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ALTERATIONS IN PURINE NUCLEOTIDE BIOSYNTHESIS INDUCED BY 2-AMINO-6-CHLOROPURINE*

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Abstract—Exposure of sarcoma 180 ascites cells to 2-amino-6-chloropurine (6-ClG) *in vivo* resulted in several metabolic lesions in the pathways of purine nucleotide biosynthesis. Pronounced inhibition of the incorporation of glycine-2-^{14}C into the guanine and adenine nucleotides of the nucleic acids, coupled with blockade of the utilization of 4-amino-5-imidazolecarboxamide-2-^{14}C solely for the formation of guanine nucleotides, indicated the presence of a site of inhibition on the pathway of purine nucleotide formation *de novo*. The conversion of hypoxanthine-8-^{14}C to nucleic acid guanine was markedly decreased by 6-ClG, whereas the utilization of this labeled precursor for the formation of nucleic acid adenine was not lowered by this agent. Under these conditions neither the incorporation of xanthine-8-^{14}C nor of guanine-8-^{14}C into the guanine moiety of the nucleic acids was depressed, indicating that 6-ClG or a derivative thereof inhibited the conversion of inosine 5′-phosphate to xanthosine 5′-phosphate. Blockade of this site was confirmed by the finding that 2-amino-6-chloropurine ribonucleoside 5′-phosphate inhibited the activity of inosine 5′-phosphate dehydrogenase. Molar equivalent levels of 2-amino-6-chloropurine ribonucleoside and 2-amino-6-chloropurine ribonucleoside triacetate caused a degree of inhibition of the conversion of hypoxanthine-8-^{14}C to guanine nucleotides comparable to that produced by 6-ClG. 2-Amino-6-iodopurine ribonucleoside was at least as active an inhibitor of the formation of nucleic acid guanine as the chloro-derivatives.

6-MERCAPTOPURINE (6-MP), 6-thioguanine (TG), and 6-chloropurine (6-ClP) are purine analogs that possess potent antineoplastic and immunosuppressive properties. Both purinethiols, as mononucleotides, induce multiple metabolic blocks on the pathways of purine nucleotide biosynthesis.[1–6] The enzymatic sites of blockade include: phosphoribosylpyrophosphate (PRPP) amidotransferase, inosine 5′-phosphate (IMP) dehydrogenase, adenylosuccinate lyase, and guanosine 5′-phosphate (GMP) and IMP pyrophosphorylase by both 6-MP and TG; GMP: adenosine triphosphate phosphotransferase by TG; and adenylosuccinate synthetase by 6-MP. In contrast, the halogen-substituted purine 6-ClP is considerably more selective with regard to inhibition of the formation of purine nucleotides, since inhibitions of IMP dehydrogenase[7–11] and GMP reductase[12] are the only known enzymatic blocks in the anabolic routes produced by this agent after its conversion to 6-chloropurine mononucleotide.[13]

Although the halogenated guanine analog, 2-amino-6-chloropurine (6-ClG), which relates to 6-ClP in a manner analogous to that of TG to 6-MP, has been available,

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little is known of the metabolic alterations produced by this agent. In relatively high doses 6-ClG has been reported to inhibit the growth of adenocarcinoma 755.[14] With regard to known biochemical effects, the ribonucleoside of 6-ClG did not serve as a substrate for the enzyme-catalyzed extracellular exchange that occurs between purine bases and ribonucleosides,[15] but did serve as a substrate for the dechlorinase activity of adenosine deaminase of rat heart.[16,17]

This report describes isotopic studies designed to elucidate some of the sites of blockade of purine nucleotide biosynthesis induced by 6-ClG in sarcoma 180 ascites cells.

MATERIALS AND METHODS

Experiments were performed on female CD-1 Swiss mice (9–11 weeks old) obtained from Charles River Breeding Laboratories, North Wilmington, Mass. These animals were inoculated i.p., 6 days before use, with 0·1 ml of ascites cell suspension prepared by centrifugation (1600 *g*) for 2 min followed by dilution of the cell pack 1:15 with isotonic saline.[18]

Inhibitors were homogenized in absolute ethanol (adjusted so that the final concentration of the drug solution was 5 per cent with respect to ethanol) and 2–3 drops of 20 per cent aqueous Tween 80, and then made up to volume with isotonic saline. Each ascites tumor-bearing animal was given a single i.p. dose of 6-ClG or a related compound and, at selected time intervals thereafter, either 100 μg glycine-2-^{14}C ($4{\cdot}9 \times 10^4$ cpm/μg), 90 μg 4-amino-5-imidazolecarboxamide-2-^{14}C ($1{\cdot}5 \times 10^4$ cpm/μg), 50 μg hypoxanthine-8-^{14}C ($1{\cdot}1 \times 10^4$ cpm/μg), 50 μg guanine-8-^{14}C ($1{\cdot}0 \times 10^4$ cpm/μg), 50 μg xanthine-8-^{14}C ($2{\cdot}6 \times 10^4$ cpm/μg), or 50 μg adenine-8-^{14}C ($2{\cdot}6 \times 10^4$ cpm/μg) was administered by i.p. injection to each mouse and 1 hr was allowed for metabolic utilization. The cells were then collected, and the nucleic acid purines and acid-soluble adenine were isolated and analyzed as described previously,[19,20] except that radioactivity was measured with a Packard Tri-Carb liquid scintillation spectrometer. The composition of the phosphor solution has been reported.[21]

IMP dehydrogenase was partially purified from sarcoma 180 ascites cells by centrifugation of sonicates at 104,000 *g* and ammonium sulfate fractionation of the supernatant; the precipitate that formed between 20 and 40 per cent saturation with $(NH_4)_2SO_4$ contained most of the enzymatic activity. Enzymatic activity was determined by monitoring the change in absorbancy at 340 mμ with time, a measure of the net production of NADH.[22] Assays were conducted at 37°; each cuvette contained 2·3 μmole IMP, 0·28 μmole NAD, 100 μmole KCl, 100 μmole Tris Cl (pH 8·0), and 0·1 ml of enzyme preparation (1–3 mg protein).

RESULTS

Several isotopic precursors of purine nucleotides were employed to detect sites of inhibition by 6-ClG on the pathways involved in the biosynthesis of adenine and guanine nucleotides; the results obtained are shown in Table 1. The conversion of glycine-2-^{14}C to purine nucleotides was used as a measure of the *de novo* biosynthetic route. Essentially equal inhibition of the incorporation of radioactive glycine into both guanine and adenine of the nucleic acids was induced by 6-ClG. The finding that the incorporation of 4-amino-5-imidazolecarboxamide-2-^{14}C into the adenine nucleotides was not depressed by 6-ClG indicated that blockade of the *de novo* pathway

occurred prior to the formation of 4-amino-5-imidazolecarboxamide ribonucleotide. Inhibition of the utilization of 4-amino-5-imidazolecarboxamide-^{14}C for the formation of nucleic acid guanine was produced by 6-ClG, suggesting the presence of an additional metabolic block subsequent to the formation of IMP. 6-ClG inhibited the incorporation of hypoxanthine-8-^{14}C into nucleic acid guanine, whereas the utilization

TABLE 1. INCORPORATION OF LABELED PRECURSORS INTO THE NUCLEIC ACID PURINES OF SARCOMA 180 ASCITES CELLS TREATED WITH 2-AMINO-6-CHLOROPURINE*

Isotopic substrate	6-ClG	cpm/μmole $\times 10^{-2}$		
		NA† guanine	NA adenine	AS adenine
Glycine-2-^{14}C	−	6·8 ± 0·7	7·1 ± 0·5	191·6 ± 15·2
	+	1·6 ± 1·1	2·1 ± 0·5	51·5 ± 14·7
4-Amino-5-imidazole-carboxamide-2-^{14}C	−	48·8 ± 7·5	60·0 ± 7·1	753·7 ± 53·3
	+	6·4 ± 0·3	66·1 ± 1·0	1241·8 ± 106·7
Hypoxanthine-8-^{14}C	−	11·4 ± 2·0	11·1 ± 1·2	259·8 ± 27·6
	+	1·3 ± 0·1	13·8 ± 3·5	420·5 ± 29·1
Xanthine-8-^{14}C	−	13·6 ± 0·8	1·0 ± 0·2	11·2 ± 1·7
	+	12·5 ± 1·1	1·2 ± 0·3	18·5 ± 2·6
Guanine-8-^{14}C	−	24·0 ± 4·0	1·6 ± 0·3	51·4 ± 6·4
	+	20·7 ± 4·0	1·6 ± 0·3	47·8 ± 9·3
Adenine-8-^{14}C	−	3·0 ± 0·5	48·6 ± 5·5	1159·0 ± 68·1
	+	1·2 ± 0·1	47·2 ± 12·0	1337·9 ± 32·0

* Mice bearing 6-day implants of sarcoma 180 ascites cells received a single i.p. dose of 88 mg 6-ClG/kg. One hr later, either 100 μg glycine-2-^{14}C ($4·9 \times 10^4$ cpm/μg), 90 μg 4-amino-5-imidazolecarboxamide-2-^{14}C ($1·5 \times 10^4$ cpm/μg), 50 μg hypoxanthine-8-^{14}C ($1·1 \times 10^4$ cpm/μg), 50 μg xanthine-8-^{14}C ($2·6 \times 10^4$ cpm/μg), 50 μg guanine-8-^{14}C ($1·0 \times 10^4$ cpm/μg), or 50 μg adenine-8-^{14}C ($2·6 \times 10^4$ cpm/μg) was administered by i.p. injection to each mouse and 1 hr was allowed for metabolic utilization. Each figure represents the mean value (± the S.E.) of results obtained from the separate analyses of cells from 3–8 mice.

† NA, nucleic acid; AS, acid-soluble.

of both xanthine-8-^{14}C and guanine-8-^{14}C for the formation of nucleic acid guanine was not depressed. Since 6-ClG did not retard the conversion of these radioactive substrates to adenine of the nucleic acids, it appeared that 6-ClG inhibited the conversion of IMP to xanthosine 5′-phosphate. It is of interest that xanthine-8-^{14}C was an effective precursor of the nucleic acid purines in this system; this phenomenon would appear to be due in part to the relatively low xanthine oxidase activity of this ascites neoplasm (unpublished observations).

To test this premise, the effects of 2-amino-6-chloropurine ribonucleoside 5′-phosphate (6-ClGMP) on the activity of IMP dehydrogenase of sarcoma 180 were determined. At a concentration of $2·3 \times 10^{-3}$ M IMP and $2·8 \times 10^{-4}$ M NAD, 50 per cent inhibition of enzymatic activity was produced by a level of $3·7 \times 10^{-4}$ M 6-ClGMP. 2-Amino-6-chloropurine ribonucleoside and 6-ClG at concentrations up to $4·6 \times 10^{-3}$ M and $3·8 \times 10^{-3}$ M, respectively, did not inhibit the activity of this enzyme.

The conversion of adenine-8-^{14}C to guanine nucleotides in intact sarcoma 180 ascites cells was inhibited by 6-ClG (Table 1), whereas the utilization of this radioactive precursor for the formation of adenine nucleotides was unaffected.

The concentration of 6-ClG required to inhibit partially the incorporation of hypoxanthine-8-^{14}C into the guanine moiety of the nucleic acids in intact sarcoma 180

ascites cells is shown in Fig. 1. The data presumably reflect inhibition of IMP dehydrogenase by 6-ClGMP. Maximum inhibition by 6-ClG was achieved at a dose of 22 mg/kg. The duration of the blockade of this pathway induced by the guanine analog was ascertained with a relatively large dose of 6-ClG (88 mg/kg); these results are depicted in Fig. 2. Maximum inhibition (approximately 80 per cent) of the incorporation of hypoxanthine-^{14}C into the guanine of nucleic acids persisted for up to 3 hr after

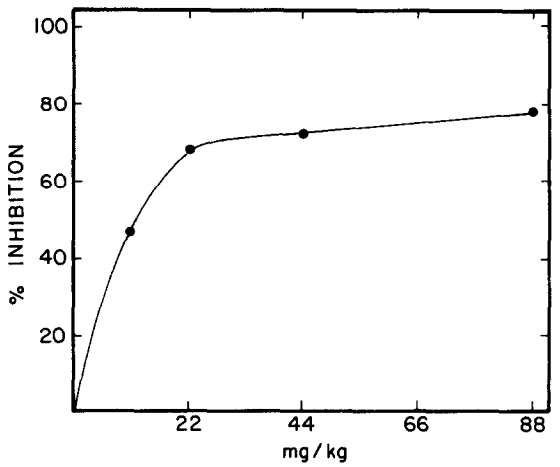

FIG. 1. Effect of various levels of 2-amino-6-chloropurine on the incorporation of hypoxanthine-8-^{14}C into nucleic acid guanine of sarcoma 180 ascites cells. Tumor-bearing mice were injected i.p. with various doses of 6-ClG. One hr later, 50 μg hypoxanthine-8-^{14}C ($1{\cdot}1 \times 10^4$ cpm/μg) was administered by i.p. injection to each mouse and 1 hr was allowed for metabolic utilization. Each point represents the mean value of results from the separate analyses of cells from 4–8 mice.

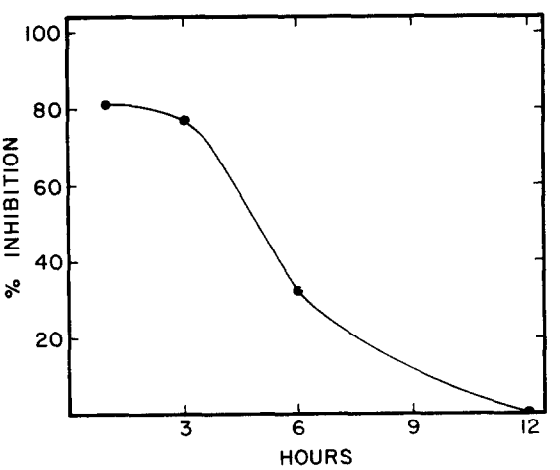

FIG. 2. Duration of inhibition of the incorporation of hypoxanthine-8-^{14}C into nucleic acid guanine of sarcoma 180 ascites cells by 2-amino-6-chloropurine. Tumor-bearing mice were injected i.p. with a single dose of 88 mg 6-ClG/kg. At selected time intervals thereafter, 50 μg hypoxanthine-8-^{14}C ($1{\cdot}1 \times 10^4$ cpm/μg) was administered by i.p. injection to each mouse and 1 hr was allowed for metabolic utilization. Each point represents the mean value of results from the separate analyses of cells from 4–8 mice.

the drug was administered. Rapid recovery from inhibition occurred; thus, when the isotopic substrate was administered 6 hr after 6-ClG, only about 30 per cent inhibition remained. By 12 hr after the administration of the analog, no retardation of the conversion of hypoxanthine to guanine nucleotides was observed.

The effects of some halogenated purine ribonucleoside analogs on the inhibition of the incorporation of hypoxanthine-8-^{14}C into the nucleic acid guanine of sarcoma 180 were determined (Table 2). The results obtained with 2-amino-6-chloropurine ribonucleoside and 2-amino-6-chloropurine ribonucleoside triacetate were comparable to

TABLE 2. EFFECT OF 6-HALOGENATED PURINE RIBONUCLEOSIDES ON THE INCORPORATION OF HYPOXANTHINE-8-^{14}C INTO THE NUCLEIC ACID PURINES OF SARCOMA 180 ASCITES CELLS*

Inhibitor	(mg/kg)	cpm/μmole $\times 10^{-2}$		
		NA† guanine	NA adenine	AS adenine
None		12·9 ± 1·5	14·4 ± 2·9	238·6 ± 20·7
2-Amino-6-chloro-	39	4·4 ± 0·6	11·1 ± 1·5	201·9 ± 11·6
purine ribonucleoside	78	3·1 ± 0·7	17·6 ± 3·6	259·1 ± 10·5
	156	1·5 ± 0·3	17·0 ± 2·9	233·1 ± 21·6
2-Amino-6-chloro-	110·5	3·3 ± 0·3	17·4 ± 1·3	229·6 ± 8·1
purine ribonucleoside triacetate	221	1·8 ± 0·2	14·6 ± 0·5	182·9 ± 7·8
2-Amino-6-iodopurine	50·8	3·5 ± 0·4	11·2 ± 0·7	223·3 ± 16·0
ribonucleoside	101·5	1·0 ± 0·1	12·6 ± 0·4	217·7 ± 28·8
	203	1·4 ± 0·3	12·1 ± 1·6	166·5 ± 13·1

* Mice bearing 6-day implants of sarcoma 180 ascites cells received a single i.p. dose of one of the indicated 6-halogenated purine ribonucleosides. One hr later, 50 μg hypoxanthine-8-^{14}C ($1{\cdot}1 \times 10^4$ cpm/μg) was administered by i.p. injection to each mouse and 1 hr was allowed for metabolic utilization. Each figure represents the mean value (± the S.E.) of results obtained from the separate analyses of cells from 3–4 mice.
† NA, nucleic acid; AS, acid-soluble.

those produced by molar equivalent concentrations of 6-ClG. Furthermore, 2-amino-6-iodopurine ribonucleoside caused a degree of blockade of the formation of guanine nucleotides as intense as that produced by the chloro-derivatives. The incorporation of hypoxanthine into adenine nucleotides was not inhibited by either the 6-chloro- or 6-iodo-derivatives.

It was of interest to determine the effects of 6-ClG on the biosynthesis of purine nucleotides in sarcoma 180/TG ascites cells, a subline resistant to both the growth-inhibitory effects and the blockade of guanine nucleotide formation that are induced by TG, 6-MP and 6-ClP.[23,24] In contrast to results obtained with other purine analogs,[23] 6-ClG caused marked inhibition of the biosynthesis of nucleic acid guanine in this variant (Table 3). The pattern of inhibition paralleled that observed in the parent purine analog-sensitive neoplasm, sarcoma 180.

DISCUSSION

Although many similarities exist between 6-MP and TG with regard to the sites of inhibition induced by these agents on the metabolic pathways involved in the biosynthesis of purine nucleotides,[1–6] marked differences exist between 6-ClP and 6-ClG. The biochemical effects of 6-ClG in sarcoma 180 ascites cells, detailed in the present study, and those of 6-ClP, summarized from results obtained earlier[9] under similar

TABLE 3. INCORPORATION OF LABELED PRECURSORS INTO THE NUCLEIC ACID PURINES OF SARCOMA 180/TG ASCITES CELLS TREATED WITH 2-AMINO-6-CHLOROPURINE*

Isotopic substrate	6-ClG	cpm/μmole × 10^{-2}		
		NA† guanine	NA adenine	AS adenine
Glycine-2-^{14}C	−	18·7 ± 1·5	16·9 ± 0·9	336·7 ± 31·5
	+	3·3 ± 0·7	6·2 ± 0·7	149·8 ± 8·9
Hypoxanthine-8-^{14}C	−	18·5 ± 3·0	15·9 ± 3·1	204·9 ± 29·9
	+	10·0 ± 1·5	19·5 ± 2·2	370·5 ± 33·9
Adenine-8-^{14}C	−	6·0 ± 0·7	32·5 ± 2·4	488·3 ± 69·7
	+	2·5 ± 0·6	30·4 ± 2·0	537·8 ± 43·0

* Mice bearing 6-day implants of sarcoma 180/TG ascites cells received a single i.p. dose of 88 mg 6-ClG/kg. One hr later, either 100 μg glycine-2-^{14}C ($4·9 \times 10^4$ cpm/μg), 50 μg hypoxanthine-8-^{14}C ($1·1 \times 10^4$ cpm/μg), or 50 μg adenine-8-^{14}C ($2·6 \times 10^4$ cpm/μg) was administered by i.p. injection to each mouse and 1 hr was allowed for metabolic utilization. Each figure represents the mean value (± the S.E.) of results obtained from these parate analyses of cells from 4 mice.

† NA, nucleic acid; AS, acid-soluble.

conditions, are compared in Table 4. Since these results were obtained with approximately molar equivalent quantities of each halogenated purine, it appears that, in general, 6-ClG is the more potent inhibitor of purine nucleotide metabolism. The finding that 6-ClG inhibits the incorporation of glycine-2-^{14}C into both the adenine

TABLE 4. SUMMARY OF THE EFFECTS OF 2-AMINO-6-CHLOROPURINE (88 mg/kg) AND 6-CHLOROPURINE (80 mg/kg) ON THE BIOSYNTHESIS OF PURINE NUCLEOTIDES IN SARCOMA 180 ASCITES CELLS

Isotopic substrate	Percent of control specific activity			
	NA* guanine		NA adenine	
	6-ClG	6-ClP	6-ClG	6-ClP
Glycine-2-^{14}C	8	25	29	91
4-Amino-5-imidazole-carboxamide-2-^{14}C	13	39	110	118
Hypoxanthine-8-^{14}C	24	46	124	143
Guanine-8-^{14}C	86	106	89	84
Adenine-8-^{14}C	40	192	117	113

* NA, nucleic acid.

and guanine moieties of the nucleic acids, coupled with the lack of retardation of the incorporation of 4-amino-5-imidazolecarboxamide into the adenine of the nucleic acids, indicates that this agent or a derivative thereof inhibits the *de novo* biosynthesis of purine nucleotides. Alternatively, although less likely, the 6-ClG-induced inhibition of IMP dehydrogenase might result in the accumulation of a quantity of IMP that is sufficient to result in a feedback blockade of the *de novo* biosynthetic pathway. In contrast to these observations, 6-ClP decreased the rate of incorporation of the two isotopic tracers only into the guanine nucleotides, thereby eliminating the possibility

of the presence of a 6-ClP-induced block on the purine nucleotide biosynthetic pathway *de novo*. Both halogenated purines caused retardation of the utilization of hypoxanthine-^{14}C for the synthesis of guanine nucleotides, while the conversion of guanine-^{14}C to nucleotide forms was unaffected. Thus, enzymatic blockade by both agents occurs as shown in this report for 6-ClG, and earlier for 6-ClP,[7–11] at the level of IMP dehydrogenase. The treatment of ascites cells with 6-ClG resulted in a stimulation of the incorporation of radioactive 4-amino-5-imidazolecarboxamide, hypoxanthine, and xanthine into acid-soluble adenine nucleotides, suggesting a diversion of these metabolic tracers into the adenine nucleotide pool as a result of the inhibition of IMP dehydrogenase by 6-ClGMP.

Measurement of the effects of the purine analogs on the conversion of adenine-8-^{14}C to guanine nucleotides resulted in the elucidation of an interesting difference in the mode of action of these agents. Whereas 6-ClP caused a stimulation of the utilization of adenine for the guanine of the nucleic acids, 6-ClG caused marked inhibition. Since both halogenated purines inhibit the activity of IMP dehydrogenase, an enzymatic reaction known to be involved in the biosynthetic sequence by which adenine is converted to guanine nucleotides, the results obtained with 6-ClP allow the suggestion of the presence of an alternate route in these cells that circumvents the blockade of IMP dehydrogenase induced by this agent. The finding of a 6-ClG-induced decrease in the rate of conversion of adenine-8-^{14}C to nucleic acid guanine implies the presence of an additional site sensitive to 6-ClG, or more probably to a nucleotide derivative thereof.

Since 6-iodopurine has been shown to be only about one-third as active as 6-ClP as an inhibitor of the incorporation of glycine-2-^{14}C into nucleic acid guanine,[25] a process that presumably is an expression of blockade of IMP dehydrogenase, it was of interest that 2-amino-6-iodopurine ribonucleoside was at least as potent an inhibitor as the chloro-derivatives of the formation of guanine nucleotides. Because available evidence suggests that IMP, GMP and analogs thereof may occupy similar sites on IMP dehydrogenase,[11,26] corresponding orders of reactivity with regard to the halogen substituent were expected for both the 6-halo- and 2-amino-6-halo-purine series; no explanation is currently available for these divergent activities.

The pattern of inhibition by 6-ClG of the incorporation of the various isotopic substrates used as probes to monitor purine nucleotide formation in sarcoma 180 was also obtained in the purine-analog resistant neoplasm sarcoma 180/TG. This latter tumor is insensitive both to the growth-inhibitory properties of purine analogs and to the ability of these antimetabolites to cause blockade of the synthesis of nucleic acid guanine.[23,24] The mechanism of resistance in this variant appears to involve the catabolism of the purine nucleotide derivatives, presumed to be the active inhibitory forms of the purine analogs, by a phosphohydrolase.[24,27] The effectiveness of 6-ClG as an inhibitor of purine nucleotide formation in sarcoma 180/TG implies that the levels of 6-ClGMP present in these neoplastic cells are sufficient to inhibit metabolic processes. The mechanism by which 6-ClG induces blockade of guanine nucleotide synthesis under conditions that do not allow inhibition by several other purine analogs is under study in this laboratory.

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REFERENCES

1. J. F. HENDERSON and H. G. MANDEL, *Adv. Pharmac.* **2**, 297 (1963).
2. R. W. BROCKMAN, *Adv. Cancer Res.* **7**, 129 (1963).
3. R. W. BROCKMAN and E. P. ANDERSON, *A. Rev. Biochem.* **32**, 463 (1963).
4. J. F. HENDERSON, *Prog. exp. Tumor Res.* **6**, 84 (1965).
5. A. C. SARTORELLI, *Prog. exp. Tumor Res.* **6**, 228 (1965).
6. G. B. ELION and G. H. HITCHINGS, *Adv. Chemother.* **2**, 91 (1965).
7. A. C. SARTORELLI, J. R. AKERS and B. A. BOOTH, *Biochem. Pharmac.* **5**, 238 (1960).
8. A. C. SARTORELLI and B. A. BOOTH, *Archs. Biochem. Biophys.* **89**, 118 (1960).
9. A. C. SARTORELLI and B. A. BOOTH, *Biochim. biophys. Acta* **55**, 214 (1962).
10. A. HAMPTON, *J. biol. Chem.* **238**, 3068 (1963).
11. J. H. ANDERSON and A. C. SARTORELLI, *Fedn Proc.* **26**, 730 (1967).
12. A. HAMPTON, A. NOMURA and L. W. BROX, *Fedn Proc.* **25**, 750 (1966).
13. A. HAMPTON and A. R. P. PATERSON, *Biochim. biophys. Acta* **114**, 185 (1966).
14. F. M. SCHABEL, JR., J. A. MONTGOMERY, H. E. SKIPPER, W. R. LASTER, JR. and J. R. THOMSON, *Cancer Res.* **21**, 690 (1961).
15. A. R. P. PATERSON, *Can. J. Biochem.* **43**, 257 (1965).
16. H. P. BAER, G. I. DRUMMOND and E. L. DUNCAN, *Fedn. Proc.* **25**, 786 (1966).
17. H. P. BAER, G. I. DRUMMOND and E. L. DUNCAN, *Molec. Pharmac.* **2**, 67 (1966).
18. G. A. FISCHER and A. C. SARTORELLI, *Meth. med. Res.* **10**, 247 (1964).
19. G. A. LEPAGE, *Cancer Res.* **13**, 178 (1953).
20. A. C. SARTORELLI, *Meth. med. Res.* **10**, 285 (1964).
21. A. C. SARTORELLI and B. A. BOOTH, *Cancer Res.* **25**, 1393 (1965).
22. B. MAGASANIK, H. S. MOYED and L. B. GEHRING, *J. biol. Chem.* **226**, 339 (1957).
23. A. C. SARTORELLI, H. F. UPCHURCH, A. L. BIEBER and B. A. BOOTH, *Cancer Res.* **24**, 1202 (1964).
24. A. L. BIEBER and A. C. SARTORELLI, *Cancer Res.* **24**, 1210 (1964).
25. A. C. SARTORELLI, B. A. BOOTH and R. K. ROBINS, *Biochem. Pharmac.* **11**, 1017 (1962).
26. J. H. ANDERSON and A. C. SARTORELLI, *Fedn Proc.* **25**, 745 (1966).
27. J. E. BROWN and A. C. SARTORELLI, *Fedn Proc.* **25**, 453 (1966).