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EXPERIMENTAL ARTICLES

Interaction of Bacteria with Mercuric Compounds

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Abstract—We investigated the interaction of mercuric compounds with the bacteria *Corynebacterium ammoniagenes, Micrococcus luteus*, and *Mycobacterium smegmatus* capable of producing hydroxylamines (R-NOH) and 2-*C*-methyl-D-erythritol-2,4-cyclopyrophosphate (MECP), which are prone to form free radicals. The interaction of these substances with Hg^{2+} ions and their dynamics during the mercuric poisoning of bacteria was studied by EPR and NMR. Under stress conditions induced by lowering pH or generation of active oxygen species, the bacteria and, especially, their mutants with enhanced sensitivity to oxidative stress, were found to respond to exposure to 1–3 μg/ml $HgCl_2$ and *p*-chloromercuribenzoate by a several-fold increase in their viability. The data obtained were interpreted in terms of the involvement of the sulfhydryl groups of bacterial surface proteins in this phenomenon. The interaction of bacteria with mercuric compounds may affect the pathogenesis of tuberculosis and other diseases.

Key words: mercury, oxidative stress, mycobacteria, corynebacteria

Heavy metals are widely used in chemistry and engineering. There is increasing interest in the biotechnology of extraction of rare metals from primary and secondary raw materials [1]. On the other hand, heavy metals are very toxic compounds, which comprise the essential part of anthropogenic pollutants [2, 3]. High concentrations of heavy metals in soil can affect bacterial communities and change the proportion between soil bacteria and microscopic fungi in favor of the latter by as high as ten times [4, 5]. There is evidence that increased concentrations of titanium and zirconium may be an indication of the occurrence of charbon foci in the environment [6]. Mercury is an extremely hazardous chemical element because of its volatility in the metal state and ability to form numerous very toxic volatile organic compounds under the action of bacteria present in soil and other environments [7].

It should be noted that the observation of the beneficial effect of some mercuric compounds on yeasts [8] did not shake the belief that mercuric compounds are extremely toxic to animals [9]. Although healthy human organisms are found to contain mercury in amounts of about 20 μg/g dry weight, nothing is known about the useful effects of mercuric compounds in animals.

As for microorganisms, there are at least five ways by which they defend themselves from the action of mercury [9]: (1) the lowering of the cell wall permeability to mercury, (2) the formation of insoluble mercuric sulfides, (3) the conversion of mercuric compounds to metal mercury, (4) the conversion of nonvolatile mercuric compounds to volatile ones by means of methylation, and (5) the rapid excretion of mercury from cells into the medium. According to data available in the literature, some bacteria are able to diminish the toxicity of potassium tellurite by reducing it to elemental tellurium [10]. Other observations suggest the involvement of intracellular phosphates in the detoxification of heavy metals by yeasts [1]. Of interest is the putative detoxifying role of 2-*C*-methyl-D-erythritol-2,4-cyclopyrophosphate (MECP) [11], whose concentration in cells exposed to oxidative stress exceeds the concentrations of other cellular phosphates detectable by ^{31}P NMR. Theoretically, the redox potentials of cellular compounds prone to the formation of free radicals, such as lysodektose, ammonigenin, and especially their hydroxylamines, are sufficient for their direct interaction with mercuric ions and potassium ferricyanide [12]. For comparison, below are given the redox potentials of hydrogen, mercuric ions, and ferricyanide:

$$1/2H_2 = H^+ + e^- \quad 0.00\ V,$$

$$Fe(CN)_6^{4-} = Fe(CN)_6^{3-} + e^- \quad -0.36\ V,$$

$$1/2Hg_2^{2+} = Hg^{2+} + e^- \quad -0.91\ V.$$

This work was undertaken to study the interaction of some bacterial metabolites (lysodektose, ammonigenin, MECP, etc.) with mercuric ions and to investigate their effect on bacterial cultures subjected to oxidative stress or the action of other factors promoting the biosynthesis of these metabolites.

MATERIALS AND METHODS

Bacterial cultures used in this study were as follows: *Pseudomonas putida* M19-3, *Mycobacterium* sp. CHM

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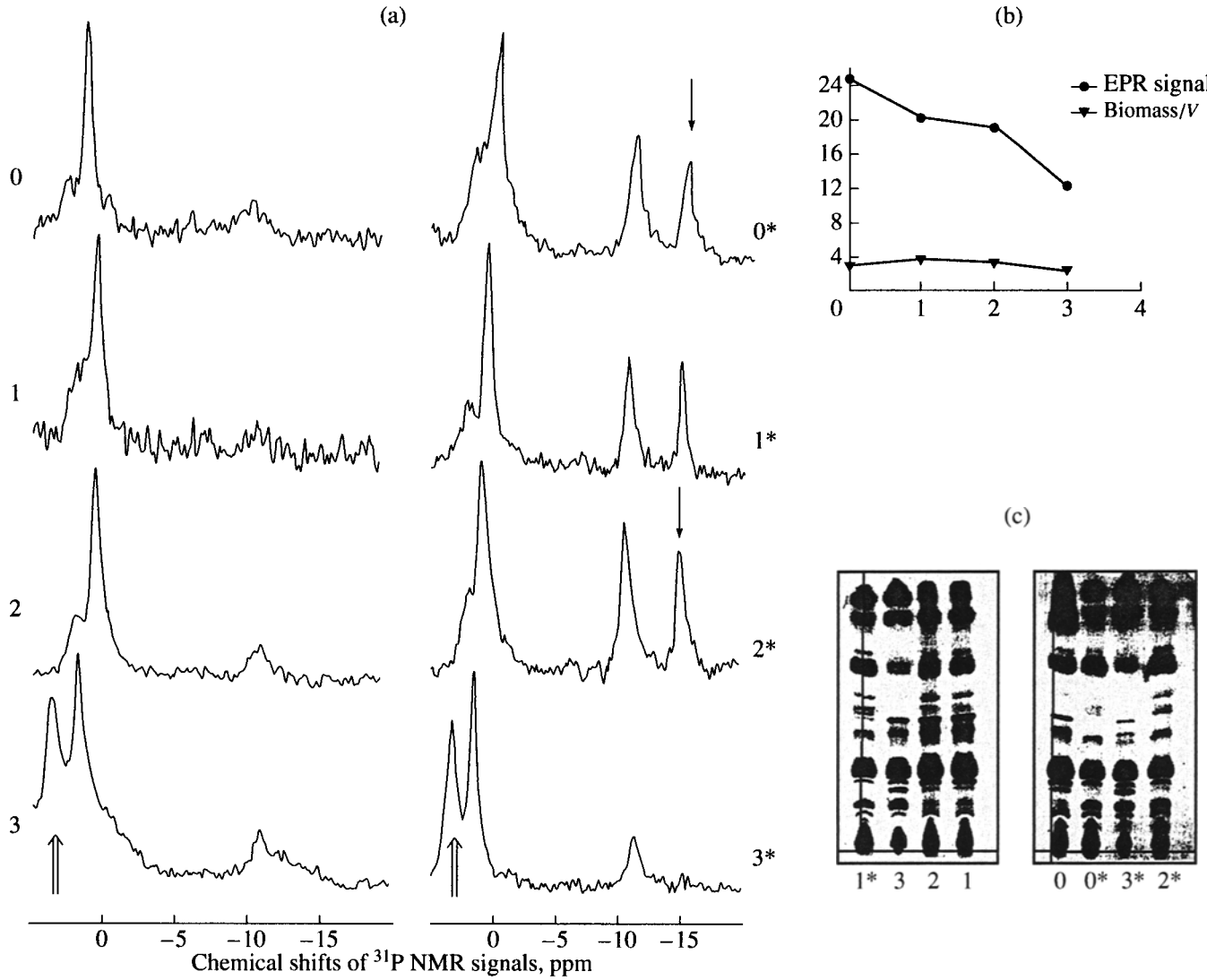

Fig. 1. Effect of $HgCl_2$ on the phosphorus, lipid, and carbohydrate metabolism of *C. ammoniagenes* by the data of (a) ^{31}P NMR, (b) EPR, and (c) TLC. $HgCl_2$ was added in the late logarithmic growth phase. After 17 h of incubation in the presence of $HgCl_2$, cells were harvested by centrifugation and resuspended in D_2O. A portion of this cell suspension was analyzed by ^{31}P NMR. Another portion of the suspension was treated with 50% methanol to extract R-NOH, which was detected by EPR after conversion to the free radical state with $K_3Fe(CN)_6$. The third portion of the suspension was treated with a chloroform–methanol mixture to extract lipids, which were separated on silica gel plates and visualized in an atmosphere of iodine vapors. Numerals 0, 1, 2, and 3 indicate $HgCl_2$ concentrations of 0, 1, 10, and 100 μM, respectively. The asterisk notes that the medium was supplemented with 50 μg/ml benzyl viologen.

21-1, and *M. luteus* B2, which were obtained from the Collection of Microorganisms of the Institute of Molecular Genetics, Russian Academy of Sciences, as well as *Corynebacterium ammoniagenes* ATCC 6872, *C. ammoniagens* E-1 [13], *Escherichia coli* XL-1, *E. coli* DH 10B, and *E. coli* CA-18.

Basal medium A for the cultivation of bacteria contained (g/l) peptone, 10; yeast extract (Sigma), 3; and NaCl, 5. The pH of the medium was adjusted to the required values with NaOH or HCl solutions. In some experiments, we used TGE agar (Merck) and Lab M nutrient broth E.

MECP in bacterial cultures was determined by ^{31}P NMR as described earlier [14]. To induce MECP biosynthesis, liquid logarithmic-phase cultures were supplemented with benzyl viologen and glucose to final concentrations of 50 μg/ml and 1%, respectively, and cultivation was continued for the next 17–20 h. The biomass was then harvested by centrifugation and suspended in D_2O or extracted with 50% methanol. To study the interaction of MECP with mercury in vitro, this compound was isolated from *C. ammoniagenes* cells and purified by trice-repeated chromatography on Dowex 1X-4 anion-exchange resin, as described earlier [14].

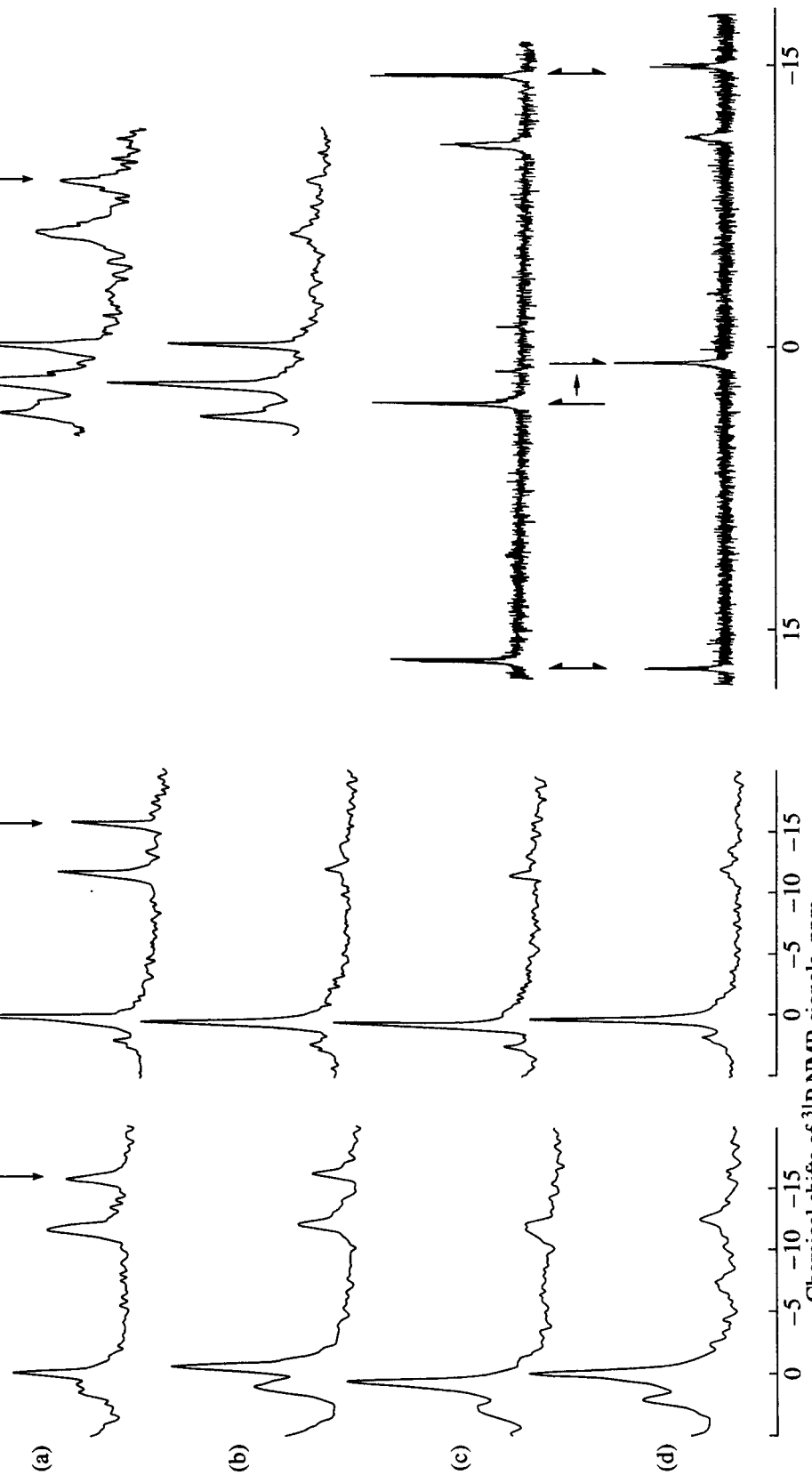

Fig. 2. ^{31}P NMR study of the in vitro interaction of $HgCl_2$ with MECP, its derivative, and (1) *M. luteus*, (2) *P. putida*, and (3) *Mycobacterium* sp. cells bearing mercury resistance plasmids. (a) Standard medium supplemented with 50 μg/ml benzyl viologen; (b) standard medium; (c) standard medium with 10 μM $HgCl_2$; and (d) standard medium with 50 μg/ml benzyl viologen and 10 μM $HgCl_2$. 3c and 3d show, respectively, the spectrum of isolated MECP (signals at −10.7 and −15 ppm) with an equal amount of 2-*C*-methyl-D-erythritol-2,4-cyclophospho-4-phosphate (signals at +17 and +3.5 ppm) and the spectrum of the same specimen treated with $HgCl_2$ (in this case, signal at +3.5 ppm shifted to +0.96 ppm).

The EPR signals of radical-producing metabolites in cell extracts were recorded with a PE-1306 radiospectrometer at room temperature in the presence and absence of potassium ferricyanide or mercuric chloride. EPR signals were referred to the third and fourth signals of external Mn^{2+}/MgO standard.

RESULTS AND DISCUSSION

The addition of $HgCl_2$ to a late-logarithmic-phase *C. ammoniagenes* culture and its subsequent incubation for 17 h under normal conditions or under oxidative stress induced by the addition of 50 μg/ml benzyl viologen to the medium led to considerable changes in the phosphorus, lipid, and carbohydrate metabolisms of this bacterium, as is evident from ^{31}P NMR, EPR, and TLC data (Fig. 1). Mercuric ions suppressed the growth of *C. ammoniagenes* and decreased the cellular content of ammonigenin (Fig. 1). Low mercury concentrations in the medium did not affect benzyl viologen–induced MECP synthesis, whereas 0.1 mM $HgCl_2$ in the medium decreased the cellular content of MECP by 5 times, as can be seen from the decrease in the intensity of NMR signal with a chemical shift of –15 ppm. In this case, the cellular content of phosphorylated sugars with the signals detected at +3 to +5 ppm rose severalfold. Both oxidative stress and exposure to mercuric compounds intensified the biosynthesis of a minor phospholipid with $R_f = 0.15$ and led to some other metabolic changes. The effects of oxidative stress and mercuric compounds were additive. It is difficult to decide which of these changes are adaptive and which are pathological; in our opinion, however, the decrease in the cellular content of MECP and ammonigenin suggests that either compound fulfils protective functions.

The three strains of *P. putida, Mycobacterium* sp., and *M. luteus* isolated from natural sources according to their increased mercury resistance turned out to carry plasmids responsible for this resistance [15, 16]. All these strains intensified MECP biosynthesis in response to the addition of 50 μg/ml benzyl viologen to the medium. This effect of benzyl viologen could be prevented by the addition of $HgCl_2$ to the medium at concentrations of up to 100 μM (Fig. 2). Like mutant *C. ammoniagenes* strain E-1 with enhanced sensitivity to oxidative stress, the Hg-resistant strains of *Mycobacterium* sp. and *M. luteus* were able to produce some amounts of MECP under normal growth conditions.

As shown earlier, the complexation of MECP with Cd^{2+} and BO_4^{3-} ions changes the chemical shift of ^{31}P NMR signals from –10.7 to –10.0 ppm and from –14.5 to 15.6 ppm, respectively. In the present work, we failed to reveal any changes in the ^{31}P NMR signals of MECP in response to the addition of $HgCl_2$ in amounts equivalent to or even higher than the amounts of Cd^{2+} and BO_4^{3-} in the aforementioned experiments (Fig. 2, spectra C-3 and D-3). In this case, the ^{31}P NMR signals

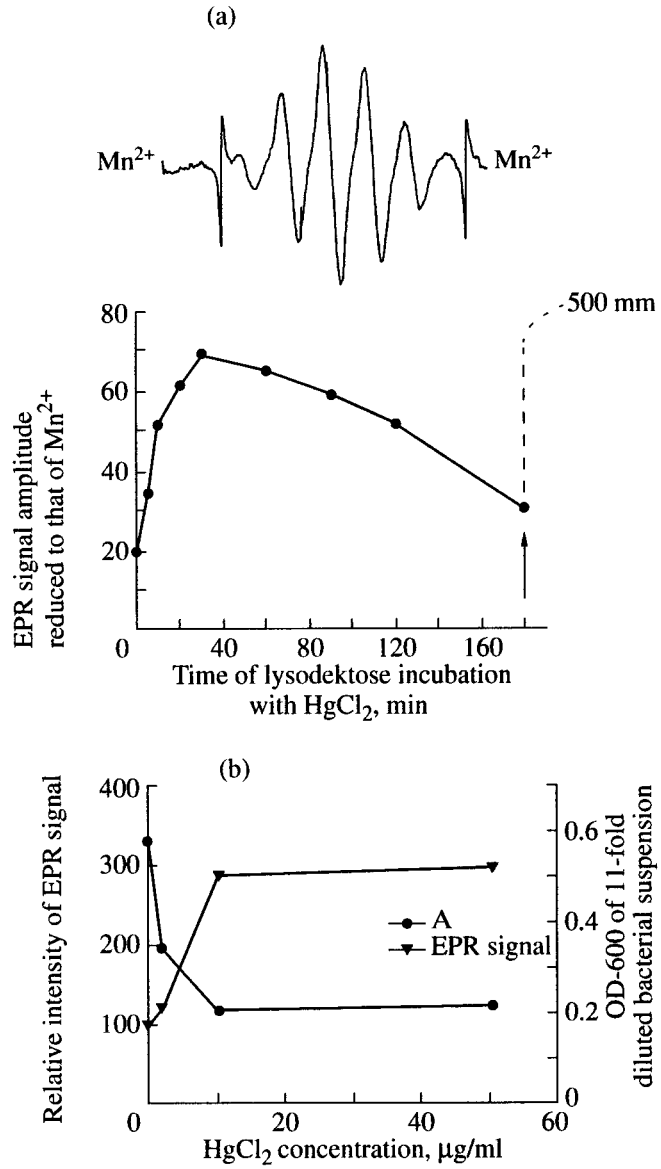

Fig. 3. Formation of the free radicals of lysodektose in *M. luteus* extract to which 20 mM $HgCl_2$ was added, and lysodektose content in *M. luteus* cells treated with $HgCl_2$. Panel (a) shows the EPR signal of lysodektose (a sextet between Mn^{2+} signals); the addition of 10 mM potassium ferricyanide (indicated by the arrow) gave rise to a 100-fold increase in the signal amplitude. Panel (b) shows changes in the optical density (600 nm) of the 11-fold diluted bacterial suspension incubated for 4.5 h with different concentrations of $HgCl_2$ (the optical density of diluted bacterial suspension before the addition of $HgCl_2$ was 0.26) and the content of lysodektose in cells per unit optical culture density.

of the spontaneous derivative of MECP, 2-*C*-methyl-D-erythritol-2,4-cyclophospho-4-phosphate, changed from +3 to +0.9 ppm, suggesting some changes in the valent angles of P–O bonds around P atoms.

Both ordinary and Hg-resistant *M. luteus* strains were able to synthesize lysodektose, which was detected by EPR after its oxidation with ferricyanide

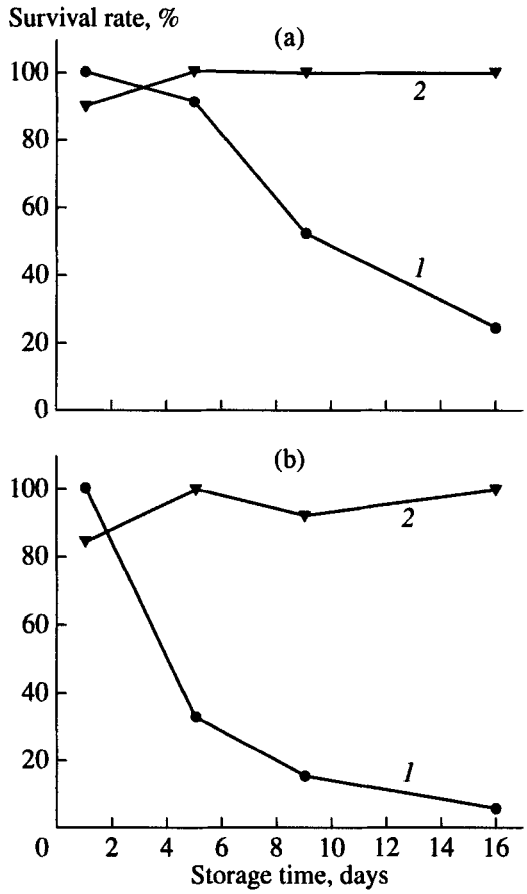

Fig. 4. Survival rates of *C. ammoniagenes* strains stored at 4°C for different times, estimated as the relative number of 2-day colonies grown on (*1*) agar medium A and on (*2*) agar medium A supplemented with 1 μg/ml $HgCl_2$. Panels (a) and (b) show the survival rate of, respectively, original strain ATCC 6872 and mutant strain E-1 with increased sensitivity to oxidative stress.

into the free radical form. The cellular content of lysodektose in the Hg-resistant *M. luteus* strain considerably increased in the presence of $HgCl_2$, whereas strain growth was suppressed (Fig. 3). In vitro experiments with the lysodektose extracted from non-Hg-resistant *M. luteus* cells showed that as little as 1–2% of the lysodektose present in the medium really react with $HgCl_2$ to form Hg_2^{2+}. Such a low reactivity of lysodektose with $HgCl_2$ can be explained by the low ability of this salt to dissociate in solutions (with pK = 13.2, the concentration of Hg^{2+} ions in $HgCl_2$ solutions is about 10^{-7} M). Therefore, although the redox potential of mercury is higher than that of ferricyanide, the nonenzymatic interaction of lysodektose and mercuric ions is weak and cannot serve for their detoxification.

Thus, the Hg-resistant bacterial strains studied possess three factors possibly responsible for their Hg resistance: plasmids encoding mercury reductases, radical-producing hydroxylamines, and mechanisms of induction of MECP synthesis operating under conditions of oxidative stress. The chemical interaction of MECP and free radicals with mercuric ions may only be a subsidiary mechanism of their detoxification. As for the enzymatic mechanisms of mercury interaction with MECP and free radicals, they are still to be studied.

Another approach to the study of the possible involvement of MECP in the detoxification of mercuric ions lies in the comparison of the mercury resistance of the parent *C. ammoniagenes* strain ATCC 6872 and its mutant E-1, exhibiting enhanced resistance to oxidative stress [13] and ability to synthesize large amounts of MECP under normal growth conditions. The mercury resistance of strain 6872 was estimated under both normal growth conditions and conditions promoting MECP biosynthesis, i.e., in the presence of benzyl viologen in the cultivation medium. For this purpose, we estimated the number of viable cells in overnight bacterial cultures grown in liquid medium to the stationary phase and stored at 4°C in a refrigerator. Viable cells were enumerated by plating the 0.1-ml aliquots of diluted bacterial cultures on agar medium A (1.5% Ferak bacto agar) and estimating the number of colonies grown on this medium after 2-day incubation at 30°C. Mercuric chloride present in this medium at a concentration of 5 μg/ml and higher strongly suppressed the growth on both parent and mutant strains. The number of viable cells in liquid bacterial cultures stored at 4°C gradually decreased (the decrease in viability was more pronounced in the case of mutant E-1), if this number was estimated as the number of colonies grown on agar medium without mercuric chloride (Fig. 4, curve *1*). At the same time, the viability of the stored bacterial cultures seemed not to change, if viable cells were enumerated using agar medium containing sublethal $HgCl_2$ concentrations (1–2 μg/ml) (Fig. 4, curve *2*).

Such an unexpected observation brought up a number of questions: What is the range of physical, chemical, and physiological factors responsible for the increased viability of bacterial cultures in the presence of sublethal mercury concentrations? Is this phenomenon species-specific? Can it be observed in nature (in soils, waters, during the contact of pathogenic microorganisms with plants and animals, etc.)? What is the molecular mechanism of activation of bacterial growth by mercury? It should be noted that the beneficial effect of mercuric compounds on the population of microscopic fungi in soil has been known for a long time and was accounted for by the death and, hence, decreasing competition of soil bacteria and other soil inhabitants, which are less mercury-resistant than microscopic fungi.

Of interest is the fact that the sublethal concentrations of mercury stimulated the growth of colonies from bacterial cells stored in the cold only when agar medium A was used for colonial growth. However, if the bacteria preliminarily stored in the cold were plated onto TGE agar or Lab M nutrient agar, they exhibited high sensitivity to 1 μg/ml $HgCl_2$ (data not presented).

This observation can be explained by assuming that medium A contains a hypothetical toxic compound (HTC), whose detrimental effect on bacteria is stronger if they were preliminarily stored in the cold. Microamounts of mercury in the medium may bind this HTC, thus improving growth conditions. The support to this assumption comes from the fact that mercury efficiently binds to thiol compounds, which are known to inhibit bacterial growth in some nutrient media [17]. It should, however, be noted that the inhibitory concentrations of the known thiol compounds (1–10 mM) are about 1000-fold higher than the stimulatory concentrations of mercuric chloride. Therefore, either HTC is a so far unknown supertoxic thiol compound present in the medium, or we must further assume that, for instance, the stimulatory effect of mercury is due to some cellular constituents that behave differently when bacteria grow on agar medium A, TGE agar, or Lab M agar. Such hypothetical cellular constituents may be intracellular low-molecular-weight sulfhydryl compounds (e.g., glutathione), which are known to be involved in the protection of cellular enzymes against mercuric poisoning [18]. The SH-groups located at different depths from the cell surface can be distinguished by comparing the effects of Hg^{2+} ions and *p*-chloromercuribenzoate (*p*-CMB) [19]. In our experiments, both $HgCl_2$ and *p*-CMB at concentrations of 1 and 2 μg/ml beneficially influenced the growth of bacterial colonies on agar media. Therefore, the hypothetical intracellular sulfhydryl compounds must be located close, if at all, to the cell surface.

The more pronounced beneficial effect of mercury on mutant E-1 with increased sensitivity to oxidative stress than on the parent *C. ammoniagenes* strain suggested the involvement of oxidative stress in the phenomenon discussed. In view of this, we simulated oxidative stress by adding different concentrations (0–15 μg/ml) of benzyl viologen to TGE agar.

As can be seen from the table, mercuric chloride exerted the most pronounced stimulatory effect on colonial growth on medium A, when the concentration of benzyl viologen was 2–3 μg/ml and the pH of the medium was 6.5 (colonies showed no growth at pH 5.0 and grew well at pH 8, irrespective of the presence of mercuric chloride at stimulatory concentrations of 1–2 μg/ml). Under these conditions, electron transfer via the respiratory chain is probably suppressed; this must lead to a discharge of electrons from quinones to oxygen with the formation of superoxide radicals at concentrations sufficient to cause oxidative stress. The stress can give rise to disulfide bridges in the proteins which are involved in bacterial reproduction (for instance, in the proteins that govern water partition [20]) and thus inactivate them. Binding to one of the sulfur atoms of disulfide bridges, mercuric ions break them, thus restoring the conformational flexibility and activity of proteins vital to bacterial cells. Increasing the concentration of mercuric ions in the medium brings about a blockade of the second sulfur atom of the disulfide bridges, which must lead to the complete inhibition of the respective proteins and ultimately to growth cessation.

Effect of mercuric chloride on bacterial growth on agar media (CFU per petri dish estimated as the mean of triplicate platings from 10^6-fold culture dilutions)

Culture grown on medium A at pH 6.5	$HgCl_2$, μg/ml			
	0	1	2	4
Fresh log-phase *C. ammoniagenes*	29	78	110	82
Myc. smegmatis stored at 4°C for 3 days	5	285	236	2
M. luteus stored at 4°C for 3 days	18	350	241	0
C. ammoniagenes stored at 4°C for 80 days	0	170	166	–
	Benzyl viologen, μg/ml			
	0	1	2	
	0	182	188	–

C. ammoniagenes stored at 4°C for 20 days and plated onto TGE agar containing $HgCl_2$ in amounts (μg/ml)	Benzyl viologen, μg/ml				
	0	1	2	3	4
0	350	390	310	5	0
0.3	340	345	350	370	19
0.8	380	335	425	280	0

It should be noted that the presence of mercuric ions in agar medium A with pH 6.5 not only considerably raised the number of grown colonies (see table and Fig. 5) but also increased their size in the early cultivation terms: the mean diameter of 2-day colonies grown on the mercury-containing medium A was more than 1 mm, whereas the majority of 2-day colonies grown on medium A without mercury had diameters close to 0.1 mm (data not shown). After 3–5 days of cultivation, differences in the colonies grown in the presence and absence of mercuric chloride essentially diminished. The stimulatory effect of mercury on the growth of colonies on medium A with pH 6.5 was observed in experiments with not only cultures stored in the cold but also with a fresh logarithmic-phase *C. ammoniagenes* culture and stationary-phase *Myc. smegmatis* and *M. luteus* cultures. On the other hand, we failed to demonstrate the stimulatory effect of mercuric chloride on the growth of *Myc. smegmatis* on TGE agar in the presence of benzyl viologen and on the growth of three *E. coli* strains, XL-1, DH-10B, and CA-18, on medium A with pH 6.5. In other words, we demonstrated the stimulatory effect of mercury with only a few bacterial species cultivated on the special medium under specific conditions. It should, however, be noted that it is these conditions (oxidative stress, acidic pH values, and cold shock) under which pathogenic bacteria occur in host organisms or nature.

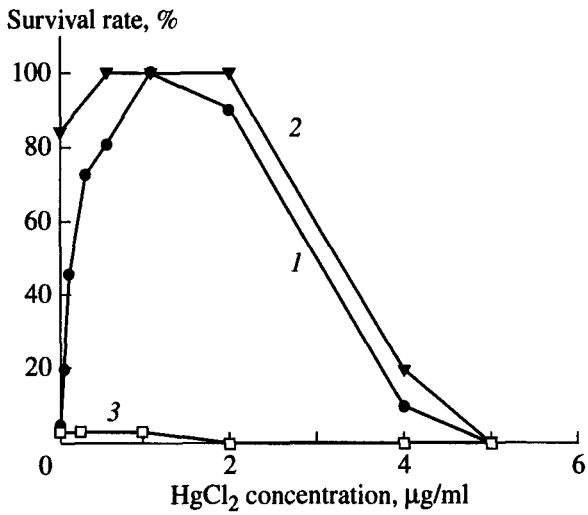

Fig. 5. Survival rates of *C. ammoniagenes* strain E-1 stored at 4°C for 30 days, estimated as the relative number of 2-day colonies grown at 30°C on agar medium A with pH (*1*) 6.5, (*2*) 7.5, and (*3*) 5.5 in the presence of different concentrations of $HgCl_2$. Each petri dish was plated with a 0.1-ml aliquot of 10^6-diluted culture; 100% survival rate corresponds to 30015 CFU/plate.

In ecological experiments, we did not reveal any stimulatory effect of mercury on bacteria present in water samples taken from the Moscow River, although we observed some stimulatory effect of mercury on bacteria present in aqueous extracts of soil samples taken in the Moscow region and Germany. Noteworthy are the facts that some bacteria remain viable after the sorption of considerable amounts of mercuric compounds and that mercuric oinments, which are widely used in treatment of skin diseases, are not recommended for tuberculosis patients. Therefore, the suggestion that mercury activates *Mycobacterium tuberculosis* is not improbable.

Furthermore, the concentrations of mercury in waters do not usually exceed 0.0001 μg/ml, although mercury can be found in fish in an amount of 0.2 μg/ml (0.2 mg/kg) and even 1 μg/ml. A daily consumption of 3 μg ethyl mercury per g body weight during 3 months can lead to death. Thus, the mercury concentrations discussed in this paper are comparable with those which humans may accidentally receive in their every-day life; therefore, the activation of pathogenic bacteria by mercury can really be hazardous to humans.

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REFERENCES

1. Korenevskii, A.A., Sorokin, V.V., and Karavaiko, G.I., Interaction of Rare-Earth Metal Ions with *Candida utilis* Cells, *Mikrobiologiya*, 1997, vol. 66, pp. 198–205.
2. Avtsyn, A.P., Zhavoronkov, A.A., Rish, M.A., and Strochkova, L.S., *Mikroelementozy cheloveka* (Microelement Diseases in Humans), Moscow: Meditsyna, 1991, pp. 1–496.
3. Burkhardt, C., Insam, H., Hutchinson, T.C., and Reber, H.H., Impact of Heavy Metals on the Degradative Capabilities of Soil Bacterial Communities, *Biol. Fertil. Soils*, 1993, vol. 16, pp. 154–156.
4. Letunova, S.V., Geochemical Environments and Soil Microbial Complexes, *Tez. dokl. XI Vsesoyuzn. konf. Mikroelementy v biologii i ikh primenenie v sel'skom khozyaistve i meditsine* (Proc. XI All-Union Conf. "Microelements in Biology, Agriculture, and Medicine"), 1990, pp. 54–55.
5. Ranjard, L., Richaume, A., Joster-Monrozier, L., and Nazaret, S., Response of Soil Bacteria to Hg II in Relation to Soil Characteristics and Cell Location, *FEMS Microbiol. Ecol.*, 1997, vol. 24, pp. 321–331.
6. Skorokhodov, Yu.M., Microelement Levels as Indicators of Charbon Foci, *Tez. dokl. XI Vsesoyuzn. konf. Mikroelementy v biologii i ikh primenenie v sel'skom khozyaistve i meditsine* (Proc. XI All-Union Conf. "Microelements in Biology, Agriculture, and Medicine"), 1990, pp. 82–83.
7. Jernelov, A. and Martin, A.-L., Ecological Implications of Metal Metabolism by Microorganisms, *Annu. Rev. Microbiol.*, vol. 29, pp. 61–77.
8. Rissanen, K. and Miettinen, J., Use of Mercury Compounds in Agriculture and Its Implications, *Mercury Contaminations in Man and His Environment*, Vienna: Int. Atomic Energy Agency, 1972, pp. 5–34.
9. Osborn, A.M., Bruce, K.D., Strike, P., and Ritchie, D.A., Distribution, Diversity and Evolution of the Bacterial Mercury Resistance *mer* Operon, *FEMS Microbiol. Rev.*, 1997, vol. 19, pp. 239–262.
10. Trutko, S.M., Suzina, N.E., Duda, V.I., Akimenko, V.K., and Boronin, A.M., Involvement of the Respiratory Chain of Bacteria in the Reduction of Potassium Tellurite, *Dokl. Akad. Nauk*, 1998, vol. 358, pp. 836–838.
11. Ostrovsky, D., Shashkov, A., and Sviridov, A., Bacterial Oxidative Stress Substance is 2-*C*-Methyl-D-Erythritol-2,4-Cyclopyrophosphate, *Biochem. J.*, 1993, vol. 295, pp. 901–902.
12. Biniukov, V., Kharatian, E., Ostrovsky, D., and Shashkov, A., Lysodektose, a New Trisaccharide from *Micrococcus lysodeikticus* Which Is Transformed into an Aminoxyl Free Radical with a Loss of an Electron, *Free Rad. Res. Commun.*, 1989, vol. 14, pp. 91–95.
13. Lysak, E.I., Demina, G.R., Matveeva, E.V., Ogrel', O.D., Trutko, S.M., and Ostrovskii, D.N., Biosynthesis of 2-*C*-

Methyl-D-Erythritol-2,4-Cyclopyrophosphate in Bacteria, *Mikrobiologiya*, 1999, vol. 68, pp. 5–13.

14. Shchipanova, I.N., Kharat'yan, E.F., Sibel'dina, L.A., Ogrel', O.D., and Ostrovskii, D.N., About the Nature of the Novel Macroergic Compound of Bacteria Synthesized in Response to Oxidative Stress, *Biokhimiya*, 1992, vol. 57, pp. 862–872.
15. Bogdanova, E.S., Mindlin, S.Z., Kalyaeva, E.S., and Nikiforov, V.G., The Diversity of Mercury Reductases Among Mercury Resistant Bacteria, *FEBS Lett.*, 1988, vol. 234, pp. 280–282.
16. Bogdanova, E.S. and Mindlin, S.Z., Occurrence of Two Structural Types of Mercury Reductases Among Gram-Positive Bacteria, *FEMS Microbiol. Lett.*, 1991, vol. 78, pp. 277–280.
17. Zeng, H., Snavely, I., Zamoreno, P., and Javor, G.T., Low Ubiquinone Content in *E. coli* Causes Thiol Hypersensitivity, *J. Bacteriol.*, 1998, vol. 180, pp. 3681–3685.
18. Latinwo, L., Donald, C., Ikediobi, C., and Silver, S., Effects of Intracellular Glutathione on Sensitivity of *E. coli* to Mercury and Arsenite, *Biochem. Biophys. Res. Commun.*, 1998, vol. 242, pp. 67–70.
19. Sennett, G. and Rosenberg, L., Transmembrane Transport of Cobalamine in Prokaryotic and Eukaryotic Cells, *Annu. Rev. Biochem.*, 1981, vol. 50, pp. 1053–1086.
20. Kuwahara, M., Gu, Y., Ishibashi, K., Maruno, F., and Sasaki, S., Mercury-Sensitive Residues and Pore Size in AQP3 Water Channel, *Biochemistry*, 1997, vol. 36, pp. 13973–13978.