4. Shigeshi Toyoshima, Seizaburo Kano, and Takeo Ueda: Arsenical Chemotherapeutic Drugs. XIV.[1] Studies on the Mode of Antibacterial Action of Organic Arsenical Compounds. (1).

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In the previous report[2], it was shown that several arsenical compounds exerted perceptible effect against various bacteria. In contrast to evidence for the reaction of organic arsenicals against various enzyme-systems in spirochetes and trypanosomes, little attempt has been made to explain the mode of antibacterial action of these arsenicals. This paper describes the antagonistic action of thiol compounds and the so-called sulfanil amide-antagonists on antibacterial action of organic arsenicals, and the inhibitory action of organic arsenicals against succinic dehydrogenase of bacteria.

Materials and Methods

In order to examine the antagonistic action on antibacterial organic arsenicals, oxophenarsine hydrochloride (Oxo), *p*-aminophenylarsonous acid (NH_2-AsO), and *p*-hydroxyphenylarsonous acid (OH-AsO) were employed as organic arsenicals, and cysteine hydrochloride, sodium thioglycollate, and BAL, as thiol compounds. *p*-Aminobenzoic acid, procaine hydrochloride, ethyl *p*-aminobenzoate, bouillon, and peptone were used as sulfanilamide-antagonists, and *Ecsherichia coli communis*, for the bacterium. For the basal medium a synthetic medium was employed. To determine the antagonistic action of the above chemicals, minimal bacteriostatic concentrations of the arsenicals alone and arsenicals mixed with antagonists were investigated after incubation at 37° for 48 hours.

Inhibitory actions of organic arsenicals on succinic dehydrogenase of the bacterium were investigated by employing 2-carboxydiphenylarsinous acid anyhdride (As-2), diphenylarsinous acid (As-16), 4-hydroxydiphenylarsinous acid (As-17), 4,4′-dihydroxydiphenylarsinous acid (As-18), and 4-carboxydiphenylarsinous acid (As-26) as organic arsenicals. *Staphylococcus aureus* (Terashima strain), *Ecsherichia coli communis*, *Fleximа dysenteriae* (Komagome BIII strain), and *Eberthella typhosa* (Kyodai strain) were used as sources of enzyme.

After incubation in bouillon medium at 37° for 40 hours, the bacteria were centrifuged at 3,000 r.p.m. for 10 minutes. The resulting residue was washed three times by recentrifugation after resuspension in 0.85% saline solution. The final suspension of bacteria in 0.85% saline salution was employed as source of enzyme. The activity of succinic dehydrogenase was determined by the methylene blue technique of Thunberg. The reaction components were as follows: 1.0 cc. of enzyme solution and 2.0 cc. of *M*/15 phosphate buffer of pH 8.0, in main tube, and 0.25 cc. of *M*/2,000 methylene blue and 0.5 cc. of *M*/10 sodium succinate in side bulb. The tube was evacuated and kept in a water bath at 37° for 5 minutes, after which the reactants were mixed by tilting. Each of the organic arsenicals was added into the final suspensions of the bacteria and left to react for 2 hours.

Results

The results of the experiments, described above, are illustrated in Table I by minimal bacteriostatic concentration and in Table II by reduction time of methylene blue.

From Table I, it is evident that while thiol compounds decreased the antibacterial effects of the arsenicals, sulfanilamide-antagonists did not, but even increased the minimal bacteriostatic concentration of arsenicals.

Table II shows that organic arsenicals inhibited succinic dehydrogenase of the bacteria in various concentrations and these inhibitory effects paralleled the antibacterial strengths of these arsenicals.

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1) Part XIII: This Bulletin, 1, 252 (1953).

2) T. Ueda, S. Toyoshima, K. Takahashi: This Bulletin, 1, 16, 25 (1953); K. Takahashi, S. Toyoshima, T. Ueda: *Ibid.*, 1, 20 (1953).

Table I.

Antagonistic Action of Thiol Compounds and Sulfanilamide-Antagonists against Organic Arsenical Compounds

Antagonist	Antagonist concentration added to medium (Mol.)	Minimal bacteriostatic concentration (Mol.)			
		Oxo	NH_2-AsO	OH-AsO	Sulfathiazole
p-Aminobenzoic acid	10^{-1}	10^{-4}	10^{-4}	10^{-4}	10^{-3}
	1	10^{-4}	10^{-4}	10^{-4}	10^{-2}
	10	10^{-4}	10^{-3}	10^{-5}	10^{-2}
Procaine	1	10^{-4}	10^{-4}	10^{-4}	10^{-3}
	10	10^{-4}	—	—	10^{-2}
Ethyl *p*-aminobenzoate	1	10^{-4}	10^{-4}	10^{-4}	10^{-5}
	10	10^{-4}	10^{-4}	10^{-4}	10^{-5}
Cysteine	10^{-1}	10^{-3}	10^{-4}	10^{-4}	10^{-5}
	1	10^{-3}	10^{-3}	10^{-4}	10^{-5}
	10	—	10^{-3}	10^{-3}	10^{-5}
Thioglycollic acid	10^{-1}	10^{-3}	10^{-4}	10^{-4}	10^{-5}
	1	10^{-3}	10^{-3}	10^{-3}	10^{-5}
	10	10^{-2}	—	—	10^{-5}
BAL	1	10^{-2}	10^{-2}	10^{-4}	10^{-5}
Bouillon	—	10^{-4}	10^{-3}	10^{-4}	10^{-2}
Peptone	—	10^{-3}	10^{-2}	10^{-4}	10^{-3}

Table II.

Influence of Organic Arsenicals on Succinic Dehydrogenase of Various Bacteria

Bacteria	Contrast (Bacteria only)		Arsenical compound	Anti-bacterial activity *in vitro* (Mole)	Concentration of arsenicals		
	without substrate	with substrate			$10^{-5.5}$ mole	10^{-6} mole	10^{-7} mole
Staphylococcus aureus			As- 2	10^{-6}	A	B	50 min.
			As-16	10^{-8}	A	60 min.	25 min.
	B	20 min.	As-17	10^{-7}	A	B	D
			As-18	10^{-4}	A	E	23 min.
			As-26	10^{-5}	A	F	25 min.
Esche ichia coli communis			As- 2	10^{-3}	A	A	A
			As-16	10^{-6}	A	A	A
	A	22 min.	As-17	10^{-6}	A	A	A
			As-18	10^{-4}	A	A	D
			As-26	10^{-3}	A	A	E
Fleximа dysenteriae			As- 2	10^{-6}	A	A	A
			As-16	10^{-6}	A	A	E
	A	21 min.	As-17	10^{-7}	A	A	E
			As-18	10^{-4}	A	A	E
			As-26	10^{-3}	A	A	D
Eberthera typhosa			As- 2	10^{-5}	A	A	A
			As-16	10^{-3}	A	A	E
	B	20 min.	As-17	10^{-8}	A	A	A
			As-18	10^{-4}	A	A	60 min.
			As-26	10^{-3}	A	A	25 min.

In Table II, A~F represent rate of discoloration of methylene blue (M.B.) solution:

	Approximate % discoloration of M.B.
A	0 ~ 1
B	1 ~ 5
C	5 ~ 20
D	20 ~ 90
E	90 ~ 99
F	more than 99%

Discussion and Conclusion

The relationship between arsenical drugs, mainly belonging to primary arsenical compounds, and thiol compounds to form thioarsenites, has been discussed in numerous reports by Voegtlin, Dyer, and Leonard[3], Rosenthal and Voegtlin[4], Eagle[5], Recall and Wilson[6], etc. They demonstrated that thiol compounds such as cysteine, glutathione, thioglycollic acid, and BAL inhibited the trypanocidal and spirocidal action of trivalent arsenicals *in vitro* and *in vivo*. Concerning the mode of spirocidal and trypanocidal action of arsenicals, it has hitherto been concluded that they invade the thiol enzyme-systems of spirochetes and trypanosomes, in conformity with the chemotherapeutic interferance phenomena of thiol compounds. At present, it appears that the major effect of various sulfanilamides may be due to its prevention of the syntheses of pteroyl compounds, derivatives of *p*-aminobenzoic acid, etc., in bacteria, in comformity with the action of sulfanilamide-antagonists, such as those of *p*-aminobenzoic acid and its derivatives[7~9]. From these findings, it may be said that the mode of antibacterial action of arsenical drugs might be learned from the antagonistic action on arsenical drugs.

As described in the experimental part, it was observed that antibacterial effects of the arsenicals were decreased by thiol compounds, but not by sulfanilamide-antagonists.

From these findings, it may be deduced that the arsenicals affect thiol enzymes of the bacterium, but not the respiratory enzymes, which was shown to be invaded by sulfanilamides, and not by the prevention of the syntheses of pteroyl compounds by the bacterium.

The effects of organic arsenicals against thiol enzymes such as cholinesterase[10~11], adenosine triphosphosphatase[12], glutathione[12], kathepsine[13~14], urease[15], and papain[16] in body tissue of spirochetes and trypanosomes, have been reported by numerous authors. However, the inhibitory action of organic arsenicals against thiol enzymes of bacteria has hardly been studied.

As shown in the experimental part, it was observed that the arsenicals clearly inhibited succinic dehydrogenase of bacteria in various concentrations and their antibacterial activities are considered parallel to these inhibitory effects, which suggests that the antibacterial action of organic arsenicals is at least partially due to the inhibitory effects against succinic dehydrogenase of bacteria.

From this point of view, it may be concluded that organic arsenical drugs as described in Part XI and XII are of promise for chemotherapeutic use against various bacteria resistant to sulfanilamide drugs.

Further work on this problem is in progress.

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Summary

(1) The antibacterial action of organic arsenical compounds was inhibited by thiol compounds but not by sulfanilamide-antagonists.

(2) The activity of succinic dehydrogenase of various bacteria was inhibited by organic arsenical compounds. This inhibitory effect was considered parallel to the bacteriostatic activities of organic arsenical compounds.

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5. Takeo Ueda and Tadakazu Tsuji: Arsenical Chemotherapeutic Drugs. XV. Studies on Arsenical Compounds as Anthelmintics.

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Many informations, regarding the trypanocidal, spirocidal, and antibacterial activities of the arsenical drugs, have been gained, but up to date no observations have been reported regarding their anthelmintic activities.

Studies to determine the most suitable type of chemical structure among the arsenical compounds and the most appropriate method of the anthelmintic tests for them were undertaken before proceeding with their detailed studies. Several arsenical compounds were examined as to their anthelmintic properties by the usual method. This paper describes the anthelmintic activities of several arsenical compounds against *Ascaris lumbricoides.*

Compounds Used to Test Anthelmintic Action Three series of arsenical compounds, viz. the diarylarsinic acids, the arylarsenous acids and the diarylarsinous acids, as shown in the following tables, were tested for their anthelmintic properties . All of the compounds are known. Among them, the diarylarsinic acids and the diarylarsinous acids were prepared by Ueda-Takahashi method[1].

Anthelmintic Action According to the Lamson-Nakamura method[2], these arsenical compounds were examined as to their anthelmintic activities by observing the kinetic state of *Ascaris lumbricoides* exposed in dilution of 1 : 1,000 Bunge's solution of the chemicals at 38°.

Results are given in Tables I, II, and III. Numbers under the heading "Paralysis" denote the time after which the ascarid showed no movement without outside stimulus, and under "Death", the time after which the ascarid showed no movement under any stimulus.

As shown in the tables, none of the diarylarsinic acids showed any action, though some of the arylarsenous acids and the diarylarsinous acids exerted considerable effect: Phenylarsenous acid (No. 16), 4-tolylarsenous acid (No. 17), 4-hydroxyphenylarsenous acid (No. 18), and 4-hydroxydiphenylarsinous acid (No. 22) showed marked ascaricidal effect and 3-nitro-4-hydroxyphenylarsenous acid (No. 19), 3-amino-4-hydroxyphenylarsenous acid (No. 20), diphenylarsinous acid (No. 21), 4-nitrodiphenylarsinous acid (No. 23), 4-chlorodiphenylarsinous acid (No. 24), 2-carboxydiphenylarsinous acid

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