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# STUDIES ON THE MODE OF ACTION OF PSICOFURANINE*

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**Abstract**—The mode of action of the antibiotic psicofuranine was studied using *E. coli B*. The inhibition of bacterial growth by psicofuranine was only transitory and the cells were able to overcome it after an extended lag phase. The inhibitory action of psicofuranine could be reversed by guanine and its derivatives; other purines and pyrimidines were inactive as reversing agents. Cells growing in the presence of psicofuranine excreted xanthosine into the medium. The measurements of purine synthesis from glycine-1-$^{14}$C in whole cells under the influence of psicofuranine showed a decreased isotope incorporation into guanine with a simultaneous increase in the radioactivity of xanthine. No effect on the incorporation of glycine-1-$^{14}$C into adenine was observed. The same results were obtained when the conversion of hypoxanthine-8-$^{14}$C into adenine and guanine as measured in inhibited cells. In the presence of psicofuranine, conversion of xanthine-8-$^{14}$C into both guanine and adenine was inhibited. These results showed that psicofuranine inhibited the conversion of xanthosine-5′-phosphate to guanosine-5′-phosphate.

PSICOFURANINE was isolated from culture media of *Streptomyces hygroscopicus var. decoyinine*[1] and found to possess antibacterial[2] and antitumor[3] properties. Its structure was established as 6-amino-9-D-psicofuranosylpurine[4] and it may, therefore, be visualized as a nucleoside analogue, containing the anomalous hexose, psicose, instead of the normally occurring pentose. Hanka[5] found that the inhibitory effect of psicofuranine on growth of *S. aureus* could be reversed by guanine, guanosine and guanosine-5′-phosphate† at concentrations of from 5 to 10 μg/ml. Other purines and pyrimidines showed some reversal effect at much higher levels. These results led Hanka[5] to the suggestion that psicofuranine interfered with the synthesis of G5′P from X5′P.

This communication presents conclusive evidence that in *E. coli B*, psicofuranine inhibits the conversion of X5′P to G5′P.

## METHODS

Cultures of *E. coli B* were maintained on Difco Nutrient Agar slopes. Bacteria were grown in the minimal salts-glucose medium, described by Davis and Mingioli.[6] In the growth experiments, 4 ml of sterile medium were placed into sterile colorimeter tubes and each tube was inoculated with 1–2 × $10^7$ cells from a liquid medium culture

* 6-Amino-9-D-psicofuranosylpurine.

† The following abbreviations are used: G5′P, guanosine-5′-phosphate; X5′P, xanthosine-5′-phosphate; I5′P, inosine-5′-phosphate; A5′P, adenosine-5-phosphate; ATP, adenosine-triphosphate; GTP, guanosinetriphosphate.

grown for 12 hr. The tubes were incubated at 37 °C and growth was followed by measuring the light transmittance at 520 mμ in a Bausch and Lomb colorimeter. Viable cell counts were made by plating 1 : $10^6$ and 1 : $10^7$ dilutions of the cell suspensions three times each and counting the colonies after 24 hr of incubation.

Xanthosine, which was excreted into the medium by inhibited cells, was isolated and identified in the following manner: After removal of cells by centrifugation, all ultraviolet light absorbing material from 1000 ml of growth medium was adsorbed on 3 g of activated charcoal (Darco G-60) and eluted with a mixture of 65% water, 25% ethanol and 10% concentrated ammonium hydroxide. Five elutions, 150 ml each, were made to recover 98 per cent of the ultraviolet light absorbing material from the charcoal. The combined eluates, after filtration through Celite, were evaporated to dryness in a rotary flash evaporator at 35 °C and the dry crystals were dissolved in 10 ml of water containing 0·25 ml of concentrated ammonium hydroxide. The solution was poured onto a column of Dowex-1 (chloride form, 8·3 cm × 1 cm) and the resin was eluted with 0·1 M $NH_4Cl$, adjusted to pH 9·5 with ammonium hydroxide.[7] The eluate was collected in 10 ml fractions and analyzed for absorption at 260 mμ. Separation of the components in the eluate from charcoal was also achieved by paper chromatography, using a *n*-butanol–ammonia solvent system.[8] For the paper rechromatography of the isolated xanthosine and its separation from xanthine, the *iso*amyl alcohol–$Na_2HPO_4$ mixture of Carter[9] was used. Spots of xanthosine were detected by examining the paper chromatograms in ultraviolet light and by spraying with a solution of sodium metaperiodate and Schiff's reagent.[10] Free pentose, resulting from hydrolysis of xanthosine with 1 N HCl for 30 min in boiling water bath, was detected on paper chromatograms by its reaction with the anilinephthalate reagent of Partridge.[11]

Experiments with radioactive tracers were made by techniques similar to those of Tomisek *et al.*[12, 13] Two Erlenmeyer flasks containing 20 ml of washed cell suspension in the growth medium (transmittance 20 per cent) were incubated for 30 min at 37 °C. Two milligrams of psicofuranine in 1 ml of water were added to one flask. The control flask received 1 ml of water. The incubation was continued for 10 min, after which radioactive substrates were added to both flasks. The cells were allowed to assimilate the added radioactive compounds for 10 min and their metabolism was stopped by pouring the cell suspension onto 10 g of ground ice in a 50 ml centrifuge tube. Cells were sedimented by centrifugation at 0 °C and washed twice with 2 ml of ice-cold physiological saline. The sedimented and washed cells were then hydrolyzed for 60 min in 3 ml of 1 N HCl in a boiling water bath. This treatment liberated the purine bases from nucleic acids and nucleotides. The resulting suspension was centrifuged at 10,000 × *g* for 10 min and the sedimented residual proteins were washed twice with 1 ml of water and dried *in vacuo*. The washings were combined with the original 1 N HCl extract and dried *in vacuo* over NaOH. The dry residues were dissolved in 0·2 ml of water. Separation of adenine, guanine, hypoxanthine and xanthine in this solution was achieved by two-dimensional paper chromatography,[14] using *iso*propanol-HCl[15] as the first and *n*-butanol-$NH_3$[8] as the second solvent system. Since the bacteria in the amount used did not contain enough hypoxanthine and xanthine to permit their detection by ultraviolet light absorption, carrier hypoxanthine and xanthine were applied on the chromatograms prior to the extracts. Purines were eluted from the paper with 5 ml of 0·1 N HCl and their absorption spectra determined in a

recording spectrophotometer (Cary, model 11). Aliquots of the eluates were taken for determination of radioactivity. Owing to the short incubation period, no spectrophotometrically demonstrable increase in total adenine and guanine concentration was observed after quantitative elution of the respective spots. Therefore, the radioactivity measurements were calculated as counts per minute in each purine and comparison was made by expressing the counts in each individual purine as percentage of the total radioactivity in the four purine bases. Specific activities of adenine and guanine were calculated after quantitative elution of the respective spots on paper chromatograms with 0·1 N HCl and measurements of ultraviolet light absorption at 260 mμ for adenine and at 250 mμ for guanine. The extinction coefficients used were those given by Wyatt.[14] Location of the spots on the paper chromatograms was also detected by radioautography. Developed chromatograms were left in contact with Kodak no-screen X-ray film for a period of from 2 to 3 weeks.

Radioactive carbon ($^{14}C$) was measured in a Packard tri-carb liquid scintillation spectrometer. The counting solution was a mixture of toluene and absolute ethanol (7 : 3) with 0·4% 2 : 5-diphenyloxazole and 0·01% 1 : 4-di-[2-(5-phenyloxazolyl)] benzene as the phosphors. Protein was determined by the optical method of Warburg and Christian.[16]

Glycine-1-$^{14}C$ (14·7 μc/mg), hypoxanthine-8-$^{14}C$ (5·4 μc/mg) and xanthine-8-$^{14}C$ (1·6 μc/mg) were purchased from Volk Radiochemical Company and used without dilution. Adenine, guanine, hypoxanthine, xanthine, guanosine and xanthosine were products of Nutritional Biochemical Corporation. The other purine and pyrimidine derivatives were purchased from California Corporation for Biochemical Research. Xanthosine-5′-phosphate was synthesized from guanosine-5′-phosphate by diazotation and hydrolysis of the diazonium compound.[17, 18]

## RESULTS

Growth curves of *E. coli B* in the presence of increasing concentration of psicofuranine are shown in Fig. 1. It may be seen that with increasing concentrations of

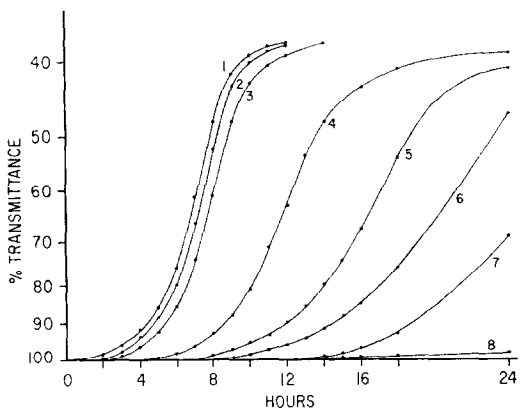



FIG. 1. Growth curves of *E. coli B* in the presence of increasing concentrations of psicofuranine. Concentrations of psicofuranine per ml: 1 = 0; 2 = 5 μg; 3 = 10μg; 4 = 50 μg; 5 = 75 μg; 6 = 100 μg; 7 = 150 μg; 8 = 200 μg.

psicofuranine a longer lag phase occurred before the onset of growth. With relatively low concentrations of the antibiotic (5–10 μg/ml) the cells eventually overcame the inhibition completely and grew at the same rate as the control cells. With higher concentrations of psicofuranine, the growth rate during the logarithmic phase was inhibited, the extent of inhibition being dependent on the concentration of the inhibitor. When psicofuranine was added to a growing culture of *E. coli B* at the beginning of the logarithmic phase in the concentration 100 μg/ml, which was inhibitory to the inoculum for at least 7 hr (Fig. 1), it produced only a partial inhibition of growth, as shown in Fig. 2. Microscopic examination of cultures growing in the

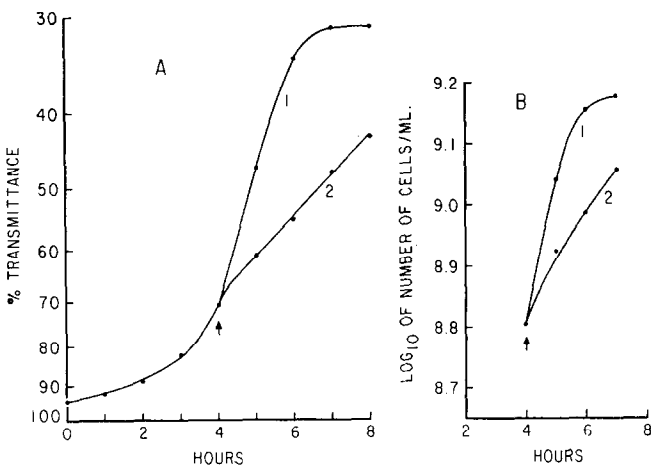



FIG. 2. Effect of psicofuranine on bacterial growth when added at the beginning of the logarithmic phase of growth, measured by light transmittance of the cell suspension and by viable cell counts. Two test tubes with 4 ml of medium were inoculated with $2 \times 10^7$ cells and at the time indicated by the arrow, psicofuranine (100 μg/ml) was added to one test tube. From this point on, samples were taken for the viable cell count determination. A = light transmittance measurements; B = viable cell counts; curve 1 = without psicofuranine; curve 2 = with added psicofuranine.

presence of psicofuranine failed to reveal any elongated or filamentous forms of the cells. Viable cell counts, determined in the experiment shown in Fig. 2, confirmed that light transmittance measurements were indeed measurements of the growth of the bacterial population.

Attempts to prevent the inhibitory effect of psicofuranine on the growth of *E. coli B* by purines, pyrimidines, and their derivatives are summarized in Table 1. The data show the striking superiority of guanine and related compounds over the other substances in their ability to abolish the effect of psicofuranine. Results of a detailed reversal study with guanine, present in the medium in the concentration range from 0 to 10 μg/ml, are shown in Fig. 3. Similar results were obtained with guanosine and G5'P as reversing agents.

Excretion of an ultraviolet light absorbing substance into the medium by cells growing in the presence of psicofuranine was noticed when the absorption spectra of the growth medium with 100 μg/ml of the psicofuranine were determined at zero time and after 24 hr of incubation; at this time the bacterial growth was identical with the maximal growth of the control. Both spectra are reproduced in Fig. 4. Subtracting

TABLE 1. PREVENTION OF THE INHIBITORY ACTION OF PSICOFURANINE (100 μg/ml) ON THE GROWTH OF *E. coli B* BY PURINES AND PYRIMIDINES PRESENT IN THE MEDIUM
(The growth was measured after 8 hr.)

| Reversing compound (10 μg/ml) | Growth in % of the control |
|---|---|
| Adenine | 14·5 |
| Guanine | 102 |
| Hypoxanthine | 2 |
| Xanthine | 4 |
| Cytosine | 4 |
| Uracil | 2 |
| Thymine | 4 |
| Adenosine | 4 |
| Guanosine | 100 |
| Inosine | 4 |
| Xanthosine | 4 |
| Cytidine | 1 |
| Uridine | 1 |
| Adenosine-5′-phosphate | 6 |
| Guanosine-5′-phosphate | 102 |
| Cytidine-5′-phosphate | 2 |
| Uridine-5′-phosphate | 2 |
| Deoxyadenosine | 4 |
| Deoxyguanosine | 75 |
| Deoxycytidine | 4 |
| Thymidine | 2 |
| Deoxyadenosine-5′-phosphate | 4 |
| Deoxyguanosine-5′-phosphate | 62·5 |
| Deoxycytidine-5′-phosphate | 4 |
| Thymidine-5′-phosphate | 2 |
| No additions | 2 |

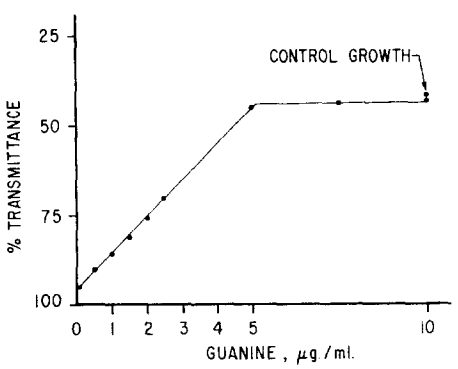



FIG. 3. Prevention of the inhibitory action of psicofuranine on growth of *E. coli B* by guanine. Concentration of psicofuranine was 100 μg/ml. The concentrations of guanine in the medium ranged from 0 to 10 μg/ml. Growth was measured after 8 hr.

the original spectrum at zero time from the final one after 24 hr, a difference spectrum was obtained which closely resembled that of xanthosine. No ultraviolet absorbing materials were excreted into the growth medium by the control cells. Isolation and identification of xanthosine was made from 1000 ml of growth medium of an inhibited

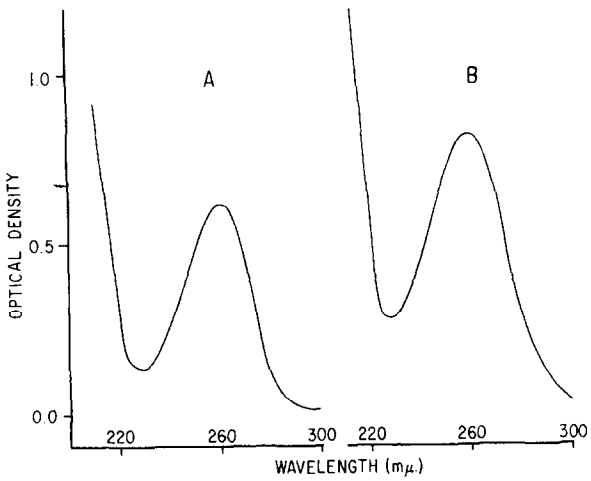



FIG. 4. Accumulation of an ultraviolet light absorbing material in the growth medium by cells growing in the presence of psicofuranine. A, ultraviolet light absorption spectrum of the medium with 100 $\mu$g of psicofuranine per ml at the beginning of the growth experiment (0 hr); B, ultraviolet light absorption spectrum of the medium after 24 hr of incubation. Media were diluted 1 : 10 with $H_2O$.

culture by charcoal adsorption and column chromatography of the charcoal eluate on Dowex-1. The elution pattern from the column is shown in Fig. 5. Component *A* was identified as psicofuranine and component *B* as xanthosine. Paper chromatography of the charcoal eluate in the *n*-butanol–$NH_3$ solvent system revealed only two spots: that of psicofuranine with $R_f = 0{\cdot}18$ and that of xanthosine with $R_f = 0{\cdot}02$. Identity of xanthosine was established by the following criteria: ultraviolet light absorption spectrum in 0·001 N HCl and in 1 N NaOH; paper chromatography in the *iso*amyl alcohol–$Na_2HPO_4$ system; hydrolysis with 1 N HCl and identification of resulting xanthine and ribose by paper chromatography. A control sample of xanthosine was

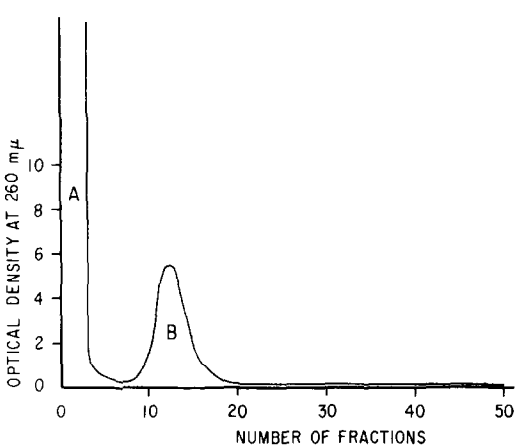



FIG. 5. Separation of psicofuranine and xanthosine on Dowex-1. The column was eluted with 0·1 *M* $NH_4Cl$ (pH adjusted to 9·5 with $NH_4OH$). Peak A = psicofuranine; peak B = xanthosine.

TABLE 2. EFFECT OF PSICOFURANINE ON THE INCORPORATION OF GLYCINE-1-$^{14}$C INTO PURINE BASES OF *E. coli B*

Two Erlenmeyer flasks, each containing 20 ml of bacterial suspension (transmittance 20 per cent), were incubated for 30 min; then psicofuranine was added to one flask in the final concentration of 100 μg/ml. After 10 min, 5μc of glycine-1-$^{14}$C were added to both flasks and the incubation was continued for an additional 10 min. Washed cells were hydrolyzed in 1 N HCl in a boiling water bath and the purines isolated by two-dimensional paper chromatography.

| | Without psicofuranine | | | With psicofuranine | | |
|---|---|---|---|---|---|---|
| | Counts/min | % of the total purine radioactivity | Specific activity | Counts/min | % of the total purine radioactivity | Specific activity |
| Total purines | $3{\cdot}455 \times 10^5$ | 100 | — | $2{\cdot}375 \times 10^5$ | 100 | — |
| Adenine | $1{\cdot}535 \times 10^5$ | 44·5 | $6{\cdot}20 \times 10^4$ counts/min per μM | $1{\cdot}090 \times 10^5$ | 46 | $4{\cdot}40 \times 10^4$ counts/min per μM |
| Guanine | $1{\cdot}745 \times 10^5$ | 50·5 | $6{\cdot}48 \times 10^4$ counts/min per μM | $0{\cdot}603 \times 10^5$ | 25·4 | $2{\cdot}23 \times 10^4$ counts/min per μM |
| Hypoxanthine | $0{\cdot}0765 \times 10^5$ | 2·2 | — | $0{\cdot}066 \times 10^5$ | 2·7 | — |
| Xanthine | $0{\cdot}0985 \times 10^5$ | 2·8 | — | $0{\cdot}616 \times 10^5$ | 26 | — |
| Protein | — | — | $6{\cdot}44 \times 10^4$ counts/min per mg | — | — | $6{\cdot}03 \times 10^4$ counts/min per mg |

TABLE 3. EFFECT OF PSICOFURANINE (100 $\mu$g/ml) ON THE CONVERSION OF HYPOXANTHINE-8-$^{14}$C INTO OTHER PURINE BASES IN *E. coli B*; HYPOXANTHINE-8-$^{14}$C (5 $\mu$C) WAS ADDED TO EACH FLASK

(For experimental details, see legend to Table 2.)

| | Without psicofuranine | | | With psicofuranine | | |
|---|---|---|---|---|---|---|
| | Counts/min | % of the total purine radioactivity | Specific activity | Counts/min | % of the total purine radioactivity | Specific activity |
| Total purines | $3{\cdot}58 \times 10^5$ | 100 | — | $2{\cdot}01 \times 10^5$ | 100 | — |
| Adenine | $1{\cdot}66 \times 10^5$ | 46·4 | $6{\cdot}64 \times 10^4$ counts/min per $\mu$M | $0{\cdot}97 \times 10^5$ | 48·3 | $3{\cdot}88 \times 10^4$ counts/min per $\mu$M |
| Guanine | $1{\cdot}47 \times 10^5$ | 41·2 | $5{\cdot}47 \times 10^4$ counts/min per $\mu$M | $0{\cdot}525 \times 10^5$ | 25·1 | $1{\cdot}61 \times 10^4$ counts/min per $\mu$M |
| Hypoxanthine | $0{\cdot}113 \times 10^5$ | 3·2 | — | $0{\cdot}095 \times 10^5$ | 4·7 | — |
| Xanthine | $0{\cdot}326 \times 10^5$ | 9·1 | — | $0{\cdot}420 \times 10^5$ | 20·9 | — |

TABLE 4. EFFECT OF PSICOFURANINE (100 $\mu$g/ml) ON THE CONVERSION OF XANTHINE-8-$^{14}$C INTO OTHER PURINE BASES IN *E. Coli B*; XANTHINE-8-$^{14}$C (2·5 $\mu$C) WAS ADDED TO EACH FLASK

(For experimental details, see legend to Table 2.)

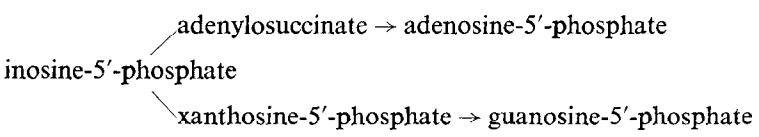
| | Without psicofuranine | | | With psicofuranine | | |
|---|---|---|---|---|---|---|
| | Counts per min | % of the total purine radioactivity | Specific activity | Counts per min | % of the total purine radioactivity | Specific activity |
| Total purines | $10{\cdot}65 \times 10^4$ | 100 | — | $6{\cdot}07 \times 10^4$ | 100 | — |
| Adenine | $4{\cdot}00 \times 10^4$ | 37·6 | $2{\cdot}99 \times 10^4$ counts/min per $\mu$M | $1{\cdot}12 \times 10^4$ | 18·5 | $0{\cdot}864 \times 10^4$ counts/min per $\mu$M |
| Guanine | $5{\cdot}35 \times 10^4$ | 50·2 | $3{\cdot}45 \times 10^4$ counts/min per $\mu$M | $2.04 \times 10^4$ | 33·6 | $1{\cdot}36 \times 10^4$ counts/min per $\mu$M |
| Hypoxanthine | $0{\cdot}27 \times 10^4$ | 2·5 | — | trace | — | — |
| Xanthine | $1{\cdot}026 \times 10^4$ | 9·6 | — | $2{\cdot}91 \times 10^4$ | 48 | — |

analyzed simultaneously and no discrepancy in the properties of the two substances was found.

The effect of psicofuranine on the synthesis *de novo* of purine bases in *E. coli B* from glycine-1-$^{14}$C is summarized in Table 2. It may be seen that in the presence of psicofuranine, the synthesis of the purine ring, measured by the incorporation of radioactive carbon from glycine-1-$^{14}$C, was inhibited by 31 per cent and the distribution of the isotope between the four purine bases was changed. While the percentage incorporation of the isotope into adenine remained practically unchanged, a significant decrease in the radioactivity of guanine, together with an increase in the radioactivity of xanthine was observed. Table 2 also shows that the incorporation of glycine-1-$^{14}$C into proteins was only slightly inhibited in the presence of psicofuranine under these experimental conditions. Table 3 shows the effect of psicofuranine on the conversion of hypoxanthine-8-$^{14}$C into other purines in *E. coli B*. The results are analogous to those obtained when glycine-1-$^{14}$C was used as tracer. The percentage radioactivity incorporated into adenine was the same as the control, the radioactivity of guanine was decreased and the radioactivity of xanthine increased, both to an extent comparable to the previous experiment. Conversion of xanthine-8-$^{14}$C into other purines was studied similarly and the results are shown in Table 4. In this case, the conversion of xanthine to both adenine and guanine was inhibited by the presence of psicofuranine.

## DISCUSSION

The last stages of the biosynthesis of purine bases at the nucleotide level[19, 20] are schematically shown below:




The reversal of the inhibitory action of psicofuranine on bacterial growth specifically by guanine and its derivatives, the other purines and pyrimidines being ineffective in this respect, could indicate the inhibition of G5′P synthesis from X5′P. Detailed experiments with guanine in concentrations varying from 0 to 10 $\mu$g/ml showed (Fig. 3) that with 5 $\mu$g/ml of guanine, a complete reversal of the inhibition by psicofuranine was achieved and larger amounts of guanine in the medium had no additional effect on growth. When the nutritional requirements of the purineless mutant *E. coli B*-96 (obtained from Dr. S. Friedman, New York Medical College, New York) were examined, it was found that 7·5 $\mu$g of guanine per ml ensured maximal growth. Burton[21] in his work with this mutant supplemented the medium with 7 $\mu$g/ml of guanine. Considering that (a) in the mutant, the added guanine must also provide for the adenine requirements of the cells and that (b) the postulated block in the synthesis of G5′P might not be absolute, then the two figures (5 $\mu$g/ml with inhibited cells and 7·5 $\mu$g/ml with the mutant) are in reasonable agreement. Additional support for the assumption that psicofuranine inhibited the amination of X5′P [22] comes from the observation that the cells growing in the presence of psicofuranine excreted xanthosine into the medium. Accumulation of xanthosine in the medium was reported by Magasanik and Brooke[23] with an *Aerobacter aerogenes* mutant which specifically lacked the X5′P aminase. Apparently, the cells disposed of the accumulating X5′P by excreting it as the nucleoside. It is probable that other nucleotides are also

excreted as nucleosides, since Friedman and Moat[24] found accumulation of 5-aminoimidazole riboside and inosine in the medium by biotin-deficient yeast.

The data obtained with glycine-1-$^{14}$C and hypoxanthine-8-$^{14}$C as isotopically labeled precursors of adenine and guanine are entirely in agreement with the assumption that psicofuranine inhibits the amination of X5′P to G5′P. The percentage distribution of the radioactive carbon from both tracers (Tables 2 and 3) into the purine bases showed a decrease in the radioactivity of guanine with a simultaneous increase in the radioactivity of xanthine; the radioactivity of adenine remained the same. However, the total synthesis of the purines was inhibited (31 per cent in the glycine-1-$^{14}$C experiment and 42 per cent in the hypoxanthine-8-$^{14}$C experiment) and this was reflected in the specific activities of both adenine and guanine. It may be noted that the decrease in specific activities of guanine was considerably larger than the decrease in the specific activities of adenine. This result lends support to the suggestion made by Magasanik[25] that the relative concentrations of ATP and GTP control the synthesis of A5′P and G5′P, respectively, from the common precursor I5′P. ATP has been shown to participate in the amination of X5′P to G5′P [22] and to inhibit the reductive deamination of G5′P to I5′P. [26] On the other hand, GTP is required for the condensation of I5′P with aspartic acid to adenylosuccinic acid, an immediate precursor of A5′P. [27] Consequently, the inhibition of G5′P synthesis would result in a decrease in GTP level and a subsequent decrease in the synthesis of A5′P. The data, showing decreases in the specific activities of adenine and guanine, are in harmony with this theoretical conclusion.

The data obtained with xanthine-8-$^{14}$C (Table 4) again showed that in the presence of psicofuranine, the synthesis of G5′P was inhibited with the resulting accumulation of X5′P. The percentage incorporation of the isotope into adenine in the presence and absence of psicofuranine followed that expected from the properties of the isolated enzymes, I5′P dehydrogenase[28] and X5′P aminase.[22] Both enzyme reactions are practically irreversible and therefore the conversion of X5′P to A5′P must be via G5′P, followed by reductive deamination to I5′P,[26] condensation with aspartic acid and hydrolysis to A5′P. Thus the inhibition of X5′P aminase by psicofuranine should not only decrease the conversion of xanthine-8-$^{14}$C to guanine but also the subsequent conversion to adenine. The relative radioactivities of adenine (37·6 per cent in the absence and 18·5 per cent in the presence of psicofuranine) agreed with this interpretation.

Magee and Eberts[29] studied the effect of psicofuranine on the incorporation of glycine-1-$^{14}$C into the purines and proteins of the Walker carcinoma of the rat. They observed decreases in specific activities of adenine and guanine comparable to those found in *E. coli B* (Table 2) and a pronounced decrease in the specific activity of the proteins. As Table 2 shows, no marked change in the specific activity of proteins in *E. coli B* was noted in the presence of psicofuranine. This apparent difference between the tumor and the microbial system can be explained by the fact that the bacterial suspension used was more concentrated (transmittance 20 per cent) than the culture at the maximal growth (transmittance approximately 30 per cent). Thus, no growth was possible and presumably only exchange reactions between the labeled glycine and the protein were taking place.

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