INFLUENCE OF DIOXIDINE ON THE CONTENT OF PROTEIN AND NUCLEIC ACIDS IN *Staphylococcus aureus*

I. N. Degtyarev

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The method of synthesis of dioxidine (1,4-di-N-hydroxy-2,3-dihydroxymethylquinoxaline) was developed in our institute [1].

The biological activity of dioxidine was first studied on 16 species of bacteria in experiments *in vitro* and on models of experimental acute bacterial infections of white mice [2]. On the basis of our investigations, followed by a clinical study, dioxidine has been recommended for the treatment of acute bacterial infections.

The preparation possesses a broad spectrum of antibacterial action and is active with respect to bacteria resistant to antibiotics and sulfanilamides.

The mechanisms of the action of preparations of the quinoxaline series on the bacterial cells are obscure. The purpose of this work was to study the influence of dioxidine on the nucleic acid and protein content in *Staphylococcus aureus*.

For the experiments we used 24-, 48-, and 72-h-old cultures of *Staphylococcus aureus* (Zhaev strain), which were grown on meat–peptone broth, pH 7.5, at 36°. Dioxidine was added to the meat–peptone broth in bacteriostatic concentrations of 0.01 and 0.05%.

For a study of the influence of dioxidine on the protein and nucleic acid content in various fractions of the *Staphylococcus* culture, we conducted a fractionation including the following steps.

The *Staphylococcus* culture was centrifuged at 4000 rpm for 40 min. The precipitate of bacterial cells was washed twice with physiological solution, then with Tris–HCl buffer (pH 7.5), and triturated several times with liquid nitrogen to break down the cell walls. The homogenate obtained was centrifuged at 12,000 rpm for 40 min on an MSE centrifuge. The supernatant obtained was a cytoplasmic extract.

The protein concentration was determined by the Lowry method [3] and the nucleic acid concentration by the Spirin method [4] in the suspension, centrifugate, and intracellular

TABLE 1. Influence of Dioxidine on the Protein and Nucleic Acid Content in *Staphylococcus aureus* (in comparison with control without dioxidine)

Dioxidine concentration, %	Suspension		Centrifugate		Intracellular extract	
	protein	ΣDNA + RNA	protein	ΣDNA + RNA	protein	ΣDNA + RNA
0,01	96	94	92	76	79	54
0,05	89	62	88	44	55	23

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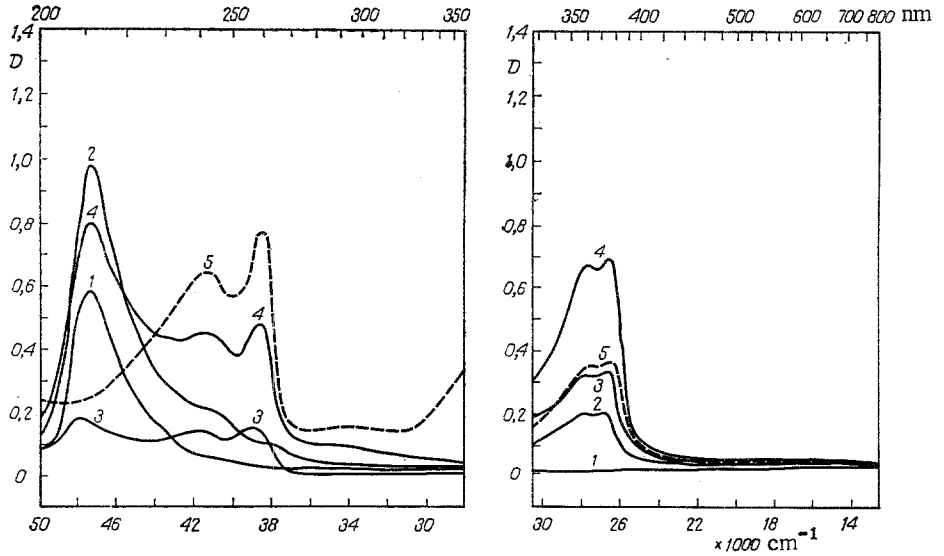

Fig. 1. Absorption spectra of dioxidine in centrifugates of *Staphylococcus* culture. 1) Without preparation; 2, 3, 4) in the presence of 0.01, 0.05, and 0.1% concentrations of the preparation; 5) in meat–peptone broth (0.0007% dioxidine).

extract. The data obtained, treated statistically, are reliable. Table 1 presents only the arithmetic means from five to six determinations. It must be noted that the determination of the concentration of nucleic acids by specific methods was not possible, since in the presence of dioxidine, the diphenylamine and orcinol reagents gave a dark color.

As can be seen from Table 1, dioxidine in a 0.01% concentration has practically no influence on the level of protein in the suspension and centrifugate. Increasing the concentration of the preparation to 0.05% also has no appreciable influence on the level of protein. Under the action of a 0.01% dioxidine concentration in the suspension, the level of nucleic acids is almost unchanged, while when the concentration of the preparation is increased to 0.05%, the level of nucleic acids decreases to 62% in comparison with the control. In the centrifugate, dioxidine in a concentration of 0.01% lowers the level of nucleic acids to 76%, and in a concentration of 0.05%, to 44% in comparison with the control.

For a judgment of the observed changes, it was necessary to determine the behavior of dioxidine in a cell culture, grown in the presence of the preparation. We studied the spectral behavior of dioxidine in centrifugates of 24-, 48-, and 72-h-old cultures on a Specord recording spectrophotometer. It was established that in the presence of various concentrations of the preparation, the centrifugates have absorption spectra characteristic of dioxidine, regardless of the age of the culture. The characteristic peaks are observed at 240, 260, and 360 nm, just as in a control sample of dioxidine in meat–peptone broth (Fig. 1). The concentration of the preparation in the centrifugate is very close to the initial value (0.01-0.04%).

Thus, in a centrifugate of a culture of *Staphylococcus*, dioxidine maintains the nature of its spectral behavior, and in this case a substantial decrease in the amount of nucleic acids is observed, which evidently occurs on account of a disruption of the enzymatic processes of nucleic acid synthesis in *Staphylococcus*. Therefore, the fraction of cytoplasmic supernatant in which the basic enzymatic functions of the bacterial cell, proteins and nucleic acids, are localized was of special interest [5].

Analyses showed that substantial changes in the protein and nucleic acid content are observed in this fraction (see Table 1). Thus, under the influence of dioxidine in a concentration of 0.01%, the level of nucleic acids was lowered almost twofold, and when the dioxidine concentration was increased to 0.05%, the level of nucleic acids was lowered by more than fourfold in comparison with the control. The same pattern is also traced with respect to proteins. Dioxidine in a concentration of 0.05% almost halves the protein level. Since the presence or absence of dioxidine in the intracellular supernatant permits a

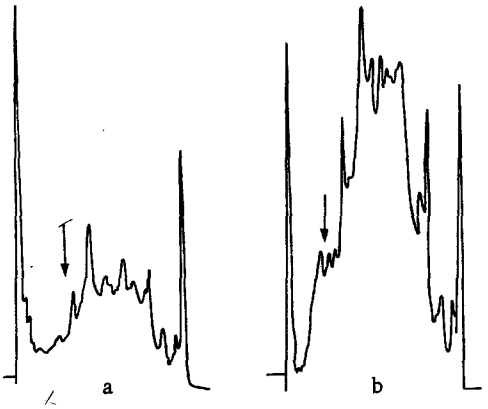

Fig. 2. Densitograms of the protein spectrum of *Staphylococcus*. a) Without the preparation; b) in the presence of 0.1% dioxidine.

judgment of the penetration of the preparation through the cell walls and membranes, it was necessary to determine the content of the preparation in this fraction. Dioxidine was determined by measuring the absorption at 360 nm. The concentration of the preparation was calculated according to a calibration curve. The presence of dioxidine could be established in the fraction with an initial content of the preparation 0.05%. It was established that dioxidine penetrates into the cell, and only 1/100 of the initial concentration is detected in the cytoplasmic fraction. The penetration of dioxidine into the bacterial cytoplasm permitted a study of its action on the composition of intracellular proteins, which is specific for each microbe. For this purpose we used the method of disk electrophoresis in polyacrylamide gel, using the reagents and instrument of Reanal. Electrophoresis was conducted at pH 8.3 in Tris–glycine buffer. The samples for electrophoresis contained from 50 to 150 μg protein. Staining for protein was performed with Amido Black, and for nucleoproteins with Pyronine Y. The gels were scanned on a Chromoscan-200 instrument from the English firm Joyce Lobel. The densitograms obtained (Fig. 2) show that in the presence of high concentrations of dioxidine (0.1%) in the intracellular extract, there are changes in the zone of high-molecular-weight nucleoproteins, which are characterized by the appearance of three new peaks, which are absent on the control densitogram. The results obtained show that when *Staphylococcus* is cultured in the presence of dioxidine, there are substantial biochemical changes in the subcellular fractions, which are evidence of a substantial disruption of the processes of nucleic acid and protein biosynthesis in *Staphylococcus*.

As a result of a study of the spectral behavior of dioxidine in various fractions, it can be suggested that the action of dioxidine on the microbial cells is developed according to the type of the so-called DNA-tropic effect, based on the ability of the substances to form strong compounds with bacterial DNA. The complexes that arise in this case interfere in bacterial DNA synthesis and, changing it, disrupt the growth, reproduction, and mechanism of resistance of the bacterial cell [6, 7].

It is known from the literature that many compounds are capable of forming complexes with DNA — derivatives of the phenothiazine and acridine series, preparations from the quinoline group, actinomycin, daunomycin, etc. [8, 9]. The study of the ability of dioxidine to form complexes with DNA will be a problem for further investigation.

Thus, it has been established that dioxidine penetrates through the cell walls and is detected in the cytoplasmic fraction. A decrease in the amount of nucleic acids under the action of the preparation is observed in the subcellular fractions. Under the influence of dioxidine there is a change in the composition of the intracellular nucleoprotein complex of *Staphylococcus*.

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EFFECTS OF ARYLFURAN DERIVATIVES ON THE ACTIVITY OF PYRIDOXAL ENZYMES

N. I. Fadeeva, T. A. Gus'kova, G. N. Pershin, and I. N. Degtyareva

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The presence of antimicrobial activity was established earlier in a series of arylfuran derivatives [1-3]. To determine the relationship between the chemical structure, antimicrobial activity, and inhibiting effect on enzymes, we studied a series of derivatives of 5-aryl-2-bromoacetylfurans and 5-arylfuryl-2-glyoxals.

Reactions of transamination of aspartic acid and alanine, which are catalyzed by pyridoxal enzymes [4, 5], were selected as the enzyme model. At present enzymatic transamination is considered as a most important link in the processes of assimilation and dissimilation of nitrogen, in the coupling of the amino acid, carbohydrate, and fat metabolism of the living cell [6].

The establishment of the pathway of biosynthesis of many amino acids has provided new data on the role of the processes of transamination in amino acid biosynthesis. Enzymatic transamination consists of a reversible transfer of amino groups and a hydrogen atom by amino transferases from α-, β-, γ- or δ-amino acid to oxo acids, which have a carbonyl group in one of these positions.

A number of authors attribute physiological significance chiefly to two reactions, which are catalyzed by L-aspartate-2-oxoglutarate aminotransferase (EC 2.6.1.1) (AST) and L-alanine-2-oxoglutarate aminotransferase (EC 2.6.1.2) (ALT).

We studied the inhibiting effect of arylfuran derivatives on the activity of AST and ALT and their germistatic activity in experiments *in vitro*.

The substances studied were synthesized in the laboratory of heterocyclic compounds of the All-Union Scientific-Research Institute of Pharmaceutical Chemistry, (VNIKhFI) and kindly provided us for the investigation.

METHODS

The activity of AST and ALT was determined in standard acetone preparations, prepared from various strains of tuberculosis mycobacteria by the Umbreit colorimetric method in our modification, according to the amount of pyruvic acid formed as a result of the enzymatic reaction [7].

The activity of AST and ALT was determined in the presence of various concentrations of the preparation (10^{-2}-10^{-4} M) and in the initial suspension (without the preparation). The enzyme and preparation were incubated for 1 h at 37°.

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