191~193°. *Anal.* Calcd. for $C_{13}H_{18}O_7N_3AsS_2$: As, 16.15. Found : As, 16.75.

3-Nitro-4-benzyloxyphenyl-bis(α-amino-α-carboxyethyl)dithioarsenite—Yellow powder. *Anal.* Calcd. for $C_{19}H_{22}O_7N_3AsS_2$: As, 13.78. Found : As, 13.75.

3-Nitro-4-hydroxyphenyl-bis(2-carboxyphenyl)dithioarsenite—Yellow powder, m.p. 160~162° (decomp.). *Anal.* Calcd. for $C_{20}H_{14}O_7NAsS_2$: As, 14.42. Found : As, 15.01.

3-Nitro-4-methoxyphenyl-bis(2-carboxyphenyl)dithioarsenite—Slightly yellow powder, m.p. 170~171°. *Anal.* Calcd. for $C_{21}H_{16}O_7NAsS_2$: As, 14.01. Found : As, 14.20.

3-Nitro-4-benzyloxyphenyl-bis(2-carboxyphenyl)dithioarsenite—Slightly yellow powder, m.p. 198°(decomp.). *Anal.* Calcd. for $C_{27}H_{20}O_7NAsS_2$: As, 12.25. Found : As, 12.40.

3,5-Dinitro-4-hydroxyphenyl-bis(2-carboxyphenyl)dithioarsenite—Yellow powder, m.p. 185~187°. *Anal.* Calcd. for $C_{20}H_{13}O_9N_2AsS_2$: As, 13.27. Found : As, 13.50.

2) 1.0 cc. of thioglycolic acid was added into a solution of 0.02 mole of arsene oxide dissolved in 40 cc. of water by adding alkali, and boiled for several mins. After cooling, 3.0 cc. of 10% $BaCl_2$ was added cautiously into the reaction mixture. A precipitate, produced by neutralization with ammonia water in the presence of methyl red, was filtered and washed with water and EtOH.

3-Nitro-4-hydroxyphenyl-[barium bis(carboxymethyl)]dithioarsenite—Yellow powder, m.p. > 255°. *Anal.* Calcd. for $C_{10}H_8O_7AsBaS_2$: As, 14.50. Found : As, 15.01.

3-Nitro-4-methoxyphenyl-[barium bis(carboxymethyl)]dithioarsenite—Yellow powder. *Anal.* Calcd. for $C_{11}H_{10}O_7NAsBaS_2$: As, 14.08. Found : As, 14.20.

3-Nitro-4-hydroxyphenyl-(sodium α,β-dicarboxyethyl)dithioarsenite—0.23 g. of 3-nitro-4-hydroxyphenylarsene oxide was mixed with 0.3 g. of thiomalic acid, emulsified by adding 5 cc. of water. Yellow precipitate thus produced was filtered, washed with water, and dried *in vacuo*. The dried compound was redissolved in calculated amount of NaOH solution and dried at 37~40° under a diminished pressure. Red plates. Yield, 0.4 g. *Anal.* Calcd. for $C_{14}H_{12}O_{11}NAsNa_2S_2$: As, 13.54. Found : As, 13.50.

Summary

1. Derivatives of 3-nitro-4-hydroxyphenylarsene oxide were synthesized on the basis that the parent compound exerted a remarkable antibacterial activity among the phenylarsonous series.

2. 3-Nitro-4-hydroxyphenyl-bis(α,β-dicarboxyethyl)dithioarsenite was selected as the most effective against *Shigella dysenteriae*, especially that of sulfa-resistant strains.

(Received June 25, 1954)

---

69. Seizaburo Kano and Shigeshi Toyoshima : Arsenical Chemotherapeutic Drugs. XVII.[1]. Resistance Development and Cross-Resistance of *Shigella dysenteriae* to 3-Nitro-4-hydroxyphenyl-bis(α,β-dicarboxyethyl)dithioarsenite and 3-Amino-4-hydroxydiphenyl-(α,β-dicarboxyethyl)-thioarsinite.

(*Pharmaceutical Institute, Keio-Gijuku University**)

As described in Part XVI and XI of this series[1,2], it was found that 3-nitro-4-hydroxyphenyl-bis(α,β-dicarboxyethyl)dithioarsenite, named Nitrosen, and 3-amino-4-hydroxydiphenyl-(α,β-dicarboxyethyl)-thioarsinite, named Diarsen, possessed strong activities against *Shigella dysenteriae*. Subsequently, resistance development and cross-resistance of these two drugs were investigated by the authors. This paper is concerned with development of bacterial resistance to Nitrosen and Diarsen and cross-resistance between dihydrostreptomycin, terramycin, chloramphenicol, nitrofuracin, and these arsenical drugs.

* Shinano-machi, Shinjuku-ku, Tokyo (加納晴三郎, 豊島 滋).

1) T. Ueda : Arsenical Chemotherapeutic Drugs. XVII; Part XVI : This Bulletin, **2**, 301(1954).

2) T. Ueda, S. Toyoshima, K. Takahashi : *Ibid.* **1**, 25 (1953).

Resistance Development of *Shigella dysenteriae*: The experimental procedures are described in the following. For the study of resistance development, *S. dysenteriae* (Komagome B III) was used. The starting inoculum was one drop of the 48-hour culture of the test bacterium grown in broth. Subsequently, one drop inoculum was taken from the tube and the highest concentration of the drug in which definite visible growth occurred, was determined. The development of drug resistance was observed during 30 subcultures. The development grade of resistance to the individual drug is shown with the index obtained by dividing the minimum bacteriostatic concentration in the primary culture (R_o) by that in the last subculture (R_m).

Experimental Results: The resistance obtained by *S. dysenteriae* to the six drugs are shown in Table. I.

TABLE I. Development of Resistance

Compound	R_0	R_m	R_0/R_m
Terramycin	20,000	5,000	4
Chloramphenicol	320,000	20,000	16
Nitrosen	640,000	80,000	8
Diarsen	640,000	80,000	8
Nitrofuracin	40,000	2,500	16
Dihydrostreptomycin	128,000	500	256

It is evident from Table I that dihydrostreptomycin is the substance in which the bacteria can most rapidly develop resistance, chloramphenicol and nitrofuracin are the substances in which the bacteria cannot so readily develop resistance, and terramycin and the two organic arsenicals are substances in which the bacteria hardly develop resistance. Thus, it may be said that the arsenical drugs could not be readily resisted by the bacterium.

Cross Resistance of *Shigella dysenteriae* between the Six Drugs: *S. dysenteriae* (Komagome B III) made resistant to dihydrostreptomycin, terramycin, chloramphenicol, nitrofuracin, Nitrosen, or Diarsen, were used for the tests. To determine the degree of cross-resistance developed, cross-resistance index was obtained by dividing minimum bacteriostatic concentration in the sensitive bacteria (R_o) by that in the resistant bacteria (R_m). The experimental results are shown in Table II.

TABLE II. Cross-Resistance of *Shigella dysenteriae*

Drug / Resistant Strain	Terramycin	Chloram-phenicol	Nitrosen	Diarsen	Nitro-furacin	Dihydro-strepto-mycin
Terramycin-resistant	4	0.5	2	4	0.25	0.5
Chloramphenicol-resistant	0.5	16	2	2	0.13	16
Nitrosen-resistant	1	1	8	4	0.13	0.5
Diarsen-resistant	1	2	2	8	1	0.5
Nitrofuracin-resistant	1	2	4	4	16	1
Dihydrostreptomycin-resistant	0.5	0.13	0.5	1	0.13	256

It is evident from Table II that the five resistant strains of bacteria, except the dihydrostreptomycin-resistant, are slightly resistant to Nitrosen and Diarsen. However, this experimental result may not be so significant for clinical use of these organic arsenicals, since various bacteria do not develop resistance to terramycin and chloramphenicol in practical clinical use, and these organic arsenicals may be seldom used for patients with terramycin- or chloramphenicol-resistant bacteria. From this fact it is suggested that the mode of action of terramycin, chloramphenicol, and nitrofuracin should resemble those of organic arsenicals.

Absorption of Arsenicals by Drug-resistant Bacteria: In the studies reported by Leonard and Smith[3], it has been shown that arsenic in arsenical drugs was absorbed into

3) Voegtlin, Dyer, Leonard: Public Health Repts.(U.S.), **38**, 1882(1923); Voegtlin, Smith: J. Pharmacol., **15**, 475(1920).

the cell body by arsenical-sensitive trypanosoma, but arsenical-resistant trypanosoma do not absorb arsenic through the cell membrane into the cell body. From this finding it is concluded that resistance development of trypanosoma caused the change of absorption characteristics of the cell membrane for the drugs. On the basis of these findings, the absorption grade of arsenic in both drug-sensitive and drug-resistant bacterial membrane was surveyed by the authors. The experimental results are shown in Table III.

TABLE III. Absorption Grade of Arsenic in Resistant and Sensitive Bacteria

Strain resistant to / Absorption grade(γ)	Diarsen	Nitrosen	Terra-mycin	Nitro-furacin	Chloram-phenicol	Dihydro-strepto-mycin	Sensitive bacteria
Vol. added to culture medium (γ)	100	100	100	100	100	100	100
Residue in supernatant (γ)	60	50	62	62	38	35	30
Vol. absorbed into bacterial body (γ)	35	49	38	42	60	65	70
Absorption into bacterial body (%)	35	49	38	42	60	65	70
Min. bacteriostatic concn. by Diarsen	80,000	160,000	160,000	160,000	320,000	640,000	640,000

For microdetermination of arsenic, the modified Magnason's method improved by the authors was used. This method will be described in detail in the next part of this series.

It is evident from these experimental data that the sensitive bacteria absorbed certain amount of arsenic, but the resistant bacteria showed smaller absorption of arsenic into the cell body and this decreased grade paralleled the resistance of the bacteria for the arsenical tested.

Thus, it may be assumed that the antibacterial effect of the arsenical drugs might depend on the absorption of arsenic by the bacteria.

Summary

1. *Shigella dysenteriae* does not develop resistance against 3-nitro-4-hydroxyphenyl-bis(α,β-dicarboxyethyl)thioarsenite(Nitrosen)and 3-amino-4-hydroxydiphenyl-(α,β-dicarboxyethyl)-thioarsenite (Diarsen).

2. A higher concentration of these arsenicals are required for the inhibition of the strains resistant to other drugs, except that of dihydrostreptomycin.

3. Bacteria resistant to arsenicals is unable to absorb the arsenic in such drugs into its cell.

(Received June 25, 1954)