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INCORPORATION OF THE PURINE MOIETIES OF GUANOSINE AND INOSINE ANALOGS INTO NUCLEOTIDE POOLS OF HUMAN ERYTHROCYTES*

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Abstract—The metabolism of several purine ribonucleoside analogs in human erythrocytes was studied. When suspensions of erythrocytes were incubated with 6-thioguanosine (6-TGR) in the presence of dithiothreitol, the 5'-mono, di- and triphosphate ribonucleotides of 6-thioguanosine were synthesized. Although the formation of the monophosphate nucleotide occurred quite early in the incubation period, the di- and triphosphate nucleotides appeared only after more prolonged incubation. When 6-TGR was present at 1·0 mM in the incubation medium, the quantities of nucleotides produced were approximately 0·23 μmole 6-thioGMP, 0·05 μmole 6-thioGDP and 0·17 μmole 6-thioGTP/ml of erythrocytes after 2 hr of incubation. In contrast to the findings with 6-thioguanosine, incubation of erythrocytes with 6-thioinosine (6-mercaptopurine ribonucleoside, 6-MPR) or 6-selenoguanosine (6-SeGR) led to the formation of only the respective 5'-monophosphate ribonucleotides. The triphosphate derivatives were not formed even after incubation of as long as 24 hr. The quantities of the analog ribonucleotides/ml of erythrocytes produced during 2 hr of incubation were 0·66 μmole 6-thioinosine-5'-monophosphate (6-thioIMP) and 0·33 μmole 6-selenoguanosine-5'-monosphate (6-SeGMP). It is proposed that the analog nucleosides are first split by purine nucleoside phosphorylase (PNPase), liberating the corresponding free base, which then reacts with 5-phosphoribosyl-1-pyrophosphate (PRPP) and the salvage enzyme, hypoxanthine–guanine phosphoribosyltransferase (HGPRTase) to form analog monophosphate nucleotides.

The nucleotide profiles of human erythrocytes, when compared with those of nucleated cells, are relatively simple and consist predominately of nucleotides of adenine [1–4]. Most samples of human erythrocytes examined contained only small quantities of guanine nucleotides, despite the fact that these cells possess the complete enzymic pathway for the conversion of guanine or guanosine to GTP [5]. Recently, with the aid of high pressure liquid chromatography, this laboratory has shown that when human erythrocytes are incubated with guanosine in the presence of potassium phosphate buffer, GDP and GTP form readily, and concentrations of GTP can be reached that exceed the ATP level by several-fold [6].

In an earlier study, this laboratory reported that a number of adenosine analogs are readily incorporated into the nucleotide pools of human erythrocytes, presumably after an initial reaction with the enzyme, adenosine kinase [7]. In addition, the reactions of guanine, 8-azaguanine, 6-mercaptopurine, 6-thioguanine and 6-selenoguanine with human erythrocytic hypoxanthine–guanine phosphoribosyltransferase (HGPRTase)‡ were shown to be markedly influenced by pH in a manner which suggests that the unionized form of the purine or purine analog is the active substrate [8]. At low pH values, all of these compounds were similar in substrate activity. Furthermore, these compounds were all substrates for purine nucleoside phosphorylase [9–12]. Investigations in the laboratory of Hershko *et al.* [13, 14] have shown that in a number of mammalian tissues, including erythrocytes, the enzyme PRPP synthetase was highly responsive to the concentration of orthophosphate and that maximal rates of PRPP synthesis were achieved in intact erythrocytes at orthophosphate concentrations in the range of 20–30 mM.

Earlier attempts to demonstrate the formation of nucleotides from ribonucleoside analogs that contain free sulfhydryl groups, such as 6-thioguanosine and 6-thioinosine, were unsuccessful, apparently because the thiopurine derivatives reacted readily with the denatured proteins during inactivation by trichloracetic or perchloric acids [10]. It was also learned that this phenomenon could be prevented by the addition of a thiol reagent such as dithiothreitol (DTT) during the extraction procedure. In view of these observations, it was decided to reconsider the question of incorporation of analogs of guanine and hypoxanthine into the nucleotide pools of fresh human erythrocytes when in-

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‡ Abbreviations used: HGPRTase, hypoxanthine–guanine phosphoribosyltransferase; PRPP, 5-phosphoribosyl-1-pyrophosphate; DTT, dithiothreitol; 6-MP and 6-MPR, 6-mercaptopurine and 6-thioinosine; 6-TG and 6-TGR, 6-thioguanine and 6-thioguanosine; 6-SeG and 6-SeGR, 6-selenoguanine and 6-selenoguanosine; 6-thioIMP, 6-thioinosine-5'-monophosphate; 6-thioGMP, 6-thioGDP and 6-thioGTP, 6-thioguanosine-5'-mono, di- and triphosphate; 6-SeGMP, 6-selenoguanosine-5'-monophosphate; 6-MeSeGR, 6-methylselenoguanosine; α-SeGdR and β-SeGdR, α- and β-6-seleno-2'-deoxyguanosine; and PCA, perchloric acid.

cubated with the ribonucleoside analogs in the presence of 50 mM potassium phosphate and 2 mM DTT. It has been found that, under these conditions, intact human erythrocytes can synthesize relatively large amounts of nucleotides of 6-thioguanine, 6-selenoguanine and 6-mercaptopurine. Preliminary reports of portions of this work have been presented [15, 16].

MATERIALS AND METHODS

Materials. Dithiothreitol was purchased from Cal-Biochem Corp. Snake venom 5′-nucleotidase (from *Crotalus adamanteus* venom, grade II, 18 units/mg), 6-thioguanosine and 6-thioinosine were from Sigma. Fresh blood collected in acid–citrate–dextrose medium, from which the platelet-rich plasma had been removed, was obtained from the Division of Hematological Research, Pawtucket Memorial Hospital, Pawtucket, R.I. 6-Selenoguanosine was synthesized by methods described previously [17] and supplied by Dr. S.-H. Chu of this Division. Partially purified pig heart succinate thiokinase (sp. act. about 5 units/mg of protein) was a gift from Dr. D. Baccanari of this Division. 6-Methylselenoguanosine, α- and β-6-seleno-2′-deoxyguanosine were also gifts from Dr. S.-H. Chu.

Incubation procedure. The buffy coat and the acid–citrate–dextrose medium were separated from the suspensions of human erythrocytes after centrifugation, and the erythrocytes were washed twice with isotonic saline solution. The incubation medium contained 75 mM NaCl, 2 mM $MgSO_4$, 50 mM potassium phosphate buffer (pH 7·4), 10 mM glucose and 2 mM dithiothreitol. The analog ribonucleosides were usually 1·0 mM. In all experiments, 25% erythrocyte suspensions were used unless stated otherwise. All incubations were performed in a shaking water bath (approximately 80 oscillations/min) at 37° with air as the gas phase.

Extraction procedure. Aliquots (5 ml) were removed from the incubation mixture at selected times and added dropwise with rapid stirring on a Vortex mixer into 2·5 ml of 12% cold perchloric acid containing 2 mM dithiothreitol to give a final concentration of 4% PCA. These mixtures were allowed to stand at 4° for 5–10 min. The denatured protein was removed by centrifugation. The supernatant fluids were neutralized with potassium hydroxide, using Phenol Red as the pH indicator. After removal of the precipitated potassium perchlorate by centrifugation, the neutralized extracts were stored at −20° for further study.

Nucleotide analysis. The neutralized extracts were placed on a column (1 × 10 cm) of Bio-Rad AG 1-X8 (200–400 mesh, formate form). To separate the analog nucleotides from the corresponding nucleosides and bases, the formic acid–ammonium formate gradient system described by Moore and LePage [18] and modified by Ross *et al.* [12] was used. All eluting solutions contained 2 mM dithiothreitol. Flow rate was 1 ml/min and 5-ml fractions were collected. The elution of the analog nucleotides was followed by monitoring the fractions at either 345 nm (6-thioguanosine), 360 nm (6-selenoguanosine) or 327 nm (6-mercaptopurine ribonucleoside).

Identification of the various nucleotide peaks. Enzyme peak-shift methods were used to aid in the identification of the various analog nucleotide peaks. To 5 ml neutralized erythrocytic extract, 5 units snake venom 5′-nucleotidase was added, and the extract was adjusted to 2 mM $MgSO_4$ by the addition of crystalline $MgSO_4$. The mixture was allowed to incubate at room temperature for 30 min. The nucleotides were extracted as described for other experiments. When the resultant extracts were chromatographed as described above, peaks which represent the 5′-monophosphate nucleotides of 6-TG, 6-MP and 6-SeG disappeared, and new peaks which co-chromatographed with the

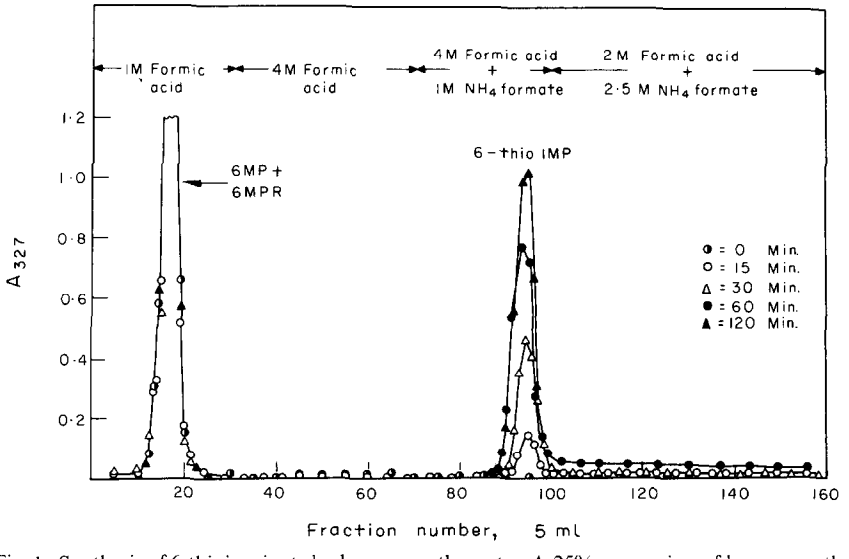

Fig. 1. Synthesis of 6-thioinosinate by human erythrocytes. A 25% suspension of human erythrocytes was incubated at 37° in a medium containing 50 mM potassium phosphate (pH 7·4), 10 mM glucose, 2 mM $MgSO_4$, 2 mM dithiothreitol and 1 mM 6-thioinosine. At the indicated time, an aliquot (5 ml) of suspension was removed and added dropwise to 2·5 ml of 12% perchloric acid with rapid stirring. The denatured protein was removed by centrifugation and 5 ml of the supernatant fluid was neutralized with KOH using Phenol Red as the pH indicator. After the removal of potassium perchlorate by centrifugation, the entire neutralized extract was analyzed by the anion exchange chromatography method of Moore and LePage [18].

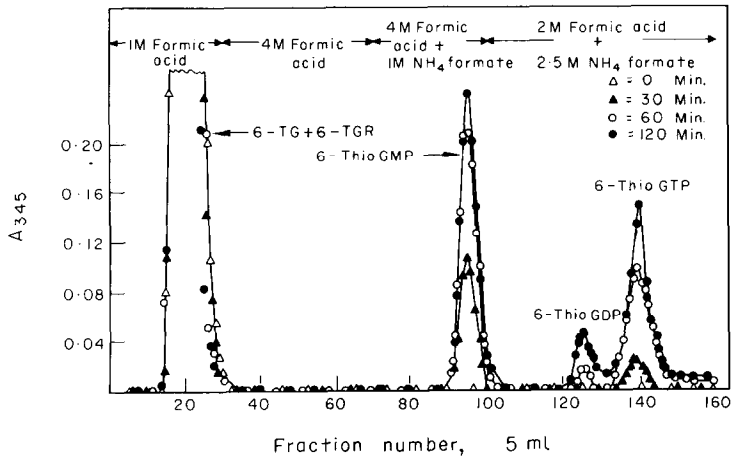

Fig. 2. Progressive synthesis of polyphosphate nucleotides of 6-thioguanosine by human erythrocytes. Conditions of incubation, extraction and analysis of analog nucleotides are as described in Fig. 1 with the exception that 6-thioguanosine is used in place of 6-thioinosine.

respective chemically synthesized analog nucleosides were formed. Chemically synthesized 6-thioIMP was eluted between fractions 92 and 100 (see Fig. 1) in this anion exchange chromatographic system which coincided with the 6-thioIMP peak observed in the erythrocytic extracts. In order to identify the 6-thioGDP and 6-thioGTP peaks, the peak-shift method with succinate thiokinase described by Nelson and Parks [19] was used, since it had been reported previously that 6-thioGTP could replace GTP in the succinate thiokinase catalyzed reaction [20]. To 5 ml neutralized erythrocytic extract containing 6-thioGDP and 6-thioGTP, 50 mM Tris-succinate (pH 7·5), 1 mM CoA and 1 μM unit succinate thiokinase were added to a final volume of 5·5 ml. The incubation was carried out at room temperature for 1 hr. The reaction mixture was treated and prepared for nucleotide analysis as described above. A control sample of the neutralized extract was treated with 0·2 ml saline instead of succinate thiokinase. The 6-thioGTP peak, which eluted between fractions 135 and 142 (see Fig. 2), was shifted to the position of the 6-thioGDP peak which eluted between fractions 123 and 132. The size of the 6-thioGMP peak was not significantly changed, and ATP added to the reaction mixture was not affected by the succinate thiokinase reaction. The control sample (incubated minus succinate thiokinase) gave an elution profile similar to that of the original extract.

RESULTS

Formation of 6-thioinosinate in human erythrocytes. The ability of intact human erythrocytes to synthesize 6-thioIMP from 6-thioinosine is demonstrated in Fig. 1. The single large peak which eluted between fractions 90 and 100 was identified as 6-thioIMP as described in Materials and Methods. The quantity of 6-thioIMP that accumulated after a 2 hr incubation period was 0·66 μmole/ml of erythrocytes. However, di- and triphosphate nucleotides of 6-mercaptopurine could not be detected even after incubation periods of 24 hr. This finding is in accord with the earlier observation that guanylate kinases purified from various sources do not react with 6-thioIMP [5, 21, 22].

In earlier experiments in which suspensions of washed human erythrocytes were incubated with 6-thioinosine and aliquots of the reaction mixtures were inactivated by addition of perchloric acid, the analog nucleoside and its derivatives were found to be bound to the acid-denatured proteins, and this binding could be prevented by addition of dithiothreitol [10]. This binding phenomenon was also observed in similar experiments with 6-thioguanosine and 6-selenoguanosine and was assumed to be due to the formation of covalent bonds with thiol groups in the denatured protein.

Formation of polyphosphate ribonucleotides from 6-thioguanosine. When human erythrocytes were incubated with 6-thioguanosine, the mono-, di- and triphosphate analog ribonucleotides were synthesized as shown in Fig. 2. After 2 hr of incubation, the quantities of ribonucleotides that accumulated were approximately 0·23 μmole 6-thioGMP, 0·047 μmole 6-thioGDP and 0·17 μmole 6-thioGTP/ml of erythrocytes, and as seen in Table 1, a total of approximately 0·54 μmole/ml of erythrocytes of 6-thioguanine ribonucleotides was formed in 3 hr.

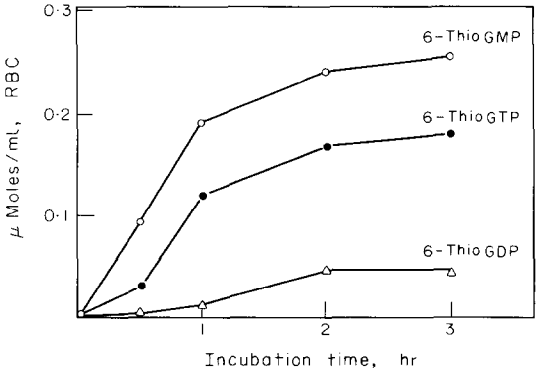

Fig. 3. Rates of synthesis of analog nucleotides from 6-thioguanosine by human erythrocytes. A 25% suspension of human erythrocytes was incubated in the presence of 1 mM 6-thioguanosine under conditions described in Fig. 1. At the times indicated, samples were extracted, prepared and analyzed as described. The concentrations of 6-thioGMP, 6-thioGDP and 6-thioGTP were estimated from peak areas of the chromatograms.

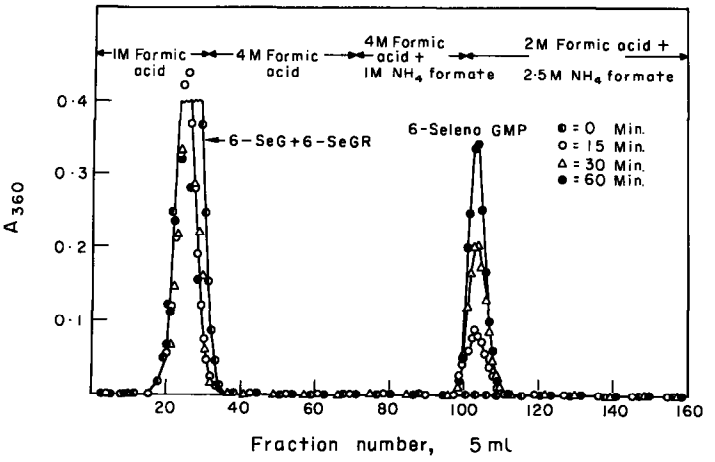

Fig. 4. Synthesis of 6-selenoguanylic acid by human erythrocytes. A 25% suspension of human erythrocytes was incubated with 1 mM 6-selenoguanosine. All conditions and procedures for extraction and analysis of nucleotides were similar to those described in legend to Fig. 1.

Figure 3 shows the time course of the synthesis of the 5'-mono-, di- and triphosphate ribonucleotides of 6-thioguanine. There was a rapid synthesis of 6-thioGMP with a delay in the formation of 6-thioGDP and 6-thioGTP. These results are in striking contrast to those observed when human erythrocytes were incubated with guanosine [6]. During the synthesis of large quantities of GDP and GTP, the concentration of GMP in erythrocytes remained so low that changes were not detected. These observations are consistent with earlier findings that the reaction velocity of 6-thioGMP with guanylate kinase is less than 0·05 per cent of that with GMP [22].

Formation of 6-SeGMP in human erythrocytes. As shown in Fig. 4, when human erythrocytes were incubated with 1·0 mM 6-selenoguanosine, there was the progressive formation of substantial quantities of 6-SeGMP. However, in contrast to the observation with 6-thioguanosine and in agreement with findings in Sarcoma 180 cells [12], formation of the di- and triphosphate ribonucleotides could not be detected during incubation periods as long as 24 hr. As seen in Table 1, approximately 0·39 μmole 6-SeGMP accumulated over a 3-hr period.

Incubation of other Se-containing ribonucleoside analogs of guanosine and deoxyguanosine with human erythrocytes. As seen in Table 1, several other analog ribonucleosides were tested for their conversion to the ribonucleotide level by human erythrocytes. Ribonucleotide formation was not seen with either 6-methylselenoguanosine or α-6-seleno-2'-deoxyguanosine. However, with β-6-seleno-2'-deoxyguanosine, the formation of about 0·11 μmole of a 6-selenoguanine-containing monophosphate nucleotide/ml of erythrocytes was observed after a 3-hr incubation period.

Table 1. Synthesis of analog nucleotides*

Analog	Nucleotide formed (μmoles/ml erythrocytes)
6-MPR	0·80
6-TGR	0·54†
6-SeGR	0·39
6-MeSeGR	0
α-SeGdR	0
β-SeGdR	0·11

* Erythrocytes were incubated with 1 mM of the analog nucleosides for 3 hr. Experimental conditions were as described for Fig. 1.

† Sum of the 5'-mono-, di- and triphosphate nucleotides.

DISCUSSION

These observations demonstrate that human erythrocytes are capable of incorporating relatively large amounts of the purine moieties of the analog ribonucleosides, 6-thioinosine, 6-thioguanosine and 6-selenoguanosine, into their nucleotide pools. Under the incubation conditions described above, quantities of analog nucleotides in the range of 0·4 to 0·8 μmole/ml of erythrocytes were formed.These amounts are impressive when one considers that the most plentiful nucleotide found normally in human erythrocytes, ATP, is usually in the concentration range of about 1·2 to 1·6 μmoles/ml of cells [1, 2]. If similar results could be obtained under conditions *in vivo*, the total amount of 6-thioinosinate formed in the erythrocytes of an adult human being (assuming a red cell mass of about 2·5 liters) would exceed 1 g. It should be noted, however, that special incubation conditions were employed, i.e. phosphate concentration, 50 mM; the addition of dithiothreitol; and the use of the ribonucleosides rather than the free bases of the purine analogs. It is doubtful whether similar rates of analog nucleotide synthesis could be achieved *in vivo* [23]. The purine antimetabolites, 6-thioguanine and 6-mercaptopurine (often administered as azathioprine), are commonly used for immunosuppression [24] and in the therapy of human malignancies. At present it is difficult to predict the potential effects of the incorporation of analogs, such as 6-thioguanine and 6-mercaptopurine, into the nucleotide pools of human erythrocytes, since there is speculation that erythrocytes may trans-

port nucleotides from organs such as the liver to other tissues [25, 26]. It is entirely possible that damage to specific tissues might be caused by the delivery of toxic nucleotides [27, 28]. However, it has been shown that 6-methylmercaptopurine ribonucleoside (6-MMPR) can accumulate in the erythrocytes of treated patients at the 5'-monophosphate nucleotide level in amounts of about 1·0 μmole/ml of cells, and with prolonged incubation high concentrations of the di- and triphosphate nucleotides are formed [23]. This analog nucleotide accumulation does not affect erythrocytic survival [29]. Therefore, incorporation of analogs such as 6-TG, 6-MP or 6-SeG into the erythrocytic nucleotide pools might have little effect on the survival of the erythrocytes while serving as a mechanism for removing potentially toxic substances from the circulation [30].

The presumed enzymic pathway in human erythrocytes by which 6-thioguanosine is converted to mono-, di- and triphosphate ribonucleotides is as follows:

(1) 6-Thioguanosine + Pi $\underset{\text{Phosphorylase}}{\overset{\text{Purine nucleoside}}{\rightleftharpoons}}$ 6-TG + Ribose-1-phosphate

(2) 6-TG + PRPP $\overset{\text{HGPRTase}}{\rightleftharpoons}$ 6-ThioGMP + PPi

(3) 6-ThioGMP + ATP $\overset{\text{Guanylate kinase}}{\rightleftharpoons}$ 6-ThioGDP + ADP

(4) 6-ThioGDP + ATP $\underset{\text{Diphosphokinase}}{\overset{\text{Nucleoside}}{\rightleftharpoons}}$ 6-ThioGTP + ADP

(5) Ribose-1-phosphate $\overset{\text{Phosphoribomutase}}{\rightleftharpoons}$ Ribose-5-phosphate

(6) Ribose-5-phosphate + ATP $\overset{\text{PRPP synthetase}}{\rightleftharpoons}$ PRPP + AMP

All of these enzymic reactions have been demonstrated in human erythrocytes [3, 5] and the approximate activities in μM units/ml of human erythrocytes of some of these enzymes are as follows: purine nucleoside phosphorylase, 17; HGPRTase, 0·3; guanylate kinase, 0·3; nucleoside diphosphokinase, 70; and PRPP synthetase, 0·35*. Speculations have been made on the occurrence in animal tissues of a guanosine kinase capable of the direct formation of GMP from guanosine through a phosphorylation reaction [31-33]. To date, this enzyme has not been demonstrated in human erythrocytes.

In addition to the enzymic steps listed above, a membrane-bound facilitated transport system for purine and pyrimidine nucleosides occurs in human erythrocytes [34–36]. It seems unlikely that this transport mechanism could be rate-limiting under the incubation conditions employed above, i.e. analog ribonucleoside concentrations of 1·0 mM. Simple diffusion should have been sufficient to account for the rates of nucleotide synthesis observed [37]. Of the enzymic reactions listed above, it seems probable that the rate-limiting step is the generation of PRPP by PRPP synthetase (Reaction 6). This enzyme is under the influence of strong allosteric regulation by the concentrations of nucleotides, inorganic orthophosphate and atmospheric oxygen [14, 15, 38–40]. In preliminary studies, it was observed that the generation of GTP is much more rapid when guanosine is used as the substrate instead of guanine, and similar results have been obtained with 6-thioguanosine and 6-thioguanine. This suggests that the synthesis of PRPP from glucose may also be rate-limiting and that the purine nucleoside phosphorylase reaction by generating ribose-1-phosphate (Reaction 1) greatly facilitates the intracellular synthesis of PRPP.

In recent studies in this laboratory [6], it was shown that when human erythrocytes were incubated with 1·0 mM guanosine, concentrations of GTP were formed that exceeded the ATP concentration by several-fold. During the synthesis of these large amounts of GTP, changes in the GMP concentration were too small to permit ready detection by high pressure liquid chromatography. The ratio of GTP to GDP was in the range of 10:1 to 5:1 and reflected the ATP to ADP ratio. These findings are in striking contrast to the observations reported above with analog-containing ribonucleosides. When human erythrocytes were incubated with 6-selenoguanosine or 6-thioinosine, only the monophosphate nucleotides, 6-SeGMP and 6-thioIMP were formed. When erythrocytes were incubated with 6-thioguanosine, initially a high concentration of the mononucleotide (greater than 0·2 mM) was produced followed by a slower synthesis of 6-thioGDP and 6-thioGTP. These observations are in excellent agreement with earlier studies of guanylate kinases isolated from various tissues, including human erythrocytes [21, 22, 41] which showed that 6-thioIMP is not a substrate for the enzyme and that, although 6-thioGMP appears to be a competitive inhibitor of GMP kinase (with a K_i similar to the K_m of GMP), it is, in fact, an alternative substrate with a very low V_{max} i.e. less than 0·1 per cent of the V_{max} with GMP. The relatively slow synthesis of 6-thioGTP was also observed in studies with Sarcoma 180 cells [19]. An intriguing question raised by these observations is: Why does the synthesis of analog nucleotides from 6-thioguanosine level off when less than half of the analog nucleoside has been consumed? Under similar conditions, the synthesis of GDP and GTP from guanosine continues until almost all of the added nucleoside is converted to nucleotides with the concentrations of GDP and GTP reaching levels that exceed those of the adenine nucleotides [15].

An observation of potential importance is that, in contrast to the results with 6-thioguanosine in both erythrocytes and Sarcoma 180 cells [12], the formation of nucleotides from 6-selenoguanosine stops at the monophosphate nucleotide level despite the finding

* Calculated from Fox and Kelley, *J. biol. Chem.* **246**, 5739 (1971).

that 6-SeGMP can undergo a very slow reaction with guanylate kinase [12]. If, indeed, the di- and triphosphate nucleotides of 6-selenoguanine are not formed *in vivo*, clearly this purine analog could not be incorporated into cellular RNA or DNA. This suggestion, if valid, has important implications at both the theoretical and applied levels. It would demonstrate that this type of guanine analog which accumulates only at the monophosphate nucleotide level can inhibit tumors to an extent that is equal to, or greater than, that seen with 6-thioguanine or its derivatives [12, 42]. If 6-selenoguanine is, in fact, very similar or identical in its antitumor action to 6-thioguanine, it indicates that the incorporation of 6-thioguanine into the nucleic acids of tumor cells [43] may not be the crucial factor in its mechanism of antitumor action. At the practical level, a serious potential hazard in the long-term use of 6-thioguanine and its derivatives as therapeutic agents for immunosuppression and/or the treatment of chronic diseases is the threat of carcinogenesis [44], mutagenesis or teratogenesis resulting from the incorporation of 6-thioguanine into cellular DNA. If 6-selenoguanine does not enter the nucleic acids, these risks should be abolished or greatly diminished.

Recently several groups, including this laboratory, have suggested that erythrocytes that contain purine analogs in their nucleotide pools might be employed in chemotherapy [27, 45, 46]. Special attention has been directed to the disease, schistosomiasis, since the causative agent, a blood fluke which feeds on erythrocytes, is deficient in the *de novo* pathway for purine biosynthesis [47] and possibly utilizes erythrocytic purine nucleotides. A report has appeared that describes a therapeutic response when tubercidin-loaded erythrocytes were infused into monkeys infected with schistosomiasis [45]. A number of other adenosine analogs have been shown to enter erythrocytic nucleotides readily [7], probably by reaction with adenosine kinase, and have been suggested for consideration if this modality of therapy becomes practical [46]. Since these worms have been shown to possess various enzymes of purine metabolism such as purine nucleoside phosphorylase and one or more enzymes related to HGPRTase, it seems likely that they should be capable of incorporating guanine or hypoxanthine analogs into their nucleotide pools, probably with detrimental effects. Therefore, if this approach becomes feasible, erythrocytes that contain substantial quantities of guanine and hypoxanthine analogs, such as 6-thioguanine, 6-mercaptopurine and 6-selenoguanine, in their nucleotide pools should be considered as likely candidates for chemotherapy.

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REFERENCES

1. E. M. Scholar, P. R. Brown, R. E. Parks, Jr. and P. Calabresi, *Blood* **41**, 927 (1973).
2. P. R. Brown, R. P. Agarwal, J. Gell and R. E. Parks, Jr., *Comp. Biochem. Physiol.* **43B**, 891 (1972).
3. R. E. Parks, Jr., P. R. Brown, Y.-C. Cheng, K. C. Agarwal, C. M. Kong, R. P. Agarwal and C. C. Parks, *Comp. Biochem. Physiol.* **45B**, 355 (1973).
4. G. R. Bartlett and G. L. Brewer, *Red Cell Metabolism and Function*, p. 245. Plenum, New York (1970).
5. R. P. Agarwal, E. M. Scholar, K. C. Agarwal and R. E. Parks, Jr., *Biochem. Pharmac.* **20**, 1341 (1971).
6. P. R. Brown and R. E. Parks, Jr., *Analyt. Chem.* **45**, 948 (1973).
7. R. E. Parks, Jr. and P. R. Brown, *Biochemistry* **12**, 3294 (1973).
8. C. M. Kong and R. E. Parks, Jr., *Molec. Pharmac.*, **10**, 648 (1974).
9. T. A. Krenitsky, *Molec. Pharmac.* **3**, 526 (1967).
10. M. R. Sheen, B. K. Kim and R. E. Parks, Jr., *Molec. Pharmac.* **4**, 293 (1968).
11. G. A. LePage, *Can. J. Biochem.* **46**, 655 (1968).
12. A. F. Ross, K. C. Agarwal, S.-H. Chu and R. E. Parks, Jr., *Biochem. Pharmac.* **22**, 141 (1973).
13. A. Hershko, A. Razin and J. Mager, *Biochim. biophys. Acta* **184**, 64 (1969).
14. A. Hershko, A. Razin, T. Shoshani and J. Mager, *Biochim. biophys. Acta* **149**, 59 (1967).
15. R. E. Parks, Jr., P. R. Brown and C. M. Kong, *Advan. Exper. Med. Biol. 41A: Purine Metabolism in Man* (Eds. O. Sperling, A. De Vries and J. B. Wyngaarden), p. 117. Plenum, New York (1974).
16. C. M. Kong and R. E. Parks, Jr., *Proc. Am. Ass. Cancer Res.* **15**, 21 (1974).
17. S.-H. Chu, *J. med. chem.* **14**, 254 (1971).
18. E. C. Moore and G. A. LePage, *Cancer Res.* **18**, 1075 (1958).
19. J. A. Nelson and R. E. Parks, Jr., *Cancer Res.* **32**, 2034 (1972).
20. S. Cha, C.-J. M. Cha and R. E. Parks, Jr., *J. biol. Chem.* **242**, 2577 (1967).
21. R. P. Miech and R. E. Parks, Jr., *J. biol. Chem.* **240**, 351 (1965).
22. R. P. Miech, R. York and R. E. Parks, Jr., *Molec. Pharmac.* **5**, 30 (1969).
23. T. P. Zimmerman, L.-C. Chu, C. J. L. Buggé, D. J. Nelson, G. M. Lyon and G. B. Elion, *Cancer Res.* **34**, 221 (1974).
24. G. H. Hitchings and G. B. Elion, *Pharmac. Rev.* **15**, 365 (1963).
25. J. F. Henderson and G. A. LePage, *J. biol. Chem.* **234**, 3219 (1959).
26. J. B. Pritchard, F. Chavez-Peon and R. D. Berlin, *Am. J. Physiol.* **219**, 1263 (1970).
27. C. G. Smith, L. M. Reineke, M. R. Burch, A. M. Shefner and E. M. Muirhead, *Cancer Res.* **30**, 69 (1970).
28. T. B. Grage, D. B. Rochlin, A. J. Weiss and W. L. Wilson, *Cancer Res.* **30**, 79 (1970).
29. D. H. W. Ho, J. K. Luce and E. Frei, III, *Biochem. Pharmac.* **17**, 1025 (1968).
30. T. L. Loo, J. K. Luce, M. P. Sullivan and E. Frei, III, *Clin. Pharmac. Ther.* **9**, 180 (1968).
31. G. A. LePage, I. G. Junga and B. Bowman, *Cancer Res.* **24**, 835 (1964).
32. K. J. Pierre and G. A. LePage, *Proc. Soc. exp. Biol. Med.* **127**, 432 (1968).
33. M. Schaffer, R. B. Hurlbert and A. Orengo, *Cancer Res.* **33**, 2265 (1973).
34. J. M. Oliver and A. R. P. Paterson, *Can J. Biochem.* **49**, 262 (1970).
35. C. E. Cass and A. R. P. Paterson, *J. biol. Chem.* **247**, 3314 (1972).
36. M. A. Pickard, R. R. Brown, B. Paul and A. R. P. Paterson, *Can. J. Biochem.* **51**, 666 (1973).
37. H. Roos and K. Pfleger, *Molec. Pharmac.* **8**, 417 (1972).
38. R. L. Switzer and D. C. Sogin, *J. biol. Chem.* **248**, 1063 (1973).
39. I. H. Fox and W. N. Kelley, *J. biol. Chem.* **247**, 2126 (1972).

40. P. C. L. Wong and A. W. Murray, *Biochemistry* **8,** 1608 (1969).
41. K. C. Agarwal and R. E. Parks, Jr., *Molec. Pharmac.* **8,** 128 (1972).
42. H. G. Mautner, S.-H. Chu, J. J. Jaffe and A. C. Sartorelli, *J. med. chem.* **6,** 36 (1963).
43. G. A. LePage and J. P. Whitecar, Jr., *Cancer Res.* **31,** 1627 (1971).
44. G. D. Stoner, M. B. Shinkin, A. J. Kniazeff, J. H. Weisburger, E. K. Weisburger and G. B. Gori, *Cancer Res.* **33,** 3069 (1973).
45. J. J. Jaffe, H. M. Doremus, H. A. Dunsford, W. S. Kammerer and E. Meymarian, *Am. J. trop. Med. Hyg.* **22,** 62 (1973).
46. R. J. Stegman, A. W. Senft, P. R. Brown and R. E. Parks, Jr., *Biochem. Pharmac.* **22,** 459 (1973).
47. A. W. Senft, R. P. Miech and D. B. Senft, *Int. J. Parasit.* **2,** 249 (1972).