Our findings therefore provide further experimental evidence for the reaction mechanism postulated by METZLER AND SNELL[1,8] for chemical transamination.

SUMMARY

Analytical investigation of chemical transamination reactions between α-keto or amino acids and phosphorylated and non-phosphorylated derivatives of vitamin B_6, has been carried out by means of chromatography and electrophoresis on filter paper. It has thus been possible to give evidence that in each reaction two strongly fluorescent intermediates are formed.

Two of these compounds have been isolated in appreciable amounts by means of electrophoresis on a cellulose column.

The spectra and chemical properties of the intermediate compounds indicate that they are Schiff-base metal chelates between pyridoxal or pyridoxal phosphate and amino acids and pyridoxamine or pyridoxamine phosphate and α-keto acids.

Evidence has, moreover, been obtained for the participation of these compounds in the transamination mechanism.

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Short Communications

Studies of bacterial resistance to 6-azauracil and its riboside*

It was previously reported that 6-azauracil (*as*-triazine-3,5-dione, AzU) is an effective inhibitor of the growth of a number of species of microorganisms and experimental neoplasms in mice[1,2,3,4]. However, in the microbial studies, resistant populations ultimately evolved in most experiments. The nature of this resistance has been investigated in *Streptococcus faecalis* (A.T.C.C. 8043) and less completely in *Escherichia coli* B and *Lactobacillus leichmannii* (A.T.C.C. 7830). When *S. faecalis* was grown in the presence of 6-azauracil-2-^{14}C** essentially no free AzU or nucleic acid-bound

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** This compound, *as*-triazine-3,5-dione-3-^{14}C, was prepared by Dr. P. K. CHANG in this department by a method to be submitted for publication.

AzU accumulated, but considerable amounts of radioactive metabolites appeared in the acid-soluble fraction of the cells. By growing large quantities of these cells in the presence of slightly inhibitory levels of AzU the major metabolite was isolated and has been characterized as azauracil riboside (AzUR)[5]. This compound appears to be identical with a small amount of a synthetically prepared ribofuranoside of AzU[5].

A strain of *S. faecalis* demonstrated to be resistant to 5 μmoles AzU/ml, was grown in the presence of 0.25 μmole AzUR/ml; ultimately a strain resistant to both AzU and AzUR was obtained. Inhibition and reversal studies on these strains are shown in Table I. As indicated, AzUR is a more potent inhibitor of *S. faecalis* than AzU. Also it is apparent that while uracil and uridine are about equally effective in overcoming the action of AzU, only uridine will nullify the inhibition produced by AzUR. The fact that AzU-resistant cells are inhibited by the riboside of AzU suggests a unique biochemical mechanism of circumventing resistance.

TABLE I

EFFECT OF AZAURACIL AND AZAURACIL RIBOSIDE ON THE GROWTH OF *S. faecalis*

	Turbidity, Klett units	
	Sensitive strain	*AzU-resistant strain*
AzU 0.0 μmole/ml		
+ Uracil 0.0 μmole/ml	115	115
AzU 0.25 μmole/ml		
+ Uracil 0.0 μmole/ml	18	105
+ Uracil 0.05 μmole/ml	68	115
+ Uracil 0.25 μmole/ml	115	115
+ Uridine 0.05 μmole/ml	47	114
+ Uridine 0.25 μmole/ml	108	115
AzUR 0.05 μmole/ml		
+ Uracil 0.0 μmole/ml	18	18
+ Uracil 0.05 μmole/ml	25	25
+ Uracil 0.25 μmole/ml	29	22
+ Uridine 0.05 μmole/ml	50	68
+ Uridine 0.25 μmole/ml	110	110

Duplicate tubes containing basal synthetic medium, thymine (0.05 μmole/ml) and the additions indicated above, were inoculated with washed cells to give a turbidity of 15 Klett units. Average turbidity values are reported after growth at 37° for 8 hours. Uracil and uridine had no significant effects on growth.

TABLE II

ACCUMULATION OF AZAURACIL METABOLITES IN THE ACID-SOLUBLE FRACTION OF GROWING *S. faecalis*

	Radioactivity (c.p.m./mg of bacteria)		
	Sensitive strain	*AzU-resistant strain*	*AzUR-resistant strain*
AzU-2-^{14}C	270,000	210	210
AzUR-2-^{14}C	234,000	—	177,000

Duplicate tubes containing basal synthetic medium, thymine (0.05 μmole/ml), uracil (0.05 μmole/ml) and the radioactive analogue (0.25 μmole/ml) were inoculated with washed cells to give a turbidity of 15 Klett units. After 8 hours at 37°, the cells were centrifuged, washed with saline, extracted with 5% TCA at 0° and aliquots plated for counting. Bacterial mass was calculated from protein determinations on an aliquot of the washed cells (1 mg protein = 7.0 mg of dry bacteria). Maximal incorporation was about 15% of the added radioactivity.

Some information on the mechanism of resistance has been obtained by following the incorporation of AzU-2-^{14}C and AzUR-2-^{14}C into the acid-soluble fraction and the incorporation of uracil-2-^{14}C and uridine-2-^{14}C into the nucleic acids of rapidly growing cells. As indicated in Table II, development of resistance to AzU is paralleled by an inability of the strain to convert the analogue to the riboside and other bound forms. Further, resistance to both AzU and AzUR does not limit the transport into the cell of AzUR which is found concentrated in the acid-soluble fraction. Conceivably, one of the other bound forms found in the acid-soluble fraction, perhaps a phosphorylated derivative, is the active inhibitor of growth. The characterization of these other compounds is now in progress. The relation of this pattern to uracil metabolism is seen in Table III.

TABLE III

INCORPORATION OF URACIL AND URIDINE INTO THE NUCLEIC ACIDS OF GROWING *S. faecalis*

	Radioactivity (c.p.m./μmole RNA-ribose)		
	Sensitive strain	*AzU-resistant strain*	*AzUR-resistant strain*
Uracil-2-^{14}C	17,000	294	130
Uridine-2-^{14}C	45,200	31,800	1,880*

Duplicate tubes containing basal synthetic medium, thymine (0.05 μmole/ml) and uracil or uridine (0.25 μmole/ml) were inoculated with washed cells to give a turbidity of 15 Klett units. After 8 hours at 37° the cells were centrifuged, washed with saline, and extracted with 5% TCA at 0°. The residues, after being washed with alcohol and ether, were extracted with hot 5% TCA to obtain nucleic acid pyrimidines. RNA-ribose was determined on an aliquot of the residues before hot TCA extraction. Maximal uracil incorporation was approximately 15%, while the incorporation of uridine approached 50% of the amount of radioactive material added.

* The degree of resistance encountered in the AzUR-resistant strain selected was less marked than that observed with the strain resistant to AzU, a circumstance attributable to the severe limitations on the concentrations of AzUR which were imposed by the supply.

Uracil incorporation into the nucleic acids is blocked in both the strain resistant to AzU and that resistant to AzUR. However, the incorporation of uridine in the AzU-resistant strain approached that observed in the sensitive strain, but was much reduced in the AzUR-resistant strain. Similar studies have been conducted with sensitive and AzU-resistant *E. coli* and *L. leichmannii* and confirm the observation that resistance to AzU is accompanied by a parallel loss in ability to convert AzU to the riboside and other metabolites and to incorporate uracil into the nucleic acids of these cells.

These data suggest that for AzU to inhibit microbial systems, a preliminary conversion to ribose-containing derivatives is necessary. A similar observation with mammalian cells (sarcoma 180) in tissue culture has been made by SCHINDLER AND WELCH[6]. Both biological and chemical preparation of larger quantities of the riboside are in progress; these should permit evaluations of this compound as a carcinostatic and antiviral agent *in vivo* and a more detailed study of the mechanism of action of AzU and its derivatives.

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