of 400 m*M* KH_2PO_4, pH 7.3, 10 μl of 25 μg/ml deoxycoformycin, 10 μl of 40 m*M* dithiothreitol, 20 μl of 50 m*M* [^{3}H]adenosine, and 60 μl of 10 m*M* homocysteine. At various times, adenosylhomocysteine was isolated by paper chromatography and its radioactivity measured.

Results. 1. The first question is: Why are investigators making so many measurements of adenosine deaminase (as well as of purine nucleoside phosphorylase and 5′-nucleotidase), what do these results mean, and why is there still disagreement about some of the conclusions that have been reached?

While the endpoint of studies measuring adenosine deaminase activity is all the same, the motives for doing such work vary widely, yet this often is not considered worthy of comment. However, it would seem that adenosine deaminase is of interest (a) as a cell subtype marker, (b) as a functional or differentiation state marker, (c) as a proliferation state marker, (d) as a target for chemotherapeutic attack, (e) as a determinant of drug sensitivity even though not the direct target, (f) as a determinant of drug catabolism, and (g) as a determinant of drug activation; perhaps there are others. All these subquestions are valid, but interpretation depends in part on clarity regarding the particular question being asked.

Despite an enormous literature, reports of measurements of adenosine deaminase, purine nucleoside phosphorylase and 5′-nucleotidase in cell extracts still show some areas of disagreement; for example, what really are the relative activities of these enzymes in T and B cells from peripheral blood? At present, reported values vary from 0.5 to 12 for the ratio of adenosine deaminase activity in T and B cells. One possible basis for these discrepancies is the enzyme assay itself; sometimes only inosine is measured as product, whereas often some (even considerable) hypoxanthine is formed even when nonphosphate buffers are used. It has also been pointed out that conclusions may vary depending on whether results are expressed on a per cell or per unit protein basis. Finally, it seems likely that what are called B cells may vary according to the preparation method used.

Few comparisons of T and B (or non-T) cell enzyme activities have used intact cells. Table I, however, shows rates of deamination of adenosine in intact lymphocytes incubated with adenosine; results of similar studies with myeloma cells are also presented. (Rates of deamination by peripheral blood granulocytes are in the range of those given by the myeloma cells.) These results differ from the common generalization that adenosine deaminase activities are greater in T than B cells, and further studies are required to resolve this question.

Another matter is the relevance of conclusions regarding peripheral T and B cells *in vivo* based on studies of cultured lymphoblast lines of T and B cell origins. In some cases T and B lymphoblasts differ enormously, e.g., with respect to adenosine deaminase activities, and these differences are much greater than any result obtained with periph-

TABLE I. ADENOSINE DEAMINASE ACTIVITY IN INTACT LYMPHOID CELLS *IN VITRO*

Donor[a]	Enzyme activity[b] T lymphocytes	Non-T lymphocytes	Plasma cells
LB	0.45	0.90	
BP	0.08	0.38	
EP	0.44	0.75	
PR	0.22	0.18	
JL	0.74	0.92	
GZ	0.45	0.93	
MB	0.92	0.91	
CP	0.59	0.54	
AH			5.26
SH			2.76
BS			5.45
NS			4.10
GD			5.34
ER			9.42

Note. Values are averages of triplicate determinations; average variation from the mean was less than 10%.

[a] T and non-T lymphocytes were isolated from blood of normal individuals and plasma cells from myeloma marrow aspirates and incubated with 0.5 m*M* [^{3}H]adenosine.

[b] Nanomoles of adenosine deaminated per 10^6 cells per hour.

eral blood cells. To what differentiation or functional states do these cultured lymphocytes correspond, if any? To what extent do they reflect the situation of resting lymphocytes at all?

Finally, very interesting studies have shown marked species differences in the enzymes of purine metabolism in lymphocytes from different animal species. What do these differences tell us about the relationship between purine metabolism and lymphocyte growth and function? To what extent are we misled by drawing conclusions regarding this relationship from studies using cells from a single species? Further investigation is needed.

2. The second question is: What do we really know about nucleosides and lymphocyte death?

Lymphocytes are unique in their sensitivity to the cytotoxic effects of a variety of nucleosides, but also in the fact that they (or at least some subtypes) are programmed for death to a particularly notable extent. What does this mean? And death for lymphocytes means not only cessation of reproductive potential, but relatively rapid lysis. There are many questions that still remain unanswered about this subject.

This field really may need to be divided into two parts, one concerned with the death of rapidly dividing cells, such as cultured lymphocytes and some classes of thymocytes; and the other concerned with the death of nongrowing lymphocytes, such as those in the peripheral blood. Whether these are one subject or two is not yet clear.

Studies of nucleosides and lymphocyte death have focused on the action of deoxyadenosine and drugs that cause its accumulation (2), and on the action of deoxyguanosine and its analogs (3).

Treatment of mice with deoxycoformycin, a potent inhibitor of adenosine deaminase, leads to the accumulation of deoxyadenosine and this potent nucleoside produces lymphoid toxicity. Proposed mechanisms of this toxicity include inhibition of ribonucleotide reductase by dATP, inhibition or inactivation of adenosylhomocysteine hydrolase by deoxyadenosine, and accumulation of single-strand breaks in DNA (2). Most studies of the mechanism of toxicity of deoxyadenosine have been carried out using cultured cell systems; here studies of this toxicity using mouse tissues *in vivo* are reported.

Treatment of mice with a range of doses of deoxycoformycin from 0.2 to 10 mg/kg produced progressive decreases in thymus weight 24 hr after administration; these ranged from 17% loss of weight at the lowest dose to 63% at the highest dose.

The concentrations of dATP and the activity of adenosylhomocysteine hydrolase were each measured in a drug-sensitive tissue, thymus, and an insensitive tissue, liver, over the same 40-fold range of deoxycoformycin doses used above (0.25 to 10 mg/kg). Table II shows that there was a dose-related increase in dATP concentrations following deoxycoformycin treatment. In thymus, 0.25 mg/kg deoxycoformycin produced only a small (13%) increase in dATP, and the maximum increase was 5.7-fold control levels. In contrast, 0.25 mg/kg deoxycoformycin produced a 3-fold increase in dATP in liver, while its concentration in livers of mice treated with

TABLE II. EFFECT OF DEOXYCOFORMYCIN TREATMENT ON dATP CONCENTRATIONS AND ADENOSYLHOMOCYSTEINE ACTIVITY IN THYMUS AND LIVER

Deoxycoformycin dose (mg/kg)	dATP concentration (pmole/μg protein)		Adenosylhomocysteine hydrolase activity (pmole/min/μg protein)	
	Liver	Thymus	Liver	Thymus
0	0.0011 ± 0.0002	0.050 ± 0.003	19.2 ± 0.17	2.74 ± 0.21
0.25	0.0032 ± 0.0002[b]	0.057 ± 0.006[a]	19.2 ± 0.15[a]	2.27 ± 0.24[a]
0.50	0.0051 ± 0.005[b]	0.080 ± 0.007[b]	20.5 ± 0.18[a]	1.79 ± 0.14[b]
1.0	0.0066 ± 0.005[b]	0.085 ± 0.007[b]	17.9 ± 0.14[a]	1.79 ± 0.15[b]
5.0	0.0132 ± 0.011[b]	0.136 ± 0.012[b]	15.7 ± 0.13[a]	1.68 ± 0.13[b]
10	0.0219 ± 0.120[b]	0.287 ± 0.027[b]	10.5 ± 0.07[b]	0.95 ± 0.09[b]

Note. CDF mice were injected intraperitoneally with deoxycoformycin, and measurements made after 24 hr. Values are means ± SD of four determinations in two experiments.

[a] $P > 0.05$.

[b] $P < 0.05$.

10 mg/kg was elevated 20-fold. Note, however, that the total dATP concentration at each deoxycoformycin dose was higher in thymus than in liver.

Table II also shows the effect of deoxycoformycin treatment on activities of adenosylhomocysteine hydrolase in homogenates of thymus and of liver; this enzyme has been reported to be inhibited by free deoxyadenosine. It may be noted first that in untreated mice the activity of this enzyme was sevenfold greater in liver than in thymus.

In liver the two lower doses of deoxycoformycin did not have any effect on adenosylhomocysteine hydrolase levels; however, the enzyme in thymus was inhibited at these doses. The maximum extent of inhibition was 45% in liver and 65% in thymus.

Other experiments showed that deoxycoformycin had no effect on ATP or cyclic AMP concentrations in these tissues.

A second area of investigation in regard to the toxicity of nucleosides for lymphocytes concerns the effects of deoxyguanosine, a subject that has received less attention. Studies of protection against deoxyguanosine toxicity, and of the metabolism of this nucleoside under conditions of toxicity, are reported here.

Dose–response relationships for deoxyguanosine toxicity were determined using both Chinese hamster ovary (CHO) cells and mouse lymphoma L5178Y cells grown in culture. The L5178Y cells were the more sensitive; 100 μM deoxyguanosine produced 20% inhibition of growth in 24 hr, whereas this concentration had no effect on CHO cells. Deoxyguanosine concentrations of 250 μM produced 100% cessation of growth in L5178Y cells, but only 30% inhibition in CHO; 1000 μM nucleoside was required for complete growth inhibition in the latter cells. Virtually complete protection against deoxyguanosine toxicity was afforded by a combination of deoxycytidine plus adenine; each afforded partial protection individually.

Table III shows that intracellular concentrations of both GTP and dGTP were elevated substantially in L5178Y cells incubated with deoxyguanosine, depending on time and deoxyguanosine concentration. The relative increase was greater for dGTP than for GTP, though the total amount of GTP formed was the greater. Other experiments have shown that added deoxyguanosine was in fact metabolized to both GTP and dGTP under these conditions.

Two questions were asked in experiments using [3H]deoxyguanosine: (a) Is the protective effect of deoxycytidine produced by prevention of the phosphorylation of deoxyguanosine? (b) To what extent is deoxyguanosine converted to dGTP through direct phosphorylation, and to what extent via conversion to guanine ribonucleotides and subsequent reduction of the ribonucleotides to deoxyribonucleotides? Data not reported here showed that deoxycytidine had no effect

TABLE III. EFFECT OF DEOXYGUANOSINE ON GTP AND dGTP CONCENTRATIONS IN LYMPHOMA L5178Y CELLS

Deoxyguanosine concentration (μM)	GTP concentration (nmole/10^6 cells)		dGTP concentration (pmole/10^6 cells)	
	7 hr	24 hr	7 hr	24 hr
0	0.6 ± 0.05	0.6 ± 0.05	13 ± 1.1	12 ± 0.08
125	1.9 ± 0.16[b]	1.6 ± 0.11[b]	17 ± 2.2[a]	16 ± 1.0[b]
250	2.8 ± 2.2[b]	2.6 ± 0.22[b]	68 ± 4.9[b]	252 ± 18.2[b]

Note. L5178Y cells were grown in the presence and absence of deoxyguanosine. Values are means ± SD of four measurements in two experiments.
[a] $P > 0.05$.
[b] $P < 0.05$.

on the conversion of deoxyguanosine to dGTP.At least some dGTP is produced via guanine ribonucleotides, but technical limitations have so far prevented quantitative estimation of this route.

Table IV shows that the concentration of adenine used to protect L5178Y cells against the toxicity of deoxyguanosine (50 μM) also prevented the increase in GTP that usually accompanied incubation with deoxyguanosine. This result presumably was due (a) to competition for phosphoribosylpyrophosphate between adenine and the guanine derived from the phosphorolysis of deoxyguanosine, and (b) inhibition of guanine phosphoribosyltransferase by the elevated ATP concentrations resulting from the metabolism of adenine. At the same time, the elevation of dGTP concentrations accompanying incubation with deoxyguanosine was also reduced, but not completely to control levels; the simplest conclusion is that ca. 80% of the dGTP formed from deoxyguanosine was synthesized via GTP, with the remainder perhaps resulting from the direct phosphorylation of deoxyguanosine.

3. The third question is: To what extent do we really understand nucleoside and nucleotide metabolism in lymphocytes?

Although we have a great deal of data on certain aspects of metabolism, particularly total activities of enzymes of purine catabolism and nucleoside salvage, there is a great deal that has not been sufficiently explored, or about which we have only sparse information.

For one thing, there really are relatively few data on ribonucleotide concentrations in lymphocytes, and even fewer for deoxyribonucleotide concentrations, though this situation is improving. (More data are available for cultured lymphoblasts, of course, but these do not represent exactly the same biological system.) Such measurements are lim-

TABLE IV. EFFECT OF ADENINE ON NUCLEOTIDE CONCENTRATIONS IN L5178Y CELLS TREATED WITH DEOXYGUANOSINE

Deoxyguanosine concentration (μM)	Adenine	ATP (nmole/10^6 cells)	GTP (nmole/10^6 cells)	dGTP (pmole/10^6 cells)
0	0	2.6 ± 0.18	0.7 ± 0.06	14 ± 1.2
0	50	4.2 ± 0.04[b]	0.6 ± 0.04[a]	12 ± 1.4[a]
250	0	2.2 ± 0.02	2.4 ± 0.19	65 ± 4.8
250	50	4.6 ± 0.04[b]	0.8 ± 0.07[b]	22 ± 2.1[b]

Note. L5178Y cells were grown for 4 hr in the presence or absence of deoxyguanosine and adenine. Values are means ± SD of four measurements in two experiments.
[a] $P > 0.05$.
[b] $P < 0.05$.

ited by the relatively small number of lymphocytes that are available in many types of experiments. In addition, the preparation or purification methods that often have to be used may lead to some nucleotide breakdown. Finally, extraction methods always need to be looked upon critically, lest they also produce breakdown. One control that may be used is the ratio of ATP concentration to that of ADP. Theoretical considerations lead to the belief that high values indicate good energy metabolism, low rates of nucleotide dephosphorylation, or both; in cultured cells ratios of between 10 and 20 are routinely measured in this laboratory. If this ratio is 5 or more (or preferably 10 or more), one can be assured that nucleotide degradation is a minor problem, or none at all. Values of 3 to 5, which often are found after Percoll or Hypaque procedures, are less desirable, but not invalidating. Values below 3, however, indicate that serious degradation of nucleotides probably has taken place.

Other questions that need much more investigation, include the following. (a) What are the functions of the catabolic enzymes that are the focus of so much work in this field (and adenosine deaminase, purine nucleoside phosphorylase, and 5′-nucleotidase are catabolic enzymes and not, as sometimes said, salvage enzymes)? To what extent are they purely catabolic, to what extent are their natural substrates extracellular or intracellular, and to what extent are they linked to salvage enzymes to provide cycles of purine catabolism and reutilization? (b) The well-deserved focusing of attention on nucleotide catabolism and its regulation has brought attention to this relatively neglected area, but the relationship of catabolism in intact cells to other aspects of nucleotide metabolism—salvage, *de novo* synthesis, interconversion, phosphorylation, nucleotide coenzymes—now needs to be studied, and in intact cells in which all these processes are going on at the same time. (c) Recent studies in other systems raise questions regarding the relationship of the activity of surface enzymes to receptors of various types, and of the relationship of adenosine physiology and pharmacology to adenosine toxicity.

All of these areas of investigation need clearly to be conducted on as many subsets, differentiation stages and functional states of lymphocytes as possible, and in several species. For biochemists, this of course raises questions of methods of preparing these cells, characterizing them and checking their purity, and of the complexity of needing sometimes to work with mixtures of cell types which are of functional importance. The potential of the cell sorter has not yet been much used for biochemical studies, and the methods customarily used to fix appropriate antibodies to lymphocytes must be investigated to see if they are unfavorable to nucleotide metabolism. In addition, the time required to collect the number of cells required for biochemical studies may result in some nucleotide breakdown.

One area that has received relatively little attention is *de novo* purine and pyrimidine nucleotide biosynthesis in lymphocytes. For many years it has been believed that nucleated bone marrow cells, of which a portion are lymphoid, cannot synthesize purines *de novo;* in addition, peripheral lymphocytes also were believed to have very low rates of this process unless subjected to mitogen stimulation. Conditions under which substantial rates of purine biosynthesis *de novo* can be demonstrated in these cell types are reported here.

In initial experiments it was not possible to demonstrate [14C]formate incorporation into ATP in preparations of nucleated mouse bone marrow cells or of human peripheral lymphocytes, when either Krebs–Ringer phosphate medium or Fisher's tissue culture medium buffered with phosphate were used. However, the medium used recently by Gordon *et al.* (4) in studies with peripheral leukocytes led to quite substantial incorporation of formate; Table V shows representative data using human lymphocytes. The most important component of this medium appears to be the carbon dioxide; however, even in its absence this medium supported formate incorporation to a greater degree than did Krebs–Ringer phosphate medium.

Table VI shows that the medium of Gordon *et al.*, in the presence of 5% carbon dioxide, stimulated radioactive formate incorporation into ATP of mouse bone marrow cells, peripheral lymphocytes (but not gran-

TABLE V. PURINE BIOSYNTHESIS *DE NOVO* IN HUMAN PERIPHERAL LYMPHOCYTES IN DIFFERENT MEDIA

Medium	Incorporation of [^{14}C]formate into ATP (dpm)
Krebs–Ringer + 25 m*M* phosphate	214 ± 14.7[a]
Krebs–Ringer + 25 m*M* phosphate + HCO_3/5% CO_2	3260 ± 226[b]
Gordon *et al.* + 25m*M* phosphate	2483 ± 186[b]
Gordon *et al.* + 25 m*M* phosphate + HCO_3/5% CO_2	9250 ± 796[b]

Note. Human peripheral lymphocytes (10^7 cells/ml) were incubated for 60 min with 1 m*M* [^{14}C]formate. The medium of Gordon *et al.* [Ref. (4)] contains 25 m*M* K_2HPO_4, 100 m*M* NaCl, 20 m*M* Hepes, pH 7.4, 10 m*M* $NaHCO_3$, 5.5 m*M* glucose, 4.0 m*M* glycine, 2.0 m*M* glutamine, and 0.4% bovine serum albumin. Results are means ± SD of duplicate determinations in four to six experiments.
[a] $P > 0.05$.
[b] $P < 0.05$.

ulocytes), and in mouse spleen erythroblasts produced following phenylhydrazine treatment. In other experiments this was also demonstrated in concanavalin A-stimulated mouse spleen lymphoblasts, even though mitogen treatment itself produces an acceleration of *de novo* purine synthesis. Further studies of this system are continuing.

Discussion. Observations that deoxycoformycin treatment elevates dATP concentrations in liver as well as in thymus provide new evidence regarding deoxyribonucleoside metabolism in the former tissue; formerly it was believed that deoxyadenosine was not phosphorylated in nongrowing, nonlymphoid cells. In addition, it becomes clear that elevated dATP concentrations alone are insufficient to produce toxicity; whether this metabolism plays no role in deoxyadenosine toxicity, or whether other factors must also be operative, remains to be investigated.

The inhibition of adenosylhomocysteine hydrolase in liver and thymus shows the potency of free deoxyadenosine as an inhibitor of this enzyme. Observations that its activity generally is lower in thymus than in liver, and that deoxycoformycin treatment produced greater inhibition in thymus, suggest that it may play a role in deoxyadenosine toxicity in this tissue; however, the difference in sensitivity is not great. Certainly, these relatively straightforward dose–response studies of biochemical parameters of deoxyadenosine toxicity have not led to clear-cut conclusions regarding its mechanism of action. The possibility that still other mechanisms of toxicity, such as accumulation of DNA strand breaks (5, 6), are important must also be seriously considered.

Deoxyguanosine was shown to be toxic to cultured murine lymphoblasts, and toxicity was associated with elevated concentrations both of GTP and of dGTP. One protecting metabolite, deoxycytidine, did not decrease dGTP concentrations or prevent deoxyguanosine phosphorylation; hence it may work by restoring dCTP concentrations, which are reduced by deoxyguanosine treatment.

The fact that adenine lowers GTP concentrations to normal and partially lowers dGTP concentrations is consistent with considerable phosphorolysis of deoxyguanosine to guanine and conversion of the latter to guanylate via guanine phosphoribosyltransferase; guanine ribonucleotides are then converted to deoxyribonucleotides. Inasmuch as adenine did not completely inhibit guanine phosphoribosyltransferase under the conditions used,

TABLE VI. PURINE BIOSYNTHESIS *DE NOVO* IN MOUSE CELLS IN DIFFERENT MEDIA

Cell type	Incorporation of [^{14}C]formate into ATP (dpm): Krebs–Ringer + 25 m*M* phosphate medium	Gordon *et al.* + 25 m*M* phosphate + HCO_3/5% CO_2
Bone marrow	196 ± 14.1[b]	44,400 ± 2800[b]
Erythroblasts	244 ± 18.2[a]	114,500 ± 6700[b]
Peripheral lymphocytes	110 ± 8.2[b]	9,100 ± 450[b]
Peripheral granulocytes	50 ± 3.6[a]	143 ± 10[b]

Mouse cell preparations (ca. 10^7 cells/ml) were incubated as described in Table I. Results are means ± SD of duplicate determinations in four experiments.
[a] $P > 0.05$.
[b] $P < 0.05$.

the possibility must be considered that all of the observed elevation in dGTP may be due to metabolism of deoxyguanosine to guanine ribonucleotides, rather than to direct phosphorylation via deoxynucleoside kinases.

The observation that lymphocytes and some other hematopoietic cells can synthesize purine nucleotides *de novo* if the appropriate incubation medium is used is quite noteworthy. Exactly why these cells are so sensitive to incubation conditions in this regard, and the precise role of carbon dioxide in stimulating purine synthesis, remain to be investigated in detail. In the latter case, one possibility is that the bicarbonate-requiring reaction in the pathway of purine biosynthesis *de novo* has a higher Michaelis constant for this substrate than in other tissues.

If lymphocytes and nucleated bone marrow cells synthesize purines *de novo in vivo*—a possibility that remains to be tested—then a new look must be taken regarding the chemotherapy of malignant lymphoid cells and regarding the possible bone marrow toxicity of drugs that inhibit this pathway.

In conclusion, the area of nucleosides and lymphocytes is a promising and rewarding area of investigation, and one with much application to important developmental, functional, pathological and pharmacological problems.

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