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# VIRULENCE OF *ENTAMOEBA HISTOLYTICA* ACCORDING TO THE STRAINS IN KOREA

## Ⅲ. AMEBICIDAL RESPONSE OF ANTIAMOEBIC AGENTS ON SEVERAL STRAINS OF *ENTAMOEBA HISTOLYTICA* IN VITRO

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## INTRODUCTION

Although a number of drugs have been used in the treatment of *Entamoeba histolytica* infections, none of them were completely satisfactory against the trophozoites in tissue phase and cyst form in the intestinal cavity at the same time. However, several another synthetics and antibiotics, which were effective both to the *E.histolytica* in intestinal and liver tissue, have been reported in recent (Kradolfer and Jarumilinta, 1965; Powell et al., 1966; Doshi et al., 1968; Khambatta, 1968, 1969; Powell, 1969).

The present study was undertaken to examine the amebicidal activities of several traditional and newly appeared drugs, in vitro, utilizing five strains of *E. histolytica*.

## MATERIALS AND METHODS

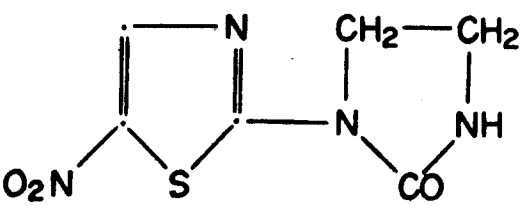
**Drugs:**

Emetine hydrochloride, carbarsone, diodoquin, chloroquine, atabrine, chloramphenicol and tetracycline were used as the traditional antiamoebic drugs, and niridazole(Ambilhar, CIBA), metronidazole(Flasinyl; Hanil Pharma. Ind. Co.) and No. 8603 substance(Fujisawa Pharma. Co., Japan) were employed as the newly appeared synthetics.

Niridazole is a nitro-thiazole derivative; 1-(5-nitro-2-thiazolyl)-2-imidazolidinone. It was shown to be active against *E. histolytica* in vitro and in experimental animals by Kradolfer and Jarumilinta (1965), and its usefulness both in intestinal and hepatic amebiasis was reported by Powell et al. (1966) and Doshi et al. (1968).

Metronidazole is a nitro-imidazole preparation; 1-β-hydroxyethyl-2-methyl-5-nitroimidazole, and it was reported to be potent as intestinal and systemic amebicide(Powell et al., 1966; Khambatta, 1968, 1969).

Followings are the structural formulas of niridazole and metronidazole.




1-(5-nitro-2-thiazolyl) 2-imidazolidinone

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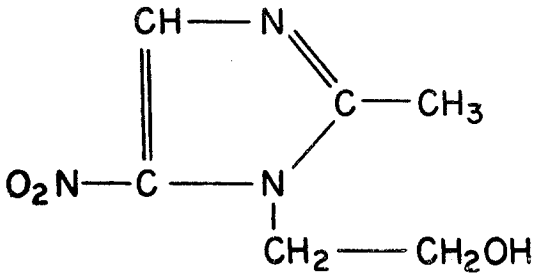

1-β-hydroxyethyl-2-methyl-5-nitroimidazole

No. 8603 substance is a new anti-protozoal antibiotic produced by a streptomycete, and it was provided through the courtesy of Dr. Sakai, H. (Research Lab., Fujisawa Pharma. Co., Japan).

**Strains of Amebae:**

Five strains of *E. histolytica* were used. They were collected from human cases of liver abscess, amebic dysentery and cyst-carriers, and maintained on the diphasic media adding calf serum and penicillin G (Cho, 1968) in association with mixed, unknown bacterial flora.

1) YS14-strain. This strain was isolated in January 1967 from a 63-year-old healthy cyst-passer's tsool(man, Cheju islander) and subcultured every other day.

2) YS15-strain. The ameba was obtained in January 1968 from the feces of a 51-year-old symptomless cyst-carrier (man, Suwon inhabitant) and maintained as above.

3) NAMBU II-strain. The strain was isolated by Dr. J.H. Cross(NAMRU No. 2, Taiwan) in 1967 from an acute amebic dysentery patient (Vietnam dweller) by rectoscopic method, and it was shared by him on September, 1968.

4) YS24-strain and 5)YS25-strain.

These strains originates from the trophozoites in liver abscess of a 33-year-old man and a 42-year-old man respectively in June 1969 at Severance Hospital, and associated with the bacteria flora of NAMRU II-strain ameba.

**Performance of the test:**

The amebicidal assay was carried out against the above five stranis of *E. histolytica* by the procedure described by Berberian et al.(1961). The modified Boeck and Drbohlav's diphasic medium (Faust et al., 1968), which consisted of coagulated egg slants covered with buffered saline (pH 7.0), was used throughout the test. Because some compounds were very sparlingly soluble in water, a carefully weighed quantity of each compound was ground in a sterile mortar and suspended in sterile buffered saline solution (pH 7.0). Serial dilutions were prepared in the same medium. Each tube contained 4.5 ml of the sample solution, and a control tube contained only the vehicle. The tubes were immediately seeded with 0.5 ml of a rich inoculum containing approximately 10,000 trophozoites of 48-hour culture of the different strains of amebae, and added with a small amount of rice starch. After 48 hours incubation at 37°C, growth of the amebae was checked microscopically. As the amebae proliferated at the bottom of the culture tubes, the supernatant fluid was decanted prior to examination, leaving approximately 0.5ml of sediments. One or more preparations were made from each concentration by placing one drop of the sediment on a microscope slide and it was covered with a 22-mm square cover slip. The entire area of the cover slip was carefully examined for trophozoites confirming the motilities of amebae under high power magnification. To determine the criteria of amebicidal action of each drug, complete abscence of living amoeba in each drug-culture mixture was conditioned by examining the amoeba parasite in three preparations of the sediment. The maximum dilution which killed all amebae after 48 hours of incubation was regarded to be the amebicidal titre.

## RESULTS

Ranges of amoebicidal activities of drugs are presented in Fig. 1. Three cycle logarithmic scale was used. With respect to range of activity against *E. histolytica*, the tested compounds fall into three distinct groups. Moderate ranges are observed at emetine hydrochloride, carbarsone and diodoquin with the dilutions of 1:5,000 to 1:20,000, 1:10,000 to 1:20,000 and 1:8,000 to 1:16,000 respectively.

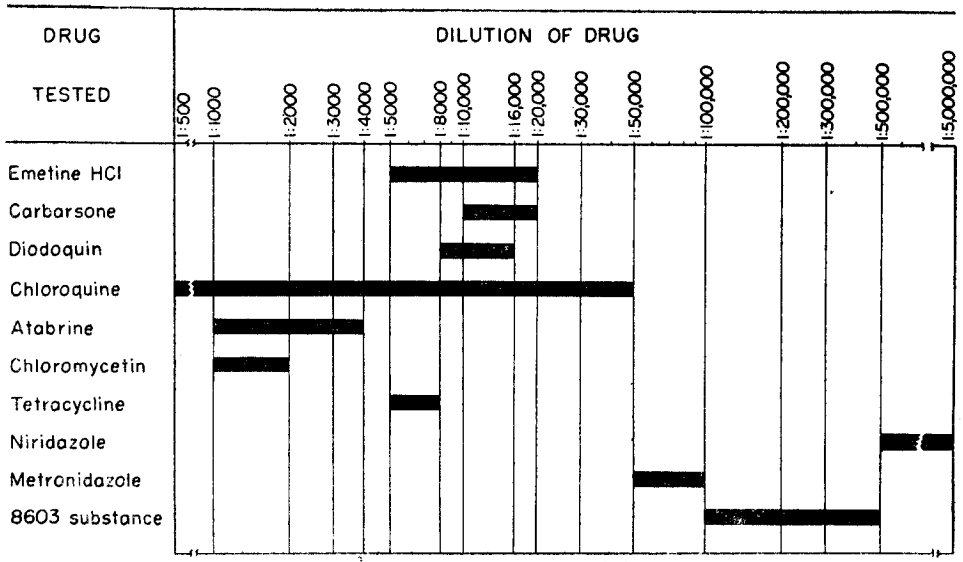


**Fig. 1.** Ranges of amoebicidal activities of drugs in vitro

Lower ranges are indicated at atabrine, chloramphenicol and tetracycline in the concentrations of 1: 1,000 to 1:4,000, 1:1,000 to 1:2,000 and 1:5,000 to 1:8,000 respectivey. Chloroquine covers relative-

**Table 1.** *Amoebicidal activity of conventional antiamoebic drugs in vitro.*

| Drug | Ameba strain | No. of exp. | Amebicidal titre | Effective drug conc. in μgm per ml |
|---|---|---|---|---|
| Emetine hydrochloride | YS 14 | 2 | 1:10,000 | 100 |
| | YS 15 | 2 | 1: 5,000 | 200 |
| | NAMRU II | 2 | 1:10,000 | 100 |
| | YS 24 | 2 | 1:20,000 | 50 |
| | YS 25 | 2 | 1:20,000 | 50 |
| Carbarsone | YS 14 | 2 | 1:20,000 | 50 |
| | YS 15 | 2 | 1:20,000 | 50 |
| | NAMBU II | 3 | 1:20,000 | 50 |
| | YS 24 | 3 | 1:10,000 | 100 |
| | YS 25 | 3 | 1:10,000 | 100 |
| Diodoquin | YS 14 | 2 | 1:16,000 | 62.5 |
| | YS 15 | 3 | 1:16,000 | 62.5 |
| | NAMRU II | 2 | 1: 8,000 | 125 |
| | YS 24 | 4 | 1: 8,000 | 125 |
| | YS 25 | 4 | 1: 8,000 | 125 |
| Chloroquine | YS 14 | 1 | 1:50,000 | 20 |
| | YS 15 | 4 | 1:50,000 | 20 |
| | NAMRU II | 3 | 1:50,000 | 20 |
| | YS 24 | 4 | 1: 500 | 2,000 |
| | YS 25 | 4 | 1: 1,000 | 1,000 |
| Atabrine | YS 14 | 2 | 1: 2,000 | 500 |
| | YS 15 | 2 | 1: 4,000 | 250 |
| | NAMRU II | 2 | 1: 1,000 | 1,000 |
| | YS 24 | 2 | 1: 2,000 | 500 |
| | YS 25 | 2 | 1: 2,000 | 500 |

ly wide range of activity, from lower to moderate, with the value of 1:500 to 1:50,000. Higher zone of activities are observed in newly appeared synthetics. Amebicidal concentration of niridazole shows 1:500,000 to 1:5,000,000, that of metronidazole is 1:50,000 to 1:100,000, and 8603 substance indicates 1:100,000 to 1:500,000.

Table 1 illustrates amebicidal activities of emetine, carbarsone, diodoquin, chloroquine and atabrine according to ameba strains. In the experiment of emetine hydrochloride, lower titres were observed at the intestine originated amebae, YS 14, YS 15 and NAMRU II-strain, with the values of 1:5,000 (=200 μgm/ml) to 1:1000 (=100 μgm/ml), whereas the liver originated amebae, YS 24 and YS 25-strain, indicated equally with 1:20,000 (= 50 μgm/ml).

On the contrary, the intestine originated amebae showed higher titres in carbarsone and diodoquin experiments with the dilutions of 1:20,000 (=50 μgm/ml) and 1:16,000 (62.5 μgm/ml), than liver originated amebae, which indicated with the concentrations of 1:10,000 (=100 μgm/ml) and 1:8,000(=125 μgm/ml)respectively.

Chloroquine was also amebicidal at higher titres in intestinal originated amebae with the dilution of 1:50,000 (=20 μgm/ml), than the liver borne amebae, which showed with 1:500 (=2,000 μgm /ml) to 1:1,000 (=1,000 μgm/ml). In atabrine, the titres of variation encountered in the intestine originated amebae. YS 14, YS 24 and YS 25 strains showed equally with the concentration of 1:2,000(=500 μgm/ml), but YS 15 strain and NAMRU II strain was 1:4,000 and 1:1,000 res-

**Table 2.** *Amoebicidal activity of antibiotics and newly appeared synthetics.*

| Drug | Ameba strain | No. of exp. | Amebicidal titre | Effective drug conc. in μgm per ml |
|---|---|---|---|---|
| Chloramphenicol | YS 14 | 2 | 1: 2,000 | 500 |
| | YS 15 | 2 | 1: 1,000 | 1,000 |
| | NAMRU II | 2 | 1: 1,000 | 1,000 |
| | YS 24 | 3 | 1: 2,000 | 500 |
| | YS 25 | 3 | 1: 2,000 | 500 |
| Tetracycline | YS 14 | 1 | 1: 5,000 | 200 |
| | YS 15 | 1 | 1: 5,000 | 200 |
| | NAMRU II | 2 | 1: 8,000 | 125 |
| | YS 24 | 2 | 1: 5,000 | 200 |
| | YS 25 | 2 | 1: 5,000 | 200 |
| Niridazole | YS 14 | 2 | 1:5,000,000 | 0.2 |
| | YS 15 | 1 | 1:1,000,000 | 1 |
| | NAMRU II | 3 | 1: 500,000 | 2 |
| | YS 24 | 4 | 1: 500,000 | 2 |
| | YS 25 | 3 | 1: 500,000 | 2 |
| Metronidazole | YS 14 | 1 | 1:100,000 | 10 |
| | YS 15 | 1 | 1:100,000 | 10 |
| | NAMRU II | 2 | 1:100,000 | 10 |
| | YS 24 | 3 | 1: 50,000 | 20 |
| | YS 25 | 2 | 1: 50,000 | 20 |
| 8603 substance | YS 14 | 1 | 1:100,000 | 10 |
| | YS 15 | 1 | 1:100,000 | 10 |
| | NAMRU II | 2 | 1:100,000 | 10 |
| | YS 24 | 3 | 1:500,000 | 2 |
| | YS 25 | 2 | 1:400,000 | 2.5 |

pectively.

Table 2 presents the amoebicidal effects of chloramphenicol, tetracyline, niridazole, metronidazole and 8603 substance, In chloramphenical, YS 14, YS 24 and YS 25-strains showed equal values of 1:2,000 as the results at atabrine, though YS 15 and NAMRU II strain showed lower titres with 1:1,000. In the tetracycline experiment, all tested ameba strains showed equally with the value of 1:5,000(=200 μgm/ml), except NAMRU II-strain, which were 1:8,000(=125 μgm/ml) at the amebicidal concentration.

In the newly appeared drugs, niridazole showed higher concentrations in cyst borne amebae (YS 14 and YS 15-strain) with the titres of 1:1,000,000 (=1μgm/ml) to 1:5,000,000 (=0.2 μgm/ml), than the trophozoite borne amebae (NAMRU II, YS 24 and YS 25-strain), which equally showed amebicidal activities at the dilution with 1:500,000 (=2 μgm/ml). In metronidazole, intestine originated amebae (YS 14, YS 15 and NAMRU Ⅱ strain) higher titres of 1:100,000(=10 μgm/ml), compared with the liver borne amebae (YS 24 and YS 25 strain, which the amebicidal concentration was 1:500,000(=2 μgm/ml).

In the experiment with 8603 substance, cyst borne amebae indicated lower titres (1:100,000=10 μgm/ml) than the trophzoite borne amebae (1:400,000 to 1:500,000) in constrast with niridazole and metronidazole, and exerted an excellent antiamoebic activities in vitro.

## DISCUSSION

Effective concentration for antiamoebic activity of drugs varies in vitro according to conditions of media tested; oxygen tension, concomitant bacterial flora and strains of *E. histolytica*.

There have been several reports in related to the ranges of amebicidal activity of drugs in vitro. Dennis et al. (1949) used Hansen's egg infusion medium for this study. The ranges for amebicidal activity of emetine hydrochloride, carbarsone, diiodo-oxyquinoline and chloroquine were at the concentration of 1:15,000 to 1:70,000, 1:35,000 to 1:40,000, 1:500 to 1:3,000 and 1:1,000 to 1:10,000 respectively. Berberian et al. (1961) reported that an effective amebicidal activity of emetine was observed at a concentration of 1:5,000, but in another test (1961) he also experienced it at 1:7,500 using Hansen's egg medium. In the present study, ranges for amebicidal activities of emetine hydrochloride, carbarsone,diodoquin and chloroquine were at the concentration of 1:5,000 to 1:20,000, 1:10,000 to 1:20,000, 1:8,000 to 1:16,000 and 1:500 to 1:50,000, and settled at almost similar titres of previous reports, though diodoquin showed higher titre. Thompson et al. (1950) reported an effective concentration of diido-oxyquinoline under aerobic condition using Balamuth egg yolk medium was 150 μgm/ml. It is an approximate value to the present data (62.5~125 μgm/ml). The diverseness of the results might be due to that diodoquin was insoluble in water, and was compelled to use it only as suspension.

Although any previous reports with atabrine, tetracycline and metronidazole were not available to compare, the present data showed lower ranges of antiamebic activities at the concentrations of 1:1,000 to 1:4,000 by atabrine and 1:5,000 to 1:8,000 by tetracycline, but higher range was observed with metronidazole at the concentration of 1:50,000 to 1:100,000.

Thompson et al. (1950) also reported that the antiamebic activity of chloramphenicol, either by aerobically or anaerobically in Balamuth egg infusion medium, was shown at the concentration of 286 to 375 μgm/ml. However, much larger concentrations (1,500~2,000 μgm/ml) were required when L.E.L. medium was used, and they considered that the lower activity in L.E.L. medium was due to the binding of the antibiotics with egg-protein and absorption on coagulated yolk. The chloramphenicol in the present study utilizing several different strains of *E. histolytica* showed the antiamebic titers at 1:1,000 (=500 μgm/ml) to 1:2,000(=1,000 μgm/ml) in the modified Boeck and Drbohlav's

diphasic medium which the 0.85% phosphate buffered saline (pH 7.0) was used as overlay. The difference between diphasic and monophasic medium seemed not the important position than the component part of liquid phase of medium for the enhancement of antiamebic activities of the drugs.

Kradolfer and Jarumilinta (1965) tested the amoebicidal activities of niridazole, emetine and entobex, using strains of carrier originated (SF and Moore strain) and dysentery originated amebae (Shaw and 80S strain), and concluded that the minimum inhibitory concentration of niridazole was 10 μgm/ml. The values were almost constant to all strains and was more active against some strains of *E. histolytica* as compared with emetine and entobex. The present data also show that amebicidal potency of niridazole is approximately five times of the above reports at the concentration with 0.2～1 μgm/ml to cyst originated amebae and 2 μgm/ml to dystentery and liver abscess originated amebae.

No. 8603 substance (Fujisawa Pharma. Co., Japan) is a white needled crystal, fairly soluble in chloroform and dichloroethane, slightly soluble in alcohol and insoluble in water, ether and hexane, and stable in alkaline, but unstable in acid. Sakai (1969) reported that the minimum inhibition concentration of this drug against *Trichomonas vaginalis* was 0.2～0.5 μgm/ml, and that against fungi and yeasts were less than 10 μgm/ml, and acute toxicity on mice was 12.5 mg/kg. In the present study, this drug was amebicidal at the concentration of 2～10 μgm/ml, and the value was closely approximated to metronidazole, which showed amebicidal activity at 10-20μgm/ml. If animal and clinical experiments for *E. histolytica* respond successfully, No. 8603 substance is anticipated as a satisfactory antiamebic drug as metronidazole. Metronidazole has been shown by several workers (Powell, 1967 and 1969; Khambatta, 1968 and 1969) to be highly active against *E. histolytica* both within the bowel and systemically, without any significant toxicity, with minimal side effects, less duration of treatment and with easier acceptibility for patient.

In the present results, ameba strains were compared arbitrarily with two groups according to their originated natures; intestine originated to liver originated amebae, or cyst borne to trophozoite borne amebae. Emetine and No. 8603 substance showed higher titres at intestine originated amebae, while, carbarsone, chloroquine and metronidazole showed lower titres than liver originated amebae. Chloramphenicol showed higher titres to liver originated than intestine originated amebae except YS 14-strain. Diodoquin and niridazole showed higher titres to trophozoite borne than cyst borne amebae. Significant differences were noted in each groups, while atabrine showed irregular titres and tetracycline showed almost equal titres between ameba strains. If it is considered that YS 24 and YS 25 strains were associated with the bacterial flora of NAMRU II strain ameba, concomitant bacterial flora is not regarded to have any significant effect upon the antiamebic activity of drugs in vitro.

Kradolfer and Jarumilinta (1964) ascribed the differences on minimum inhibitory concentration of antiamebic drugs in vitro in related to the variety of media, and test methods with several strains of amoeba, but they neglected to check the results by the differences of ameba strains. However, the data in the present study manifest the existence of considerable variety for amebicidal activity of drugs in vitro among *E. histolytica* strains.

## SUMMARY

The amebicidal activity of traditional antiamoebic drugs(emetine, carbarsone, diodoquin, chloroquine, atabrine, chloramphenicol and tetracycline) and newly appeared chemicals (niridazole, metronidazole and No. 8603 substance) were assayed by in vitro experiment using five strains of human originated *E. histolytica*. The variety of amebicidal activity of drugs by the strains were discussed.

1) Ranges of amoebicidal activity of traditional antiamoebic drugs kept almost similar titers of previous reports at the concentrations; 1:5,000 to

1:20,000 with emetine hydrochloride, 1:10,000 to 1:20,000 with carbarsone, 1:8,000 to 1:16,000 with diodoquin, 1:50,000 with chloroquine, 1:1,000 to 1:4,000 with atabrine, 1:1,000 to 1:2,000 with chloramphenicol and 1:5,000 to 1:8,000 with tetracycline. The newly appeared chemicals showed higher amebicidal titres at the concentrations; 1:500,000 to 1:5,000,000 with niridazole, 1:50,000 to 1:100,000 with metronidazole and 1:100,000 to 1:500,000 with No. 8603 substance.

2) Emetine, chloramphenicol and No. 8603 substance showed amebicidal activities at lower concentration to intestine originated amebae (YS 14, YS 15 and NAMRU Ⅱ strain) than to liver originated amebae (YS 24 and YS 25 st rain), while carbarsone, chloroquine and metronidazole showed the activity at higher concentrations.

Diodoquin showed lower amebicidal titres to trophozoite borne amebae (NAMRU Ⅱ, YS 24 and YS 25 strain) than to cyst borne amebae (YS 14 and YS 15 strain), but niridazole showed controverse results. The concentration of atabrine for amebicidal activity was not constant according to strains of the amoeba, but tetracycline showed almost settled titers.

## ACKNOWLEDGEMENTS

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=國文抄錄=

## 抗아메바藥劑의 培養痢疾아메바에 對한 株別作用

延世大學校 醫科大學 寄生蟲學教室

趙 基 穆·李 俊 永·蘇 鎭 琯

從來 使用되어온 抗아메바藥劑中 emetine, carbarsone, diodoquin, chlorquin, atabrine, chloramphenicol, tetracycline과 抗아메바新劑인 niridazole, metronidazole 및 No. 8603 substance(日本藤澤藥品會社製)를 使用하여 5株의 培養痢疾아메바에 對한 殺蟲作用을 觀察하고 아울러 藥劑의 痢疾아메바에 對한 株別 殺蟲力의 多樣性에 關하여 檢討한 바 그 結果를 要約하면 다음과 같다.

1) 從來 使用되어온 抗아메바劑의 殺蟲作用濃度限界는 現在까지의 諸報告와 比較한 바 거의 近似한 値가 觀察되었으며 殺蟲濃度稀釋値는 emetine이 1:5,000 乃至 1:20,000, carbarsone이 1:10,000 乃至 1:20,000, diodoquin이 1:8,000 乃至 1:16,000, chloroquine이 1:500 乃至 1:50,000, atabrine이 1:1,000 乃至 1:4,000, chloramphenicol이 1:1,000 乃至 1:2,000이었으며 tetracycline이 1:5,000 乃至 1:8,000이었다.

抗아메바新劑에 있어서는 一般的으로 高値의 殺蟲濃度稀釋倍數가 觀察되었으며 niridazole이 1:500,000 乃至 1:5,000,000, metronidazole이 1:50,000 乃至 1:100,000이었고 No. 8603 substance는 1:100,000 乃至 1:500,000이었다.

2) 痢疾아메바의 株別 殺蟲力에 있어서는 emetine, chloramphenicol 및 No. 8603 substance는 腸起原性아메바諸株 (YS 14, YS 15, NAMRU II株)에 對하여는 肝臟起原性아메바諸株 (YS 24, YS 25株)에 比하여 低値의 殺蟲濃度가 觀察되었으며 carbarsone, chloroquine 및 metronidazole에 있어서는 腸起原性아메바諸株에 對하여 高値이었다. diodoquin은 榮養型아메바 起原諸株(NAMRU II, YS 24, YS 25株)에 對하여는 씨스트起原性아메바諸株(YS 14, YS 15株)에 比하여 低値의 殺蟲濃度를 보였으며 niridazole에 있어서는 그와 反對이었다. atabrine은 아메바 各群間에 殺蟲濃度의 差異가 一定치 않았으며 tetracycline에 있어서는 各群間에 있어서 거의 一律的인 殺蟲濃度가 觀察되었다.