Alterations in Pyrimidine Metabolism in L5178Y Leukemia Cells Resistant to 6-Azauridine*

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SUMMARY

A clone of a subline of L5178Y (termed L5178AzUR) that requires 1500 times more 6-azauridine or 6-azauridylic acid to cause a 50 per cent inhibition of its rate of growth, as compared with that of the parent strain, has been isolated.

The metabolism of orotic acid by L5178Y and L5178AzUR has been studied in whole animals, in cells growing in culture, in resting cell suspensions, and in cell-free extracts. The extent to which uridylic acid was formed from orotic acid by whole cells or cell-free extracts was the same or only slightly greater in the resistant as compared with the sensitive line, and the inhibition of this reaction in cell-free extracts by azauridylic acid was equal in both cell lines.

Incorporation of uridine into the nucleic acids of L5178AzUR cells growing in culture was approximately 40% that with L5178Y growing under the same conditions.

The formation of phosphorylated derivatives of uridine and azauridine by resting cell suspensions and cell extracts was much lower in the AzUR-resistant strain than in the strain sensitive to the drug.

These results are compatible with the concept that in these AzUR-resistant cells there is a defect in pyrimidine metabolism at the stage of uridine phosphokinase.

The carcinostatic effects of 6-azauridine, the ribofuranoside of 6-azauracil, have been ascribed to its effects on pyrimidine metabolism (7, 10, 13, 17). Administration of this analog of uridine to mice bearing the L5178Y lymphoma resulted in a rapid accumulation of azauridine 5'-phosphate in the liver and tumor (17). This derivative, but not azauridine per se, was shown to be a competitive inhibitor of the decarboxylation of orotidine-5'-phosphate by soluble extracts of such tissues (10, 13, 17). In the livers and tumors of animals treated with azauridine the utilization of orotic acid was sharply reduced, but the incorporation of uridine into the nucleic acids was greater than that in control animals. Simultaneous with these changes in pyrimidine metabolism, it was observed that considerable amounts of orotidine accumulate in the tumors of animals treated with azauridine. Although the synthesis of pyrimidines de novo is also blocked in liver and intestinal epithelium, the available toxicological evidence (12) suggests that these tissues are relatively immune to the action of the antimetabolite.

These indications of a favorable therapeutic index suggested evaluation of the drug in human neoplastic disease, and, in preliminary trials in acute leukemias, there was evidence that azauridine can modify the course of some types of this disease, at least temporarily (4). At the same time it was recognized that prolonged treatment with this analog would probably be limited by the emergence of resistant populations of tumor cells. Earlier studies had indicated that resistant bacteria were incapable of converting azauracil and its ribonucleoside to nucleotide derivatives, and a corresponding loss in the capacity to incorporate either uracil or uridine into nucleic acids was also noted (8). It was considered desirable to investi-
gate neoplastic cells which could be made highly resistant to azauridine and be studied independently of the host. Lymphoma L5178Y was chosen because the parent line is very sensitive to azauridine (14), whereas high levels of resistance were readily achieved by growth of the neoplastic cells in vitro in the presence of increasing concentrations of the drug. The pyrimidine metabolism of the resistant subline, L5178AzUR, in mice as well as in culture, was compared with that of the parent line of cells, in an attempt not only to elucidate the mechanism of the resistance, but also to disclose possible ways of inhibiting the drug-resistant cells. Although the metabolism of orotic acid was virtually the same in the two types of resistant cells, the utilization of uridine was severely depressed in L5178AzUR cells growing either in the mouse or in culture. Similar studies on suspensions or soluble extracts of such cells incubated in vitro have confirmed this finding and have demonstrated a reduced conversion of azauridine to azauridine 5'-phosphate.

MATERIALS AND METHODS

Materials.—Azauridine prepared by a modification (9) of the procedure described by Škoda et al. (21) was generously supplied by the Squibb Institute for Medical Research. Azauridine 5'-phosphate was prepared by a chemical synthesis (11). Azauridine-2-C14 (21) was made from azauracil-2-C14, kindly prepared by Dr. P. K. Chang (2). Orotic acid-4-C14, orotic acid-7-C14, and uridine-5,6-H2 were obtained from the New England Nuclear Corporation. Orotidyl acid was isolated from cultures of Escherichia coli B (9), the growth of which was inhibited by azauracil.

Experimental procedures.—Tumors were maintained in AKR/DBA/2 mice and harvested as described previously (17). For the reproduction of L5178Y cells in culture, a modified growth medium devised to permit clonal reproduction of isolated lymphoblasts was used; the incubation conditions described in earlier reports (5) were used.

Cell number was determined with a Coulter Particle Counter, Model A. A strain of L5178Y which was resistant to azauridine was developed by serial sub-culture in media containing increasing concentrations of azauridine, in a manner similar to that used for the isolation of L5178 cells resistant to amethopterin (6). During a period of 100 days a progressive increase in resistance was observed. From the final population, composed of cells with varying levels of resistance, a probable clone of azauridine-resistant cells (L5178AzUR) was obtained by dilution technics. Although the rate of reproduction of the resistant subline was equivalent to that of cells of the sensitive clone of origin, the drug-resistant cell line reverted to lower levels of resistance when cultured in the absence of azauridine. Similar reversions to less resistant forms were detected when the resistant line was maintained in mice.

The uptake of uridine and orotic acid by growing cells was measured in 500 ml. of growth medium containing cells and the appropriate radioactive substrate. Following an initial lag phase (2–12 hours), doubling times of 11–17 hours were obtained. Cultures were then harvested by centrifugation, and the sedimented cells were washed twice with 5 ml. of the growth medium without horse serum or Krebs III buffer (15) and suspended 3 times in 5 ml. of cold 0.5 M perchloric acid and centrifuged. The residue was washed once with 5 ml. of 60 per cent ethanol and once with 5 ml. of 100 per cent ethanol; it was then extracted with 5 ml. of 5 per cent trichloroacetic acid at 100° C. for 10 minutes.

For experiments with resting cell suspensions and cell extracts, L5178Y and L5178AzUR cells were grown in 1 liter of medium from a concentration of 5 × 10^4 cells/ml to 1 × 10^6 cells/ml. These mass cultures were harvested by centrifugation at 0° C. and treated in the following manner. Cell suspensions were prepared by washing the sedimented cells in growth medium without serum. They were then incubated in this medium with orotic acid-7-C14, in Warburg flasks as previously described (13). The uptake and metabolism of labeled azauridine and uridine by whole cell suspensions, incubated in medium without serum in stopped Erlenmeyer flasks at 37° C., were followed by removing aliquots of the incubated suspensions and centrifuging in 0.7-ml hematocrit tubes. Fresh medium was carefully added to the tubes without disturbing the pack, aspirated, and the process repeated; this procedure removes radioactivity adhering to the walls of the tube and on top of the pack. The cells were then extracted 3 times with 0.7 ml. of ice-cold perchloric acid (0.5 M) to remove acid-soluble nucleotides by suspension with a bent wire rotated by a stirring motor. The precipitate was dissolved in 0.7 ml. of potassium hydroxide solution (0.2 n) for the measurement of radioactivity in the nucleic acids. All treatments were carried out in the hematocrit tube.

Cell-free extracts were prepared by suspending a washed cell preparation in 6 volumes of 0.25 M sucrose (pH 7.0) and freezing in a solid CO2-acetone bath and thawing to 0° C. 3 times. The supernatant fraction obtained after centrifuging at 105,000 × g for 60 minutes in a Spinco re-

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frigerated centrifuge was incubated with orotic acid-7-C¹⁴ in Warburg flasks, or with uridine-5,6-H² and azauridine-2-C¹⁴ in Erlenmeyer flasks. The composition of the incubation medium and the separation of labeled products on Dowex-1 have been described earlier (17).

Analytical procedures.—The incorporation of labeled orotic acid and uridine into the nucleic acids of solid tumors in mice and cells in culture was determined as previously described (17). The amount of deoxyribonucleic acid was measured by the diphenylamine method (20) in hot trichloroacetic acid extracts. The decarboxylation of orotic acid-7-C¹⁴ by cell suspensions and extracts was determined according to an earlier procedure (13). The formation of nucleotides of uridine-5,6-H² and azauridine-2-C¹⁴ was measured by Dowex chromatography of the acid-soluble fraction obtained after the addition of cold perchloric acid to the incubation mixture (17). Orotidylic acid decarboxylase activity was measured spectrophotometrically (16). Protein was determined by the biuret method. All data quoted in this paper are the average values calculated from the results of two or more experiments.

RESULTS

Characterization of resistance.—The sensitivity to azauridine of both cell lines in culture is presented on Chart 1, from which it can be seen that for the resistant line, L5178AzUR, the concentration of the analog required for inhibition was approximately 1500 times that needed with the parent strain. As anticipated, in mice this line is completely resistant to the action of tolerated levels of azauridine. The prevention of the inhibitory activity of azauridine on cells in culture is accomplished by uridine but not uracil or orotic acid. It is important to note that the resistant line required more than 600 times as much uridine to prevent the inhibition of growth by azauridine as did the sensitive strain. It has been demonstrated that the azauridine-resistant line is equally resistant to azauridyl acid. This result is taken to indicate that the 5'-phosphate is cleaved before the compound enters the cell, since the sensitivity to azauridyl acid of orotidylic decarboxylase from the two types of cell was identical, as demonstrated below.

Studies with whole animals.—The uptake of labeled orotic acid and uridine by solid L5178Y tumors in mice, in the presence and absence of azauridine, has been studied previously (17). Such experiments were repeated with mice bearing L5178AzUR as well. The incorporation of orotic acid-4-C¹⁴ is the same in the sensitive and resistant tumors (Table 1). As might be expected, inhibition of this uptake by azauridine was observed only in the sensitive tumor. On the other hand, there was considerably less incorporation of uridine-5,6-H² into the resistant, as compared with the sensitive, lymphoma. Treatment with azauridine increased the concurrent utilization of uridine in both lines, presumably by a preferential restriction of pyrimidine nucleotide synthesis de novo. These results suggest that a decreased anabolism of uridine, and hence presumably of azauridine, plays a prominent role in the mechanism of resistance.

Incorporation studies in culture.—The possibility that azauridine was degraded to inactive metabolites such as azauracil (19) in the presence of resistant cells was made very unlikely by the demonstration that a concentration of 1 X 10⁻⁴ M azauridine in the medium remained unchanged during the reproduction of L5178AzUR for a period of 48 hours. To compare the pyrimidine incorporation by sensitive and resistant L5178Y cells, cultures were grown in the presence of labeled orotic acid or uridine, with and without the presence of azauridine. Little radioactivity was found in the cold acid-soluble fraction of any of the cultured cells. However, considerable incorporation of the uridine radioactivity into the nucleic acid fractions was observed.

It is apparent from the results in Table 2 that, under these conditions of growth, exogenous orotic acid is poorly utilized by the cells during normal growth. In contrast to orotic acid, the uptake of uridine by sensitive cells was rapid in
growing cultures, whereas the uptake by resistant cells was slight. In preliminary experiments with uridine-5,6-^H possessed a specific activity 100 times higher than that used in the experiments presented in Table 2, sufficient radioactivity (2.0 \times 10^6 \text{ counts/min/\mu g DNA}) was incorporated into the sensitive cells completely to halt cell division because of radiation damage. Under these same conditions the resistant line incorporated little uridine (2.5 \times 10^3 \text{ counts/min/\mu g DNA}) and grew at a normal rate.

Studies with resting cell suspensions.—Two types of experiments were performed. The first was designed to evaluate the capacity of the two cell lines to convert orotic acid to ribonucleotides of uracil by measuring the evolution of C^14O_2 from orotic acid-7-C^14 and to study the effect of added azauridine upon this reaction. By the procedure previously described (13), it was found that evolution of C^14O_2 by L5178AzUR cells was somewhat greater than that observed with L5178Y cells (Chart 2). However, inhibition of this reaction by azauridine in the resistant subline was not seen at low levels of the antimetabolite; in fact, a slight but consistent stimulation of this reaction was noted in four experiments.

In the second type of experiment, the total entry of uridine and azauridine was found to be

| TABLE 1 |
| INCORPORATION OF LABELED OROTIC ACID AND URIDINE INTO THE NUCLEIC ACIDS OF L5178Y AND L5178AZUR IN MICE |

<table>
<thead>
<tr>
<th>TUMOR</th>
<th>OROTIC ACID-4-C\textsuperscript{14} INJECTED (COUNTS/\text{MIN/MG OF DRIED DEFATTED TISSUE})</th>
<th>URIDINE-5,6-H\textsuperscript{3} INJECTED (COUNTS/\text{MIN/MG OF DRIED DEFATTED TISSUE})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>Assuridine-treated</td>
</tr>
<tr>
<td>L5178Y</td>
<td>29</td>
<td>7</td>
</tr>
<tr>
<td>L5178AzUR</td>
<td>28</td>
<td>24</td>
</tr>
</tbody>
</table>

Groups of mice (20–22 gm. each) were given injections subcutaneously of 1 \times 10^6 cells of L5178Y or L5178AzUR and after 7 days were given intraperitoneal injections of orotic acid-4-C\textsuperscript{14} (10 \text{ µ moles, } 1 \times 10^6 \text{ counts/min}) or uridine-5,6-H\textsuperscript{3} (10 \text{ µ moles, } 6 \times 10^6 \text{ counts/min}) and killed after 4 hours. Half the mice in each group had received an intraperitoneal injection of azauridine (10 \text{ µ moles}) 1 hour before administration of the isotopically labeled compound. Radioactivity in nucleic acids was determined as described under "Materials and Methods."

| TABLE 2 |
| INCORPORATION OF LABELED OROTIC ACID AND URIDINE INTO THE NUCLEIC ACIDS OF L5178Y AND L5178AZUR CELLS GROWING IN CULTURE |

<table>
<thead>
<tr>
<th>CELL LINE</th>
<th>RADIOACTIVE PRECURSOR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OROTIC ACID-4-C\textsuperscript{14} (COUNTS/\text{MIN/MG DNA})</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
</tr>
<tr>
<td>L5178Y</td>
<td>61</td>
</tr>
<tr>
<td>L5178AzUR</td>
<td>65</td>
</tr>
</tbody>
</table>

* It should be noted that the level of added radioactivity in the cultures containing labeled orotic acid was 200 times greater than that of the cultures containing labeled uridine.

Cell suspensions (4 \times 10^4 cells/ml) were incubated in the presence of orotic acid-4-C\textsuperscript{14} (1.0 \times 10^4 \text{ counts/min/ml, } 5 \times 10^{-4} \text{ M}) and uridine (5 \times 10^{-4} \text{ M}) or of uridine-5,6-H\textsuperscript{3} (60 \text{ counts/min/ml, } 5 \times 10^{-4} \text{ M}) and orotic acid (5 \times 10^{-4} \text{ M}) for 61 hours. Half the cultures also contained azauridine (5 \times 10^{-4} \text{ M}). Because of the uridine present in all flasks, the growth of the sensitive cells was not inhibited. The cultures were harvested when the cell count had reached 4.4 \times 10^6 cells/ml and were treated as described under "Materials and Methods."
considerably higher in the sensitive than in the resistant cells (Chart 3). Fractionation of the cells by cold perchloric acid and analysis of the resulting extracts by chromatography on Dowex-1 showed the difference to be the result mainly of a lower rate of nucleotide formation by the resistant line. Incubation for an additional 10 minutes did not alter the total uptake greatly, but in the case of uridine increased the percentage present in nucleic acids, at the expense of acid-soluble nucleotides. As reported previously (13, 17), azauridine was not metabolized to any detectable degree beyond the monophosphate level. It is important to note that the level of free azauridine found in the acid-soluble fraction of the resistant cells is approximately equal to that calculated for passive diffusion, and hence permeability is not a factor under these conditions. However, the diffusion of uridine appears to be much more rapid than that of azauridine and is attended by the extensive formation of phosphorylated derivatives as soon as uridine gains entry into the cell.

Studies with cell extracts.—Extracts of L5178Y cells grown in culture rapidly decarboxylated orotic acid-7-C\textsuperscript{14} when incubated in the fortified reaction mixture described earlier (17). A slight but significant increase in this reaction was observed in the rate of C\textsuperscript{14}O\textsubscript{2} production by the extracts of resistant cells (Table 3). The inhibition of the decarboxylation by azauridine, however, was lower in the resistant strain. When azauridylic acid was added instead of azauridine, this difference disappeared. Again, these results imply that the resistant extracts have a decreased capacity to convert azauridine to its 5'-phosphate but that the nucleotide itself is an equally effective inhibitor of the enzyme from both lines. This view was confirmed by the findings that (a) uridine was phosphorylated at a lower rate by extracts of resistant cells, as compared with extracts derived from sensitive cells (Chart 4), and (b) the conversion of azauridine to azauridylic acid was also depressed in extracts obtained from the resistant line (Chart 4). Finally, the amount of orotidylic acid decarboxylase activity found in an unfortified reaction mixture was approximately the same in the two lines, and azauridylic acid inhibited this enzyme to the same degree in extracts of both types of cell (Table 4).

### Chart 2
Liberation of C\textsuperscript{14}O\textsubscript{2} from orotic acid-7-C\textsuperscript{14} by suspensions of resting L5178Y and L5178AzUR cells. Cell suspensions (200 mg. wet weight) were incubated in Warburg flasks containing 2 ml. of growth medium in the presence of orotic acid-7-C\textsuperscript{14} (1 X 10\textsuperscript{6} counts per minute/ml, 1 X 10\textsuperscript{\text{-}6} M). Azauridine (5 X 10\textsuperscript{-4} M) was present in half of the flasks in each group. Liberated C\textsuperscript{14}O\textsubscript{2} was measured after 30 and 60 minutes, respectively (IS).

### Chart 3
Permeability and metabolism of labeled uridine and azauridine by suspensions of L5178Y and L5178AzUR cells. Suspensions of each cell line (approximately 70 mg. of wet weight of cells/ml) were incubated in growth medium containing uridine-5,6-H\textsuperscript{3} (4.7 X 10\textsuperscript{6} counts per minute/ml, 5 X 10\textsuperscript{-5} M) or azauridine-2-C\textsuperscript{14} (5.3 X 10\textsuperscript{6} counts per minute/ml, 5 X 10\textsuperscript{-5} M). Aliquots were removed after 0, 5, and 10 minutes' shaking in a Dubnoff incubator at 37° C. and centrifuged at 0° C. in hematocrit tubes. The packed cell to medium ratios of uridine and azauridine concentrations and the radioactivity in the various cell fractions was determined as described under "Materials and Methods." These ratios have not been corrected for the intercellular volume of the cell pack or the volume of the cells which is impermeable to water. Preliminary experiments (R. E. Handschumacher, unpublished) indicate that these minor corrections will not alter the conclusions significantly.
DISCUSSION
The results of experiments with L5178Y and L5178AzUR growing in mice (Table 1) or in cell culture (Table 2), and with cell suspensions (Charts 2 and 3) and cell extracts (Table 3 and Chart 4), all show one common feature, namely, a blockade in the anabolism of either uridine or azauridine in resistant cells. The failure to form significant amounts of azauridylic acid, the actual inhibitor of pyrimidine synthesis de novo, would explain the capacity of L5178AzUR to grow in the presence of high concentrations of azauridine. Interference with uridine metabolism, which may occur concomitantly at these very high levels of drug, is not detrimental to such cells, since their growth in culture depends upon pyrimidine synthesis de novo, through orotic acid, for the synthesis of uridylic acid. With respect to the very poor utilization of orotic acid by growing cell cultures, preliminary experiments with the leukemic cells in vitro have indicated that they are remarkably impermeable to orotic acid, a condition which may not obtain with cells growing in the animal.

The enzymatic site at which azauridine and uridine metabolism is depressed in resistant cells appears to be uridine phosphokinase, an enzyme which catalyzes the only step which is common to the anabolism of both azauridine and uridine in animals. Inhibition of subsequent reactions of uridylic acid is unlikely, because the synthesis of nucleic acids from orotic acid is unaltered in resistant cells (Tables 1 and 2). Alteration in the permeability of the resistant cell membrane to azauridine appears to be ruled out, since both free uridine and azauridine were present in the resistant cells in amounts close to that predicted on the basis of passive diffusion. Increased destruction of azauridylic acid is unlikely, since orotidylic acid decarboxylase was equally well inhibited by azauridylic acid in sensitive and resistant lines (Tables 3 and 4). The demonstration that azauridine added to the medium is stable in the presence of resistant cells rules out the possibility that catabolism of azauridine itself may be responsible for the resistance of L5178AzUR cells.

A strong parallel is to be seen in the mechanism of resistance observed with L5178 cells and that reported for Streptococcus faecalis, with which an impairment of uracil and uridine utilization also was observed (8). The situation is also analogous to one type of fluorouracil resistance in Ehrlich ascites cells which has been described by Reichard et al. (18). That report indicated that the

<table>
<thead>
<tr>
<th>CELL LINE</th>
<th>CONTROLS (COUNTS/MIN/MG PROTEIN)</th>
<th>INHIBITOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azauridine (COUNTS/MIN/MG PROTEIN)</td>
<td>Change (PER CENT)</td>
<td>Azauridine 5'-phosphate (COUNTS/MIN/MG PROTEIN)</td>
</tr>
<tr>
<td>L5178Y</td>
<td>38,000</td>
<td>14,500</td>
</tr>
<tr>
<td>L5178AzUR</td>
<td>60,000</td>
<td>42,000</td>
</tr>
</tbody>
</table>

Cell-free extracts of each cell line (0.9-1.0 mg. protein) were incubated with orotic acid-7-C14 (1 X 10^6 counts/min/ml, 1 X 10^-4 M) in Warburg flasks, with the 0.5 ml. of the incubation mixture previously described (17). Azauridine (5 X 10^-4M) or azauridine 5'-phosphate (1.6 X 10^-6 M) was present in the indicated flasks. Liberation of C14O2 was measured (13) after 30 minutes, in a final volume of 1.5 ml.
of inhibition of the decarboxylation of orotidylic acid by this level of azauridine (Chart 2). Another quantitative discrepancy is to be found in the studies with soluble extracts from cells. In these experiments there again was evidence for an approximately tenfold reduction in the phosphorylation of azauridine and uridine, whereas the conversion of orotic acid to uridine nucleotides, as measured by the release of radioactive CO₂, was inhibited by azauridine, after its conversion to azauridylic acid, only one-half as well in the resistant as in the sensitive cell extracts. This must not be interpreted too rigorously, since inhibition of the reaction by azauridine could not be expected until azauridylic acid had been formed.

These apparent difficulties may arise from lack of understanding of the dynamic effects that tenfold differences in enzyme activity may create in a growing cell and reflect the danger of rigorous interpolation between growing cells and isolated enzyme complexes.

A final quantitative contradiction is found in a comparison of the depression of uridine incorporation into cells growing in culture with that in the same line growing as a lymphoma in mice. This difference probably can be accounted for by the contamination of lymphomas with rapidly proliferating connective and adipose tissues which are unchanged in their capacity to utilize uridine. Considering the magnitude of resistance and the fact that it was achieved by what appeared to be a many-step process, it is perhaps unreasonable to assume that a change in the level of uridine phosphokinase is the only mechanism of resistance in this line; however, of the more obvious possibilities, this alone has received consistent experimental support.

The implications of the results described in this communication, with respect to chemotherapy with azauridine, are clear. If resistance to azauridine in a neoplasm is accompanied by a decrease in uridine utilization, then, uracil analogs such as fluorouracil, which depend on the occurrence of similar anabolic reactions before a chemotherapeutic action can be exerted, will be less effective than in the sensitive line. Fluorodeoxyuridine, on the other hand, may be active, since the phosphorylation of deoxyuridine does not appear to be catalyzed by uridine phosphokinase.¹ The lack of uridine incorporation by resistant tumors might conceivably be exploited for therapeutic results (22); for example, the effectiveness of a drug which interferes with the orotic acid pathway, for the synthesis of uridylic acid, should be increased when reversal of its action by the utilization of exogenous uracil or uridine is minimized.

TABLE 4

<table>
<thead>
<tr>
<th>SOURCE OF EXTRACT</th>
<th>RATE OF DECARBOXYLATION (MMoles/MIN/ML)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No inhibitor</td>
<td>1.1</td>
</tr>
<tr>
<td>Azauridine</td>
<td>0.2</td>
</tr>
<tr>
<td>Azauridine</td>
<td>0.2</td>
</tr>
</tbody>
</table>

The cell-free extract (1.0 mg. of protein) was incubated with tris(hydroxymethyl)amino methane (0.05 M, pH 8.0) and orotidylic acid (5 X 10⁻⁷ M) at 37°C in the medium previously described, in the presence of sufficient cell-free extract to give a final concentration of 0.2 mg of protein/ml. The radioactive products formed after 30 and 60 minutes were analyzed by Dowex chromatography (17).

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PASTERNAK et al.—Pyrimidine Metabolism in Azauridine-resistant Leukemia


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