

## Virulence of *Entamoeba histolytica* According to the Strains in Korea

### II. Studies on the Pathogenicity of *Entamoeba histolytica* Strains in Rats\*

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#### ABSTRACT

The experimental infection of rats with seven strains of *Entamoeba histolytica* were carried out according to animal ages, number of inoculated amebae, rat strain differences and rat-culture passages. The rat cecal scoring technique of Neal (1951) was utilized to measure the invasiveness of the parasite. The results are summarized and concluded as follows;

1. In the infection of Sprague-Dawley strain rat with YS9-strain and NAMRU II-strain amebae, which were confirmed highly invasive to the membrane of cecum of rabbits in the previous reports (Cho, 1968; Cross, 1968), remarkable invasiveness was observed in the 30-day-old rat groups with the average cecal score above 5.0.

Although no statistical differences of virulence by the number of inoculations showed in rat groups, the cecal scores were markedly reduced in the 50,000 amebae inoculated rats.

2. The hybrid albino rats were considered unsuitable for virulence study of *E. histolytica*, since

the invasiveness of the amebae was inconsistent.

3. The virulences of YS 14 and YS16-strains from cyst carrier showed no virulence, YS 15 from cyst carrier and YS 24 from liver abscess were moderately invasive, and only YS25 from liver abscess showed highly invasive as with YS 9 and NAMRU II-strain amebae.

By rat-culture passage, YS14-strain and YS24-strain amebae showed marked increase of invasiveness.

It was presumed that the rat-culture passage should be indispensably supplemented in the studies on the virulence of *E. histolytica*.

#### INTRODUCTION

Until Neal's successful report (1951) utilizing Wistar strain rats, rats had been regarded as unsuitable hosts in the studies of infections of *E. histolytica*, and were considered to be rather refractory to infections with *E. histolytica*. After Thompson et al. (1954) did a comparative virulence study of two strains of *E. histolytica* using Sprague-Dawley strain rats, the experimental infection of rats with *E. histolytica* was renovated. It opened a new line of inquiry into some of the problems of amebiasis; chemotherapy of amebiasis, virulence of ameba strains ect. (Vincent and Neal, 1960; Bird and Neal, 1962; Singh et al., 1963;

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Kradolfer and Jarumilinta, 1965; Healy and Gleason, 1966; Neal et al., 1968; Neal and Johnson, 1968).

The present study deals with a comparison of the virulence of *E. histolytica* to Sprague-Dawley strain rats according to the ages of the animal, number of inoculated amebae, rat strain differences and rat-culture passages using seven strains of *E. histolytica*. The cecal scoring technique by Neal (1951) was applied.

## MATERIALS AND METHODS

### Strains of *E. histolytica*:

As listed in Table 1 ameba strains were obtained 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) YS9-strain. This strain was isolated in September 1966 from the feces of a 51-year-old man with liver abscess (Cheju islander), and subcultured every other day.

2) NAMRU II-strain. The strain, obtained from NAMRU No. 2 (Taiwan) through the courtesy of Dr. J. H. Cross, was isolated in 1967 from an acute dysentery patient (Vietnam dweller) by rectoscopic method, and subcultured.

3) YS14-strain. The ameba was isolated in January 1967 from a 63-year-old healthy cyst-passer's stool (man, Cheju islander), and maintained by subculture.

4) YS 15-strain The strain was collected in Janu-

ary 1968 from the feces of a 51-year-old symptomless cyst carrier (man, Suwon inhabitant), and maintained as above.

5) YS16-strain. This was isolated in February 1968 from a 45-year-old woman, healthy cyst-passer, at Severance Hospital, and subcultured.

6) YS 24-strain and 7) YS 25-strain. These strains were collected in June 1969 from trophozoites in the liver abscess of a 33, and a 42-year-old man respectively at Severance Hospital and associated with the bacterial flora of NAMRU II-strain ameba, and maintained as above.

### Animals:

Sprague-Dawley strain and hybrid rat were used. Sprague-Dawley strain has been bred in animal quarter of Yonsei University Medical College Laboratory. Hybrid was obtained from local supply houses. The animals were placed under the normal diet throughout the entire experiment.

### Inoculation technique:

The animals were inoculated with *E. histolytica* intracecally following the technique described by Jones (1946). The sediments from several media of 48-hour-old amebae were pooled and centrifuged at 1500 rpm for 5 minutes. The sediment was suspended in warmed normal saline (37°C) and spun as before. Supernate was decanted and amebae were counted by Spencer Bright-Line Improved Neubauer counting chamber, and the number of amebae per inoculum was adjusted with warmed normal saline. The rats were anesthetized with ether, and an incision was made slightly to the left of the midline of the body and the cecum

Table 1. Strains of *E. histolytica*

Source Strain	Material	Sex	Age	Locality	Date, subcultured
YS- 9	Cyst in stool of liver abscess patient	M	51	Cheju Island	Nov., 1966
NAMRU II	Trophozoite in dysentery stool	—	—	Vietnam	Jan., 1968
YS-14	Cyst from cyst carrier	M	63	Cheju Island	Jan., 1968
YS-15	"	M	49	Suwon, Kyonggi-do	Feb., 1968
YS-16	"	F	45	Severance Hospital	Feb., 1968
YS-24	Trophozoite in liver abscess	M	33	"	June, 1969
YS-25	"	M	42	"	June, 1969

was exposed. The inoculum was delivered with a 23 gauge needle and a one ml tuberculin syringe. The inoculum was done toward the bline end of the caecum from a point anterior to the junction of the caecum and colon. Abdominal muscles and skin were sutured with #1 surgical silk.

#### Scoring technique:

The rats were killed by ether anesthesia one week after inoculation and the entire caecum was removed and slit opened. The condition of the caecal contents were recorded by the procedure of Neal (1951). A portion of contents was examined for the parasite as a direct mount using saline, and it was inoculated in the culture media and examined after incubation.

After the contents were observed and scored, the caecum was agitated in a cold normal saline. Inner wall of caecum was examined and also scored by Neal's method (1951). All ulcers visualized were aspirated with Pasteur pipett and confirmed the presence of amebae in tissues. In the caecum which had lesions, the amebae were found in all cases, and occasionally the colon was involved, but was never observed from rectum.

The followings are the criteria for scoring used.

Contents: Normal	0
Slightly less solid than normal	1
Slightly mucoid	2
Mucoid, some solid matter present	3
No, solid matter, white or yellow mucus only	4
Wall: Normal	0
Slight thickening	1
Marked local thickening and contraction	2
Extensive thickening and contraction	3
Caecum shapeless, extensive ulceration with abscess formation	4

The average cecal score was calculated only from infected rats (Fig. 1). Portions of the examined ceca were fixed, sectioned and stained by Gomori's trichrome staining technique in order to determine histopathologic changes and presence of amebae in

the formed lesions (Fig. 2, 3, 4 & 5).

#### Rat-culture passage:

This study was conducted in order to investigate loss or increase of invasiveness of the organisms according to the strains by passing them by turns through rats ceca and culture.

The parasite associated with rat born bacterial flora, which was obtained at the first rat passage, was subcultured until it becomes familiar to the culture media and presents luxurial propagation, and rats cecal inoculations were followed. Then, the animals were killed and ceca were scored.

## RESULTS

### 1. Invasiveness of *E. histolytica* in rats according to the host ages

YS 9-strain and NAMRU II-strain amebae were applied to Sprague-Dawley strain rats. Each animal was inoculated equally with 100,000 organisms.

In the YS 9-strain ameba infected rats (Table 2), four groups were compared; 30-day-old (Group A1, A2), 40-day-old (Group B1, B2), 60-day-old (Group C) and 90-day-old (Group D). The weight range of group A was 29-40 grams before inoculation and increased to 40-50 grams by the time of autopsy. Numbers of animals were 5 in Group A1 and 6 in Group A2, and the rate of infection was 100 per cent respectively. Average cecal scores showed  $7.8 \pm 0.1$  in Group A1 and  $5.0 \pm 0.43$  in Group A2. Group B rats weighed 43-50 grams before inoculation and 47-57 grams at autopsy. Numbers of rats were 5 in Group B1 and 3 in Group B2, and all animals were infected. Average cecal scores showed  $4.2 \pm 0.48$  in Group B1 and  $3.4 \pm 0.22$  in Group B2. Group C weighed 8.4-84 grams before inoculation and 102-105 grams at autopsy. Two rats were infected, and average cecal score showed  $2.5 \pm 0.31$ . In group D, 5 rats weighed 160-240 grams before inoculation and the same at autopsy. Infection rate was 80 per cent and average cecal score showed  $2.8 \pm 0.32$ . In Group A, the cecal scores were all above 5.0.

**Table 2. Invasiveness of YS9-strain of *E. histolytica* in Sprague-Dawley strain rats according to ages (Number of inoculation 100,000.)**

Group	Age (days)	Weight range(gm)		No. of animals		Average cecal scores		
		Before inoculation	At autopsy	infected /inoculated	% infected	Wall	Contents	Total±SE
A1	30	29-40	40-50	5/5	100.0	3.8	4.0	7.8±0.10
A2	"	30-40	"	3/6	50.0	2.3	2.7	5.0±0.43
B1	40	43-50	47-57	5/5	100.0	1.8	2.4	4.2±0.48
B2	"	43-45	"	3/3	100.0	1.7	1.7	3.4±0.22
C	60	84-86	102-105	2/3	66.7	0.5	2.0	2.5±0.31
D	90	160-240	160-240	4/5	80.0	1.3	1.5	2.8±0.32

**Table 3. Invasiveness of NAMRU II-strain of *E. histolytica* in Sprague-Dawley strain rats according to ages (Number of inoculation; 100,000.)**

Group	Age (days)	Weight range(gm)		No. of animals		Average cecal scores		
		Before inoculation	At autopsy	infected /inoculated	% infected	Wall	Contents	Total±SE
A	20	20-24	21-33	8/8	100.0	1.6	1.8	3.4±0.29
B	30	32-45	33-45	5/6	83.3	2.6	3.0	5.6±0.36
C1	50	60-70	67-92	3/3	100.0	1.0	1.0	2.0±0.00
C2	"	52-56	"	5/6	83.3	1.0	0.0	1.0±0.40
C3	"	60-72	"	5/6	83.3	0.2	0.0	0.2±0.95

Among rats inoculated with NAMRU II-strain (Table 3), three groups were compared; 20-day-old (Group A), 30-day-old (Group B) and 50-day-old (Group C1, C2, C3).

Since Sprague-Dawley strain rats were not fully weaned up to 28-day-old, mother animal was housed together in Group A. Group A rats, including 8 animals, weighed 20-24 grams before inoculation and 21-33 grams at autopsy. Infection rate was 100 per cent, and average cecal score showed  $3.4 \pm 0.29$ . Group B rats, including 6 animals, weighed 32-45 grams before inoculation and 33-45 grams at autopsy. Infection rate was 83.3 per cent, and average cecal score showed  $5.6 \pm 0.36$ . Three rats

of Group C1, 6 rats of Group C2, 6 rats of Group C3 weighed 60-70 grams, 52-56 grams and 60-72 grams respectively before inoculation, and 67-72 grams equally at autopsy. Infection rates were 100 per cent, 83.3 per cent and 83.3 per cent in the respective groups, and average cecal scores showed  $2.0 \pm 0.0$  in Group C1,  $1.0 \pm 0.4$  in Group C2, and  $0.2 \pm 0.95$  in Group C3. A high degree of invasiveness was observed in Group B.

## 2. Virulence of *E. histolytica* in rats in relation to the number of inoculated amebae (Table 4)

The animals were of fully weaned Sprague-Dawley strain rats, which were 30-day-old and 30-40

**Table 4. Virulence of YS9-strain of *E. histolytica* in Sprague-Dawley strain rats in relation to the number of inoculated amebae**

Group	Weight range (gm)	No. of inoculated amebae (thousand)	No. of animals		Average cecal scores		
			infected /inoculated	% infected	Wall	Contents	Total±SE
A1	29-40	100	5/5	100.0	3.8	4.0	7.8±0.10
A2	30-40	"	3/6	50.0	2.3	2.7	5.0±0.43
B	30-38	50	4/5	80.0	1.5	1.8	3.3±0.66
C	36-40	200	4/5	80.0	3.0	3.0	6.0±0.38
D1	35-39	500	5/6	83.3	3.6	3.6	7.2±0.25
D2	30-35	"	5/7	71.4	1.6	1.6	3.2±0.63

**Table 5. Invasiveness of YS 9-strain of *E. histolytica* in hybrid albino rats which were inoculated with 100,000 amebae**

Group	Weight range (gm)	No. of animals		Average cecal scores		
		infected /inoculated	% infected	Wall	Contents	Total±SE
1	35-40	5/6	83.3	2.8	3.6	6.4±0.25
2	38-40	3/5	60.0	0.0	0.7	0.7±0.21
3	33-38	5/5	100.0	0.8	1.6	2.4±0.49
4	25-35	4/5	80.0	1.5	1.8	3.3±0.65
5	36-40	4/5	80.0	3.0	3.0	6.0±0.66
6	35-40	4/5	80.0	2.5	0.0	2.5±0.95

**Table 6. Virulence of *E. histolytica* strains in Sprague-Dawley strain rats**

Ameba strain	Weight range of rats (gm)	No. of animals		Average cecal scores		
		infected /inoculated	% infected	Wall	Contents	Total±SE
YS 14	22-35	3/4	75.0	1.0	0.0	1.0±0.22
YS 15	27-40	4/5	80.0	1.5	1.5	3.0±0.38
YS 16	32-43	5/5	100.0	1.2	1.0	2.2±0.23
YS 24	30-31	2/2	100.0	1.5	1.5	3.0±1.73
YS 25	20-28	3/3	100.0	3.3	3.0	6.3±0.75

grams in weight. The ameba was of YS9-strain. The rats were inoculated with 50,000 organisms in Group B, 200,000 in Group C and 500,000 in Group D1 and D2.

In Group B, including 5 rats, infection rate was 80 per cent, and average cecal score showed  $3.3 \pm 0.66$ . In Group C, including 5 rats, infection rate was 80 per cent, and average cecal score was  $6.0 \pm 0.38$ . In Group D1, including 6 rats, infection rate was 83.8 per cent, and average cecal score showed  $7.2 \pm 0.25$ . In Group D2, including 7 rats, infection rate was 71.4 per cent, and average cecal score was  $3.2 \pm 0.63$ . Comparing the results with Group A, which was inoculated with 100,000 amebae as in the preceding experiment, the average cecal scores showed no statistical differences in Group B, C and D.

### 3. Invasiveness of *E. histolytica* in hybrid albino rats (Table 5)

The animals were of fully weanlings. Each group was composed of the same mother-born rats, and each animal was inoculated equally with 100,000 trophozoites of YS 9-strain ameba.

The experiment was performed in 6 Groups.

In Group 1, including 6 rats, infection rate was 83.3 per cent, and average cecal score showed  $6.4 \pm 0.25$ . In Group 2, including 5 rats, infection rate was 60 per cent, and average cecal score showed  $0.7 \pm 0.21$ . In Group 3, including 5 rats, infection rate was 100 per cent, and average cecal score showed  $2.4 \pm 0.49$ . In Group 4, including 5 rats, infection rate was 80 per cent, and average cecal score showed  $3.3 \pm 0.65$ . In Group 5, including 5 rats, infection rate was 80 per cent, and average cecal score showed  $6.0 \pm 0.66$ . In Group 6, including 5 rats, infection rate was 80 per cent, and average cecal score showed  $2.5 \pm 0.95$ .

In general, the results with hybrid albino rats were conflicting.

### 4. Virulence of *E. histolytica* regarding ameba strains in rats (Table 6)

The rats were of the 30-day-old Sprague-Dawley strain, and each group was composed of the same mother-born rats. Each animal was inoculated with 100,000-200,000 amebae. YS 14, 15 and 16-strain amebae, originating from cyst-carriers, and YS 24 and 25-strain amebae, isolated from liver abscess, were used.

**Table 7-a. Results of rat-culture passage of *E. histolytica*; YS 14 strain**

Group	Duration on culture	Weight range of rats(gm)	No. of animals		Average cecal scores		
			infected /inoculated	% infected	Wall	Contents	Total±SE
A	17 months	22-35	3/4	75.0	1.0	0.0	1.0±0.22
	30 days						
B		27-38	2/4	50.0	2.5	3.0	5.5±0.90

**Table 7-b. Results of rat-culture passage of *E. histolytica*; YS 24 strain**

Group	Duration on culture	Weight range of rats(gm)	No. of animals		Average cecal scores		
			infected /inoculated	% infected	Wall	Contents	Total±SE
A	15 days	30-31	2/2	100.0	1.5	1.5	3.0±1.73
	30 days						
B		40-45	2/2	100.0	2.0	2.0	4.0±0.00

In the YS14-strain ameba group, the infection rate was 75 per cent, and average cecal score showed  $1.0 \pm 0.22$ . In the YS15-strain ameba group, the infection rate was 80 per cent, and average cecal score showed  $3.0 \pm 0.38$ . In the YS16-strain group, the infection rate was 100 per cent, and average cecal score showed  $2.2 \pm 0.23$ . In the YS24-strain group, the infection rate was 100 per cent, and average cecal score showed  $3.0 \pm 1.73$ . In the YS 25-strain group, the infection rate was 100 per cent, and average cecal score showed  $6.3 \pm 0.75$ . Comparing these results with YS 9-strain and NAMRU II-strain amebae of the preceding results, YS 14 and YS16-strain showed no virulence, YS 15 and YS24-strain were moderately invasive, and only YS 25-strain was determined as a highly invasive strain.

#### 5. Rat-culture passage of *E. histolytica* (Table 7-a & 7-b)

In order to study the virulent differences of ameba strains between the pathological results in animal and clinical manifestation, these experiments were performed.

The cecal score by cyst born YS 14-strain ameba had increased from  $1.0 \pm 0.22$  (Group A) to  $5.5 \pm 0.90$  at second rat passage (Group B), but no changes of invasiveness were observed in other

cyst born ameba groups (YS 15 and YS 16-strain amebae).

The cecal score by liver abscess born YS 24-strain ameba increased from  $3.0 \pm 1.73$  in Group A to  $4.0 \pm 0.0$  at the second rat passage (Group B). On the other hands, YS 9, NAMRU II and YS 25-strains showed no differences and kept their virulences showing cecal score above 5.0 by rat-culture passages.

### DISCUSSION

In the use of animals for experimental infection of *E. histolytica*, a considerable variety of the species have been employed; monkeys by Ratcliffe (1932) and Hegner (1932), dogs by Faust (1932) and Artigas & Beaver (1961), kittens by Boeck & Drbohlav (1925) and Meleny & Frye (1934), rabbits by Westphal (1941) and Tobie (1949), guinea pigs by Carrera & Faust (1949) and Taylor et al. (1950), rats by Jones (1946) and Neal (1951), and hamsters by Reinerston & Thompson (1951) and Jarumilinta & Maegraith (1962).

Species of experimental animals should be selected primarily according to the objectives of the studies, but the cost, size, attainability, infectivity and tissue reaction of animals also might not be excluded from

consideration. All the possible conditions which may affect the invasiveness of the parasite through tissues of animals are related with species, strain and age of animals and number and strain of inoculated amebae. Factors of the enhancement of susceptibility by the parasite are also considered; bacterial flora, deficiencies of nutrient substances, lowered resistance of the host, and environmental climate etc.

There have been several reports related to the age of animals and number of inoculated amebae. Neal (1951) employed 3-week-old Wistar strain rats, and Taylor et al. (1951) utilized 30–104 grams weighed Osborne-Mendel strain rats. Thompson et al. (1954) compared the 18-day-old rats weighing 31–44 grams to 22-day-old rats weighing 43–50 grams, and reported no significant differences of the infectivity between two groups. They used the amebae as suspension of 2.5 per cent gastric mucin saline solution for inoculation. Villarejos (1962) reported that the infectiveness was not enhanced by cortisone administration in 25–35 grams rats. Neal et al. (1968) used 25–35 grams Chester-Beatty strain rats for comparative study of virulence of *E. histolytica* strains.

In the present result (1) the remarkable invasiveness was observed at 30-day-old rat group weighing 30–45 grams, without any previous or simultaneous treatment by inoculation being done. YS 9 and NAMRU II-strain amebae were employed in this experiment since Cho (1968) and Cross (1968) reported their respective notable virulency.

There have been considerable reports in regard to the number of the inoculated amebae to different animals; Villarejos (1962), Cho (1968), Kradolfer & Jarumilinta (1965), Carrera & Faust (1949), Taylor et al. (1950), Krupp & Faust (1959), Isokawa (1960), Biagi-F et al. (1962), Phillips (1964), Takada (1956), Bird & Neal (1961), Neal et al. (1968), Neal & Johnson (1968).

In the present study since the Spague-Dawley strain rats began to feed from 12-day-old and became fully weanlings at 4-week-old weighing from 30–

45 grams, the maternal animal was housed unavoidably together with its litters during the experiments.

The data of result (2) suggest a sufficient number of *E. histolytica* which may cause pathological changes in rats cecum. Higher invasiveness was observed in more than 100,000 amebae inoculated groups, and 50,000 amebae inoculated group showed lower grade of cecal score. No statistical differences of invasiveness were observed among 100,000, 200,000 and 500,000 amebae groups.

Although Group D2 showed a discrepancy, the cecal score of Group D in general was regarded as more than 5.0, since the mean cecal score of Group D1 and D2 became 5.2. It is explained that the "crowding effect" among more than 100,000 amebae inoculated groups influenced the invariable invasiveness, since it was not always correlated with the increase of the inoculated number.

Read (1951) and Roberts (1966) interpreted the "crowding effect" as being a manifestation of worm competition for available host dietary carbohydrate in *Hymenolepis diminuta* infection on rats. In the present study, it seems to provide similar effect for the colonization of over-crowded *E. histolytica* on the mucus membrane of rat cecum. However, it can be presumed that the satisfactory results will be obtained by inoculating the amebae more than 100,000 to rats.

Hybrid rat has been known as unsuitable host for the infection of *E. histolytica*. Takada (1956) raised up the infectivity by previous treatment with nitrogen mustard B or by inoculating simultaneously with both *E. histolytica* and 10% mucin solution into cecum of young hybrid rats. Healy and Gleason (1966) reported that the invasiveness of *E. histolytica* in Wistar rats were more constant, while Sherman strain gave inconsistent results. The Wistar strain animals weighed from 20–35 grams at 18–21 day old, and the report by Sherman rats varied in weight from 25 to 45 grams. In the present experiment with hybrid albino rats, the cecal scores varied from 0.7 to 6.4 in spite of

the difference of weight range being only within 5 grams. For the above reason hybrid rats are to be regarded as unsuitable for the study of invasiveness of *E. histolytica*.

During the experiment, it was not available to get Wistar strain rat in Korea. However, Sprague-Dawley strain animal was considered as a competent animal for the study of virulence of *E. histolytica*.

There have been reports concerning tissue invasion of *E. histolytica*. Since Westphal (1937) infected himself and tried to confirm the evidence of host-bacteria-ameba-environmental relationships, many studies have been conducted by workers to determine the conditions causing invasion. Assuming all the clinical and experimental evidence into consideration, Meleney (1957) presumed that *E. histolytica* was normally a commensal organism living in the lumen of colon, feeding on bacteria and other fecal constituents and form cysts during its life cycle in healthy hosts. However, he defined it from other harmless intestinal amebae, by its invading ability and lytic action.

Recently, *E. histolytica* like strains, indistinguishable with typical *E. histolytica*, were isolated from human feces. The strains are Huff strain (Beaver et al. 1956), Laredo strain by Dr. F.H. Connel (Dreyer, 1961), and JA and JG strains (Entner & Most, 1965). Goldman and Cannon (1967) compared these so called "low temperature strains" with the "regular" strains of *E. histolytica* using fluorescent-antibody test and animal test, and reported that a significant antigenic and pathogenic differences were existed between the two type of amebae, even when they were both cultured at 35°C. Krupp (1966) demonstrated the immunologic differences in the above two types of ameba by immunoelectrophoretic method. Kahn and Meero-vitch (1968) did a qualitative and quantitative evaluation of the both type amebic antigens by indirect hemagglutination, gel-precipitation and immunoelectrophoresis, and found that all the strain of *Entamoeba* studied had common antigenic moieties. More precise results will be obtained when

the antigens are fractionated and the fractions are tested independently. Neal & Johnson (1968) reported that low-temperature strains were of low infectivity, and no ulceration was observed in any of the rats infected with those strains, whereas the virulent strain "Biswas" showed extensive ulceration with the high average score of 6.0, and they suggested the necessity of further studies to reveal whether the low temperature strains are restricted in a particular geographical region, because all the low temperature strains were isolated from North America. In another report, Neal et al. (1968) compared serological titres of complement fixing and hemagglutinating antibodies of the sera from *E. histolytica* infected patients with virulence of the same amebae to rats, and found that in certain instances man could harbor *E. histolytica* in the intestinal cavity without building up antibodies in the blood, while antigen could gain access to the circulation and produce its homologous antibodies when tissue invasion occurs. They also regarded as avirulent when the average cecal score was less than 2.0 and as virulent when cecal score was above 5.0, and the group of between 2.0 and 5.0 was regarded as intermediate status. From the above views, the virulences of seven strains in the present study are recognized as follows; YS 14 and YS 16-strain are avirulent, YS 15 and YS 24-strain are intermediate status, and YS 9, YS 25 and NAM-RU II-strain are virulent *E. histolytica* strains. Among those strains, avirulent and virulent strains are affirmatively corresponded to their originated sources. Although YS 24-strain from amebic liver abscess and YS 15-strain from an asymptomatic carriers are categorized as the intermediate status strains.

Bird and Neal (1962) did the survey at Bahrain, Persian Gulf, and reported that the virulence of strains in rats correlated with the past histories of the patients, with one exception when the strain was isolated from a soft stool. They supported the Neal's conclusion that strains, isolated from patient with dysentery or diarrhoea, are invasive to rats,



while those from symptomless persons are not invasive. Some of the exceptions to this generalization are always strains isolated from persons without symptoms, but whose amebae might be virulent to rats.

In order to confirm inconsistencies of the present data, the rat-culture passages were performed. In previous, Faust & Swartzwelder (1935) and Meleney & Frye (1937) observed enhancement of virulence of *E. histolytica* by continuous passage of the parasite through dogs and kittens respectively, and presumed that rapid passage from man to man in nature probably favors the development of virulency, as in malaria, typhoid and yellow fever. Chang (1945) reported that strain of *E. histolytica* may lose its infectivity and some of its pathogenicity for kittens by a long period of cultivation without encystment. It does mean that its infectivity may be restored by encystment, and that the presence of a virulent bacterial flora may not enhance its infectivity. Thompson et al. (1954) reported that repeated passage of an attenuated culture through dogs yielded a highly virulent culture. Healy & Gleason (1966) reported that two strains of *E. histolytica*, one in culture for 11 years and another in culture for 4 years, were still as highly infective and invasive similarly to the recently isolated strains. They reported also that the strain from an amebic abscess of brain showed diminished infectivity and invasiveness, but restored its virulence to a level comparable to recently isolated strains when the strain was passed rapidly through rats and culture.

In the present study, ameba strains showed no attenuation of their virulencies by cultivation during the period of not longer than three years, and the presence of virulent bacterial flora seemed not always to enhance the infectivity of *E. histolytica*. YS24-strain showed intermediate status and YS25-strain showed high virulency, while those amebae were associated with the bacterial flora of NAMRU II-strain ameba which showed high virulency.

By rat-culture passage, YS14-strain, which was avirulent in the preceding result, turned to virulent showing cecal score of 5.5, and YS25-strain, which was intermediate status, increased its virulency.

Considering these various aspects and results, it may be presumed that animal-culture passage should be submitted to another test before comparing the virulence of *E. histolytica* strains.

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—Legend for Figures—

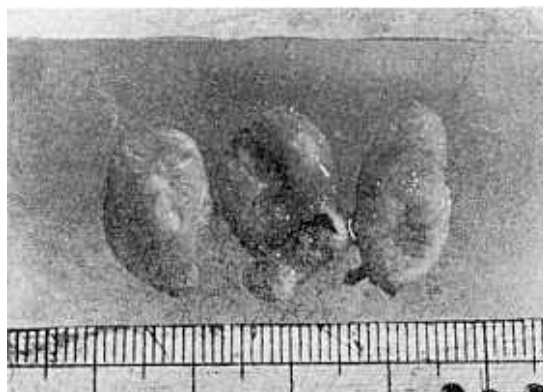


Fig. 1. Amebic lesions in the ceca of rats, sacrificed on the seventh day after inoculation (YS9-strain *E. histolytica*).

Left: cecal score 2. Middle: cecal score 3.

Right: cecal score 4.

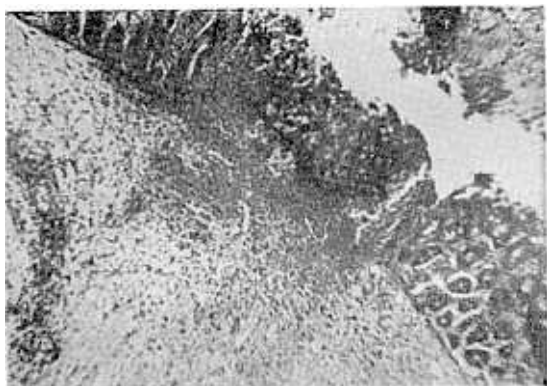


Fig. 2. Amebic ulcer in the cecum of Sprague-Dawley strain rat. ( $\times 100$ )

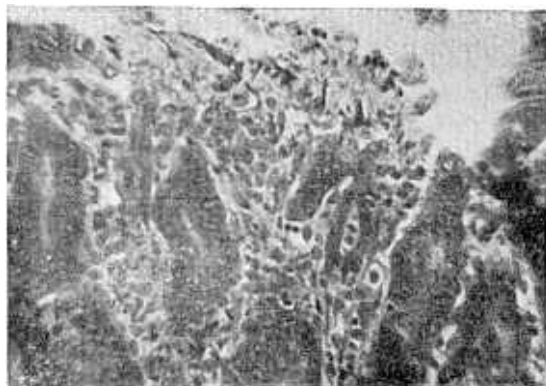


Fig. 3. Extensive mucosal and submucosal ulceration in the cecum of a rat. ( $\times 400$ )

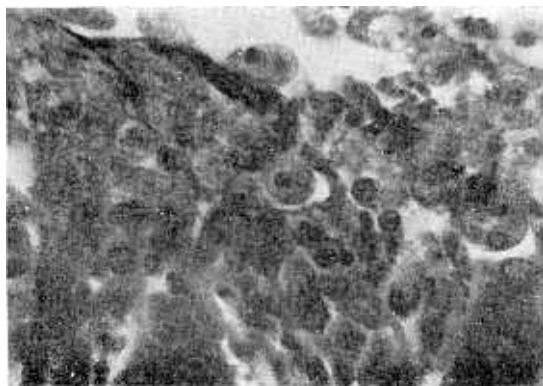


Fig. 4. High power magnification of Fig. 3. Note numerous amebae around the destroyed mucosal crypts. ( $\times 1000$ )

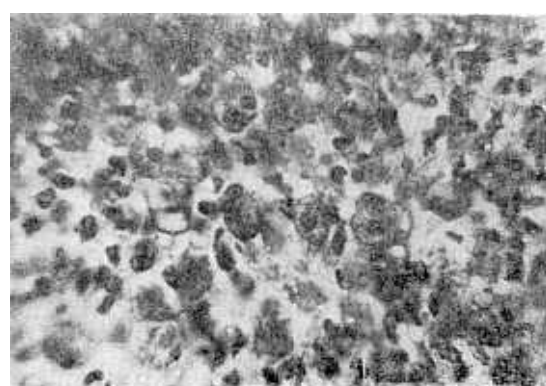


Fig. 5. Amebic invasion of the deeper portions of the mucosa of the rats cecum. ( $\times 1000$ )