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EFFECT OF 2-AMINOPURINE AND 2-AMINOPURINE 2′-DEOXYRIBOSIDE ON NUCLEIC ACID SYNTHESIS IN EHRLICH ASCITES CELLS *IN VITRO**

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Abstract—2-Aminopurine 2′-deoxyriboside has been prepared by transfer of 2′-deoxyribose from 2′-deoxythymidine to 2-aminopurine catalysed by nucleoside deoxyribosyltransferase from *L. helveticus*.

2-Aminopurine and 2-aminopurine 2′-deoxyriboside (in concentration of 2 μmoles/ml) inhibited the incorporation of $^{32}P_i$ and adenine-8-^{14}C into DNA of Ehrlich ascites tumor cells *in vitro*. Incorporation of $^{32}P_i$ into RNA was slightly inhibited by the two analogues, but the incorporation of adenine-8-^{14}C was not.

2-Aminopurine and 2-aminopurine 2′-deoxyriboside was recovered quantitatively from the acid soluble fraction of Ehrlich cells incubated with the compounds. No ribotides or deoxyribotides could be detected by the paper-chromatographic method used.

2-Aminopurine is not a substrate for a purine nucleoside phosphorylase from calf spleen.

5-Phosphoribosyl-2-aminopurine was formed in low yield from 2-aminopurine and 5-phosphoribosyl-1-pyrophosphate when incubated with a cell-free extract of Ehrlich cells.

2-Aminopurine 2′-deoxyriboside is not deaminated by an adenosine deaminase isolated from calf intestine, but is the strongest competitive inhibitor hitherto found of this enzyme.

INTRODUCTION

IT HAS been shown in this laboratory that 2′-deoxyadenosine after phosphorylation to 2′-deoxyATP inhibits DNA synthesis in Ehrlich ascites tumor cells *in vitro* by inhibiting the reduction of guanosine and cytidine phosphates to the corresponding deoxy-compounds.[1, 2] This inhibitory effect of 2′-deoxyATP has also been demonstrated in cell-free extracts of chick embryo by Reichard *et al.*[3, 4] Some analogues of 2′-deoxyadenosine which might be expected to have similar effects as 2′-deoxyadenosine have been investigated, e.g. 2′-deoxyadenosine-N^1-oxide,[5] 3′-deoxyadenosine (cordycepin),[6] 3′-deoxyadenosine-N^1-oxide (cordycepin-N^1-oxide),[7] and 8-azaadenine 2′-deoxyriboside.[8] 2′-Deoxyadenosine-N^1-oxide was slowly reduced by the cells to 2′-deoxyadenosine which then inhibits DNA synthesis by the mechanism described above. 3′-Deoxyadenosine-N^1-oxide was also reduced to the parent compound, but the inhibition of nucleic acid synthesis by 3′-deoxyadenosine[6, 7, 9 10] was exerted by mechanisms quite different from that of 2′-deoxyadenosine. 8-Azaadenine

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2'-deoxyriboside was not inhibitory to RNA or DNA synthesis, possibly because of the lack of phosphorylation and the rapid deamination.[8]

2-Aminopurine 2'-deoxyriboside (2-AP-DR) could be expected to behave metabolically similar to 2'-deoxyadenosine, and therefore to interfere with the step of reduction of ribotides to deoxyribotides. It may also be expected to interfere with other steps in nucleotide metabolism, since 2-aminopurine (2-AP) is shown by Freese[11, 12] to be mutagenic in T_4 'phage, and by Rudner[13] and Demerec[14] to give mutations in *Salmonella typhimurium*. Although it has been claimed by Kaplan *et al.*[15] that 2-aminopurine was not incorporated into DNA of *Escherichia coli*, Gottschling and Freese[16] have demonstrated that tritiated 2-aminopurine was incorporated into DNA of T_4 'phage and *E. coli*. B 97.

The present paper describes the preparation of 2-aminopurine 2'-deoxyriboside, which might be a better precursor for the corresponding triphosphate than the free base, and the effect of this compound and 2-aminopurine on nucleic acid synthesis in Ehrlich ascites cells *in vitro*. Some of the enzymatic pathways involved in a possible metabolism of these compounds have been investigated.

MATERIALS

2-Aminopurine,HNO_3 and 5-phosphoribosyl-1-pyrophosphate were purchased from Sigma Chemical Company, thymidine from Calbiochem, and the cyclohexylamine salt of the phosphoenolpyruvate, and pyruvate kinase (ATP: pyruvate phosphotransferase EC 2.7.1.40) were purchased from Boehringer, Mannheim. Ribose-1-P was a gift from Dr. H. Klenow of this institute.

Hyperdiploid Ehrlich ascites tumor cells were maintained by transfers made by interperitoneal injections of ascites fluid into mice of strain St/Eh A. Six or seven days after transplantation the mice were killed and the cells harvested.

The cell-free extract of Ehrlich cells was obtained after rupture of the cells with Tween 80 according to the method of Fischer and Harris[17] followed by centrifugation at 105,000 *g* for 60 min. This high-speed supernatant has been shown by Overgaard-Hansen[18] to catalyze the formation of 0·2 μmole AMP per min per ml extract from adenine and 5'-phosphoribosyl-1-pyrophosphate when incubated under conditions similar to those used for incubation of 2-AP and 5-phosphoribosyl-1-pyrophosphate (see Results).

Nucleoside deoxyribosyltransferase (nucleoside: purine (pyrimidine) deoxyribosyltransferase EC 2.4.2.6) was prepared from *Lactobacillus helveticus*. 23 g cells (wet wt.) were ground with two parts of aluminium oxide (Alcoa Chemicals A 301, 325 mesh) in a mortar at 0°. The paste was extracted with 60 ml 0·05 M phosphate buffer pH 7·4 and centrifuged 10 min at 10,000 *g*. After re-extraction of the precipitate with 20 ml of the same solution and centrifugation, the pooled extracts were centrifuged 30 min at 10,000 *g* and this supernatant used without any fractionation as the source of nucleoside deoxyribosyltransferase. Roush and Betz[19] have shown that a 11-fold purified nucleoside deoxyribosyltransferase from *L. helveticus* contained no hydrolytic or phosphorolytic activity towards purine deoxyribosides in the presence of deoxyribose acceptors. This is in agreement with results previously described on the preparation of 8-azaadenine 2'-deoxyriboside from thymidine and 8-azaadenine catalysed by a crude extract of *L. helveticus*.[8]

Xanthine oxidase (xanthine: O_2 oxidoreductase EC 1.2.3.2) was prepared from milk according to the method of Klenow and Emberland.[20]

Purine nucleoside phosphorylase (purine nucleoside: orthophosphate ribosyltransferase EC 2.4.2.1) was prepared from calf spleen by the method described by Price *et al.*[21] The enzyme activity was determined in a reaction mixture containing per ml; 40 μmoles Tris-HCl (pH 7·1), 2·0 μmoles hypoxanthine, 2·0 μmoles ribose-1-P, and 5 μl purine nucleoside phosphorylase. The mixture was incubated at 22° for 30 min. Every 5 min aliquots were taken, and the formation of inosine was followed by oxidation of the remaining hypoxanthine with xanthine oxidase to uric acid ($\Delta E_{292.5}$). Under these conditions 0·32 μmole inosine was formed per 60 min per μl nucleoside phosphorylase solution. One unit is defined as the amount of enzyme which forms 1 μmole of inosine per minute under the conditions described.

METHODS

Preparation of 2-aminopurine 2'-deoxyriboside (2-AP-DR)

50 mg 2-AP,HNO_3 (253 μmoles 2-AP) and 460 mg thymidine (1110 μmoles were) dissolved in 90 ml 0·05 M citrate buffer pH 6·0. 2,000 μl *L. helveticus* extract were added to the mixture, which was then incubated at 37° with shaking. After 16 hr 750 μl *L. helveticus* extract were further added, and the reaction mixture then incubated for 6 hr. The 2-AP and 2-AP-DR were quantitatively adsorbed (as revealed by the absorbancy of the solution at 305 mμ) on 3 gm of acid washed charcoal (Norit A) which was filtered on a G_4 glass filter and washed with distilled water. The charcoal was eluted on the filter with 300 ml 10% pyridine in 50% ethanol without suction. The filtrate was evaporated to dryness, and the residual solid material dissolved in a small volume of water. This solution was chromatographed on a column of Dowex-1-formate (height = 110 cm, diam. = 2·0 cm), previously equilibrated with 250 ml 4 N NH_4OH. The column was developed with a linear gradient with regard to formate [mixer: 1 l of 0·01 M ammonium formate (pH 10·3); reservoir: 1 l of 0·1 M ammonium formate (pH 7·4)]; 20 ml fractions were collected. The 2-AP-DR appeared in fractions 47–60, and a mixture of thymine and thymidine began to appear in fraction number 84. The remaining mixture of thymine and thymidine was eluted in fraction 84–89 with further 1,000 ml 0·1 M ammonium formate (pH 7·4). Finally a very small amount of unreacted 2-AP appeared in fractions 104–111. Fractions 47–60 were pooled, and the 2-AP-DR was adsorbed quantitatively on 750 mg acid washed charcoal (Norit A). After filtration on a G_4 glassfilter and wash the charcoal was eluted without suction with 300 ml 10% pyridine in 50% alcohol. The solution was evaporated to dryness, and finally dried in high vacuum over P_2O_5 for 48 hr. The overall yield of 2-AP-DR was 82 per cent of the original amount of 2-AP.

Acid hydrolysis of 2-AP-DR

1 μmole of 2-AP-DR was heated at 100° for 2 min in one ml of 0·025 N HCl and then cooled in ice. The ultraviolet material (absorbancy at 305 mμ) was adsorbed on 4 mg acid washed charcoal (Norit A), which was then washed with water and eluted with 2 × 400 μl of 10% pyridine in 50% ethanol. One third of the pooled eluates was chromatographed on Whatman 40 filter-paper in two solvents. One filter-paper was chromatographed in *n*-butanol with water[22] and one in water. Control experiments

were performed with 2-AP heated in HCl and with 2-AP-DR kept in ice instead of being heated.

Paper-Chromatographic analysis of 2-AP derivatives

The acid soluble fraction of Ehrlich cells incubated with either 2-AP or 2-AP-DR was analyzed as follows. Descending chromatography was performed on Whatman 40 filter-paper strips (width 2 cm) at 26° ± 1°. The free bases and nucleosides were first separated from each other and from the nucleotides by chromatography in *n*-butanol saturated with water.[22] The ultraviolet absorbing spots were cut out, eluted with water, and the compounds characterized by their spectra when necessary. The amounts of 2-AP and 2-AP-DR were determined from the absorbancy of the eluate at 305 mμ assuming the molar absorbancy of this wavelength to be 6,300[23] and 7,100, respectively. After treatment with *n*-butanol-H_2O, which leaves the nucleotides at the origin, the chromatogram was developed descending for 24 hr with 1 M ammonium acetate-96% alcohol (30 : 75) pH 8·4 saturated with sodium tetraborate.[24] This treatment (for 24 hr) would separate the deoxyribonucleotides from ribonucleotides, which stay at the origin, but not the deoxy-mono-, di-, and triphosphate from each other. The area of the filter-paper which possibly could contain deoxyribotides or ribotides of 2-AP was cut out, eluted with water, and the spectrum in u.v. light measured.

RESULTS

Preparation of 2-aminopurine 2′-deoxyriboside (2-AP-DR)

2-AP-DR was prepared by transfer of 2′-deoxyribose from thymidine to 2-AP catalysed by nucleoside deoxyribosyltransferase present in the crude extract of *L. helveticus*. The overall yield was 82 per cent (for details see Methods). 2-AP-DR migrated as one single spot when chromatographed on Whatman 40 filter-paper in *n*-butanol saturated with water[22] and in water. Descending chromatography was performed at 26° ± 1°, and the following R_f values were obtained in *n*-butanol saturated with H_2O; 2-AP-DR 0·49, 2-AP 0·46, adenine 0·57, 2′-deoxyadenosine 0·52, and in water: 2-AP-DR 0·64, 2-AP 0·52, adenine 0·44, and 2′-deoxyadenosine 0·58.

It seems likely that 2-AP-DR has the β-configuration, because the nucleoside deoxyribosyltransferase from *L. helveticus* catalyzes the transfer of 2′-deoxyribose between the normal bases and deoxyribosides.[19] Furthermore, 2-AP-DR is a competitive inhibitor of adenosine deaminase from calf intestine which is specific for adenine derivatives having β-configuration. α-2′-deoxyadenosine is neither substrate nor inhibitor of this enzyme.[25]

After acid hydrolysis (see Methods) of 2-AP-DR the compound migrated as 2-AP when chromatographed in the two solvents mentioned above. Presence and absence of 2′-deoxyribose in the ultraviolet absorbing spots were confirmed with the cysteine-H_2SO_4 spray.[22] When the acid reaction mixture was made alkaline the spectrum in ultraviolet light changed to that of 2-AP (disappearance of maximum at 245 mμ and increased absorbancy in the region 255 to 300 mμ).

The spectra of 2-AP-DR in acid, neutral and alkaline solution are seen on Fig. 1.

Effect of 2-AP and 2-AP-DR on nucleic acid synthesis

When Ehrlich cells were incubated in Tyrode's solution, 2-AP and 2-AP-DR only slightly inhibited the incorporation of $^{32}P_i$ into DNA and RNA even in concentrations of 6 μmoles/ml (Fig. 2). The presence of the two analogues decreased the total amounts

of radioactivity incorporated, and in most cases also the rate of incorporation, determined in the interval from one to five hr. In some experiments, however, the rate of incorporation in this time interval was less inhibited than shown on the figures, and experiments were therefore also performed in the medium of Harbers and Heidelberger.[26] In this medium 2-AP and 2-AP-DR did slightly inhibit the incorporation of $^{32}P_i$ into RNA (about 10–15 per cent inhibition), but not that of adenine-8-^{14}C (Fig. 3). The incorporation of $^{32}P_i$ into DNA was inhibited about 20–25 per cent by 2-AP and 2-AP-DR (Fig. 4). After a lag period the incorporation of adenine-8-^{14}C was inhibited about 50 per cent by 2-AP and almost completely by 2-AP-DR (Fig. 4).

Metabolism of 2-AP and 2-AP-DR

In order to investigate whether the two analogues were converted by Ehrlich cells to phosphorylated derivatives or degraded, 2-AP and 2-AP-DR were incubated in a reaction mixture containing per ml: 120 mg Ehrlich ascites cells, 30 μmoles glucose, 20 μmoles disodium succinate, 827 μl of Tyrode's solution, and either 3·26 μmoles 2-AP or 3·16 μmoles 2-AP-DR. Incubation was performed at 37° in a Dubnoff metabolic incubator with shaking. One ml aliquots were added at 0, 1, 2, and 3 hr to ice-cold 1 N PCA. The precipitate was centrifuged down, and the supernatant was neutralized with strong KOH. The $KClO_4$ precipitate was centrifuged down, the supernatant freeze-dried, and the remaining solid dissolved in a small volume of water, which was applied on filter-paper strips, chromatographed, and analyzed as described under Methods. The concentration of 2-AP was found to be constant during the incubation time with a maximal deviation from the mean value of 2·7%. Also the concentration of 2-AP-DR was constant, but the maximal deviation from the mean value was 6·9%. Deoxyribotides or ribotides of 2-AP could not be detected by the paper-chromatographic technique used. A conversion of 2–3 per cent of the added 2-AP or 2-AP-DR to phosphorylated derivatives would have been determined with certainty.

2-AP in purine nucleoside phosphorylase catalysed reaction

An attempt to prepare 2-aminopurine riboside enzymatically was made. The reaction mixture contained per ml: 50 μmoles Tris-HCl (pH 7·1); 8 μmoles 2-AP, HNO_3; 12 μmoles ribose-1-P, and 0·27 unit purine nucleoside phosphorylase. The activity of this enzyme was tested as described under Materials. Incubation was performed at 22°. Aliquots were taken every 15 min up to $2\frac{1}{4}$ hr, and assayed for P_i, which should be liberated if a reaction between 2-AP and ribose-1-P occurred. No increase in the concentration of P_i was found when determined by the method of Lowry and Lopez.[27] Because different compounds are known to interfere with this method[28] the negative results were controlled by chromatography.

A reaction mixture with a total volume of 2,225 μl contained 400 μl 0·1 M Tris-HCl (pH 7·4), 11·6 μmoles 2-AP, HNO_3, 6·67 μmoles ribose-1-P, and 1·2 units of nucleoside phosphorylase. Incubation was performed at 22° for 155 min, and then further at 37° for 90 min. The 2-AP and possible derivative was adsorbed quantitatively on 30 mg acid washed charcoal (Norit A), which was washed with water and eluted with 3 × 400 μl 10% pyridine in 50% alcohol. An amount of the pooled eluates corresponding originally to 2 μmoles 2-AP was applied on Whatman 40 filter-paper, and chromatographed at 26° in one of the following solvents: distilled water for 3 hr, *n*-butanol saturated with water[22] for 22 hr, and 1 M ammonium acetate—96%

alcohol (30 : 75) (pH 8·4) for 20 hr. On the three chromatograms only one spot corresponding to 2-AP was seen in u.v. light. The 2-AP containing spot from the chromatogram developed in water was cut out and eluted with water. No ribose could be found by the orcinol test.[29]

From the experiments presented it may be concluded that 2-AP is not a substrate for the purine nucleoside phosphorylase from calf spleen.

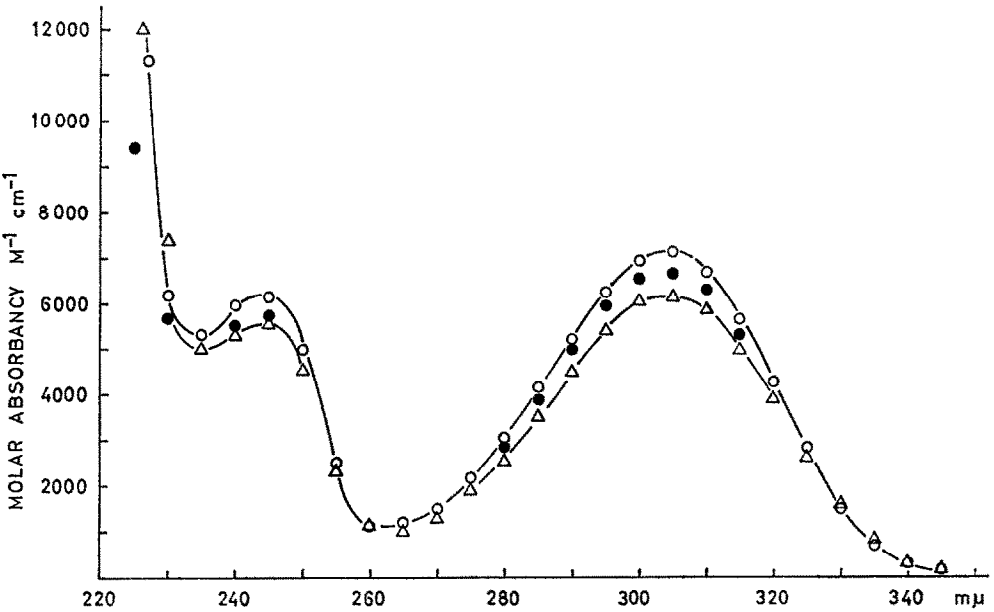

FIG. 1. 7·237 mg 2'aminopurine 2'-deoxyriboside were dissolved in H_2O to give a final concentration of 2·0 μmoles/ml. 50 μl of this solution was added to 950 μl of the following solutions: 0·05 M phosphate buffer (pH 7·4), 0·1 M acetate (pH 4·4), and 0·093 N NaOH. ○—○ 47·5 mM phosphate buffer (pH 7·4), △—△ 95·0 mM acetate buffer (pH 4·4), ●—● 88·5 mM NaOH.

Formation of 5-phosphoribosyl-2-aminopurine (2-AP-RP) from 2-AP and PRPP

In order to investigate whether 2-AP could react at all with PRPP when catalyzed by enzymes in Ehrlich cells, these compounds were incubated with a cell-free extract of the cells. The reaction mixture consisted of 21·5 μmoles phosphoenolpyruvate, cyclohexylamine salt, 12·1 μmoles 5-phosphoribosyl-1-pyrophosphate, Mg salt, 25·3 μmoles 2-AP, HNO_3, 300 μl 0·5 M Tris-HCl (pH 7·4), 5 μmoles $MgCl_2$, 38 μmoles KCl, 2·5 units pyruvate kinase, and 1,000 μl high-speed supernatant from Ehrlich ascites cells (see Materials). Total volume 1,635 μl. After incubation at 22° for 128 min 2-AP and possible derivatives were quantitatively adsorbed on 50 mg acid washed charcoal (Norit A), which was washed with H_2O and eluted with 3 × 500 μl 10% pyridine in 50% alcohol. Samples corresponding originally to about 3 μmoles 2-AP were applied on Whatman 40 filter-paper and chromatographed (descending) for 22 hr in 1 M ammonium acetate-96% alcohol (30 : 75) (pH 8·4). A spot with R_f value as AMP but very different from that of 2-AP and with blue fluorescence like that of 2-AP was cut out and eluted with water. The spectrum showed, apart from other absorption, the characteristic maximum at 305 mμ seen for 2-AP and 2-AP-DR. The amount of 2-AP-DR formed was 0·34 μmole or about 2·6% of the PRPP originally present.

Inhibition of adenosine deaminase by 2-AP-DR

2-AP-DR is not deaminated by a purified adenosine deaminase from calf intestine, but is the strongest competitive inhibitor hitherto found*. The inhibitor constant (2×10^{-6}) was one tenth of the K_m value of 2′-deoxyadenosine. 2-AP is not an inhibitor.

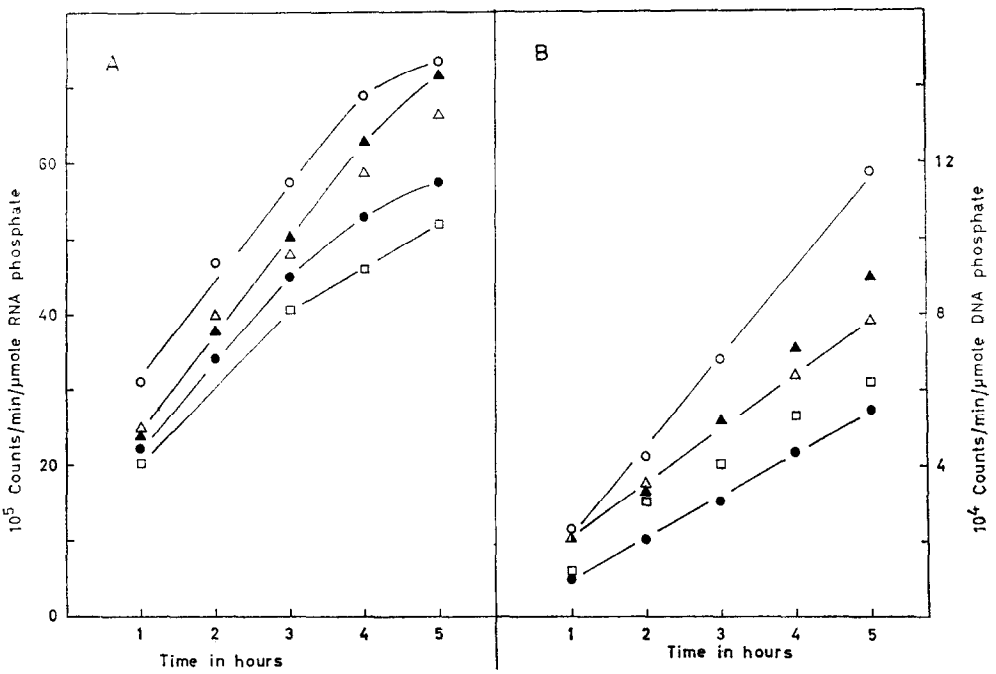

FIG. 2. Effect of 2-aminopurine (2-AP) and 2-aminopurine 2′-deoxyriboside (2-AP-DR) on the incorporation of $^{32}P_i$ into RNA (A) and DNA (B) of Ehrlich ascites cells incubated in Tyrode's solution. The reaction mixture contained per ml: 133 mg Ehrlich cells wet wt., 33 μmoles glucose, 22 μmoles disodiumsuccinate, 22 μC $^{32}P_i$ in 65 μl isotonic solution, 740 μl Tyrode's solution. Incubation was performed at 36° with shaking in a Dubnoff metabolic incubator. Incorporation into RNA and DNA was determined as previously described.[32, 5] Additions per ml: ○—○ none, △—△ 2·0 μmoles 2-AP, □—□ 6·0 μmoles 2-AP, ▲—▲ 2·0 μmoles 2-AP-DR, ●—● 6·0 μmoles 2-AP-DR.

DISCUSSION

2-Aminopurine 2′-deoxyriboside (2-AP-DR) has been prepared by transfer of 2′-deoxyribose from 2′-deoxythymidine to 2-aminopurine catalysed by nucleoside deoxyribosyltransferase from *L. helveticus* in 82 per cent yield. The spectrum of 2-AP-DR in acid, neutral and alkaline solution is shown on Fig. 1.

When Ehrlich cells were incubated in Tyrode's solution, and nucleic acid synthesis was determined by incorporation of $^{32}P_i$, 2-AP and 2-AP-DR were inhibitory to both RNA and DNA synthesis (Fig. 2). In some experiments, however, the rate of incorporation, determined in the time interval from 1 to 5 hr, was less inhibited than shown on the figures. The total incorporation in the presence of 2-AP and 2-AP-DR was, however, always less than in the control. When cells were incubated in the medium of Harbers and Heidelberger,[26] the incorporation of adenine-8-^{14}C into RNA was not inhibited by the two analogues, and the incorporation of $^{32}P_i$ was only slightly

* A Lineweaver-Burk plot will be published in a paper concerned with the specificity of this deaminase.

inhibited (Fig. 3). The incorporation into DNA of both adenine-8-^{14}C and $^{32}P_i$ was inhibited by the two analogues and more pronounced by 2-AP-DR than by 2-AP (Fig. 4). The effect of 2-AP and 2-AP-DR on DNA and RNA synthesis is quantitatively but not qualitatively different. This seems to mean that 2-AP and 2-AP-DR exert their effect as such by the same mechanism but to a different degree, or is converted to a common derivative which is the actual inhibitor. The metabolism of the compounds was therefore investigated.

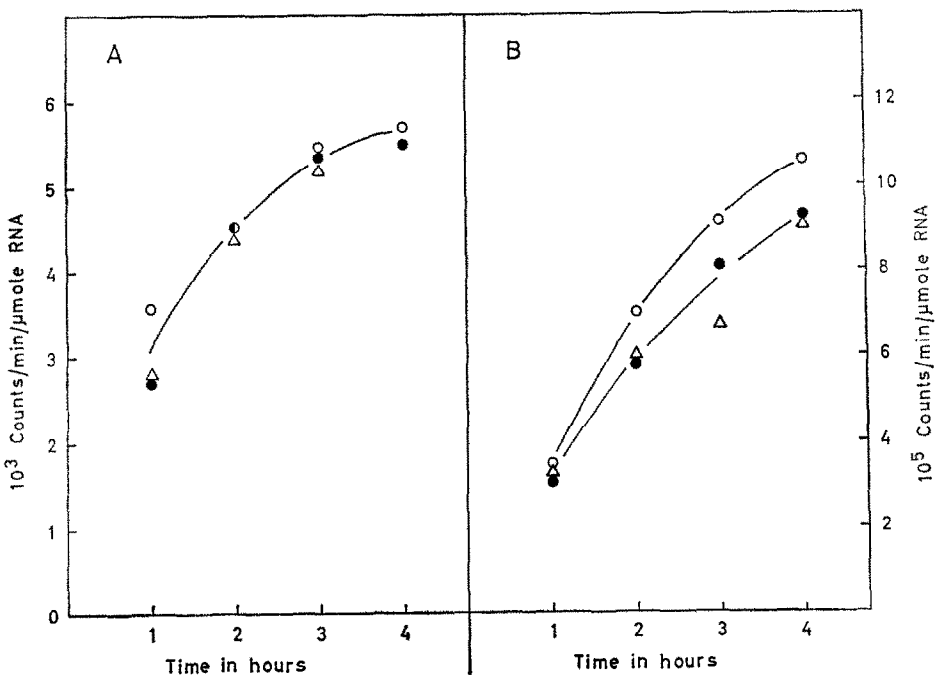

FIG. 3. Effect of 2-aminopurine (2-AP) and 2-aminopurine 2′-deoxyriboside (2-AP-DR) on the incorporation of adenine-8-^{14}C (A) and $^{32}P_i$ (B) into RNA from Ehrlich cells incubated in the medium of Harbers and Heidelberger.[26] The reaction mixture contained per ml: 85 mg Ehrlich cells, 415 μl ascites fluid, 500 μl Robinson's medium[33] containing glucose ($5{\cdot}6 \times 10^{-3}$ M), 20 mμmoles folic acid, and 5 μC adenine-8-^{14}C (8·5 μC/μmole) or 60 μC $^{32}P_i$. Incubation and determinations as for Fig. 2. Additions per ml: ○—○ none, △—△ 1·8 μmoles 2-AP, ●—● 2·0 μmoles 2-AP-DR.

The acid soluble fraction of a cell suspension incubated with one of the analogues was analysed by a paper chromatographic technique. It was found that the concentrations of both 2-AP and 2-AP-DR were constant and equal to the zero time value with a maximal deviation from mean value of 2·7 and 6·9 per cent, respectively. Deoxyribotides or ribotides of 2-AP could not be detected by the paper-chromatographic method used. A conversion of 2–3 per cent of the added 2-AP or 2-AP-DR to phosphorylated derivatives would have been determined with certainty.

Some of the enzymatic processes possibly involved in the anabolism of the two compounds were further studied under conditions which should be favourable for reaction to occur. It was found that 2-AP did react with PRPP in the presence of cell-free extract of Ehrlich cells in a very low yield (2·6 per cent) compared to that obtained with adenine as substrate. 2-AP did not react with ribose-1-P in a reaction

catalysed by purine nucleoside phosphorylase from calf spleen. The latter finding is in agreement with the stability of 2-AP-DR when incubated with Ehrlich cells. The absence of deoxyribonucleotides of 2-AP in cells incubated with 2-AP-DR is in agreement with the results that Lindberg, Hansen and Klenow obtained with a partially purified adenosine kinase from rabbit liver. The enzyme catalyses the phosphorylation of adenosine, 2.6-diaminopurine riboside, and some other analogues, but 2-AP-DR, however, is not a substrate.[30]

2-AP-DR is not deaminated by a purified adenosine deaminase from calf intestine, but is the strongest competitive inhibitor hitherto found. The inhibitor constant (2×10^{-6} M) was one tenth the K_m value of 2′-deoxyadenosine. 2-AP is not an inhibitor.

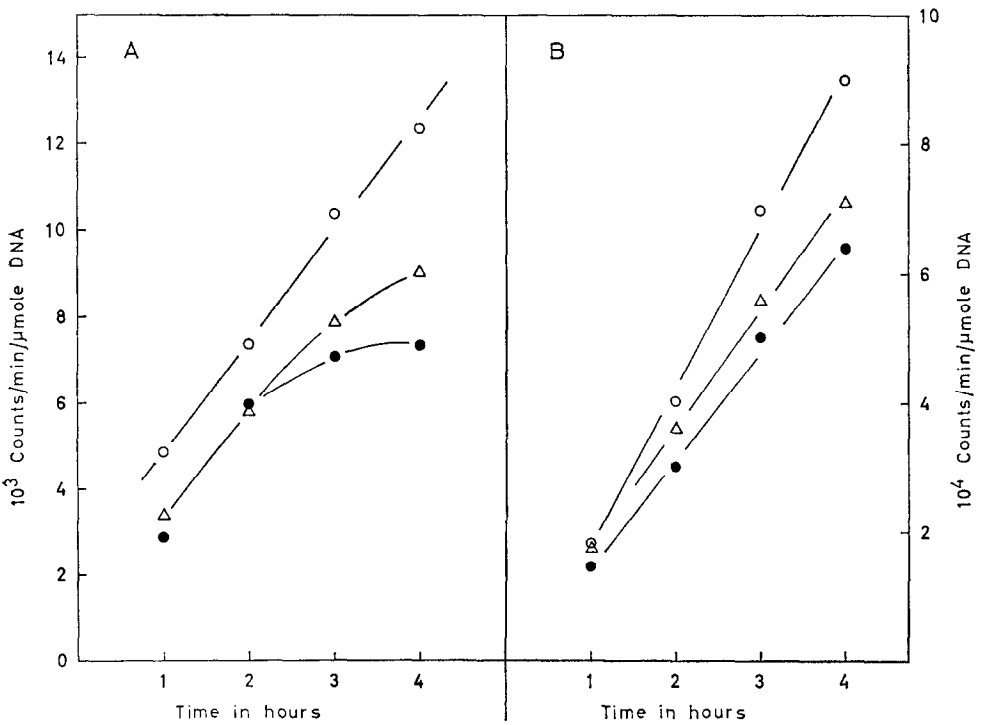

FIG. 4. Effect of 2-aminopurine (2-AP) and 2-aminopurine 2′-deoxyriboside (2-AP-DR) on the incorporation of adenine-8-^{14}C (A) and $^{32}P_i$ (B) into DNA from Ehrlich cells incubated in the medium of Harbers and Heidelberger.[26] The reaction mixture as described for Fig. 3. Incubation and determinations as described for Fig. 2. Additions per ml: ○—○ none, △—△ 1·8 μmoles 2-AP, ●—● 2·0 μmoles 2-AP-DR.

When Ehrlich cells are incubated with either 2′-deoxyadenosine[1, 5] or 3′-deoxyadenosine[6, 7] (cordycepin), phosphorylated derivatives can be isolated by the paper-chromatographic technique described. 2′-DeoxyATP inhibits the reduction of a cytidine and a guanosine phosphate to the respective deoxy compounds.[1] 3′-dADP and/or 3′-dATP inhibit RNA and DNA synthesis[6, 7] and regeneration of ATP, and 3′-dATP inhibits formation of PRPP.[9, 10] The inhibition of 2-AP and 2-AP-DR of nucleic acid synthesis seems not to require conversion to the corresponding ribotides or deoxyribotides to any appreciable extent; since 2-AP and 2-AP-DR only can be

metabolized in the cells to an extent which is within the experimental error of the paper-chromatographic method used. The inhibition found must either be exerted by 2-AP of 2-AP-DR itself or by trace amounts of phosphorylated compounds not detected by the method used. The fact that the incorporation of adenine-8-^{14}C into RNA is not inhibited by the two analogues is in contrast to the strong inhibition of the incorporation into DNA, and this indicates that the inhibited process is either the step of reduction of ribotides to deoxyribotides or one of the following steps leading to synthesis of DNA.

It is interesting to compare the highly mutagenic effect of 2-AP in *S. typhimurium*[13, 14] and phage T_4[11, 12] with the findings of Caspari and Pohley.[31] They have demonstrated that 2-AP is not mutagenic in larvae of *Ephestia kühnella* although other base analogues were. This could reflect a difference in species with regard to the effect of 2-AP. In the case of Ehrlich cells, deoxyribotides of 2-AP can only be formed—if formed—in very small amounts as demonstrated in the present paper, but the results do not exclude incorporation of 2-AP into DNA of Ehrlich cells.

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