

Factors Influencing the Pathogenicity of *Entamoeba Histolytica*

Chin-Thack Soh

This article reviews the factors associated with the pathogenicity of *Entamoeba histolytica* infection or the establishment of amoebiasis from two aspects, namely protozoan and the host. The following are the experimental results from strains collected in Korea (Ro 1967; Lee 1968; Lee 1969; Soh *et al.* 1969; Choi 1969; Cho *et al.* 1972; Chang *et al.* 1972; Soh 1981; Kim *et al.* 1983; Soh *et al.* 1984) (Table 1).

Table 1. *Entamoeba histolytica* isolated in Korea

Source	Strain
Cyst from carrier stool	YS-1,3,5,9,14,15,16 KY-15,40,41,43,44,45,48,49, 52-57,59,60
Trophozoite from dysentery stool	YS-10
Cyst in stool from liver abscess case	YS-12, KY-9
Trophozoite from liver abscess	YS-23,24,25,27 KY-9,27,37

The rat was the principal laboratory animal, although mouse, rabbit and golden hamster were also utilized when necessary. HK-9 and NAMRU-2 were used as reference strains.

PATHOGENICITY DIFFERENCES BY STRAIN

Soh *et al.* (1969) studied the virulence of strains

collected from cases of liver abscess, dysentery and cyst-carriers. The strains were maintained on diphasic medium adding calf serum and penicillin G in association with unknown bacterial flora. The history of the strains is as follows.

- 1) YS-9 strain was isolated in 1966 from the feces of a 51 year-old male with a liver abscess.
- 2) YS-14 strain was isolated from a 63 year-old healthy cyst-carrier's stool in 1967.
- 3) YS-15 strain was isolated in 1968 from the feces of a 51 year-old symptomatic cyst carrier.
- 4) YS-16 strain was from a healthy cyst-carrying 45 year-old female in 1968.
- 5) YS-24 strain was obtained from the liver abscess of a 33 years-old male in Severance Hospital in 1969.
- 6) YS-25 strain was obtained in June 1969 from the liver abscess of a 42 year-old male.
- 7) NAMRU-II strain. The strain was isolated in 1967 from an acute dysentery patient (living in Vietnam) by rectoscopic method and was shared by Dr. Cross JH, US NAMRU-2. It was used as a reference strain for the Korean strains.

Sprague-Dawley or hybrid rats were used for the experiment. The animals were fed a normal diet throughout the entire experiment, and were inoculated with *E. histolytica* intracably according to the technique described by Jones (1946). The condition of the cecal contents were recorded by the procedure of Neal (1951). A portion of the contents were examined directly, then inoculated in culture media.

The criteria for scoring were (Neal, 1951):

Contents; normal	0
slightly less solid than normal	1
slightly mucoid	2
mucoid, some solid matter present	3
no solid matter, white or yellow	4
mucus only	

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Institute of Tropical Medicine Yonsei University College of
Medicine, Seoul, Korea
Department of Parasitology, Wonkwang University College of
Medicine, Iri, Korea

Wall;	normal	0
	slight thickening	1
	marked local thickening and contraction	2
	extensive thickening and contraction	3
	cecum shapeless, extensive ulceration	
	with abscess formation	4

The scores ranged from 5.0-7.8 in 30 day-old rats, 4.2 in 40 day-old ones, 2.5 in 60 day-old ones, to 2.8 in 90 day-old ones. NAMRU-II strain exhibited a similar invasiveness according to the hosts age. The results suggest that establishment of pathological change due to amoebic infection may partly relate to the age of host.

Each experimental group was composed of Sprague-Dawley rats, with the same mother. Each animal was inoculated intracecally with 100,000 amoebic organisms belonging to strains; YS-14, YS-15 and YS-16 from the feces of cyst carriers, and YS-24 and YS-25 strain amoebae isolated from liver abscesses. Average cecal scores were 1.0 for the YS-14 strain inoculated group; 3.0 for the YS-15 strain group;

and 2.2 for the YS-16 strain group. But the scores were 3.0 and 6.3 in YS-24 and 25, respectively indicating that strains from clinical cases were more invasive than those from asymptomatic origins (Table 2).

TEMPERATURE ADAPTATIONS

Some strains of *Entamoeba histolytica* may adapt to different temperature conditions within a certain range (Cabrera and Porter, 1958). Several reports indicate that temperature adaptation is related to the virulence of the strain, though the results were diverse. Cabretra (1958) reported that the strain which adapted to lower temperatures showed higher virulence. On the contrary, Neal and Johnson (1968) observed the opposite. They found that five strains of *E. histolytica* adapted to room temperature and propagated as normal, but demonstrated a lower infectivity and produced no cecal ulceration experimentally. To clarify the foregoing discrepancies, Cho *et al.* (1972) undertook a study of the relationship between temperature adaptation and pathogenicity. The overall results suggested that the lowest critical temperature of these strains was 30°C, and survival time of the strains was not always correlated with temperature conditions.

Under three different temperatures, 37°C, 32°C and 30°C, all strains originating from non-invasive cysts and one highly invasive trophozoite strain (YS-23) showed the highest growth peak at 32°C, three strains originating from highly invasive trophozoites showed the highest peak at 37°C, but poor growth was observed at 30°C in all strains. The results suggested that strains originating from non-clinical cases were

Table 2. Pathogenicity of *Entamoeba histolytica* strains in Sprague-Dawley rats (Soh *et al.*, 1969)

Strain	Body weight (gm)	Infection/No. of Inoculation (infectivity) (%)	Cecal scores		Total
			Cecal wall	Cecal contents	
YS-14	22-35	3/4 (75.0)	1.0	0.0	1.0
YS-15	27-40	4/5 (80.0)	1.5	1.5	3.0
YS-16	32-43	5/5 (10.0)	1.2	1.0	2.2
YS-24	30-31	2/2 (100.0)	1.5	1.5	3.0
YS-26	20-25	3/3 (100.0)	3.3	3.0	6.3

Table 3. *Entamoeba histolytica* strains (Cho *et al.* 1972)

Strain	Source	Sex	Age	Collection site	Collection Date
YS-14	Cysts from cyst carrier	M	63	Cheju-island**	Jan. 1967
YS-15	Cysts from cyst carrier	M	49	Suwon, Kyonggi-Do	Jan. 1968
YS-16	Cysts from cyst carrier	F	45	Severance Hospital, Seoul	Feb. 1968
YS- 9	Cysts in stool of liver abscess patient	M	51	Cheju-island	Aug. 1966
YS-12	Cysts in stool of liver abscess patient	M	51	Cheju-island	Jan. 1967
NAMRU-2	Trophozoites in dysentery stool	—	—	Vietnam	Jan. 1968
YS-23	Trophozoites from liver abscess	M	47	Severance Hospital, Seoul	Oct. 1968
YS-24	Trophozoites from liver abscess	M	33	Severance Hospital, Seoul	June 1969
YS-27	Trophozoites from liver abscess	M	72	Korea Hospital, Seoul	Aug. 1969

Note: ** Cheju-Island = Jeju Island = Jeju-Do.

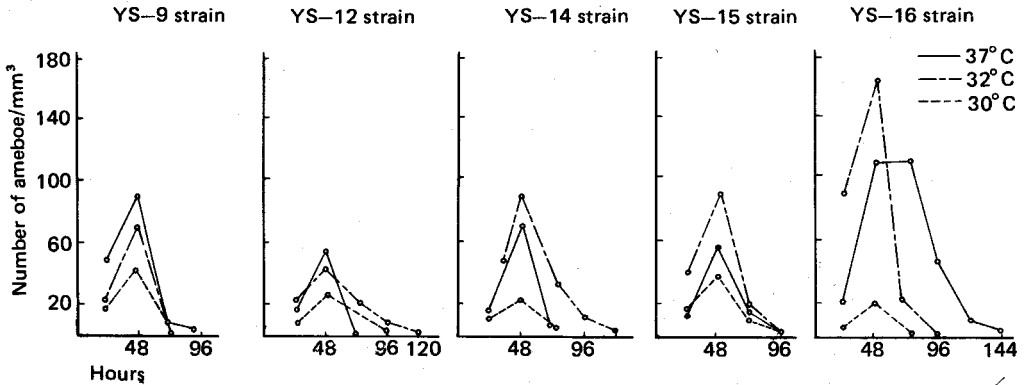


Fig. 1. Propagation curves (mean) *E. histolytica* strains of cyst originated at various temperature conditions. (Cho et al., 1972)

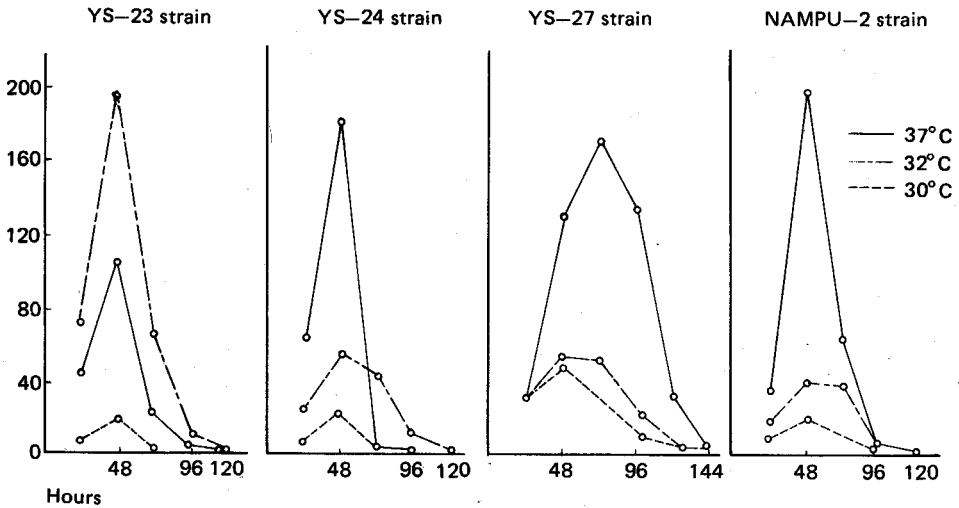


Fig. 2. Propagation curves (mean) of *E. histolytica* strains YS-23, YS-24, YS-27 and NAMRU-II of trophozoite originated at various temperature conditions. (Cho et al., 1972)

likely to be more adaptable to lower temperatures, but the strains from pathological lesions were more adaptable to propagate at body temperature of 37°C (Table 3, Figs. 1 and 2).

HEMOLYTIC ABILITY

The ingestion of red blood cells by *Entamoeba*

histolytica was first observed by Lösch in 1875, and has been recognized as a nutrient of the protozoa. But Schäffer and Iralu (1961) reported that rabbit red blood cells produced toxic substances inhibiting the propagation of *E. histolytica*. Craig (1927) found that *E. histolytica* exhibited hemolytic ability, but Schäffer and Iralu (1963) reported that the hemolytic ability of *E. histolytica* differed according to the strain. Ro (1967) studied whether red blood cells had a toxic effect on the propagation of *E. histolytica* or whether

the protozoa had the selective ability to lyse red blood cells. The strains sampled were YS-1,5,9,10.

The hemolytic ability differed with respect to strain of *E. histolytica* and YS-1 strain lysed the red cells of pig, sheep and ox, but not of rabbit, dog or man. YS-5 strain lysed the red cells of ox, but not of rabbit, dog, pig, sheep or man. YS-9 strain lysed the red cells of sheep, but not others, and YS-10 strain lysed the red cells of dog and sheep slightly, but not others. Each strain demonstrated a selective ability to hemolyse the red blood cells of different animals.

Ro (1967) found that *Bacillus subtilis* hemolysed red blood cells but showed decreased hemolysis when combined with YS1 and YS9 strains. This did not occur with YS10. *Staphylococcus aureus* showed no hemolysis when combined with YS1, 9, and 10. *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* did not hemolyse red blood cells when they were combined with *E. histolytica*. Thus, *E. histolytica* inhibited the hemolytic action of enteric bacteria, but the metabolic products of *E. histolytica* did not interfere with the hemolytic action of bacteria. The occurrence of hemolysis in medium is considered to play an important role in the propagation of amoebae. Ro (1967) added 0.2 ml of a 5% rabbit or sheep red blood cell suspension to the media. He found that rabbit red cells were hemolysed in four days, whereas sheep cells in two days. The growth of the amoebae was checked every two days for a total of 10 days. To the rabbit cell suspension medium and its control, 5,800 amoebae were added. On the second day, the numbers were 1,450/ml in the media with added red cells, but 19,600 in the control. On the 4th day, only 100/ml were found in the former but 13,700 in the latter. But after hemolysis occurred, the yields were reversed. Similar results were observed in the medium to which sheep cells had been added. The number of amoebae showed a lag phase until completion of hemolysis, then increased overwhelmingly in that group. This finding coincides with the report by Schäfer and Iralu (1961) that red blood cells inhibited the propagation of amoebae by releasing toxic substances. Thus, it is highly probable that normal red blood cells have some inhibitory action on the growth of *Entamoeba histolytica*. Nevertheless, once they are hemolysed, the cell component may contribute as nutrients to the growth.

ENZYME STUDIES

A combined cytochemical and electrons

microscopic study was conducted in order to demonstrate acid phosphatase activities in trophozoites of *E. histolytica* and *E. gingivalis* (Cho *et al.* 1973). *E. histolytica* (YS-27) strain was isolated from a liver abscess case and *E. gingivalis* (YG-215) strain was collected from the gingival crevice of a 41-year-old woman in January, 1972. The amoeba strains were maintained by subculture in a diphasic medium.

In *E. histolytica*, the reaction products were distributed evenly over the entire surface of the plasma membrane, suggesting that surface enzymes may play a role in the invasiveness of *E. histolytica*. *E. gingivalis*, however, showed no activity of acid phosphatase on the plasma membrane, except in the portion of the uroid-like structure.

In the cytoplasm of both amoebae, various reaction precipitates were observed in the vacuole limiting membrane, the vacuole membrane and its contents, and in lysosome-like structures. The contents of the vacuoles demonstrated lysosome-like structures. Enzyme activity and membrane reaction negative vacuoles were conspicuous in *E. gingivalis*, as was moderate activity on endoplasmic reticulum.

Granule-like acid phosphatase reaction products were demonstrated in the nucleoplasm of *E. gingivalis* but these were not seen in *E. histolytica*.

Electrophoretic isoenzyme patterns: The samples were collected from amoebic colitis patients, amoebic liver abscess cases, or asymptomatic cyst passers from various parts of Korea and cultured for isoenzyme studies.

Starch-gel electrophoresis for phosphoglucose isomerase (E.C. 5,3,1,9; PGI) phosphoglucomutase (E.C. 2,7,5,1; PGM), malic enzyme (E.C. 1,1,1,40; ME) and hexokinase (E.C. 2,7,1,1; HK) was performed using Maazoun's technique (Kim *et al.*, 1983). To elucidate the relation of the electrophoretic patterns of isoenzymes and clinical episodes, rats were inoculated with each of the amoebal strains in the cecal region, and then sacrificed one week after inoculation for observation of pathological changes on the cecal walls.

The results obtained were summarized as follows:

PGI appeared as single or double bands in HK-9, KY-15, 27,37,43 and 53. PGM was present as single or double bands in HK-9, KY-15, 44,45 and 53 and the patterns suggest pathogenic character.

The HK pattern showed double bands and the ME pattern exhibited a single band. These two patterns characterized *Entamoeba histolytica*.

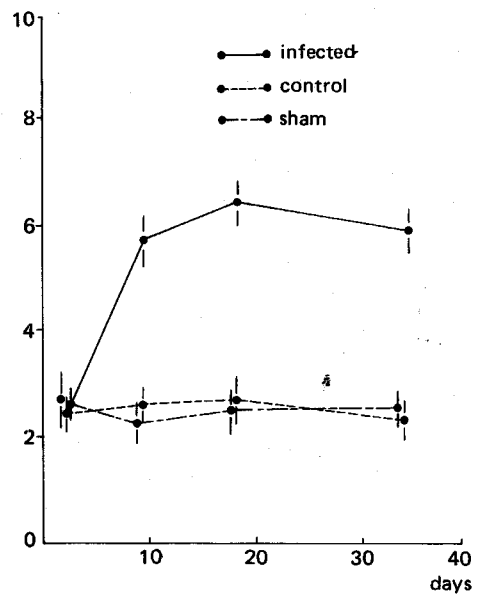
The overall results suggested that isoenzyme patterns, especially PGM, PGI and HK of the amoeba strain, contribute in the identification of *Entamoeba*

Table 4. Comparison of the number of mesenteric mast cells, and the degree of disrupted or degranulated mesenteric mast cells in mice infected with *Entamoeba histolytica*, strain YS-24 (Im *et al.* 1975)

Duration of Infection (days)	No. of Exam.	Mast cells per mm ²			Degranulation of mast cell (%)		
		Control group	Sham group	Infected group	Control group	Sham group	Infected group
2	4	55.2±7.4	53.6±4.9	50.8±1.6	18.2±3.4	18.5±3.1	16.0±3.3
9	4	52.3±4.8	54.2±5.2	85.7±5.9	16.3±2.2	18.0±2.8	61.0±7.4
18	4	55.0±6.5	53.2±4.8	87.0±4.8	18.0±2.8	16.3±3.2	70.0±3.4
34	5	50.0±4.5	55.2±7.2	89.4±6.7	19.0±3.2	15.8±2.8	71.1±6.3

histolytica from other intestinal amoebae, and identification of the pathogenic strain of *Entamoeba histolytica* from the nonpathogenic.

However, Soh *et al.* (1984) experienced different results in a follow-up study with twenty five strains of *Entamoeba histolytica* isolated in Korea. The electrophoretic patterns of the 25 strains were grouped into 12 types. All strains showed a single ME band in the same position which was characteristic of *E. histolytica*. HK revealed four bands which migrated relatively faster, but none were decisive enough to define a clinical association of the respective strain. GPI showed two bands, but neither band denoted a pathogenic association. The single band in PGM appeared in three pathogenic strains, but two strains from simple cyst passers were also recognized. Intracecal inoculation of the strains from clinical cases did not produce any noticeable pathological changes in the caecum of rats, even though the infectivity was high. As far as the results were indicated, no clearcut relationship between zymodeme pattern and pathogenicity was recognized.

**Fig. 3.** Comparison of percentage of blood eosinophils in each experimental group.

MAST CELL DISRUPTION AND EOSINOPHILIA

Mast cells may be related to allergic states in the course of *Entamoeba histolytica* infection. Im *et al.* (1975) suggested this after experimental studies. Mice weighing about 16 gm were used for sham and experimental infection groups. The sham group was injected intracurally with liquid medium which did not contain *E. histolytica*, and liquid medium containing 5,000-50,000 amebae was injected into the ceca of the experimental group after a laparotomy. Mast cells in the mesenterium and eosinophils in the peripheral

blood were examined from the first day on. Mesenteric samples from the region of the terminal ileum were fixed in methyl alcohol and stained with Pugh's solution. After seven days, ulcers were found in the cecal walls of all mice inoculated with amebae. The number of mast cells in the mesenteric tissues of the infected group increased from the first day of infection and persisted til the 34th day of the observation period. Degranulation and disruption of mast cells increased in the infected group as compared with the sham group and the control, but no difference was discerned according to strain of *E. histolytica*. Blood eosinophilia which persisted for the entire

observation period was also noted in the infected group. Thus *E. histolytica* infection may provoke the degranulation of mast cells as well as an increase in eosinophil cells. The eosinophilia was also considered to be a secondary reaction due to the degranulation of mast cells (Table 4, Fig. 3).

Five thousand of *E. histolytica* (YS-24) were inoculated into the cecum of a mouse. Two days after the infection, no difference in number of eosinophils was noticed between the experimental group and control group. But the number in the former increased to $5.8 \pm 0.6\%$ by the 9th day and lasted up to 34th day of the observation period, whereas the number in the control group remained constant at 2.4-2.5% during the same period.

NUTRITION AND INFECTION INTENSITY

Diet may be correlated with the intensity of *E. histolytica* infection (Sadun *et al.* 1952), although the relationship between the nutritional condition of the host and infectivity of *E. histolytica* has not yet been clearly defined. Choi (1969) performed an experimental study on the susceptibility or resistance to *E. histolytica* infection under varying levels of dietary proteins.

Young rats of both sexes were used for the study and were divided by diet into 4 groups

- 1) Group D ; protein depleted diet (rice powder 85% without casein)
- 2) Group L ; low protein diet (rice powder 80%, casein 5%)
- 3) Group M ; moderate protein diet (rice powder 70%, casein 15%)
- 4) Group H ; high protein diet (rice powder 60%, casein 25%)

Olive oil (4%), an inorganic salt mixture (4%), cod liver oil (2%) and yeast (5%) were added equally to each diet.

E. histolytica was inoculated into the experimental rats which had been maintained on the above formulated diets for 15 to 17 days. Amebae were pooled from a 48 hour culture and centrifuged for 5 minutes at 800-1,000 rpm. The concentrated amebae were suspended in sterile saline until a concentration of 200,000 organisms per ml. was obtained. The inoculum was injected toward the blind end of the cecum from a point anterior to the cecum and colon using a 5 ml syringe with a 23 gauge needle. The rats

were sacrificed 14 days after inoculation. The entire cecum was removed and the contents were examined for the presence of amebae in a direct saline wet mount.

The number and size of the crater-like ulcers in the ileocecal area were measured under stereomicroscope, and then histo-pathological studies were carried out. The ulcers were divided according to the severity of the ulceration;

Degree I : one pinpoint ulcer in the ileocecal area

Degree II : one or two ulcers, 1-2mm in diameter

Degree III: more than two ulcers.

The growth of the protein depleted diet group, group D, was more markedly reduced in body weight than that of any other group from the 3rd day of the diet. The amount of ingested protein did not show any difference by group statistically. The average amount of diet consumed was 30-40 gm per day per individual rat. Amebae were found in the contents of the ileocecal area of the rats: 100% in group D, 85.7% in group L, 73.6% in group M and 44.4% in group H. Generally the γ -globulin level increased in all groups after the inoculation of amebae, especially in the hyperprotein diet group, and the value of total serum protein in group D (6.87 gm%) was the lowest of any group.

Histologically, the percentage of ulceration was 60.0% in group D and 21.4% in group L. In groups M and H there was only one case in each showing cecal ulceration. In this way the cecal ulceration rate and infectivity were increased in the low protein diet group as compared to the groups fed a high protein diet. The above results suggest that the low level of protein diet retard the growth of the host and decrease the resistance of the host to amebic infection.

STRESS AND HORMONAL INFLUENCES ON PATHOGENICITY

Some biochemical and biophysical factors of the host may influence the infectivity and pathogenicity of parasites. Teodorovic *et al.* (1963) presented some evidence for an adrenal effect. He observed a marked exacerbation of *E. histolytica* pathogenicity in mice treated with corticosteroids. Conversely, Villarejos (1962) reported that cortisone did not increase the susceptibility of rats to amebic infection. Solomon (1964) reported that testosterone promoted the susceptibility of the gonadectomized animal to

Table 5. Development of amebic ulcers in rat intestines after treatment with hormones (Lee, 1968)

Hormone	Treated		Control	
	Number of rats	Number of rats which developed ulcers (%)	Number of rats	Number of rats which developed ulcers (%)
Castrated, control	12	8 (65)	5	3 (60)
Castrated, testosterone injected	13	13 (100)		
Ovariectomized, control	4	2 (50)	2	1 (50)
Ovariectomized, ergosterone injected	8	4 (50)		

Table 6. Development of amebic ulcers in rat intestine s after exposure to shaking stress (Lee, 1968)

Number of ulcers in the ilco-cecum	Number of rats with ulcer formation		
	Shaking stress, non-infected	Shaking stress, infected	Normal, control
25~	0	0	0
20~24	0	1	0
15~19	2	4	1
10~14	0	1	0
5~ 9	1	1	0
1~ 4	6	8	2
0	1	0	2
Total number of rats	10	15	5
Total number of ulcers	51	117	23
Average no. of ulcers per rat	5.1	7.8	4.6

Table 7. Size of amebic ulcers in rat intestines compressed with surgical forceps (Lee, 1968)

Size of ulcer (mm)	Number of ulcers		
	Compressed, control (%)	Compressed, infected (%)	Normal, control (%)
4~5	4 (4.4)	2 (1.8)	0
2~3	33(36.3)	50(43.9)	1 (5.5)
0.5~1	54(59.3)	62(54.3)	17(94.5)
Total	91(100)	114(100)	18(100)

parasitic infection in comparison with the effect of ergosterone. Lee (1968) tried a study to elucidate whether physical, sex hormonal, and toxic bacterial stimulation affected intestinal infection with *E. histolytica*.

YS-9 strain isolated from a dysentery case was used. The rats were castrated or oophorectomized. Animals, sham-operation or operation but without hormones, were used as control animals. As sex hormones, testosterone and ergosterone were used.

Table 8. Number of amebic ulcers by previous infection of *Shigella dysenteriae* in rats (Lee, 1968)

Number of ulcers in the ilco-cecum	Number of rats with ulcers after exposure to		
	<i>S. dysenteriae</i> only	<i>S. dysenteriae</i> and <i>E. histolytica</i>	No. exposure
25~	0	2	0
20~24	1	1	0
15~19	0	3	0
10~14	2	1	0
5~ 9	2	1	1
1~ 4	5	8	1
0	0	0	0
Number of rats	10	16	4
Total number of ulcers	74	148	12
Average number of ulcers per rat	7.4	9.3	3.0

Testosterone propionate, 50 mg/ml, was injected intramuscularly, a total of four times every other day before and after inoculation with 150,000 organisms. Estradiol benzoate, 2 mg/ml, was used as a female hormone, a total of four times every other day before and after inoculation with 200,000 organisms.

In the testosterone treated, 10 and more ulcers were observed in 4 among 13 rats. Next was the castrated control group, and the least was in the normal control group. The average number of ulcerations per rat was 9 in the testosterone treated group, 4 in the castrated group, and none in the normal control group. Ulcers above 2mm in diameter were predominantly found in 45.5% of the experimental animals. However, no appreciable effect was observed on infectivity and pathogenicity in the ergosterone treated group, on the contrary (Table 5).

The effect of shaking stress was also observed. All the animals, except a normal control group, were given shaking stress before their inoculation with amebae. They were put into a cage on the shaking machine (Arthur H. Thomas Co.), and stressed four hours daily for a week. Rats in the shaking stress group showed more severe pathological changes than in the control group of non-shaking stress.

In another experiment, the exposed ceca of laparotomized rats were compressed with surgical forceps to produce congestion, then inoculated intracecally with 300,000 parasites. The direct physical damage to the ileocecum also showed enhancement of the infectivity. Thus, Lee (1968) found that stress or physical damage caused more pathological changes

(Table 3, 6 and 7).

Previous infection with enteric bacteria also enhanced the pathogenicity. Lee (1968) observed the effect of a previous infection of *Shigella dysenteriae* in rat on amebic infection. When rats, infected orally with a 1.0 ml of *S. dysenteriae* suspension one week previously, were inoculated intracecally with 300,000 amebae, the susceptibility to *E. histolytica* was strongly enhanced (Table 8). The pathological changes in these rats were more numerous than in the mechanical stress-only group and in the merely infected group. The average number of ulcerations per rat was nine, whereas it was seven in the stress-only rats and three in the infection-only rats.

ESTABLISHMENT OF AN AMOEBIC LIVER ABSCESS

To establish a liver abscess in an amoebic infection, some predisposed factors were proven experimentally. Besides mechanical injury, chemical damage was found to provide a favorable condition for establishment. Im and Kim (1976) administered hepato-toxic agents to animals followed by inoculation with *E. histolytica*. The inoculation were given 3 hours after toxic agent administration later in the case of thioacetamide and 24 hours later in the case of Carbon tetrachloride.

Entamoeba histolytica strains YS-27 and YS-37 were

inoculated directly into the livers of mice, rats, golden hamsters, and intravenously into the superior mesenteric vein of rabbits.

Six to 11 days after inoculation, the animals were sacrificed and the livers were examined. In the healthy control group, no amoebic abscess was produced in mice, rats or rabbits; except in golden hamsters.

Vacuolization of the hepatic lobule was seen 3 hours after the injection of thioacetamide, and mild fatty degeneration was seen 24 hours after the carbon tetrachloride injection.

All rats pretreated with thioacetamide and carbon tetrachloride formed abscesses in the liver. The results suggested that hepato-toxic agents highly predisposed the formation of an abscess by the invading amoeba or by some other micro-organism within a short period of time.

SUMMARY

In summarizing the results of the experimental studies up to the present, it is conjectured that the pathogenicity of *Entamoeba histolytica* or establishment of amoebiasis is not unique but differs by strain and age of *Entamoeba histolytica* and the age of the host. A non-virulent strain is more likely adapt to as low a temperature as 32°C. This is not so in the strains which originated from clinical cases. Iso-enzyme patterns may roughly characterize pathogenic strains from non-pathogenic. Red blood cells may contribute as nutrients for growth of *Entamoeba histolytica* only after they have been hemolysed, but they are toxic to the amoebae as long as they remains intact. A low protein diet and stress may facilitate the establishment of amoebiasis; male sex hormones or previous infection by enteric bacteria provide a more advantageous condition than the female; and hepato-toxic agents will accelerate amoebic hepatitis.

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