

BIOCHEMICAL EFFECTS AND METABOLIC TRANSFORMATIONS OF 5-AZACYTIDINE IN *Escherichia coli*

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5-Azacytidine (1- β -D-ribofuranosyl 5-azacytosine) is a new antimetabolite with distinctly antibacterial effects. Complete inhibition of growth of *Escherichia coli* brought about by $1.2 \cdot 10^{-6}M$ 5-azacytidine can be removed by simultaneous addition of uridine, cytidine or thymidine, but not of uracil or cytosine. At increased temperature 5-azacytidine is decomposed and loses its biological activity. In a bacterial medium and in an *E. coli* cell-free extract 5-azacytidine is readily metabolized whereby ribosyl N-formylbiuret and ribosyl biuret are formed as decomposition products of the unstable 5-azauridine, accompanied by 5-azauracil, N-formylbiuret, biuret and other unidentified substances. After pre-incubation 5-azacytidine with the cell-free extract of *E. coli* brings about a slight inhibition of uridine phosphorolysis and a slight inhibition of cytosine deaminase activity. The mechanism of the striking inhibitory effect of 5-azacytidine is discussed in view of the incorporation of 5-azacytidine into nucleic acids.

Attempts to elucidate the mechanism of biological action of synthetic analogues of nucleic acid precursors have provided a number of fundamental data. At this Institute the synthesis and biological effects of the aza-analogues of nucleic acid pyrimidine precursors have been investigated for a number of years^{1,2}. 5-Azacytidine, a new antimetabolite of nucleic acid biosynthesis was synthesized here recently³ and was found to possess marked antibacterial and cancerostatic properties⁴. The study of cancerostatic effects of 5-azacytidine revealed that it has a specific effect on the lymphatic and hematopoietic systems causing a pronounced decrease in the number of circulating lymphocytes and of mature myeloid cells of bone marrow⁵.

The present communication is intended to contribute to the understanding of the inhibitory effect of 5-azacytidine using a microbial system. The growth of *E. coli* in a simple mineral medium and the possibility to study the antagonism between the inhibitor and the natural precursors of nucleic acids should yield fundamental information about the nature of the inhibitory effect of the substance in question.

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Experimental

Reagents. 5-Azauracil, 5-azacytosine, N-formylbiuret, 5-azauridine³ (this designation is used here in spite of the fact that the substance does not possess the usual nucleosidic structure), 5-azacytidine³ and 5-deoxyazacytidine⁶ were prepared at this Institute. α -D-Ribose 1-phosphate and 2-deoxy- α -D-ribose 1-phosphate were obtained from Calbiochem. Ribosyl N-formylbiuret and ribosyl biuret were prepared enzymatically from 5-azauracil and ribose 1-phosphate as described earlier⁷. The pyrimidine precursors of nucleic acids were of analytical purity. 5-Azacytidine-4-¹⁴C (1 mC/mmmole) was prepared by Dr J. Morávek, uracil-2-¹⁴C (9.9 mC/mmmole), uridine-G-¹⁴C (6.3 mC/mmmole), cytidine-G-¹⁴C (1.4 mC/mmmole) and cytosine-2-¹⁴C (3 mC/mmmole) were obtained from the Institute for Research, Production and Application of Radioactive Isotopes in Prague, thymine-2-¹⁴C (9.5 mC/mmmole) was from Amersham.

Cultivation of *E. coli* and preparation of the cell-free extract. As a test of growth inhibition the growth of a bacterial culture was estimated after 16 h of cultivation at 37°C. The growth took place in 10 ml synthetic medium containing glucose⁸ in test-tubes provided with Kapsenberg stoppers; inoculation was carried out with a single drop of a 24-h-old *E. coli* culture. When the reversal of inhibition was estimated the inhibitors and precursors of nucleic acids were added to the medium immediately before inoculation. The cell-free extract was prepared from a culture aerated for 8 h at 37°C in 5 l synthetic medium. After centrifugation and washing the bacteria were crushed in a cooled (−40°C) bacterial press⁹. The crushed cells were suspended in 50 ml 0.1M Tris-HCl buffer of pH 7.4 and the cell debris was centrifuged (12000 g, 20 min, 3°C). The supernatant was divided in small lots and kept at −15°C, the protein content was determined according to Lowry and co-workers¹⁰.

Determination of uridine phosphorylase, thymidine phosphorylase and cytosine deaminase activities. Incubation was carried out at 37°C in $2 \cdot 10^{-2}$ M Tris-HCl buffer of pH 7.4 in a total volume of 1 ml. The cell-free extract of *E. coli* was added in 0.1–0.3 ml amounts. Uracil, uridine, thymine or thymidine concentrations were $2 \cdot 10^{-4}$ M, that of cytosine $2.4 \cdot 10^{-4}$ M, those of magnesium chloride, ribose 1-phosphate and deoxyribose 1-phosphate $4 \cdot 10^{-4}$ M. The antimetabolites were added in the solution to a final concentration of $1.4 \cdot 10^{-3}$ M after adjusting the pH to 7.0. After definitive time intervals 0.1–0.2 ml samples of the incubation mixture were placed on Whatman No 1 paper without deproteinizing. Chromatography took place in mixtures of n-butanol–acetic acid–water (10 : 1 : 3), isobutyric acid–water–ammonia (66 : 33 : 1.5) or ethyl acetate–water–formic acid (60 : 35 : 5).

Assay of metabolic transformations of 5-azacytidine. Incubation took place in $2 \cdot 10^{-2}$ M Tris-HCl buffer or in 10^{-1} M phosphate buffer of pH 7.4 at 37°C; 5-azacytidine concentration was selected within the range of 10^{-4} – 10^{-3} M. For the investigation of *metabolic changes* of 5-azacytidine a cell-free extract of *E. coli* was used and incubation lasted 5–60 min. For the identification of substances formed during incubation of 5-azacytidine their instability in alkaline solution was made use of. In this case substances were formed that had been identified earlier during the investigation of metabolic transformation of 5-azauridine⁷. For this reason the chromatographically isolated compounds were heated for 5–30 min in a boiling-water bath in 1N-NH₄OH. In order to determine the effects of substances that are formed by *spontaneous decomposition* of 5-azacytidine 5-azacytidine was aseptically incubated 24 h in Tris-HCl buffer and subsequently treated with a cell-free extract from *E. coli*.

Determination of radioactivity. The position of radioactive compounds on chromatograms was determined from an automatic record (Friessecke-Hoepfner) of samples analyzed in parallel. Radioactive zones were eluted with water onto planchets and their radioactivity measured after drying in a methane-flow proportional 2π scaler in an infinitely thin layer. Radioactivity of whole cells was measured on membrane filters. Bacteria from 2 ml medium were washed with 5 ml 5% trichloroacetic acid, twice with 10 ml distilled water and the filters dried for 10 min at 100°C.

Results

CHARACTER OF INHIBITION OF *E. coli* GROWTH BY 5-AZACYTIDINE

In order to obtain some basic information about the efficiency of the new synthetic analogues of nucleic acid pyrimidine precursors the inhibition of *E. coli* growth was measured. The inhibitory effect of new and some previously tested^{1,2} aza-analogues of uracil and cytosine is shown in Table I. It appears that with 6-azapyrimidines the bases are more effective than the corresponding ribonucleosides. Similarly, 5-aza-uracil is a more potent inhibitor of growth than the unstable 5-azauridine⁷. A surprising exception is formed here by 5-azacytidine which shows the highest bacteriostatic effects of all the substances tested and for *E. coli* it thus represents the most powerful synthetic anomalous pyrimidine ribonucleoside inhibitor. The nature of *E. coli* growth inhibition by 5-azacytidine and 5-deoxyazacytidine may be seen in Fig. 1. 5-Azacytidine is about 20 times more powerful in inhibiting growth than is 5-deoxyazacytidine. Full inhibition of growth ensues in the presence of $1.2 \cdot 10^{-6}$ M 5-azacytidine or $2.5 \cdot 10^{-5}$ M 5-deoxyazacytidine.

In view of the instability of 5-azauridine⁷ it appeared useful first to determine the stability of 5-azacytidine. The stability of 5-azacytidine was evaluated on the basis of inhibition of *E. coli* growth. The results shown in Fig. 2 reveal how the biological effect of 5-azacytidine depends on temperature and on the length of previous incubation in a bacterial medium. It follows from the character of the curves that 5-azacytidine is readily decomposed. The decomposition proceeds readily at the boiling temperature but at low temperature a solution of 5-azauracil is relatively stable. It appears likely that in common with the situation with 5-azauridine⁷ the 5-azapyrimidine ring of 5-azacytidine is spontaneously opened and the biological activity lost.

E. coli growth inhibition due to 5-azacytidine can be suppressed by simultaneous addition of uridine, cytidine or deoxyuridine and deoxycytidine and by higher concentrations of thymidine. Uracil and cytosine do not remove the inhibition due

Table I
Antibacterial Effects of some Azapyrimidines in *E. coli*

Antimetabolite	50% Growth Inhibition		Antimetabolite	50% Growth Inhibition	
	μg/ml	M		μg/ml	M
5-Azauracil	3	$2.6 \cdot 10^{-5}$	5-Azauridine	250	$1.0 \cdot 10^{-3}$
5-Azacytosine	280	$2.6 \cdot 10^{-3}$	5-Azacytidine	0.06	$2.4 \cdot 10^{-7}$
6-Azauracil	7	$6.2 \cdot 10^{-5}$	6-Azauridine	1300	$5.3 \cdot 10^{-3}$
6-Azacytosine	14	$1.2 \cdot 10^{-4}$	6-Azacytidine	520	$2.1 \cdot 10^{-3}$

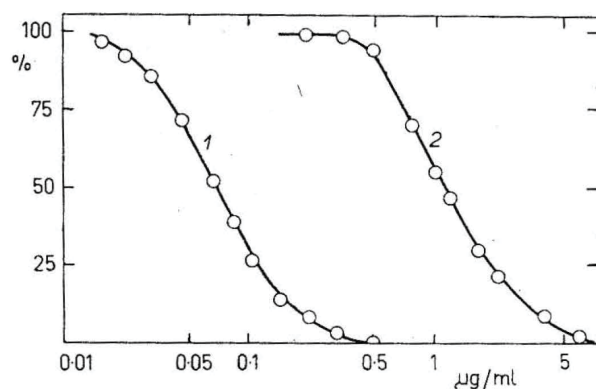


Fig. 1

Inhibition of *E. coli* Growth by 5-Azacytidine (1) and 5-Deoxyazacytidine (2)
Growth is expressed as % of uninhibited culture.

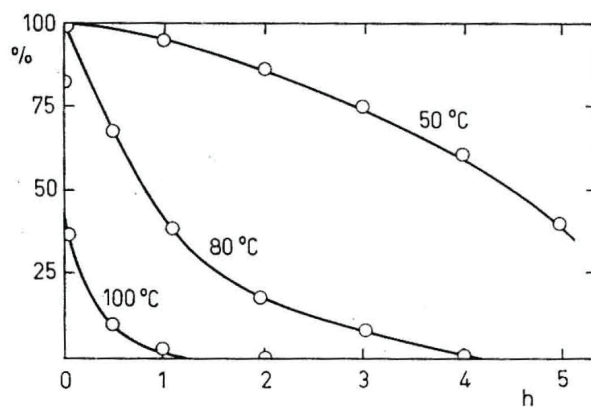


Fig. 2

Decrease in Biological Activity of 5-Azacytidine after Pre-Incubation at Higher Temperature

100 µg 5-azacytidine/ml heated aseptically in the synthetic medium at pH 7.2. In 60 min intervals samples were withdrawn and after dilution to 1 µg/ml growth inhibition assayed.

Table II

Antagonism between 5-Azacytidine and the Pyrimidine Precursors of Nucleic Acids
Cultivation of *E. coli* for 16 h at 37°C. Concentration of 5-azacytidine 1 µg/ml.

Pyrimidine Precursor	Culture Growth, %			
	5-azacytidine : pyrimidine precursor			
	1 : 100	1 : 10	1 : 1	1 : 0.1
Uracil	18	18	10	0
Uridine	100	100	55	6
Deoxyuridine	100	73	44	0
Cytosine	11	8	5	0
Cytidine	100	100	55	4
Deoxycytidine	100	55	22	0
Thymidine	91	49	3	0

to 5-azacytidine even at high concentrations (Table II). This finding is somewhat surprising since inhibition of *E. coli* growth brought about by any of the azapyrimidines shown in Table I with the exception of 5-azacytosine¹¹ can be suppressed by an addition of uracil and cytosine. Thymine and purine precursors of nucleic acids were always without effect. The inhibitory effects of 5-deoxyazacytidine can be removed by adding both uridine and cytidine, or uracil and cytosine.

In the presence of $4 \cdot 10^{-7} \text{ M}$ 5-azacytidine *E. coli* cells display pronounced morphological changes manifested by the formation of filamentous forms. At lower concentrations of the antimetabolite ($1.6 \cdot 10^{-7} \text{ M}$) the filamentous forms of cells do not occur although growth is inhibited.

On simultaneously adding uridine or cytidine (final concentration $4 \cdot 10^{-5} \text{M}$) to a culture heavily inhibited by 5-azacytidine ($4 \cdot 10^{-6} \text{M}$) no formation of filamentous cell forms was observed. An addition of uracil or cytosine did not prevent the formation of the filamentous forms. 5-Deoxyazacytidine did not cause any filamentous forms to be produced even at the concentration of $4 \cdot 10^{-5} \text{M}$.

Possibilities to enhance the inhibitory effects of 5-azacytidine were also considered. On applying simultaneously 6-azauracil or 6-azauridine with N-formylbiuret which has an inhibitory effect during the first stages of formation of nucleic acid pyrimidine precursors¹² a marked potentiation of the biological activity of both antimetabolites was observed¹³. According to our unpublished findings biological activity is also increased on combining N-formylbiuret with 6-azacytosine and 6-azacytidine. By measuring the inhibition of *E. coli* growth it was found that the combination of N-formylbiuret with 5-azacytidine (and similarly with 5-azauracil or 5-azacytosine) does not result in any potentiation of inhibitory effects. On applying simultaneously 5-azacytidine and 6-azauracil no increase in the inhibitory activity of either of the antimetabolites could be observed.

In addition to 5-azacytidine and 5-deoxyazacytidine 1- β -D-glucopyranosyl 5-azacytosine and 1- β -D-ribosepyranosyl 5-azacytosine were synthesized³. Neither of the substances inhibits *E. coli* growth at a concentration of 1 mg/ml.

TRANSFORMATIONS OF 5-AZACYTIDINE IN THE COURSE OF CULTIVATION

When the pronounced antibacterial activity of 5-azacytidine was established it appeared to be of interest to find out by what mechanism the growth of *E. coli* is inhibited. During the first phase the changes were investigated which 5-azacytidine undergoes during cultivation. The medium after inhibition with 5-azacytidine was analyzed and it was found that even during the first minutes after addition 5-azacytidine-4-¹⁴C undergoes metabolic changes. The medium was found to contain new radioactive substances which were analyzed in analogy with the degradation products of 5-azauridine⁷, and ribosyl biuret, 5-azauracil and biuret were found among the products. After 30 min of cultivation no 5-azacytidine (10^{-4}M) can be detected in the medium. Fig. 3 shows the level of 5-azauracil found in the medium of *E. coli* during cultivation in the presence of 5-azacytidine.

It was further analyzed what changes 5-azacytidine undergoes in *E. coli* cells. To this end 5-azacytidine-4-¹⁴C was added to a final concentration of 10^{-4}M to an aerated culture at the beginning of the logarithmic phase of growth and samples were withdrawn after definite time intervals to be filtered through membrane filters and their radioactivity determined. In order to remove the nucleotide pool cells on the filters were washed with ice-cold 5% trichloroacetic acid and distilled water. It follows from Fig. 4 that within a short interval after adding 5-azacytidine-4-¹⁴C a marked rise of intracellular radioactivity can be observed. A simultaneous addition of cytidine and 5-azacytidine-4-¹⁴C (10 : 1) resulted in a removal of growth inhibition and in a 4.7-fold decrease of intracellular radioactivity.

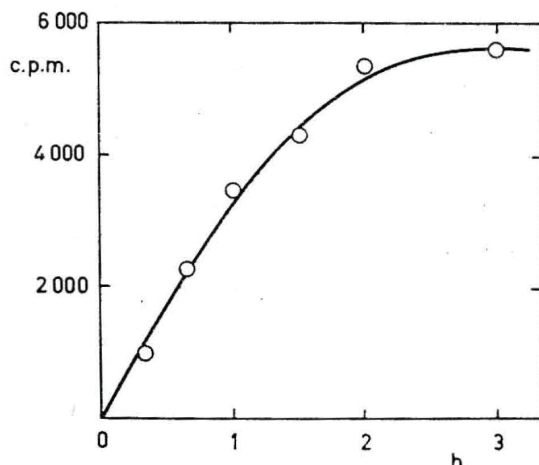


Fig. 3

Level of 5-Azauracil in *E. coli* Medium During Cultivation with 5-Azacytidine
Cultivation of *E. coli* under aeration at 37°C, 5-azacytidine (10^{-4} M, 0.2 mC/ml) added at the beginning of the logarithmic phase of growth. 0.1 ml medium samples were analyzed.

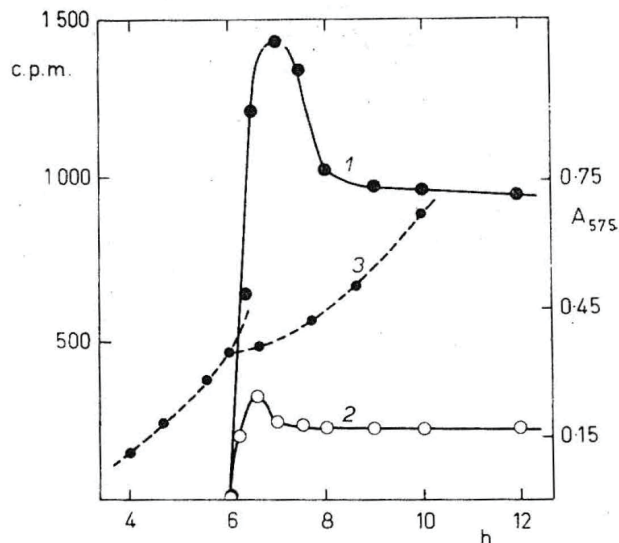


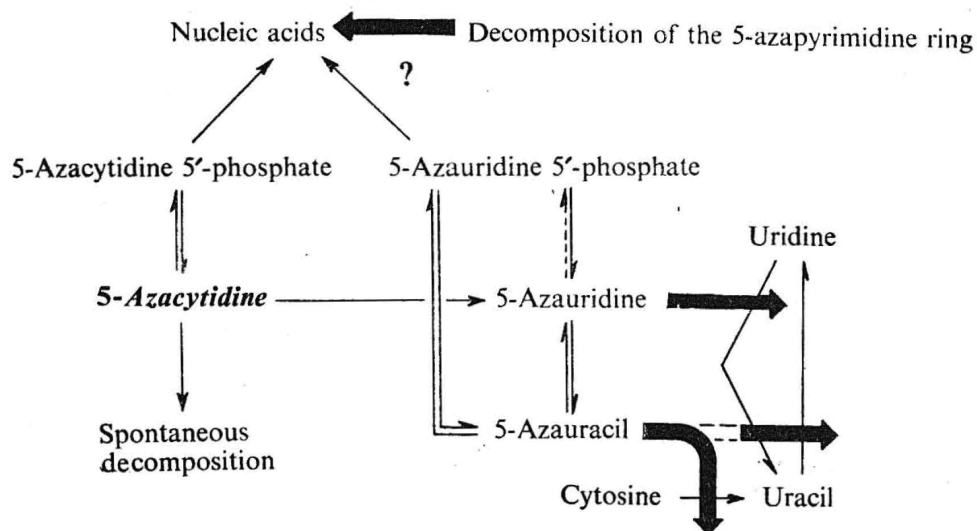
Fig. 4

Radioactivity of *E. coli* Cells during Cultivation with 5-Azacytidine-4- ^{14} C
5-Azacytidine-4- ^{14} C ($6.2 \cdot 10^4$ counts/min/ml) added after 6 h of growth to an aerated culture at 37°C. 1 Radioactivity of cells in 2 ml medium in the presence of 10^{-4} M-5-azacytidine, 2 in the presence of 10^{-4} M 5-azacytidine + 10^{-3} M cytidine, 3 growth curve in the presence of 10^{-4} M 5-azacytidine.

It appeared useful to determine what part of total radioactivity contained in the low-molecular fraction of inhibited cells is due to 5-azacytidine. Cells were extracted after different periods of growth in the presence of 5-azacytidine with 0.2N-HClO₄ under cooling with ice. Chromatographic analysis of the extracts obtained failed to detect 5-azacytidine after 1 h after beginning of growth of *E. coli* with 5-azacytidine. It did reveal a greater amount of ribosyl biuret, traces of ribosyl N-formylbiuret and of a substance which appears to be chromatographically identical with 5-azacytidine 5'-phosphate prepared enzymatically in a cell-free extract from mouse liver using 5-azacytidine and adenosine 5'-triphosphate¹⁴.

METABOLIC CHANGES OF 5-AZACYTIDINE IN A CELL-FREE EXTRACT OF *E. coli*

In order further to elucidate the transformations of 5-azacytidine taking place in *E. coli* cells during cultivation a cell-free extract was used. In view of the fact that 5-azacytidine undergoes spontaneous decomposition it was necessary to consider this factor together with metabolic transformations of 5-azacytidine. It was found in orientation experiments that substances formed by spontaneous decomposition of 5-azacytidine do not undergo any further metabolic changes and do not possess antibacterial effects toward *E. coli*.



In investigating the anabolic changes of 5-azacytidine it was established at first whether 5-azacytidine is phosphorylated. 5-Azacytidine 5'-phosphate could not be detected in a cell-free extract from *E. coli* with certainty. It was found that under the incubation conditions 5-azacytidine is rapidly metabolized. The incubation mixture was found to contain ribosyl N-formylbiuret and ribosyl biuret formed through decomposition of unstable 5-azauridine⁷, and 5-azauracil. Changes in the amounts of these substances in the course of incubation are apparent from Table III.

Relationships between the individual metabolites formed in the course of incubation from 5-azacytidine are rather complex. The transiently formed 5-azauridine can

Table III

Time Dependence of Metabolic Transformations of 5-Azacytidine in a Cell-Free Extract of *E. coli*

Incubated in $2 \cdot 10^{-2}$ M Tris-HCl buffer of pH 7.4, 1.3 mg protein, 10^{-3} M 5-azacytidine-4- ^{14} C ($6.65 \cdot 10^5$ counts/min/ml). Values obtained after chromatographic separation of 0.1 ml sample of incubation mixture.

Duration of Incubation min	5-Azacytidine + 5-Azauridine counts/min	Ribosyl N-Formylbiuret counts/min	Ribosyl Biuret counts/min	5-Azauracil counts/min
5	58 120	4 876	4 380	1 100
10	49 960	5 464	8 020	2 380
15	41 750	7 010	11 600	3 860
20	34 640	10 115	13 125	4 845
30	28 260	12 300	18 600	7 660
45	20 750	14 500	21 720	8 520
60	15 000	17 515	22 700	9 805

exist during the first phase both as an usual ribonucleoside and in the cyclized form³. It is not known whether one of the 5-azauridine forms is preferentially decomposed⁷ while the other more readily undergoes enzymatic deribosidization.

INHIBITION OF SOME ENZYMATIC SYSTEMS IN THE PRESENCE OF 5-AZACYTIDINE

Due to metabolic transformations of 5-azacytidine both in *E. coli* cells *in vivo* and in the cell-free extract *in vitro* new biologically active substances are formed (*e.g.* 5-azauracil^{11,15}, N-formylbiuret¹³). For this reason an application of 5-azacytidine can be expected to result in a polyvalent inhibitory effect. In view of structural similarities between 5-azacytidine and cytidine we took up a study of cytidine deaminase activity. However, in the cell-free extract of *E. coli* the metabolic changes of cytidine are not affected by the presence of 5-azacytidine.

Significant changes can be observed in the activity of uridine phosphorylase. While the synthesis of uridine from uracil and ribose 1-phosphate is not affected a phosphorolytic cleavage of uridine is diminished in the presence of 5-azacytidine (Table IV). Similarly the phosphorolysis of thymidine is slightly diminished in the presence of 5-azacytidine (by 16%). In analogy to the synthesis of uridine from uracil and ribose 1-phosphate an increased level of thymidine (and of β -thymine riboside) formed by the reaction of thymine with deoxyribose 1-phosphate can be found in the presence of 5-azacytidine.

In view of the metabolic changes of 5-azacytidine it appeared to be of interest in what way the inhibition of uridine phosphorylase will change with proceeding incubation. It follows from Fig. 5A that during short-term incubation (up to 5 min) no inhibition of uridine phosphorylase is apparent in the presence of 5-azacytidine.

Table IV

Inhibition of Uridine Phosphorolysis in the Presence of 5-Azacytidine

Incubation for 20 min at 37°C in $2 \cdot 10^{-2}$ M Tris-HCl buffer of pH 7.4, 1.8 mg protein. $2 \cdot 10^{-4}$ M uracil-2-¹⁴C ($1.1 \cdot 10^5$ counts/min/ml) or uridine-G-¹⁴C ($5.6 \cdot 10^4$ counts/min/ml); $4 \cdot 10^{-4}$ M ribose 1-phosphate and MgCl₂. Analysis of 0.2 ml incubation mixture.

5-Azacytidine M	Uracil counts/min	Uridine counts/min	Reacted Substrate %	Inhibition %
Synthesis of uridine				
—	10 470	11 560	52.3	—
10^{-3}	9 210	12 300	57.2 ^a	0
Phosphorolysis of uridine				
—	5 180	5 920	46.6	—
10^{-3}	4 090	7 310	35.9	23

^a Uridine level is by 9% higher than in the incubation mixture without 5-azacytidine.

For this reason it was assumed that the actual inhibitor is not 5-azacytidine but rather the transiently formed 5-azauridine. In order to verify this view the cell-free extract was pre-incubated with 5-azacytidine before estimating the uridine phosphorylase inhibition. It follows from the results obtained (Fig. 6) that the inhibition of

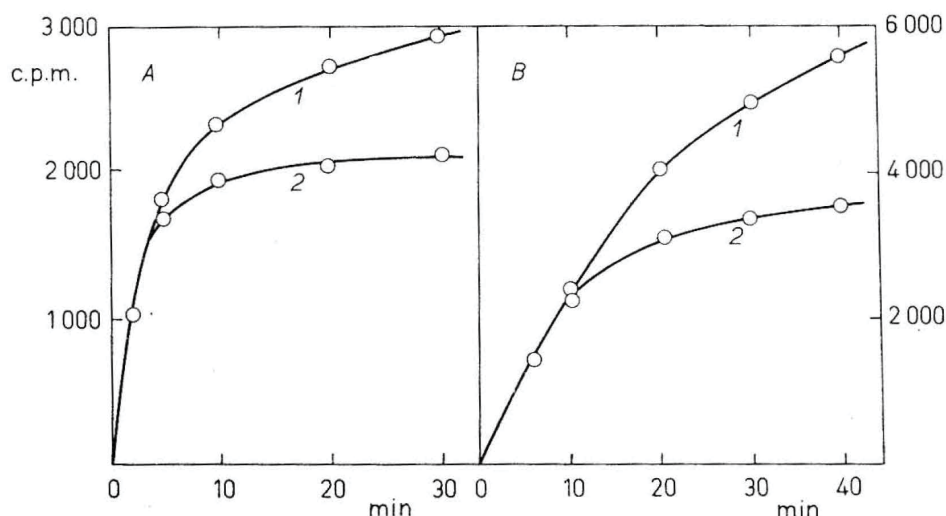


Fig. 5

Inhibition of some Enzymatic Systems in a Cell-Free Extract of *E. coli* during Incubation with 5-Azacytidine

A Activity of uridine phosphorylase: $2 \cdot 10^{-4}$ M uridine-G- ^{14} C ($4 \cdot 3 \cdot 10^4$ counts/min/ml) 2.0 mg protein. B Activity of cytosine deaminase: $2 \cdot 10^{-4}$ M uracil-2- ^{14} C ($3 \cdot 2 \cdot 10^4$ counts/min/ml), 1.8 mg protein. Incubation in $2 \cdot 10^{-2}$ M Tris-HCl buffer of pH 7.4 at 37°C. 1 Without inhibitor, 2 with 10^{-3} M 5-azacytidine.

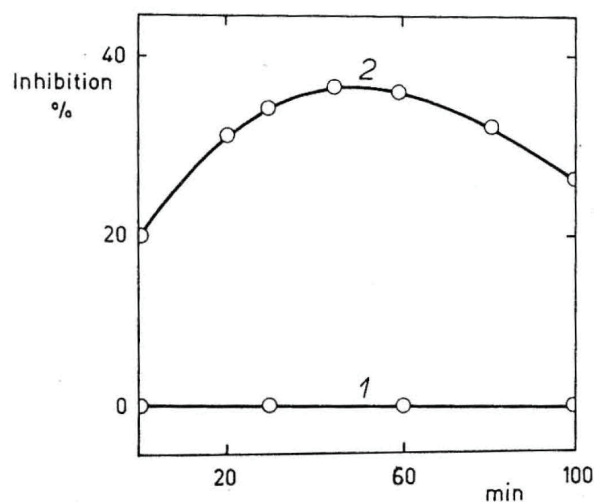


Fig. 6

Dependence of the Inhibition of *E. coli* Uridine Phosphorylase on the Length of Pre-Incubation of a Cell-free Extract with 5-Azacytidine

Incubated for 20 min at 37°C in $2 \cdot 10^{-2}$ M Tris-HCl buffer of pH 7.4, $2 \cdot 10^{-4}$ uridine-G- ^{14} C ($2 \cdot 8 \cdot 10^4$ counts/min/ml). 1 Activity of uridine phosphorylase after pre-incubation of the cell-free extract without 5-azacytidine (52% phosphorolysis of uridine to uracil), 2 after pre-incubation with 10^{-3} M 5-azacytidine.

uridine phosphorylase rises somewhat for a certain period of 5-azacytidine pre-incubation. This situation persists probably until the deamination of 5-azacytidine is overcompensated by spontaneous cleavage of the 5-azauridine formed which results in a decreased inhibition of uridine phosphorylase activity. Pre-incubation of the cell-free extract without 5-azacytidine does not affect the activity of uridine phosphorylase.

During phosphorolysis of uridine in the presence of 5-azacytidine in a phosphate buffer no inhibition of uridine phosphorylase activity takes place, in contrast with the application of Tris-HCl buffer. This phenomenon is probably connected with a different type of metabolic changes of 5-azacytidine in the two buffers.

Table V

Metabolic Transformations of Cytosine in the Presence of 5-Azacytidine
Incubated for 20 min at 37°C in $2 \cdot 10^{-2} \text{M}$ Tris-HCl buffer of pH 7.4, 2.0 mg protein, $4 \cdot 10^{-4} \text{M}$ cytosine-2- ^{14}C ($9.5 \cdot 10^4$ counts/min/ml). Analysis of 0.2 ml incubation mixture.

5-Azacytidine M	Cytosine counts/min	Uracil counts/min	Uridine counts/min	Uridine % Total Radioactivity	Deamination of Cytosine %
—	518	17 850	1 045	5.3	97.3
$1 \cdot 10^{-3}$	1 425	14 370	3 540	18.3	92.7
$2 \cdot 10^{-3}$	1 885	12 220	5 550	28.2	90.4
$4 \cdot 10^{-3}$	2 220	10 690	6 460	33.4	88.6

In the presence of 5-azacytidine a decreased formation of uracil from cytosine was observed. It follows from Fig. 5B that cytosine deaminase activity is lowered in the presence of 5-azacytidine similarly as with uridine phosphorylase only after a longer period of incubation with 5-azacytidine (10 min). It was observed before that cytosine deaminase is markedly inhibited by 5-azauracil and 5-azacytosine¹¹. It is assumed that the inhibition of cytosine deaminase observed in the presence of 5-azacytidine is due to 5-azauracil formed from 5-azacytidine during incubation. Metabolic changes of cytosine in the presence of increasing concentrations of 5-azacytidine are apparent from data in Table V.

Discussion

5-Azacytidine is a new antimetabolite with pronounced antibacterial and cancerostatic effects^{4,5}. The mechanism of the antibacterial action of 5-azacytidine is polyvalent due to spontaneous and enzymatic transformations of the compound. 5-Azauridine formed by deamination of 5-azacytidine is readily further transformed to the biologically active 5-azauracil^{13,15}. The main inhibitory effect of 5-azacytidine does not consist in its metabolic transformation to 5-azauridine or 5-azauracil even if a

certain analogy exists between the mentioned 5-azapyrimidines. A substantial part of the overall biological activity of 5-azacytidine may be due to higher anabolites of 5-azacytidine or its incorporation into nucleic acids.

With regard to the site of inhibition by 5-azacytidine it is of interest that the inhibition of *E. coli* growth brought about by 5-azacytidine can be removed by simultaneous application of the antimetabolite with uridine or cytidine but not with uracil or cytosine. Analogously, the formation of filamentous cell forms in the presence of 5-azacytidine can be prevented by simultaneous addition of uridine or cytidine but not of uracil or cytosine. It appears likely that the differences in the ability to remove the inhibitory effects of 5-azacytidine depend on the rate with which the individual pyrimidine bases or nucleosides can be utilized for nucleic acid synthesis.

The inhibitory effect that can be expected in *E. coli* on applying 5-azacytidine can be schematically represented as follows. 5-Azaauridine and 5-azaauracil formed from 5-azacytidine slight inhibit uridine phosphorylase, 5-azaauracil blocks the activity of cytosine deaminase. For the sake of explaining the pronounced inhibitory effect of 5-azacytidine it is assumed that during the first minutes after addition of 5-azacytidine *E. coli* cells effect a deamination as well as a phosphorylation of 5-azacytidine to 5-azacytidine 5'-triphosphate which is in common with animal systems¹⁴ incorporated into nucleic acids. The main inhibitory effect of 5-azacytidine apparently consists in the spontaneous decomposition of the 5-azapyrimidine ring incorporated in a nucleic acid molecule. Spontaneous decomposition of the 5-azapyrimidine ring was demonstrated for the first time with 5-azaauracil¹³ and later at the nucleoside or nucleotide level with 5-azaauridine or 5-azaauridine 5'-phosphate⁷.

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Резюме

А. Чигак и Ф. Шорм: Биохимическое действие и метаболические превращения 5-азацитидина у *Escherichia coli*. 5-Азацитидин (1- β -D-рибофуранозил-5-азацитозин) — новый анти-метаболит, обладающий выразительным антибактериальным действием. Полное ингибирование роста *Escherichia coli*, вызываемое 5-азацитидином в концентрации $1,2 \cdot 10^{-6}$ М, возможно устранить одновременной добавкой уридина, цитидина, или тогда как урацил или цитозин не оказывают никакого влияния. При повышенной температуре 5-азацитидин разлагается и теряет биологическую активность. В питательной среде бактерий и в бесклеточном экстракте *E. coli* 5-азацитидин легко дезаминируется, причем образуются рибозил-N-формилбиурет и рибозилбиурет в качестве продуктов разложения неустойчивого 5-азауридина; далее получают 5-азаурацил, N-формилбиурет, биурет и дальнейшие неидентифицированные вещества. После предварительного инкубирования с 5-азацитидином в бесклеточном экстракте из *E. coli* имеют место ингибирование фосфоролиза уридина и тимидина и слабое ингибирование цитозиндеаминазной активности. Обсужден механизм выразительного тормозящего действия 5-азацитидина с точки зрения возможного включения 5-азацитидина в нуклеиновые кислоты.