PREFACE

According to historians of medicine in Korea amebiasis has been one of the most important protozoan infection since the dawn of history, its prevalence being high particularly during summer months. It is common knowledge that intestinal amebae can be the cause of disease not only in the large bowel but in other organ systems as well. Amebic liver abscess and other forms of extra-intestinal amebiasis are not uncommon in this country, yet in spite of its importance for public health only very little effort has been made to bring it under control either individually or at community level. Several factors are thought to contribute to the high prevalence, mainly unsanitary condition and utilization of night soil, contamination of food and drinking water, unsanitary life styles, utilization of night soil in vegetable growing, the abundance of flies spreading the infection, *etc.* In any case, the only ultimate conclusion is that amebiasis must be brought under control and eradicated in the entire country.

Realizing this the author and his associates have placed research in control of ***Entamoeba histolytica*** infection as well as infection with other pathogenic species of Endamoebidae high on their list of priorities for over two decades. Research activities have been governed by the health needs of our nation. Due to the limited amount of time and limited number of colleagues available it turned out that comprehensive coverage of the entire subject matter was not possible and it was necessary to utilize results of research by other authorities in order to remedy the insufficiencies and supplement the missing portions. They were found to be highly valuable for this purpose. Nevertheless, I am still not satisfied that all pertinent aspects of the task have been solved realizing that overall reviews are not enough in order to sufficiently clarify all the problems of amebiasis in the country. I can only hope that continuous future efforts will bring about the full final solution.

Lastly, I would like to express my deep gratitude to all the associates who have been cooperating and contributing since the early sixties. The present publication may not have been possible without their dedicated help. My sincere appreciation is due the following colleagues (alphabetically):

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INTRODUCTION

A number of species of ameba are found inhabitating man as parasites or commensals. Amebiasis(amoebiasis) is the term generally referring to the disease condition due to *Entamoeba histolytica* infection. For this reason, *Entamoeba histolytica* will mostly be reviewed in the text although some other amebae found in Korea which have pathogenic potential will be included in the discussion. Taxonomically, all these amebae belong to order Amoebina of phylum Protozoa. Order Amoebina is characterized by having endoplasm and most of its species are cyst producers. Reproduction is exclusively asexual, usually by binary fission. They inhabit fresh or salt water in the free-living state, but some of them are parasitic to human beings and animals as endozoa. The order has four families: Naegleriidae, Amoebidae, Endamoebidae and Paramoebidae(Table 1). Within the families, some of species which belong to Naegleriidae, Amoebidae and Endamoebidae have been recognized as human parasites. At present, *Entamoeba histolytica*, *E. coli*, *E. gingivalis*, *Iodamoeba bütschlii*, *Endolimax nana* and *Dientamoeba fragilis* of Endamoebidae are regarded as parasites or commonsals of man in Korea.

Table 1. Classification of the order Amoebina (Kudo, 1954)

Family Naegleriidae	
Genus *Naegleria*	*N. fowleri*, *N. gruberi*,
Genus *Trimastigamoeba*	*T. philippinensis*
Family Amoebidae	
Genus *Amoeba*	*A. proteus*, *A. discoides*, *A. dubia*, *A. verrucosa*, *A. striata*, *A. guttula*, *A. limicola*, *A. spumosa*, *A. vespertilo*, *A. gorgonia*, *A. radiosa*
Genus *Diamoeba*	*D. mirabilis*
Genus *Pelomyxa*	*P. palustris*, *P. carolinensis*, *P. illinoisensis*
Genus *Vahlkampfia*	*V. limax*, *V. patuxent*
Genus *Hartmannella*	*H. hyalina*
Genus *Acanthamoeba*	*A. castellani*, *A. hyalina*, *A. culbertsoni*
Genus *Sappinia*	*S. diploidea*
Family Endamoebidae	
Genus *Endamoeba*	*E. blattae*, *E. thomsoni*, *E. disparata*, *E. majestus*, *E. simulans*, *E. sabulosa*, *E. pellucida*
Genus *Entamoeba*	*E. histolytica*, *E. coli*, *E. gingivalis*, *E. gedoelsti*, *E. equi*, *E. bovis*, *E. ovis*, *E. caprae*, *E. polecki*, *E. debliecki*, *E. venaticum*, *E. cuniculi*, *E. cobayae*, *E. muris*, *E. citelli*, *E. gallinarum*, *E. testudinis*, *E. barreti*, *E. terrapinae*, *E. invadens*, *E. ranarum*, *E. phallusiae*, *E. minchini*, *E. apis*, *E. thomsoni*, *E. aulastomi*, *E. paulista*
Genus *Iodamoeba*	*I. bütschlii*, *I. suis*

Genus *Endolimax*	*E. nana, E. caviae, E. gregariniformis, E. clevelandi, E. ranarum, E. blattae*
Genus *Dientamoeba*	*D. fragilis*
Genus *Martinezia*	*M. bazei*
Genus *Dobellina*	*D. mesnili*
Genus *Schizamoeba*	*S. salmonis*
Genus *Hydramoeba*	*H. hydroxena*
Family Paramoebidae	
Genus *Paramoeba*	*P. pigmentifera, P. schaudinni*

ENTAMOEBA HISTOLYTICA

BIOLOGY

Morphology

1) Light microscope

Shape and size are not unique, but differ according to stage in life-cycle: trophozoite, precyst and cyst. For identification, the fixed and stained specimen is preferable over the fresh specimen. Iron-hematoxylin stained preparation is used most commonly. Since the ameba is microscopic in size, morphological identification is usually performed under light microscope or electron microscope with the fixed form. For the reader's sake of convenience the description of *E. histolytica* under the light microscope is referred to "Amebiasis" by Anderson *et al.* (1953). There is considerable variation in the morphology of the three stages and it is necessary for the observer to be familiar with these variations in order to distinguish *Entamoeba histolytica* from the other intestinal amebae.

The morphology in iron-hematoxylin stained preparation is as follows;

a) *Trophozoite:* The trophic forms of *E. histolytica* range in size from 6 to 40 μm. The majority of specimens encountered have a diameter between 15 to 25μm. The small race strains of this species are less than 10 μm in size. In stools from patients with severe dysentery forms reaching 40 μm in diameter may occasionally be observed. The cytoplasm usually does not contain inclusions other than red blood corpuscles. Occasionally, a few bacteria, fragments of tissue cells or other foreign matter may be present (Fig. 1).
It is not unusual to find in a preparation containing large numbers of trophozoites without ingested red blood cells in their endoplasm. In regions where severe dysentery does not occur this may be often seen.

The presence of erythrocytes and absence of other inclusions are important diagnostic features which aid in distinguishing *E. histolytica* from *E. coli.* The differences in the details of the nuclear structure of these two amebae are of great help in diagnosis. The centrally located endosome of *E. histolytica* is smaller than the eccentrically situated larger structure occurring in *E. coli.* There is a smaller amount of peripheral chromatin on the inner aspect of the nuclear membrane of *E. histolytica* in comparison with the larger amount present in *E. coli.* The appearance of the nucleus as a whole is much more delicate in the former. The nucleus of some of the larger strains of *E. histolytica* may have the peripheral chromatin concentrated in a crescent mass on the nuclear membrane. This characteristic is a helpful diagnostic aid since it rarely, if ever, occurs with *E. coli.*

b) *The precystic stage:* Precystic amebae are smaller in size than the trophozoites and do not contain any cytoplasmic inclusions. The nuclear structure is essentially the same as in the active form although not seldom it may closely resemble that of *E. coli.* Chrom-

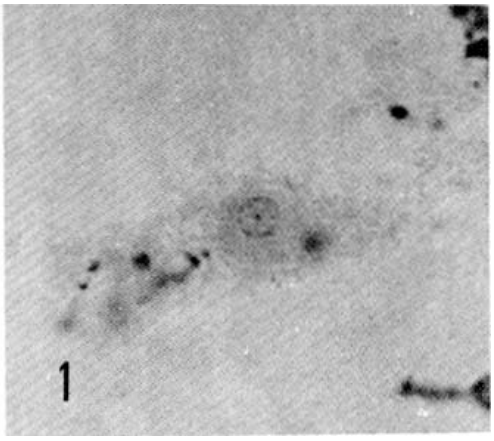


Fig. 1.

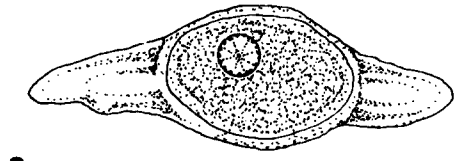


Fig. 2.

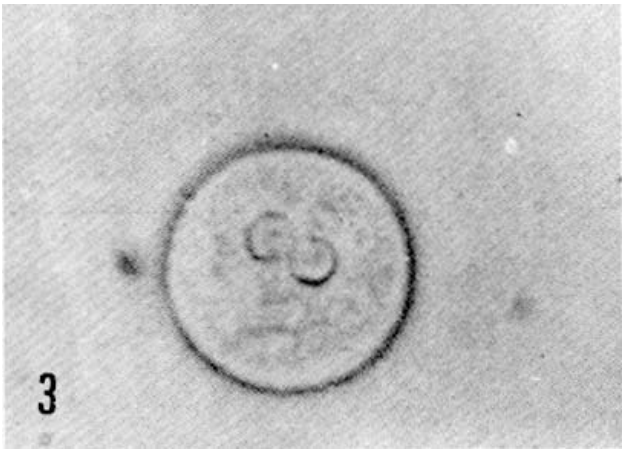


Fig. 3.

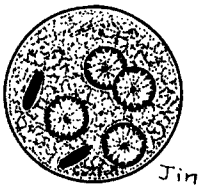
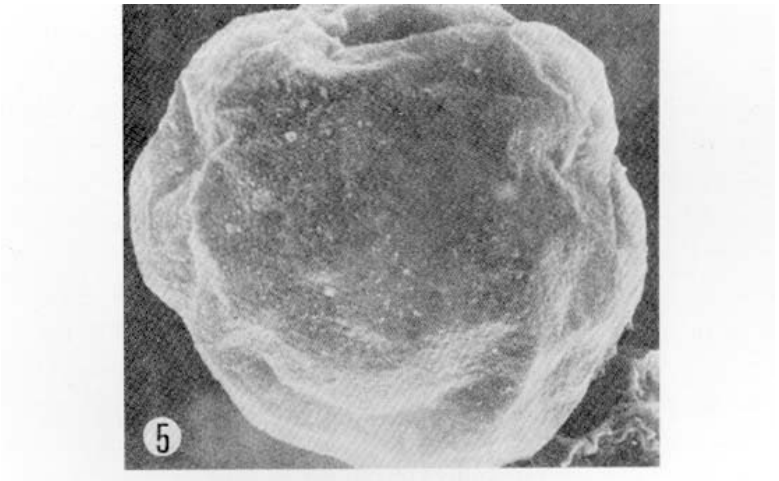


Fig. 4.

Fig. 1, 2. *E. histolytica* trophozoites. Showing the nuclear structures in photo and the diagram.

Fig. 3, 4. *E. histolytica* cysts. Showing the distinct two nuclei in photo and four nuclei in the diagram.



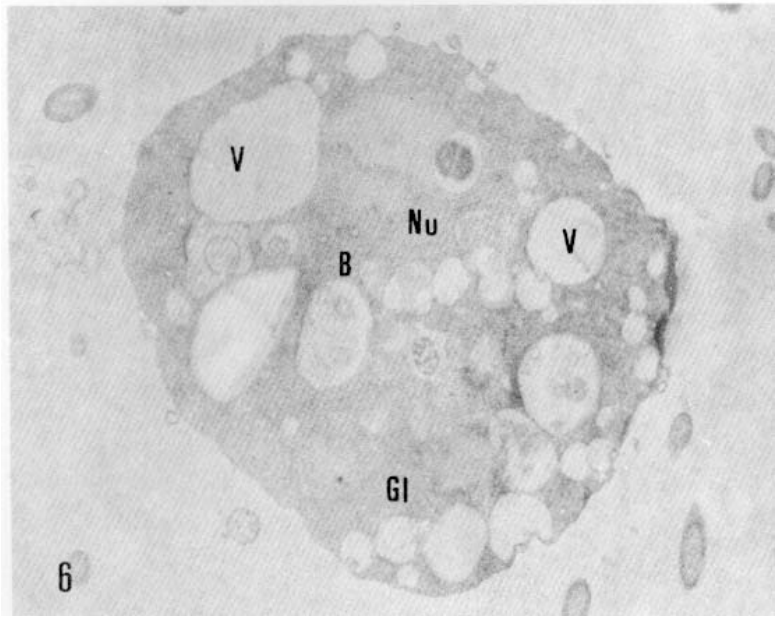


Fig. 5. Scanning electron micrograph of *E. histolytica* cyst.

Fig. 6. Ultrastructure of *E. histolytica* trophozoite. Showing diffusely scattered cellular inclusions. (B: botton body, Gl: glycogen, Nu: nucleus, V: vacuole)

atoid bodies are present occasionally. The precystic stage of intestinal amebae is often confusing from the standpoint of differential diagnosis. However, trophozoites, cysts, or both are usually also present and diagnosis should be based on observation of these stages.

c) The cystic stage: The cysts of *E. histolytica* may vary widely in size. The diameter of the cysts ranges from 5 to 23 μm. In each case, however, the size tends to remain approximately the same although two or more distinct "size strains" or races may occur in the same specimen. Usually the large cyst strains with an average diameter of 12 to 14 μm can be differentiated from the small cyst strains with an average diameter of 6 to 7μm (Fig. 3, 4).

Following the development of the cyst membrane the glycogen is concentrated in a single mass in the shape of a comparatively large sphere, but of smaller size than in the corresponding cyst of *E. coli*. The outline of the glycogen vacuole is not defined as clearly as that of *Iodamoeba bütschlii*. As nuclear division progresses the glycogen tends to become more dispersed. Chromatoid bodies are frequently present in the uninucleate cysts although they may not appear until a later stage in the development of the cyst. As the cysts mature the chromatoid bodies tend to disappear. They stain intensely black with iron-hematoxylin staining and appear as bars with bluntly rounded ends or as spheres. Usually a few large rods are present, although in the uninucleate and binucleate cysts the chromatoid material is abundant and may be present in masses of varied form and size resembling the chromatoid bars at times. In some preparations where all cysts present are fully mature no chromatoid bodies may be observed. Chromatoid material is particularly abundant in small-strain cysts.

Although the mature cyst of *E. histolytica* typically contains four nuclei, occasionally forms with eight nuclei are found. The structure of the nucleus is the same as that which occurs in the trophic form. The peripheral chromatin may be distributed in a fine uniform layer on the nuclear membrane, but more often it is concentrated in a number of small plaques. The crescent mass noted in some trophozoites likewise occasionally appears in the cystic stage of *E. histolytica*. The endosome is small and usually in a central position, and may be compact, irregular, or dispersed in granules. When examining certain stained fecal preparations it will be noted that many of the cysts of *E. histolytica* are in the uninucleate or binucleate stage. This circumstance may be of aid in differential diagnosis because uninucleate cysts of *E. coli* are encountered very seldom. In preparation containing large numbers of uninucleate cysts with nuclei of the coli or histolytica type the organisms belong in all probability to the latter species.

2) Fine structure

a) General finding: Although several investigations on the ultrastructure of *E. histolytica* were carried out (Fletcher *et al.*, 1962), no attempt has yet been made to characterize the micro-morphology of the trophozoites in the tissue invading phase. Recently, El-Hashimi and Pittman (1970) reported that the structural difference between the trophozoites obtained from the human colon and those cultivated *in vitro* was the presence of a fuzzy

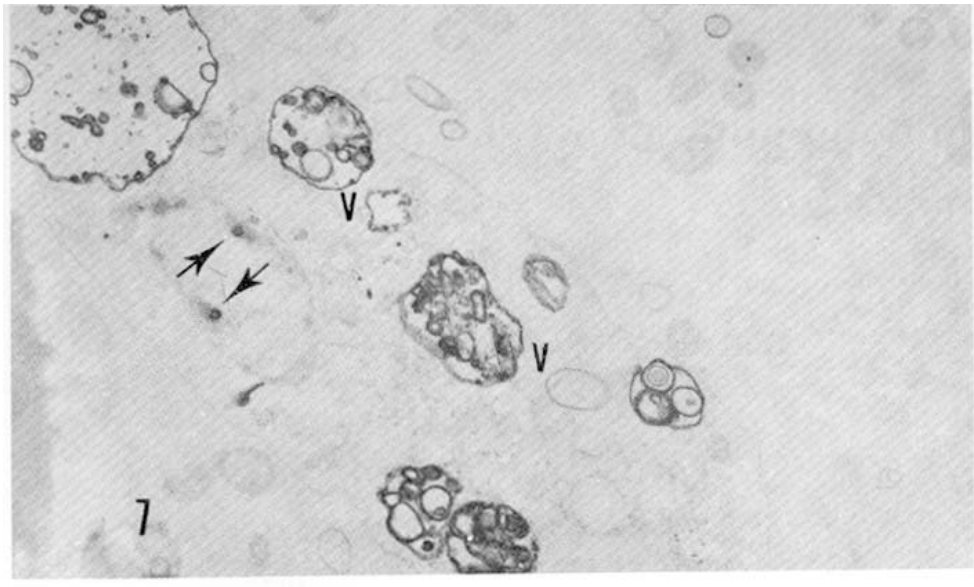
coat on the amebae originating from the colon. Cho *et al.* (1972 a) observed the structural transformation of invading amebic trophozoites in the intestinal mucosa of rabbit.

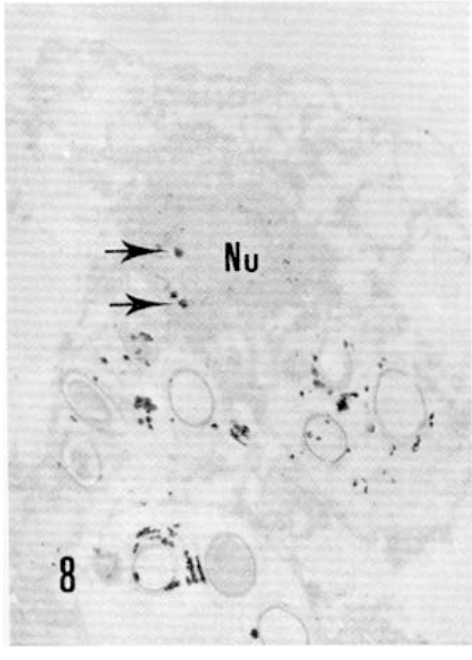
The trophozoites observed by Cho *et al.* (1972 a) were of irregular shape. Small cytoplasmic projections were observed on portions of plasmalemma which were hypothetically considered as origin of pseudopodia. Food vacuoles varied greatly in size and usually contained ingested bacteria, starch grains, and some sort of membrane covered whorl. Plasmalemma was smoothly surfaced and coated on the outside with a pale grey homogenous layer. Electron dense hollow rods radiated in rosette form from a central mass of fine granules, and some were distributed in clusters irregularly in the cytoplasm. Bundles of the crystalloid aggregation were prominent in the cytoplasm. In the cytoplasm, electron dense substances were distributed as well as fine dense granules, short filaments and rosette-like glycogen particles. These substances were grouped into four different categories: a) irregular shaped dense pigment bodies, b) spherical smaller granules, c) moderately electron-dense ellipsoidal rods, and d) moderately electron dense spherical granules. The nucleus was oval or round in shape. There were regularly spaced nuclear pores and patches of peripheral chromatin lined the inner surface of the nuclear membrane. Much larger, round and highly electron dense bodies were in the periphery of the nucleus (Fig. 5, 6).

b) Enzyme Activity: Although a number of reports has been published on the fine structures of *E. histolytica*, only a limited amount of work has been done on the functional aspect of the fine structures. Carrera and Changus (1948) demonstrated acid phosphatase activity in *E. histolytica*, and Eaton *et al.* (1970) demonstrated enzyme-containing organelles equipped with a thread-like trigger on the surface of *E. histolytica*. They suggested that in the Genus *Entamoeba* lysosome-like structures might be responsible for initiating damage to the host tissue cells. Cho *et al.* (1973) attempted to compare localization of acid phosphatase active sites of *E. histolytica* and that of *E. gingivalis* by means of electron microscopic cytochemistry, and to elucidate the relationship between this enzyme and the newly found fine structures.

E. histolytica (YS-27 strain) was isolated from a liver abscess of a 72 year-old male, and *E. ginigivalis* (YG-215 strain) was collected from the gingival crevice of 41 year-old female. The ameba strains were maintained by subculture on diphasic medium and the same strains were used throughout the study. In *E. histolytica* the reaction products were distributed evenly over the entire surface of the plasma membrane, whereas *E. gingivalis* showed no acid phosphatase activity on the plasma membrane, except in the uroid-like structured portion. In the cytoplasm various reaction product precipitates were observed in vacuoles, and vacuole limiting membranes in both amebic species and their contents, and lysosome-like structured vacuoles with strongly enzyme active contents, but reaction negative membranes were conspicuous in *E. gingivalis*. The endoplasmic reticulum showed moderate acid phosphatase activity which was absent in *E. histolytica* (Fig. 7, 8, 9).

c) Ultrastructure in immune serum: The active form of *E. histolytica* is immobilized in





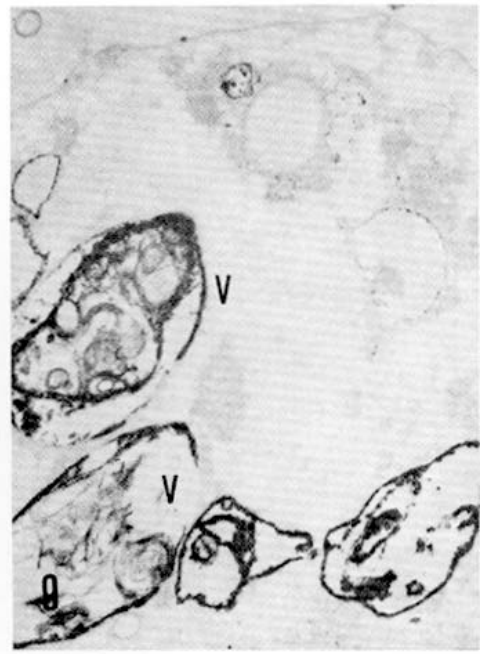
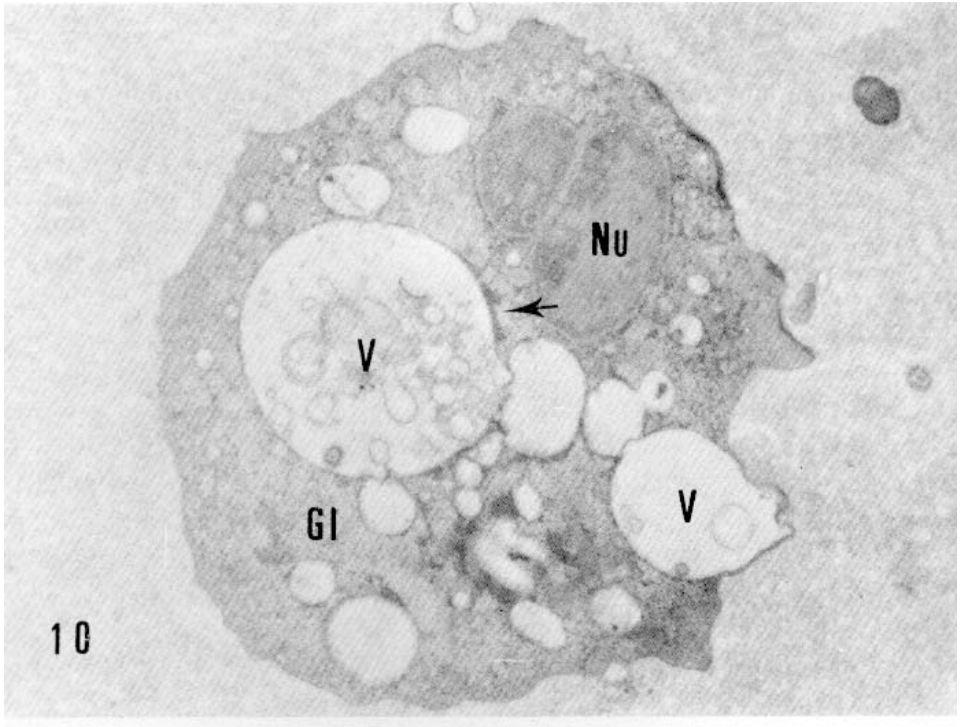


Fig. 7. Normal *E. histolytica*. Showing acid phosphatase activity. The reaction is observed in the botton bodies (arrow) and vacuoles. (×9,900)

Fig. 8, 9. Same features as above in control. (×10,500 and ×13,200)



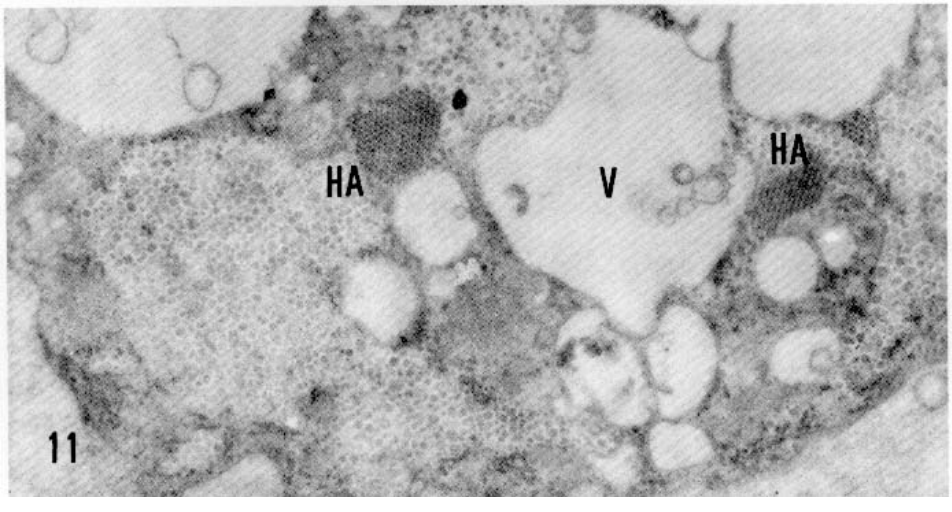


Fig. 10. Beginning stage of immobilization 30 minutes after reaction with patient's serum. (×9,000)

Fig. 11. Immobilized *E. histolytica*. Helical aggregates (HA) are shown. (×21,000)

immune serum, withdrawing pseudopodia. Even though a considerable amount of work on micromorphology of *E. histolytica* either in culture media or in its tissue phase has been accomplished (Cho and Soh, 1969), no report is avilable on the ultrastructure of *E. histolytica* in the immune serum, except Chung *et al.* (1976) who examined the fine structure during its immobilization. Trophozoites of *E. histolytica*, strain YS-9, were isolated in 1966 from the pus of a liver abscess and subcultured in modified diphasic medium (Cho, 1968). For experimental purposes the amebae were collected from 4~5 media after being cultured for 48 hours at 37°C. The media were centrifuged at 1,000 rpm for 5 minutes and 0.5 ml of the sediment was pipetted into a new test tube. The serum was obtained from an amebiasis case (immobilization test positive rate 80%, IFA titer 1:64, IHA titer 1:32,000) and kept in deep freezer at -30°C. Before use the serum was thawed at room temperature and 0.5 ml put into the test tube and mixed with the trophozoites. The tube was incubated 37°C and a part of the mixture was withdrawn after 30, 60 and 90 minutes. The general morphology of the amebae was examined under the light microscope and then under the electron microscope. The amebae, fixed in glutaraldehyde, were washed several times with low-speed centrifugation (500 rpm). The sediments were pre-embedded in 2% warm agar, and put into Gomori medium (Eranko *et al.*, 1952) at 37°C for an hour. Finally the sample was washed again with 0.2M cacodylate buffer containing 7.5% sucrose, and post-fixed in 1% osmium tetroxide in cacodylate buffer at 0~4°C for 30 minutes. The amebae obtained directly from culture media were used as control in the study. In the control group helix structures (short helical rods) were scattered throughout the cytoplasm. The ribonucleoprotein particles comprising the helix structure averaged 20nm in diameter. The amebae in the serum were immobilized after about 30 minutes of exposure then recovered again in about 60~90 minutes. At the beginning stage of the immobilization, helical aggregates (chromatoid bodies) associated with vacuoles appeared abundantly in the cytoplasm, but tended to aggregate gradually in the peripheral region of the cell, specially during the period of immobilization. Each parallel array of aggregates measured about 45 nm in width. When the organism regained motility pseudopodia appeared again, but helical aggregates disappeared and numerous helix structures were again observed in the cell periphery. Distribution of glycogen particles remained unchanged, and acid phosphatase activity was noted both in the immobilized and the control amebae. Activity was markedly noticeable in the vacuoles and botton bodies in the nucleus(Fig. 10, 11).

Cultivation

Since Boeck and Drbohlav (1925) reported their successful cultivation of *Entamoeba histolytica* in Lock-Egg-Serum (L.E.S.) medium, scores of investigators have devised and described their own new or improved methods how to grow this parasite in an artificial environment. The numerous and continuous devices indicate, nevertheless, that a wholly satisfactory culture for growth and propagation of this amebic species has not yet been reached, and the reason for this thought to be the liquid portion of the culture. Various

integrates which may influence the propagation of amebae in the liquid portion of the media have been tested; horse serum, human or rabbit whole blood, egg white, extract or infusion of liver, beef extract, yeast extract, trypticase *etc.* The greater growth potential of mammalian serum from newborn animals as opposed to adult ones has been recognized by several workers. Pederson (1944) showed that serum from newborn calf contained very little α- and β- globulin and the α_1- globulin fraction contains large amounts of mucoprotein, fetuin, different from the serum albumins and globulins. The newborn calf serum has been widely used in tissue culture because of its low cell toxicity and low antibody levels.

Cho (1968) tried to improve several procedures for preparing egg slant and overlay of modified diphasic medium using calf, cow, rabbit, dog, pig and human sera. He found that the propagation of amebae was most luxuriant on the 3rd day of culture in the group to which two drops (=2/15ml) of calf serum were added. When 1,600 I.U. of penicillin G were added to the calf serum medium, more abundant growth of amebae occured than when other amounts of penicillin G were added. The amebae which grew in the calf serum added media produced the typical amebic lesions in the cecum of rabbits on 7 and 12 days after the experimental inoculation. He concluded that calf serum was excellent for the culture of *E. histolytica* because it provided richer harvest of the parasite with less serum.

Chung *et al.* (1970) observed that the mixed cultivation of *E. histolytica* and *T. hominis* was not satisfactory in the modified diphasic medium due to unbalanced propagation of the latter.

Virulence

To clarify the pathogenicity as related to size, Lim *et al.* (1965) analysed the cysts from 59 fecal samples of chronic amebic infection patients and compared the clinical signs with size of cysts, but found no evidence that large race (9.0~10μm) only was responsible for amebic colitis and the small race (6.1~7.2μm) was not a tissue invader. There is still considerable dispute whether small race *E. histolytica* is as invasive as large race.

Ignoring size difference, Soh *et al.* (1969) studied the the virulence of the strains collected in laboratory. From human cases of liver abscess, amebic dysentery and cyst-carriers they were maintained on the diphasic medium adding calf serum and penicillin G in association with unknown bacterial flora.

1) YS-9 strain. This strain was isolated in September 1966 from the feces of a 51 year-old male with liver abscess and subcultured every other day.

2) YS-14 strain. The ameba was isolated in January 1967 from a 63 year-old healthy cyst-passer's stool.

3) YS-15 strain. The strain was collected in January 1968 from the feces of a 51 year-old symptomatic cyst carrier.

4) YS-16 strain. Isolated in February 1968 from a healthy cyst-passing 45 year-old female.

5) YS-24 strain. The strain was isolated in June 1969 from the liver abscess of a 33 year-old male in Severance Hospital.

6) YS-25 strain. The strain was obtained in June 1969 from the liver abscess of a 42 year-old male.

7) 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. It was used as a reference strain for the Korean strains.

Sprague-Dawley strain and hybrid rat were used for experiment. The animals were fed with normal diet throughout the entire experiment, and were inoculated with *E. histolytica* intracecally according to the technique described by Jones (1946). The sediments from several media with 48 hours old amebae were pooled and centrifuged at 1,500rpm for 5 minutes, suspended in warmed normal saline (37°C) and spinned.

The amebae were counted with the 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 abdomen and the cecum was exposed. The inoculum was delivered with a 23 gauge needle and a standard tuberculin syringe. The inoculum was injected toward the blind end of the cecum from a point anterior to the junction of the cecum and colon. For scoring, the rats were sacrificed by ether anesthesia one week after inoculation and the entire cecum was removed and slit open. The condition of the cecal contents were recorded by the procedure of Neal (1951). A portion of contents was examined directly, then inoculated in culture media.

After the contents were observed, then scored by Neal's method. The ceca were agitated in cold saline. All ulcers on the inner wall were aspirated with a Pasteur pipette and examined the presence of amebae in the tissues. In the ceca with lesions the amebae were found in all cases, and occasionally the colon was also involved, but involvement was never observed in the rectum.

The criteria for scoring were:

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
	cecum shapeless, extensive ulceration with abscess formation	4

The average cecal score was calculated from infected rats only. Portions for examination were fixed, sectioned and stained by Gomori's trichrome staining technique in order to examine histopathologic changes and presence of amebae in the formed lesions. Rat culture passage was conducted in order to investigate loss or increase of invasiveness of the organisms according to strain by passing them in turn through the ceca and cultures. Invasivenesses of YS-9 strain and NAMRU-II strain in the Sprague-Dawley rat were differentiated by host age. The ages were 30, 40, 60 and 90 days, and the highest cecal score was observed in 30 day-old rats.

The scores were 5.0~7.8 in 30 day-old rats, 4.2 in 40 day-old ones, 2.5 in 60 day-old ones, and 2.8 in 90 day-old ones. NAMRU-II strain showed similar invasiveness according to host age. The results suggest that establishment of pathological change due to amebic infection may depend upon host factors such as age and nutrition.

Virulence of the strains was examined in the weaned Sprague-Dawley rats. Each experimental group was composed of rats with the same mother. Each animal was inoculated intracecally with 100,000 amebic organisms belonging to strains YS-14, YS-15 and YS-16 which originated in cyst carriers, and YS-24 and YS-25 strain amebae 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; 2.2 for the YS-16 strain group; 3.0 for the YS-24 strain group, and 6.3 for YS-25 strain group. Comparing these results with YS-9 strain and NAMRU-II strain amebae, YS-14 and YS-16 strains showed no virulence, YS-15 and YS-24 strains were moderately invasive, and only YS-25 strain was determined as a highly invasive strain. The results suggest that the strains which originated from clinical amebiasis are more invasive than the strains from cyst-passers in general (Table 2). But even strain YS-14 which had the score of 1.0 showed marked increase to 5.5 at second animal passage. This suggests that *E. histolytica* itself is essentially pathogenic, but likely to be attenuated by conditions such as artificial culture, host resistance and some other unidentified factors.

However, Chiba and Kuwabara (1930) reported that *Entamoeba histolytica* isolated from a dysentery stool and cultured for two years still possessed pathogenicity in cat. They also reported that there was no difference in the pathogenicity between culture form and amebae directly from dysentery stool. Nevertheless, the overall descriptions suggest that there

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

Strain	Body weight (gm)	Infection/No. of inoculation (infectivity) (%)	Cecal scores		
			Cecal wall	Cecal contents	Sum
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	20-28	3/3 (100.0)	3.3	3.0	6.3

might be no essentially definable virulent or avirulent strains of *E. histolytica*. Instead the pathogenicity is considered to be depending on the existing conditions of micro-environment in the host.

Temperature adaptation

Some strains of *Entamoeba histolytica* may adapt in 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 are diverse. Cabrera (1958) reported that the strain which adapted to lower temperature 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 showed lower infectivity and produced no cecal ulceration experimentally. To clarify the foregoing discrepancies, Cho *et al.* (1972b) undertook a study on 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 to temperature conditions.

Under three different temperatures, 37°C, 32°C and 30°C, all strains originated from non-invasive cysts and one highly invasive trophozoite strain (YS-23) showed the highest growth peak at 32°C, three strains originated 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 originated from non-clinical cases are likely to be more adaptable to lower temperatures, but the strains from pathological lesions are more adaptable to propagate at body temperature of 37°C (Table 3, Fig. 12, 13).

Table 3. Strains of *Entamoeba histolytica* (Cho *et al.*, 1972b)

Strain \ Source	Material	Sex	Age	Place collected	Associated bacterial flora	Date collected
YS-14	Cyst from cyst carrier	M	63	Cheju-Island**	own*	Jan. 1967
YS-15	Cyst from cyst carrier	M	49	Suwon, Kyonggi-Do	own	Jan. 1968
YS-16	Cyst from cyst carrier	F	45	Severance Hospital	own	Feb. 1968
YS- 9	Cyst in stool of liver abscess patient	M	51	Cheju-Island	own	Aug. 1966
YS-12	Cyst in stool of liver abscess patient	M	51	Cheju-Island	own	Jan. 1967
NAMRU-Ⅱ	Trophozoite in dysentry stool	—	—	Vietnam	—	Jan. 1968
YS-23	Trophozoite in liver abscess	M	47	Severance Hospital	YS 9	Oct. 1968
YS-24	Trophozoite in liver abscess	M	33	Severance Hospital	YS 14	June 1969
YS-27	Trophozoite in liver abscess	M	72	Korea Hospital, Seoul	own	Aug. 1969

Note: *own... patient own self intestinal flora, — ···unknown
**Cheju-Island=Jeju Island=Jeju-Do.

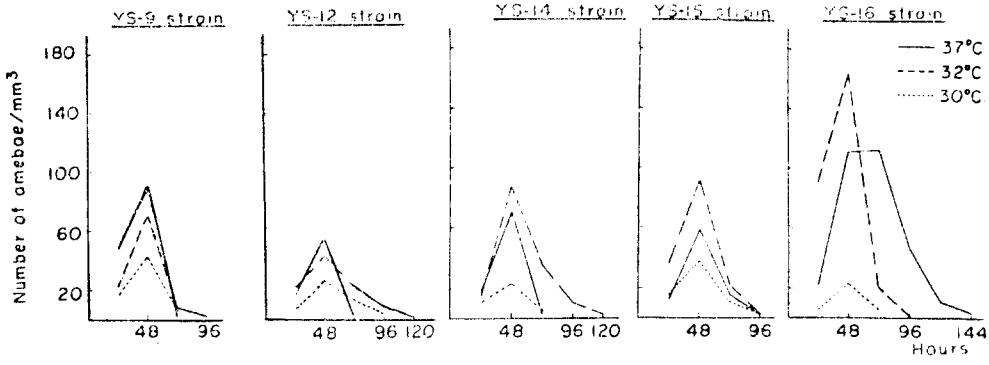


Fig. 12. Propagation curves (mean) of cyst originated. *E. histolytica* strains at various temperature conditions.

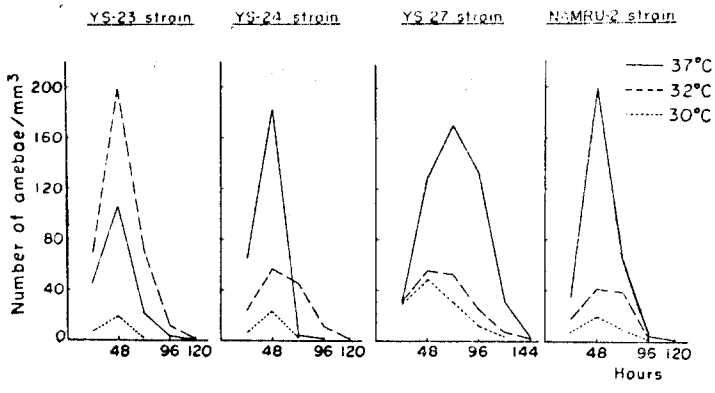


Fig. 13. Propagation curves (mean) of *E. histolytica* strains YS-23, YS-24, YS-27 and NAMRU-II at various temperature conditions.

Hemolytic ability

The ingestion of red blood cells by *Entamoeba histolytica* was first observed by Lösch in 1875, and they have been recognized as a nutrient of the protozoa. But Shaffer and Iralu (1961) reported that red blood cells of rabbit produced toxic substances inhibiting the propagation of *E. histolytica*. Craig (1927) found that *E. histolytica* had hemolytic ability, but Shaffer and Iralu (1963) reported that hemolytic ability of *E. histolytica* differed according to strain. Ro (1967) studied whether red blood cells had toxic effect on the propagation of *E. histolytica* or whether the protozoa had selective ability to lyse red blood cells. The source of the strains sampled were;

YS- 1, originated from a simple cyst carrier in Severance Hospital and had been cultured for one year.

YS- 5, collected from a cyst carrier in Jeju-Do province and had been cultured for 6 months.

YS- 9, collected from a cyst carrier in Jeju-Do province and had been cultured for 3 months.

YS-10, collected from an acute dysentery case in Severance Hospital and had been cultured for 3 months.

The hemolytic ability was different according to strain of *E. histolytica* and animal. YS-1 strain lysed the red cells of pig, sheep and ox, but not of rabbit, dog and man. YS-5 strain lysed red cells of ox, but not of rabbit, dog, pig, sheep and man. YS-9 strain lysed red cells of sheep, but not others, and YS-10 strain lysed red cells of dog and sheep slightly, but not others. In this way, each strain showed selective ability to hemolyse red blood cells of different animals.

Hemolysis in medium is considered to play an important role in propagation of the ameba. Ro (1967) added 0.2ml of 5% rabbit or sheep red blood cell suspension to the media. In the experiments he found that rabbit red cells were hemolysed in four days, whereas sheep cells in two days. The growth of the amebae was checked every two days

Table 4. Growth of *Entamoeba histolytica* in modified diphasic medium to which rabbit red blood cells were added (Ro, 1967)

Group	Day	0	2	4	6	8	10
Control	1	5800	19800	14150	1200	50	0
	2	5800	20050	13600	1400	100	0
	3	5800	18950	13350	1150	0	0
	M±S.D.	5800±0	19600±470.82	13700±327.96	1250±122.48	50±40.83	0±0
Experi-mental	1	5800	1550	150	24650	11650	1750
	2	5800	1500	100	24750	11950	1500
	3	5800	1300	50	24850	13600	1550
	M±S.D.	5800±0	1450±180.01	100±40.83	24750±81.65	12400±857.32	1600±122.47

M±S.D.: mean±standard deviation

Table 5. Growth of *Entamoeba histolytica* in modified diphasic medium to which sheep red blood cells were added (Ro, 1967)

Group	Day	0	2	4	6	7	10
Control	1	5100	19150	15250	3400	150	0
	2	5100	19900	15950	3350	200	0
	3	5100	18850	16200	3600	100	0
	M±S.D.	5100±0	19300±445.34	15800±402.08	3450±122.47	150±40.83	0±0
Experi-mental	1	5100	4400	2500	3300	50	0
	2	5100	3600	2300	3250	50	0
	3	5100	5200	2250	3350	50	0
	M±S.D.	5100±0	4500±535.41	2350±122.45	3300±40.83	50±0	0±0

M±S.D.: mean ± standard deviation

until a total of 10 days. To the rabbit cell group 5,800 amebae were added. On the second day, the numbers were 1,450/ml in red cell added media but 19,600 in the non-added control. On the 4th day, only 100/ml were found in the former but 13,700 in the latter. But after the hemolysis occurred, the yields reversed. Similar results were observed in the medium to which sheep cells had been added. The number of the amebae showed lag phase until completion of hemolysis, then increased overwhelming the control group (Table 4, 5). This finding coincides with the report by Shaffer and Iralu (1961) that red blood cells inhibited propagation of amebae by releasing toxic substances. Before, mesohematin or erythrin in red blood cell was regarded as toxic to the ameba (Kämmerer, 1941; Waksman, 1947). 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 components may contribute as nutrients to the growth.

HOST-PARASITE RELATIONSHIP

Mast cell

Mast cells may be related to allergic states in the course of *Entamoeba histolytica* infection. Im *et al.* (1975) suggested it after experimental studies. Mice weighing about 16gm were used for experimental groups; sham and experimental infection. The sham group was injected intracecally with liquid medium only which did not contain *E. histolytica*, and in the experimental group 5,000~50,000 amebae containing liquid medium were injected into the ceca of mice after laparotomy. Mast cells in mesenterium and eosinophils in 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 cecal walls in all the mice inoculated with amebae. The number of mast cell in mesenteric tissues of the infected group increased from the first day of the infection and persisted up to the 34th day of the observation period. Degranulation and disruption of mast cells increased in the infected group compared with sham operation group and the control, but no difference was discerned according to strain of

Table 6. Comparison of numbers of the mesenteric mast cells, and of 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 cell per mm^2			Degranulation of mast cell (%)		
		Control group	Group of sham operation	Infected group	Control group	Group of sham operation	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

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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

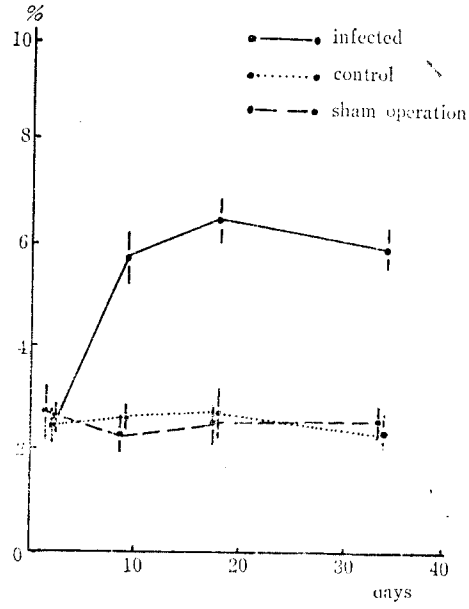

Fig. 14. Comparison of percentage of blood eosinophils in each experimental group.

E. histolytica. Blood eosinophilia was also noticed in the infected group which persisted during the entire observation period. Thus *E. histolytica* infection may provoke the degranulation of mast cells as well as an increase of the eosinophil cells. The eosinophilia was also considered to be a secondary reaction due to degranulation of mast cells (Table 6, Fig. 14).

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

Nutrition

Diet may correlate 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 susceptibility or resistance to *E. histolytica* infection while under varying levels of dietary proteins.

Young rats of both sexes were used for the study and diets were divided 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 power 60%, casein 25%)

Olive oil (4%), inorganic salt mixture (4%), cod liver oil (2%) and yeast (5%) were added to each diet in the same amount. *E. histolytica* was inoculated into experimental rats which had been maintained on the above formulated diets for 15 to 17 days. Amebae were pooled from 48 hours culture and centrifuged for 5 minutes at 800～1,000rpm. The sedimented amebae were suspended in sterile saline until it contained 200,000 organisms per ml. Through a 5ml syringe with a 23 gauge needle the inoculum was injected toward the blind end of the cecum from a point anterior to the cecum and colon. The rats were sacrificed 14 days after the inoculation. The entire cecum was removed and opened. The cecal content was examined for presence of the amebae in a direct saline wet mount.

The number and size of the crater-like ulcers in the ileocecal area were measured under stereoscope, and then histo-pathological studies were carried out. The ulcers were divided into 3 degrees according to severity of the ulceration;

Degree I: one pinpoint ulcer in ileocecal area

Degree II: one or two ulcers of 1～2mm in diameter

Degree III: more than two ulcers.

As a result, the growth of the protein depleted diet group D was markedly reduced in body weight than that of any other group from the 3rd day of diet control on. The amount of ingested protein did not show any difference by group, statistically. Average amount of diet consumed was 30～40gm 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 γ-globulin level increased in all groups after the inoculation of amebae, specially in the hyperprotein diet group, and the value of total serum protein in group D (6.87gm%) was the lowest one 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 increased in the low protein diet group as compared to the groups fed on a high protein diet. The above results suggest that the low level of protein diet retarded the growth of host and decreased the resistance of host to amebic infection.

Stress and Hormone

Some biochemical and biophysical factors of 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 rat to amebic infection. Solomon (1964) reported that testosterone promoted susceptibility of the gonadectomized animal to parasitic infection in comparison with the effect of ergosterone. Lee (1968) tried to find in an experimental study whether physical, sex hormonal, and toxic bacterial stimulation affected intestinal infection with *E. histolytica*.

YS-9 strain, which was isolated from dysentery case, was used. The rats were castrated or oophorectomized. Animals, sham-operation or operation but without hormones, were used as control animal. As sex hormones, testosterone and ergosterone were used. Testosterone propionate, 50mg/ml, was injected intramuscularly, a total of four times every other day before and after inoculation of 150,000 organisms. Estradiol benzoate, 2mg/ml, was used as a female hormone, a total of four times every other day before and after inoculation of 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 ulceration 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. In ergosterone treated group the number of animals with less than 10 ulcers was similar in each group (Table 7).

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 of the shaking stress group resulted a 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) experienced that stress or physical damage caused more pathological changes (Table 8, 9).

Previous infection with enteric bacteria also enhanced the pathogenicity. Lee (1968) observed the effect of previous infection of *Shigella dysenteriae* in rat to amebic infection.

Table 7. Development of amebic ulcers in rat intestine after treatment with hormones(Lee, 1968)

Hormone	Treated		Control	
	Number of rat	Number of rat which developped ulcer (%)	Number of rat	Number of rat which developped ulcer (%)
Castrated, control	12	8 (65)	5	3(60)
Castrated, testosterone injected	13	13(100)		
Ovarectomized, control	4	2 (50)	2	1(50)
Ovarectomized, ergosterone injected	8	4 (50)		

Table 8. Development of amebic ulcers in rat intestine after exposure to shaking stress(Lee, 1968)

Number of ulcer in ileo-cecum	Number of rat 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 rat	10	15	5
Total number of ulcer	51	117	23
Average no. of ulcer per rat	5.1	7.8	4.6

Table 9. Size of amebic ulcer in rat intestine compressed with surgical forceps (Lee, 1968)

Size of ulcer(mm)	Number of ulcer		
	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)

Table 10. Number of amebic ulcers after previous *Shigella dysenteriae* infection in rat (Lee, 1968)

Number of ulcers in ileo-cecum	Number of rat with ulcer after exposore to		
	S. dysenteriae only	*S. dysenteriae* and *E. histolytica*	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
No. of rat	10	16	4
Total number of ulcer	74	148	12
Average No. of ulcer per rat	7.4	9.3	3.0

When rats which had been infected orally with 1.0ml of *S. dysenteriae* suspension one week previously were inoculated intracecally with 300,000 amebae, the susceptibility to *E. histolytica* was strongly enhanced (Table 10). 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.

SYMPTOMATOLOGY (AMEBIASIS)

Historical outline

Historical information about amebiasis in Korea is rather limited in spite of its high prevalence. It is said that the term "E-Jil(痢疾)" or "Kojuri(蠱症痢)", which denotes dysentery, was introduced from China, probably during the Ki-Ja dynasty, 1,000 B.C. (Sato, 1913). The first mention of "E-Jil" appeared during the Koryo dynasty (918~1392 AD), namely that King Shin Jong (1197~1204) suffered from "E-Jil" in 1202 A.D. (Chun, 1975). Ja Pa Soh, a county chief, died on September 8, 1424 A.D. because of E-Jil

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Historical information about amebiasis in Korea is rather limited in spite of its high prevalence. It is said that the term "E-Jil(痢疾)" or "Kojuri(蠱症痢)", which denotes dysentery, was introduced from China, probably during the Ki-Ja dynasty, 1,000 B.C. (Sato, 1913). The first mention of "E-Jil" appeared during the Koryo dynasty (918~1392 AD), namely that King Shin Jong (1197~1204) suffered from "E-Jil" in 1202 A.D. (Chun, 1975). Ja Pa Soh, a county chief, died on September 8, 1424 A.D. because of E-Jil

(Clanbook of family Soh). Je-Ma Lee, born in 1836 A.D. made several recommendations on treatment of dysentery in his book titled "Dong E Soo-Se Bo-Won". However, no scientific criterion to define the cause of the dysentery was employed until the microscope was introduced into the country. That was probably the late part of the 19th century. Dr. H.N. Allen, a missionary of the Presbyterian Board of Missions, saved the life of a nobility. In gratitude to Dr. Allen, King Go-Jong permitted him in April 1885 to open a hospital, Gwang Hei Weon. This became the first hospital for western medicine in Korea. Microscopy was supposedly utilized in this hospital in order to examine pathogenic agents. In medical literature, Shiga (1911) was the first to describe dysentery with scientific knowledge, although several extensive reports followed.

According to the active agents two kinds of dysentery have been known in Korea, namely bacillary and amebic. Hitherto, amebic dysentery was believed more prevalent in Korea than dysentery of bacillary origin (Table 11). But scientific data suggest that prevalence of dysentery of amebic origin is much lower than that of bacillary origin. Yamaguchi (1913) classified the dysentery cases in Government Hospital in Seoul, and found that amebic dysentery cases were far less frequent than those of bacillary origin. In 1912, he studied 110 cases of dysentery, and found that 93(81.5%) were of bacillary etiology and only 17(15.5 %) of amebic etiology. Analysing 251 dysentery cases in 1913 (January~October), he found 231(91.6%) bacillary and 20(7.8%) amebic cases. However, it should be kept in mind that *Entamoeba histolytica* causes not only dysentery, but also various other types of gastrointestinal symptoms and extra-intestinal disease(Fig. 15, 16). Moon *et al.* (1964) examined 253 diarrheic stools of army soldiers in Wonju and found only 5 cases of bacillary origin, whereas 38 cases were of amebic origin. Similar results were obtained by Chang *et al.* (1964). His results suggest that amebiasis should not be considered a negligible disease entity in Korea.

Regarding extra-intestinal amebiasis, Moriyasu (1913) and Kim (1914) first reported liver abscess formation as a complication of amebic dysentery. Subsequently, Ludlow (1926) diagnosed a greater number of liver abscess cases for a decade and elucidated more clearly

Table 11. Incidence of bacillary and amebic dysentery (Chun, 1975)

Reporter	Place	Year	No. of dysentery cases	
			Bacillary	Amebic
Takida	All country	1926-30	5,188	20
Sato	All country	1911-12	198	45
Matsumoto	Daegu	1911	14	3
Ishihara & Sano	Seoul	1927-31	246	14
Subia & Chiba	Seoul	1934-38	434	6
Kim & Seuk	Seoul	1939-46	1,131	25
Moon *et al.*	Wonju	1964	5/253 dys.	38/253 dys.
Chang *et al.*	Busan & Nonsan	1964	0/711 dys.	38/711 dys.

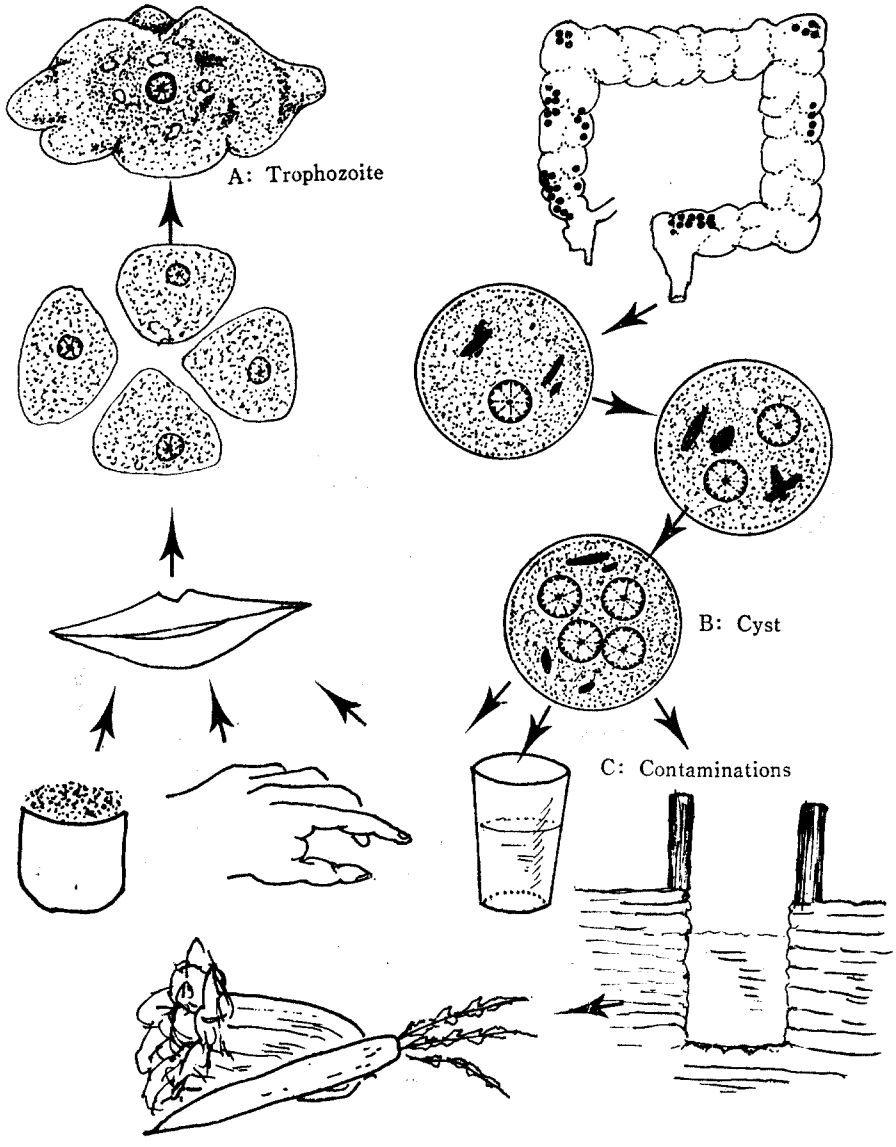


Fig. 15. Life cycle of *E. histolytica.*

the pathogenicity of the protozoa in liver abscess in Korea. During the years of 1927~1934, a number of studies concerning culture method, pathogenicity, epidemiology and treatment were performed by Chiba(1931), Kuwabara(1930, 1932), Nagahana(1935) and by their co-workers. Regarding the prevalence it is presumed that the number of actual amebiasis cases may be greater than the reported.

In general, amebiasis has a rather insidious and mild symptoms compared to bacillary dysentery and is chronic rather than acute. For this reason the patients usually find medicine from a herbist or drug store instead of visiting a modern clinic. Such factors may bring about the statistically less incidence of amebic dysentery than of bacillary dysentery.

Intestinal Amebiasis

In spite of its high prevalence there are few formal statistical data on intestinal amebiasis. The symptoms may vary from asymptomatic to fulminant dysentery which is characterized by diarrhea with blood and mucus in stool. In this chapter, clinical cases only will be reviewed. Yoon and Choi (1966) analysed 85 cases of amebic dysentery in children. They found that the incidence was higher during summer months among 70 cases; January: 5, February and March: 1 each, April: 2, May: 3, July: 4, August: 8, September: 16, October, November and December: 7 each. The initial manifestation was diarrhea in all cases associated with fever in 70% at cases, vomiting 36.5%, dehydration 35.4%. Stool examination disclosed blood and mucus from the onset in 38.8% and mucus only in 27%. The stool frequency ranged from six to 10 times per day in 76% of all the patients. Among physical findings, leucocytosis (51.8%), generalized edema (25.9%) and hepatomegaly without abscess (3.5%) were observed in that order. Intestinal amebiasis cccasionally develops to the point where surgical intervention is indicated. Yun (1968) reported one case of amebic granuloma. The case was a 60 years old female who had had complaints of tenesmus or colitis before. On examination a tumor of child's fist size was palpated seven cm above the anus. The surface was eroded and *E. histolytica* trophozoites were isolated from the submucosa following biopsy. Yun (1968) also reported that other types of surgical amebiasis such as cecal amebiasis, amebic anal fistula, and perforation of colon were not uncommon in clinic (Fig. 17, 18).

Byoun and Hong (1968) also analysed the physical signs and clinical findings in 48 cases of amebic colitis who were admitted to St. Mary's Hospital in Seoul during 5 consecutive years from 1963 on. All cases with *E. histolytica* positive stools were included (Tables 12, 13, 14, 15). They complained of clinical symptoms, but these were variable. Children complained more of diarrhea, abdominal pain with distention, and dehydration, whereas the adult group showed nutritional deficiency, anemia, diarrhea and neurological symptoms. Most frequent symptoms were diarrhea, abdominal pain, mild fever and nausea in that order. Tenderness in the lower abdomen was noted in about one half of the cases. Sigmoidoscopic examination disclosed ulcerative changes mostly in descending and sigmoid colon in 50%. About one third of the cases complained of mild fever, 37% had leucocytosis and erythrocyte sedimentation rate was increased in 21%. Complications were: liver abscess (12.5%), intestinal perforation (2.1%), empyema (2.1%) and arthritis (2.1%). Antiamebic treatment resulted in satisfactory outcome in all cases except for one case with fatal outcome from intestinal complication.

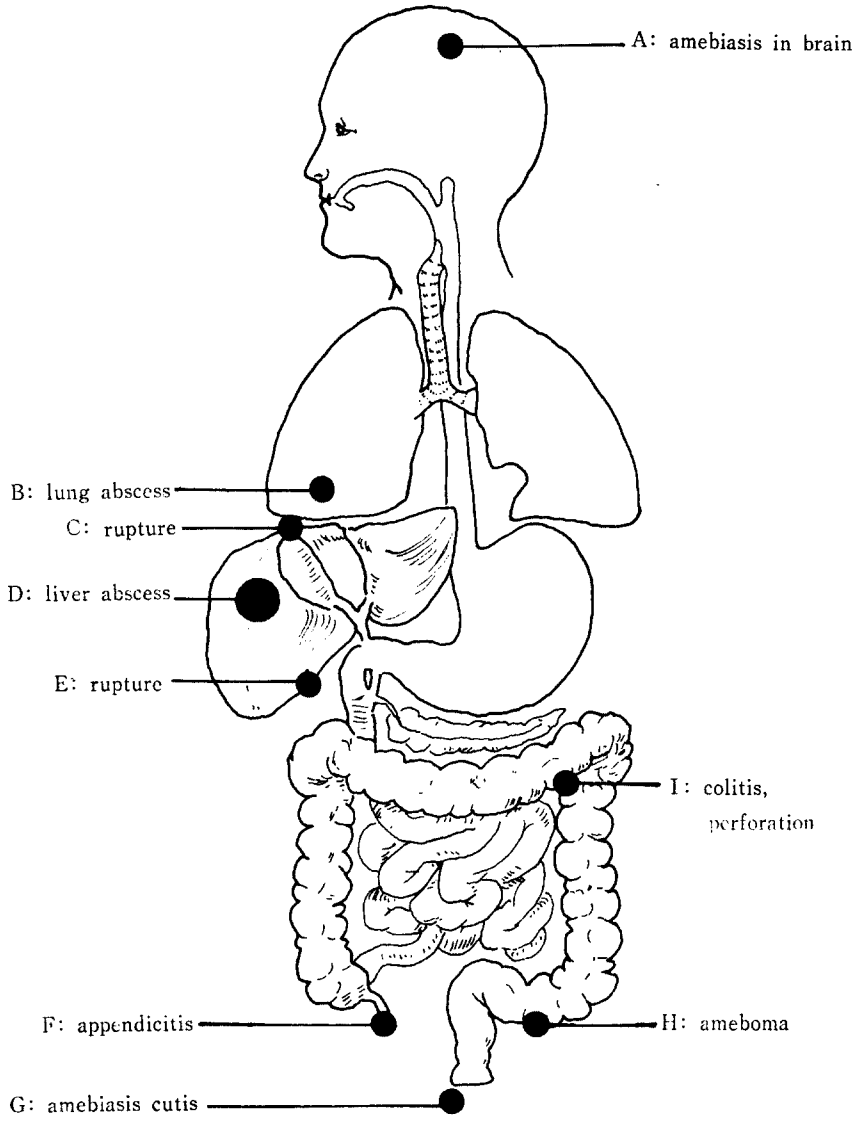
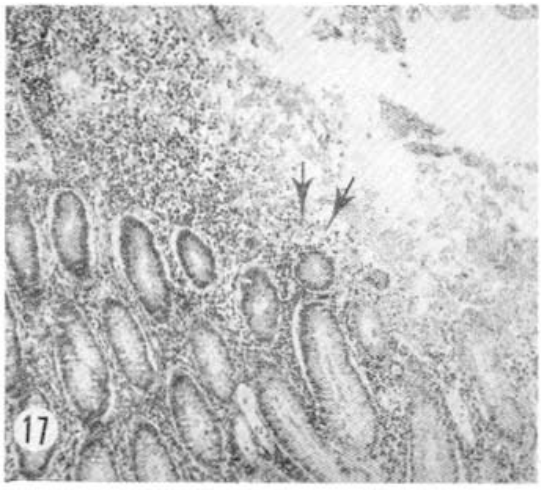
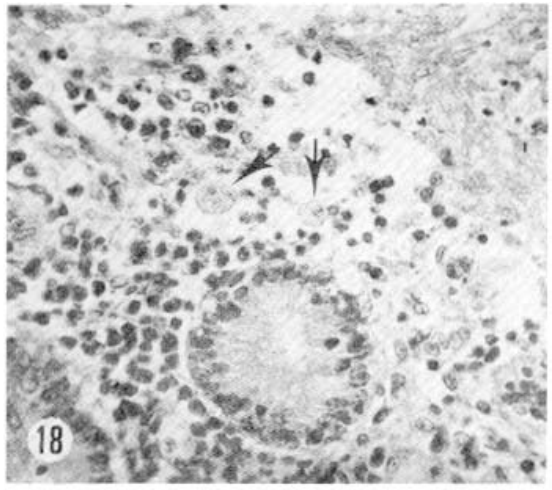
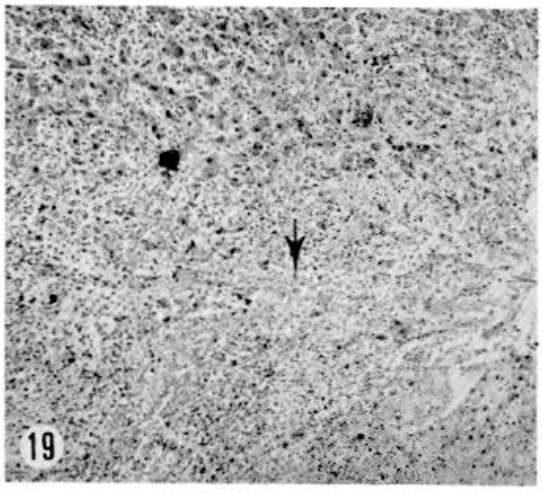


Fig. 16. Complications of amebiasis.







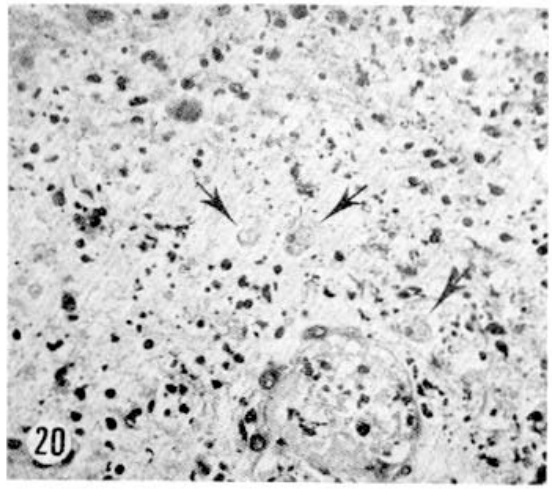


Fig. 17, 18. Chronic amebic ulcer of the colon involving submucosa (Fig. 17). Showing solitary ameba (arrow) and high power view (Fig. 18). (H-E, ×100, ×450)

Fig. 19, 20. Amebic liver abscess. Invaded ameba (arrow) in the parenchyma of the liver (Fig. 19) and the high power view (Fig. 20). (H-E, ×100, ×450)

Table 12. Subjective symptoms in 48 cases of amebic colitis (Byoun and Hong, 1968)

Symptoms	No. of patients(%)	Symptoms	No. of patients(%)
Fever	22(46)	Constipation	3(6)
Abdominal pain	27(56)	Arthralgia	4(8)
Nausea	13(27)	Nervousness	4(8)
Vomiting	10(20)	Insomnia	3(6)
Diarrhea	40(83)	Myalgia	3(6)

Table 13. Objective symptoms in 48 cases of amebic colitis (Byoun and Hong, 1963)

Signs	No. of patients(%)	Signs	No. of patients(%)
Abdominal distension	8 (16)	Mass	2 (4)
Tenderness	24 (50)	Palpable liver	6 (12)
Muscle rigidity	4 (8)	Dehydration	4 (8)

Table 14. Stool nature and frequency of defecation in 48 cases of ambic colitis (Byoun and Hong, 1968)

	No. of patients (%)			No. of patients(%)	
Stool nature	bloody	11 (28)	Frequency of defecation	2	7(18)
	mucous and bloody	18 (45)		3～ 5	21(52)
	mucous	7 (17)		6～ 8	6(15)
	watery	4 (10)		9～11	4(10)
				12～14	2 (5)

Table 15. Physico-pathological findings in 48 cases of amebic colitis (Byoun and Hong, 1968)

		Number	%
C.B.C.	R.B.C.≤ 3,5mil/mm^3	15	31.2
	Hb ≤ 11gm%	10	20.8
	W.B.C. 9000-13000mm^3	8	16.6
	>13000mm^3	10	20.8
	E.S.R. (Wintrobe)≥20 mm/hr	10	20.8
Sigmoidoscopic findings	Hyperemia	3	25
	Erosion or ulceration	3	25
	Normal	6	50
Barium enema study	Mucosal irregularity	4	30.8
	Ulceration	1	7.7
	Spastic	1	7.7
	Normal	7	53.8
Location of ulcer	Ileocecum	1	16.7
	Descending colon	3	50
	Sigmoid and rectum	2	33.3
Complications	Liver abscess	6	12.5
	Intestinal perforation	1	2.1
	Empyema	1	2.1
	Hepato-bronchial fistula	1	2.1

Hepatic Amebiasis

Hepatic involvement in *E. histolytica* infection is frequently considered to be a complication of intestinal amebiasis, and is relatively common in Korea (Fig. 19, 20). Even though the pathological criteria are not detailed yet, the clinical manifestations can be divided into two types; hepatic enlargement or hepatomegaly and hepatic abscess.

1) Hepatomegaly

Amebic hepatitis is known as a form of early regional hepatic necrosis associated with massive invasion of the affected area by *Entamoeba histolytica* (Carrera, 1950). Thus, I will define hepatomegaly as a hepatitis state, since no definite diagnosis of amebic hepatitis without abscess has been reported by any autopsy. A few reports on amebic hepatitis or hepatomegaly in Korea are available. Lah(1960) analysed 25 clinical cases who were thought to have amebic hepatitis by symptomatic pattern and good response to antiamebic drugs. The main and the most frequent subjective symptoms were, in order of frequency: dyspepsia (60%), upper abdominal pain and/or discomfort (60%), constipation (52%), low degree fever (36%), diarrhea (32%), right upper quadrant pain (28%), vomiting (20%), nausea (12%) and headache (12%). Objectively, hepatomegaly with tenderness and colonic thickening, and swelling with tenderness were invariably seen. Laboratory examination showed mild leucocytosis (ranging up to 19,000/mm^3, average of 8,800/mm^3) and almost normal liver function tests (only one case with abnormal B.S.P. retention, 3 cases with clinical jaundice). Cho *et al.* (1967) made an extensive study in Jeju-Island where pigs were raised with human night soil, and open-well water was used for drinking and laundry. A total of 738 fecal specimens were examined during the years of 1965~1966 with an average percentage incidence of 24.3% for *E. histolytica*. With this in mind the hepatomegaly cases in two villages, Sinwom-Ri and Yongheung-Ri, were examined (Table 16). In Sinwom-Ri, 17 (11.3%) individuals had hepatomegaly among the 150 examined and over half of them were positive for *E. histolytica* cysts in feces. In Yongheung-Ri there were 79 (37.1%) hepatomegaly cases among the 213 examined. The hepatomegaly cases showed 59.7% *E. histolytica* cyst positive by fecal examination. In Sinwom-Ri the hepatic enlargement cases in males were more than three times as frequent as in females, even though there were no significant differences in the incidence according to sex and age. Among 61 hepatomegaly cases, 54% had history of diarrhea within past

Table 16. Prevalence of hepatomegaly in Jeju-Do (Cho *et al.*, 1967)

*Village	No. of examination	Hepatomegaly	
		Cases	E.h. positive
Sinwom-Ri	150	17(11.3%)	0(52.9%)
Yongheung-Ri	213	79(37.1%)	40(59.7%)

* Prevalence of *E. histolytica* (E.h.)in both village: 171/738 (24.3%)

several years, and about 10% had experienced some kind of hepatic disorder.

At the time of physical examination, they complained of various general symptoms; fatigue, headache, shoulder pain, and so on. The main gastrointestinal symptoms were nausea, capricious appetite, diarrhea or soft stools and abdominal pain in decreasing order. In eighty three percent of them palpation showed soft hepatic margin and about 44% complained of tenderness in the liver. Urobilinogen test and cephalin-cholesterol flocculation test were 23.8% and 45.6% positive respectively. The overall findings suggest that the hepatomegaly is presumably related to the infection with *E. histolytica*. In any case, there should be no doubt that amebae are introduced into the liver via portal vein from the intestinal foci. Fifty four percent of the cases had a history of diarrhea or mucoid stool during the previous year and 6.8% of them suffered from certain hepatic disorders. The facts also suggest that the hepatic enlargement is most likely related to the intestinal amebiasis.

2) **Hepatic abscess**

A considerable number of amebic liver abscess cases has been reported in Korea (Ludlow, 1923, 1926; Roh and Kim, 1948; Lah, 1960; Yoon, 1965; Sun *et al.*, 1966; Hong *et al.*, 1968). Roh and Kim (1948) analysed 159 cases of amebic abscess admitted to Severance Hospital during 1921-1947. The age range was 21-50 years and 140 were male. Thus, males predominate in the incidence. It is probable that the higher rate of alcohol consumption in males creates favorable conditions for the amebic invasion of the liver. They found that 83.7% of the 159 cases were alcohol consumers and 116 cases confessed a past history of dysentery. Min (1971) also examined the charts of the 178 cases of liver abscess who were admitted to Severance Hospital during the years 1955 till 1970. According to etiological classification 102 cases (57.3%) were amebic and 76 cases (42.7%) were pyogenic origin. Amebic abscess was diagnosed by symptoms, parasitological examination, nature of the pus and drug response. *E. histolytica* trophozoites were found only in 16 cases (15.7%) out of 102, but even in the negative cases the chocolate-brown color and anchovy paste nature suggested amebic origin. Among the 102 cases, 85 or 83.3% were male and 17 or 16.7% were female. Peak incidence was noticed in the 30-40 years of age group, ranging from 30.4% to 32.4%. The main symptoms were pain in the right upper quadrant, in 85.3%, then followed fever and chills. Jaundice was noted in 6 cases. Leucocytosis of 10,000-20,000 and above were found in 75.5%. Ninety four cases (92.1%) had right lobe involvement and only 6 cases (5.9%) left lobe involvement. Roh and Kim (1948) also reported that in 154 out of 159 amebic abscess cases the abscess was in the right lobe, and leucocytosis was 11,035 on the average. In order to elucidate liver abscess by etiological agent, Hong *et al.* (1968) investigated 64 liver abscess cases. The largest group distribution was in ages of 30-39. *E. histolytica* trophozoites were found only in the abscess samples of six cases of Group 1 (Table 17,18).

By bacterial culture, *Escherichia coli* was found in two cases and paracolon group from one case in the same group. This suggests that amebic abscess is likely to be con-

Table 17. Clinical category, age group and sex distribution of amebiasis in Che-ju Island (Hong *et al.*, 1968)

Group	Clinical Category	Total No.	Sex	Age						
				0-0	10-19	20-29	30-39	40-49	50-59	60-
1	Liver abscess,	6	M 6			1	2		2	1
	E. histolytica present in liver		F							
2	Liver abscess,	13	M 13			3	4	2	3	1
	E. histolytica present in stool		F							
3	Liver abscess,	45	M 41		1	9	12	6	5	8
	E. histolytica absent		F 4	1		1	1			1
4	Hepatomegaly,	14	M 12			2	6	2		2
	E. histolytica present in stool		F 2			1			1	
5	Hepatomegaly,	91	M 78		6	19	27	12	13	1
	E. histolytica absent		F 13			2	3	2	4	2
6	Asymptomatic,	14	M 8	1	3	3			1	
	E. histolytica present in stool		F 6		4			1		1
7	Control	55	M 31		11	10	6	3		1
			F 24		18	4	1		1	
	Total	238	M189	1	21	47	57	25	24	14
			F 49	1	22	8	5	3	6	4

* M: male, F: female

Table 18. Bacteriological examination of liver abscess in Che-ju Island (Hong *et al.*, 1968)

Group	No. examined	Fungi	Cocci	Gram negative enteric bacilli					Total (%)
				Escherichia coli	*Alkaligenes faecalis*	*Aerobacter aerogenes*	Paracolon group	Unidentified	
1	6	—	—	2			1		3(50)
2	13	—	—			2		3	5(38.5)
3	45	—	—	8	6	2	1	2	19(42.2)
Total	64	—	—	10	6	4	2	5	27(42.1)

taminated with enteric bacteria. In the remained 58 cases, no amebae were detected in the abscess specimen; 13 cases of group 2 revealed the protozoa only in stool, and were negative in 45 cases of group 3, but enteric bacteria were found in 38.5% of cases in group 2 and 42.2% of cases in group 3. These results suggest that bacteriological and parasitological examination is of use only in order to differentiate amebic and pyogenic abscess. In such a confusing case serological tests will be applied as supportive. By ameba immobilization reaction the first and second group showed 100% positive while the third group showed 83.3% positive.

3) Establishment of amebic liver abscess

Amebae might enter the liver by various routes; blood and lymph vessels or directly from the adjacent portions of the colon. Entry via portal vein is recognized as the most probable. Amebae reach the lumina of the large branches of the portal vein, then the

smaller ramifications of the veins and the adjacent sinusoids of the hepatic lobules. Carrera (1950) pointed out the evidence for early thrombus formation and a mild degree of local inflammation of the blood vessel wall which may relate to Arthus phenomenon. It is possible that the amebae, once in the portal blood stream, serve as foci around which small thrombi develop. The thrombus is usually composed of fibrin filaments, leucocytes and the amebae. The hepatic cells in the periportal areas around these veins show a variable degree of degeneration and necrosis which gradually increases in severity toward the central portions of the hepatic lobules and finally results in an abscess (Fig. 21).

Mechanical and chemical damages are encountered as predisposing factors for formation of amebic abscess. Hepatic injury by migrating larvae of *Toxocara canis* (Krupp, 1956) or introduction of insolubable matter such as glass particles into liver tissues (Gogler and Knight, 1974) may provide a precondition for abscess formation by the invaded amebae. Chemical damage also provides a favorable condition for their establishment. Roh and Kim (1948) found that 83.7% of the 159 abscess cases were identified as heavy drinkers or habitual alcoholics. Im and Kim (1976), associates of the author, succeeded in establishing amebic liver abscess experimentally by administering hepato-toxic materials before infecting with amebae. These results shall be reviewed briefly. White mice weighing about 20gm, rats about 100gm, rabbits weighing about 1500gm and golden hamsters weighing about 40gm were used as experimental animals, regardless of sex. In this experiment *E. histolytica* YS-27 and YS-37 strains isolated from pus of a amebic liver abscess cases in Severance Hospital were used. The amebae were maintained by subculture in modified diphasic medium (Faust and Russell, 1964) until use; strain YS-27 was kept in the laboratory for 76 months and strain YS-37 for 42 months.

On experiment, the media were centrifuged at 500-1000 rpm for 5 minutes to separate the supernatant. The separated supernatant was used as control to study the pathogenicity of concomitant bacteria. After the experimental animal was anesthetized with ether, the abdomen was opened through a right upper paramedian incision, then *Entamoeba histolytica*,

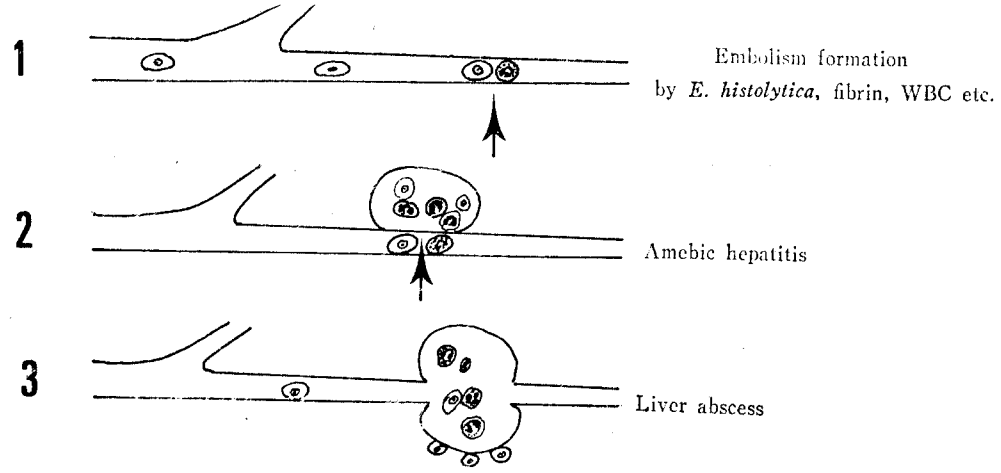


Fig. 21. Possible processes of amebic liver abscess formation.

5×10^4 to 18×10^4, were injected directly into the hepatic parenchyma of white mouse, rat and golden hamster. Bleeding was controlled using a gelfoam sponge. In rabbits, after induction of anesthesia, a low midline incision was made and *Entamoeba histolytica*, 15×10^4, were injected via the superior mesenteric vein. The animals were divided into 3 subgroups; control group with saline injection only, group injected with concomitant bacteria and group injected with amebae. Thioacetamide and carbon tetrachloride were used as hepatotoxic agents. Four percent thioacetamide was injected intramuscularly in the thigh at the dose of 0.25 mg/100gm of body weight, and the amebae were inoculated 3 hours later. Ten percent carbon tetrachloride in liquid paraffin was introduced to the stomach via nasogastric tube, the dose of 0.1mg/100gm of body weight, and amebae were injected 24 hours later. Six to 11 days after the inoculation the animals were sacrificed and examined for parasitological and pathological findings. Separately, thioacetamide and carbon tetrachloride were given to healthy rats in the same amounts as above, and the excised liver examined 3 hours and 24 hours after the administration for any pathological change of the organ. For parasitological and pathological examinations, pus from the liver was diluted with normal saline and examined for the presence of the amebae under microscope. The abscess mass and the surrounding liver tissues were fixed with 10% formaldehyde, and stained with hematoxylin and eosin for pathological examination.

The results show that, susceptibility to amebae was different in various animal species, and production of abscess was influenced by pathcgenicity of amebae and the functional status of the liver. The susceptibility to *E. histolytica* was different by animals (Table 19). In the liver of rat which had received intramuscular injection of thioacetamide no notable change was observed in three hours. The surface was smooth and normal in color, but microscopic pictures showed vacuolization around the central vein and infiltration of inflammatory cells, especially lymphocytes were seen in the periportal and centrilobular areas (Table 20, 21). Twenty four hours following carbon tetrachloride injection into rats, the surface of the liver seemed normal but the color changed to brownish yellow. Microscopic findings showed congestion and mild fatty degeneration. In mice, which were injected with 5×10^4 of YS-37 strain and 10×10^4 of YS-27 strain, no abscess was found in the liver of the former on the 9th and the latter on the 11th post-operative day.

Table 19. Development of amebic liver abscess in various experimental animals (Im and Kim, 1976)

Experimental animals	No. of exam.	Duration (day)	Inoculum		Abscess formation
			Strain	Amount	
Mouse	6	9	YS-37	5×10^4	0/6
Rat	5	11	YS-27	15×10^4	0/5
Rabbit	2	—*	YS-27	15×10^4	all dead
	3	—*	YS-37	18×10^4	all dead
Golden hamster	4	6	YS-27	10×10^4	2/3

* Died within 24 hours

Table 20. The effect of thioacetamide upon the development of amebic liver abscess in rats (Im and Kim, 1976)

Hepatotoxic agent	No. of rat	Duration (day)	Inoculum		Abscess formation
			Strain	Amount	
None	8	10	YS-27	10×10^4	1/8
Thioacetamide	6	10	saline	0	0/6
	8	10	concomitant bacteria	0	4/8
	8	8	YS-27	15×10^4	8/8
	8	10	YS-27	15×10^4	8/8

Table 21. The effect of carbon tetrachloride upon the development of amebic liver abscess in rats (Im and Kim, 1976)

Hepatotoxic agent	No. of rat	Duration (day)	Inoculum		Abscess formation
			Strain	Amount	
Carbon tetrachloride	4	10	saline	0	0/4
	4	11	concomitant bacteria	0	1/4
	6	11	YS-27	15×10^4	6/6

In rabbits which were injected with 15×10^4 of YS-27 and 18×10^4 of YS-37 via superior mesenteric vein, death occurred in all cases within 24 hours, but the reason could not be clarified. In golden hamsters, amebic liver sbscess was found after injection of YS-27 strain, 10×10^4, on the 6th postoperative day. For further experiment, rat which is non-susceptible to ***Entamoeba histolytica***, was selected to test the influence of hepatotoxic agents. Livers of the rats treated with thioacetamide and carbon tetrachloride abscess were examined. The control group, which was not pretreated with thioacetamide but injected with YS-27 strain, 15×10^4, showed one tiny abscess in one out of eight rats on the 10th postoperative day. In the saline injected group, those which were pretreated with thioacetamide showed no evidence of abscess on the 10th postoperative day. But the group injected with amebae, YS-27 strain which was pretreated with thioacetamide showed liver abscess in all the rats at the 8th and 10th postoperative day. In the thioacetamide pretreated group bacteria injection produced abscess in 4 out of 8 rats by the 10th post-operative day, the size was much smaller than that of amebic abscess. The average size of the amebic abscess was 219.4mm^3, and 120.0mm^3 in case of bacterial abscess. In the carbon tetrachloride pretreated rats, all 6 rats showed abscess formation on the llth post-operative day, but no abscess was produced by injection of only physiological saline in the carbon tetrachloride pretreated group. In the animals with concomitant bacteria injection, abscess was found in one out of four rats. Liver tissues of golden hamster which was injected with YS-27 strain showed infiltrations of neutrophil leucocytes, other polymorphonuclear leucocytes, lymphocytes and macrophages. Proliferative changes were also noticed in the abscess wall. Perivascular infiltration with chronic inflammatory cells

and macrophages were also noted in the hepatic parenchyma. Trophozoites of *Entamoeba histolytica* were found in the abscess wall.

The overall results suggest that chemical hepatic injuries might dispose of and quicken the formation of amebic liver abscess even in the less susceptible animal such as rat, and concomitant micro-organisms enhance the virulence of amebae to establish abscess in the liver, but at a non-significant level. Clinical experience may endorse a part of the experimental results.

Pulmonary amebiasis

Pulmonary amebiasis may involve lung abscess, empyema and pleurisy due to infection with *E. histolytica*. There are two infection routes in primary pulmonary amebiasis. The amebae are transported to lung directly via middle or inferior hemorrhoidal vein, then establish amebic embolism and pneumonitis. In secondary pulmonary amebiasis the amebae spread through the thoracic cavity by rupture of a liver abscess or via hepatic vein to lung. About 10~20% of the liver abscesses were complicated by pulmonary abscess (Kim, 1963), whereas primary pulmonary amebiasis is reported relatively in rare in Korea. Kim (1963) reported nine cases of pulmonary amebiasis; one primary and eight secondary. He detected the ameba in 60~75% of patient sputa.

Liver abscess may rupture and ameba in the pus spread to juxtaposition. Ludlow (1923) observed it twenty cases out of 100 amebic liver abscess cases; in nine cases to thoracic cavity, in one case to thoracic wall, in five cases to bones of the rib cage, and in five cases to abdominal cavity. Due to its anatomical position the majority of the abscesses is formed in the upper part of the right lobe of the liver, and the amebae reach the thoracic cavity passing through the diaphragm. The main complications of amebic liver abscess are pericarditis (Kim, 1964; Park *et al.*, 1966) and lung abscess (Choi, 1968). Kang *et al.* (1967) reported a case of an eight year-old boy with traumatic rupture of an amebic abscess in the right lobe into the abdominal cavity after a traffic accident. During surgery he found the ruptured cavity measuring 5.0×6.0 cm. Inside of the cavity, dark red-colored mucus still remained together with a small gas containing cyst from an amebic lung abscess in the posterior portion of the right lower lobe.

Other Extra-Intestinal Amebiasis

It is conceivable that there might exist extra-intestinal amebiasis other than liver and lung abscess, but only a few reports are available in the literature; three cases of amebiasis in uterine cervix and vagina by Lee *et al.* (1965); five cases of amebic pericolitis together with one case of coxitis and one case of amebic psoitis by Kim (1962); and one case of polyarthritis due to amebic colitis by Kim *et al.* (1968). Metastasis to brain, kidney, spleen, oösalphinx, ovary, testis and skin have been reported elsewhere but no reference is available from Korea.

EPIDEMIOLOGY

Prevalence

About 26 helminthic species and 20 protozoan species have been found so far to be parasites of man in Korea (Soh, 1973) (Table 22), and *Entamoeba histolytica* is the popularly known pathogenic species in the country among the protozoa. The incidence of *E. histolytica* has been reported by several investigators. Kessel (1925) found a 41% prevalence by four successive direct smears from 208 examinees, and Choi (1926) reported 1.5% positives among 2,000 fecal samples by single direct smear, whereas 30.2% in Seoul area were positive after six repeated examinations. Chiba (1931) also obtained similar results. Soh *et al.* (1961) reported 4.3% of 10,320 fecal samples and to be positive for *E. histolytica* found no special age or seasonal differences among them, but Yoon and Choi (1966) reported that clinical cases were predominant during summer.

Recently, Kim *et al.* (1971) reported 6.4% prevalence by direct smear method, zinc sulfate flotation method and formalin-ether concentration method among 2,250 fecal specimens which were collected from ten localities in different provinces (Fig. 22, Table 23). Each specimen was examined three times under microscope. Overall figures show that prevalences ranged between 3.3% and 9.9%; the lowest in Gyeonggi-Do and the highest in Gyeongsang Bug-Do, and national average was 6.4%. But the prevalence rates found varies according to reporter, examination method and

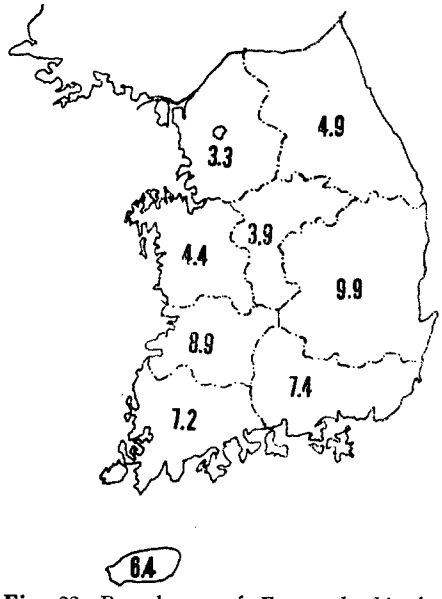


Fig. 22. Prevalences of *Entamoeba histolytica* infection in Korea (Kim *et al.*, 1971).

Table 22. Protozoa found in human subjects in Korea (Soh, 1973)

Class	Phylum protozoa
Rhizopoda	*Entamoeba histolytica, E. coli, E. gingivalis, Endolimax nana, Iodamoeba bütschlii, Dientamoeba fragilis*
Flagellates	*Giardia lamblia, Trichomonas hominis, T. tenax, T. vaginalis, Enteromonas hominis, Chilomastix mesnili, Embadomonas* sp.
Ciliates	*Balantidium coli*
Sporozoa	*Isospora hominis, Plasmodium vivax, Pl. malariae, Pl. falciparum, Toxoplasma gondii, Pneumocystis carinii*

Table 23. Prevalence of intestinal protozoa according to age and sex (Kim *et al.*, 1971)

Age (year) and Sex	No. Exam.	Positive No. (%)	Prevalence (%)									
			*E.h.	E.c.	E.n.	G.l.	T.h.	C.m.	I.b.	E. h.	D.f.	Iso
Under 4	143	40(28.0)	3.5	8.4	6.9	6.3	0.7			0.7	0.7	
M	86	22(25.6)	3.5	9.3	7.0	4.7	(1case)					
F	57	18(31.6)	3.5	7.0	7.0	8.8				(1case)	(1case)	
5~ 9	348	76(21.8)	4.9	12.6	3.2	6.9	1.1	0.9		0.9		
M	170	31(18.2)	4.1	1.2	2.9	6.5	0.6	0.6		0.6		
F	178	45(25.3)	5.6	13.5	3.4	7.3	1.7	1.1		1.1		
10~19	651	210(32.3)	6.6	21.2	8.6	5.4	1.1	0.5	0.6	0.2		
M	335	109(32.5)	6.3	20.9	8.1	5.1	0.3	0.6	0.6			
F	316	101(32.0)	7.0	21.5	9.2	5.7	1.9	0.3	0.6	(1case)		
20~29	170	76(44.7)	7.1	20.0	13.5	5.9	1.2	1.8	1.2	1.8	0.6	
M	75	27(36.0)	4.0	9.3	10.7	8.0	1.3		1.3			
F	95	49(51.6)	9.5	28.4	15.8	4.2	1.1	3.2	1.1	3.2	(1case)	
30~39	339	140(41.3)	5.6	24.8	11.8	4.4	0.9	0.6	0.9	1.2		
M	143	50(35.0)	3.5	18.9	9.8	4.9	2.1			0.7		
F	196	90(45.9)	7.1	29.1	13.3	4.1		1.0	1.5	1.5		
40~49	353	140(39.7)	7.6	2.8	14.7	3.4	0.9	0.3	0.6	0.6		0.3
M	177	53(29.9)	5.1	14.7	10.2	3.4				0.6		
F	176	87(49.4)	10.2	29.0	19.3	3.4	1.7	(1case)	1.1	0.6		(1case)
50~	246	104(42.3)	8.9	29.3	13.8	3.7	1.6		0.8	0.8		
M	118	48(40.7)	9.3	22.9	11.9	5.9	2.5		0.8			
F	128	56(43.8)	8.6	35.2	15.6	1.6	0.8		0.8	1.6		
Total	2,250	786(34.9)	6.4	20.5	10.0	5.1	1.1	0.5	0.6	0.7	0.1	0.04
M	1,104	340(30.8)	5.3	16.8	8.3	5.3	0.9	0.3	0.4	0.3	0.1	0
F	1,146	446(38.9)	7.5	24.1	11.7	4.9	1.2	0.8	0.8	1.1	0.1	0.1

* E.h.: *Entamoeba histolytica*, E.c.: *Entamoeba coli*, E.n.: *Endolimax nana*, G.l.: *Giardia lamblia*, T.h.: *Trichomonas hominis*, C.m.: *Chilomastix mesnili*, I.b.: *Iodamoeba bütschlii*, E.h.: *Enteromonas hominis*, D.f.: *Dientamoeba fragilis*, Iso.: *Isospora* species

Table 24. Prevalence of *Entamoeba histolytica* infection in Korea

Reporter	Place	Year	No. exam.	Intestinal protozoa general, %	*E. histolytica* cyts, %	Method of examination
Kessel	Seoul	1925	208		41	
Choi	Seoul	1926	2,000		30.2	6 repeated exam.
Takemura	Seoul	1933	216	39.4	18	1 exam.
Okinami	Seoul	1942	96	40.6	18	
Chang *et al.*	Seoul	1951	169	32.9	4.8	concentration method
	Choonchon	〃	262	35.5	5.0	
	Daejon	〃	80	32.5	1.3	
	Gwangju	〃	45	40.0	11.1	
	Mokpo	〃	44	34.1	4.6	
	Busan	〃	93	32.3	4.3	
	Daegu	〃	73	32.9	8.2	
	Jeju	〃	37	45.7	9.9	
Soh *et al.*	Seoul	1958~1961	10,320	22.3	4.3	formalin-ether conc. method
Kim	Jeju	1967	466	53.9	24.4	direct smear method
Kim *et al.*	whole country	1971	2,250	34.9	6.4	direct and conc. method
Cho *et al.*	Ullung-Do	1973	695	58.7	5.5	

area even when in the same province. Nevertheless, the general trend in recent years has been one of declining incidence rates compared to several decades ago (Table 24). There is still a high prevalence in some provinces, such as Jeju-Do. Environmental factors and traditional habits of the inhabitants may cause to maintain such a constancy.

Age and Sex

In a nationwide survey of the cystic stage of *Entamoeba histolytica* Kim *et al.* (1971) found a higher prevalences of 8.9% in the 50 years of age and above group(Table 25). Kim(1967) reported a higher prevalence in 31~60 years of age groups with rates of 31.6~34.8% in Jeju-Do than the young age groups(Table 26). The number of clinical case also increases in adult age. Sato(1913) summarized the amebiasis cases admitted to government hospitals. In a total of 21 cases 16 were 50 years of age and over. But Yoon and Choi(1966) found that infants less than two years of age suffered already from amebic dysentery in 28 cases(33%) out of a total of 85 below 15 years of age(Table 27).

Table 25. Prevalence of *Entamoeba histolytica* infection by age and sex in Korea (Kim *et al.*, 1971)

Age (year)	No. examined	Positive rate, %	Sex	
			Male	Female
4	143	3.5	3.5	3.5
5~9	348	4.9	4.1	5.6
10~19	651	6.6	6.3	7.0
20~29	170	7.1	4.0	9.5
30~39	339	5.6	3.5	7.1
40~49	353	7.6	5.1	10.2
50~	246	8.9	9.3	8.6
Total	2,250	6.4	5.3	7.3

Table 26. Prevalence of *Entamoeba histolytica* infection by age in Jeju-Do (Kim, 1967)

	Male		Female		Total	
Age	No. exam.	Positive(%)	No. exam.	Positive(%)	No. exam.	Positive(%)
~5	46	9(19.6)	37	4(10.8)	83	13(15.7)
6~10	85	15(17.6)	79	14(17.7)	164	29(17.7)
11~20	145	39(26.9)	103	29(28.2)	248	68(27.4)
21~30	53	7(13.2)	44	10(22.7)	79	17(21.5)
31~40	44	12(27.3)	51	18(35.3)	95	30(31.6)
41~50	31	5(16.1)	38	19(50.0)	69	24(34.8)
51~60	17	4(23.5)	25	10(40.0)	42	14(33.3)
61~	28	8(29.6)	40	8(20.0)	67	16(23.9)
Total	448	99(22.1)	417	112(26.9)	865	211(24.4)

Prevalence of *E. histolytica* infection seems to be higher in females than in males (Soh *et al.*, 1961). The data by Kim *et al.* (1971) show the ratio: 7.5 : 5.3(Table 23). Kim (1967) analyzed 211 positive cases in Jeju-Do, and found 22.1% to be male and 26.9% female(Table 26). Yoon and Choi(1966) examined 85 cases of amebic dysentery among children and found that females exceeded males(Table 27). But judgment in this matter shall be deferred because scientific reason for the difference is yet lacking.

Table 27. Age and sex distribution of amebic dysentery cases in Busan Children's Charity Hospital (Yoon and Choi, 1966)

Sex\Age	0~2	3~6	7~10	11~15	Total
Male	8	16	8	14	36
Female	20	24	5	0	49
Total	28	40	13	14	85

On one side, Kim(1971) analysed the 102 amebic liver abscess in Jeju-Do, and found 83.3% to be male and only 16.7% females. Although no logical explanation can be offered yet, environmental factors, habits and difference of physical conditions of each sex may contribute to cause such diversities.

Occupation

The occupations of the Korean people differ somewhat from those found in the western countries, and the conditions under which they live vary materially by financial status and educational standard of the individual. No recent data according to occupation are available. Nevertheless, old literature(Sato, 1913; Mills, 1927) indicate that specific relationship between occupation and disease occurrence is not established, but is rather diverse (Table 28). However, it is noteworthy that laborer(coolie) and artisan(carpenter and low levelled technician) showed higher incidence than other groups.

Table 28. Amebic dysentery cases according to occupation. From the records of the clinical laboratory of Severance Union Medical College, 1913~1917 (Mills, 1927)

Occupation	No. examined	Amebiasis(%)	Occupation	No. examined	Amebiasis(%)
Amah	1	—	Miner	14	—
Artist	3	—	Musician	1	—
Artisan	53	1(1.9)	Nurse	63	1(1.6)
Banker	6	—	Official	75	—
Beggar	4	—	Pharmacist	2	—
Clerk	19	—	Preacher	75	1(1.3)
Conductor	2	—	Printer	8	—
Cook	5	—	Sailor	4	—
Laborer	896	19(2.1)	Soldier	1	—
Dancing Girl	1	—	Student	1,163	18(1.5)
Doctor	20	—	Tailor	3	—
Farmer	850	14(1.6)	Teacher	61	—
Housewife	1,799	24(1.3)	Not stated	1,137	26(2.3)
Innkeeper	4	—			
Lawyer	1	—	Total	7,000	115(1.6)
Merchant	729	11(1.5)			

Seasonal variation

Infection with *E. histolytica* is insidious in nature and chronic. For this reason, it is not logical to draw conclusions regarding seasonal variations. From the ecological view point it is estimated that the incidence should be high during summer months. But Soh *et al.* (1961) found only 2.5~5.2% during May to August, while 5.2~6.1% during September to December(Table 29). Clinical cases, however, are more frequent during warmer months. Sato(1913) analyzed amebic dysentery cases admitted to government hospitals and found 10 of 24 cases occurred in July and August(Table 30).

Table 29. Monthly incidence of intestinal protozoa among the out-patients of Severance Hospital, July 1, 1959~June 30, 1961 (Soh *et al.*, 1961)

Month	Total No. examined	Incidence(%)					
		*E.h.	E.c.	E.n.	G.l.	I.b.	C.m.
January	835	3.6	10.7	8.4	3.7	0.5	0.2
February	899	3.7	11.5	8.6	4.9	0.7	0.7
March	882	4.3	12.6	10.7	5.6	0.4	0.4
April	740	3.8	11.8	11.8	3.5	0.4	0.3
May	908	2.5	10.1	6.0	5.0	0.3	—
June	993	2.2	11.0	8.0	3.6	0.4	0.3
July	943	4.0	8.9	4.9	5.8	0.4	—
August	902	5.2	12.3	7.3	5.3	1.2	1.0
September	1,034	5.2	11.1	6.8	5.7	0.5	0.2
October	891	5.8	9.8	6.1	4.7	0.6	0.6
November	637	5.7	11.8	7.1	5.0	0.2	0.5
December	606	6.1	11.7	7.4	3.1	—	0.2
Total	10,320	4.3	11.1	7.7	4.7	0.5	0.4

* E.h.: *Entamoeba histolytica*, E.c.: *Entamoeba coli*, E.n.: *Endolimax nana*, G.l.: *Giardia lamblia*, I.b.: *Iodamoeba bütschlii*, C.m.: *Chilomastix mesnili*

Usually, more amebic patients are recorded during warm months. Abundance of insects, availability of raw foods and weakened physical condition in hot temperature may accelerate the higher incidence.

Table 30. Monthly cases of amebic dysentery in 10 provincial hospitals in Korea (Sato, 1913)

Month	Jan.	Feb.	Mar.	Apr.	May	Jun.	July	Aug.	Sep.	Oct.	Nov.	Dec.
Case	0	1	0	0	1	2	5	5	3	1	4	1

Environmental factor

In many parts of Korea people still utilize human excreta as fertilizer for growing vegetables. Since no sanitary sewage system and night soil treatment system are yet provided in rural areas and many of the urban cities, the high prevalence of amebiasis in the land is recognized as a matter of course. Soh *et al.*(1959) detected *E. histolytica* cysts from 10 road side soil samples even in Seoul. Infected food handlers may act an important role in the transmission of the protozoa. Flies, cockroaches and some other coprophagous animals may contaminate food and drinking water.

A high incidence of amebiasis is found in areas where pollution is poorly controlled. Brooke *et al.*(1956) found 87% of *E. histolytica* carriers in the prisoner-of-war camp in Koje Island. Kim(1967) made an extensive study in Jeju Island where the inhabitants feed hogs with their feces utilizing pig-sties as toilet(Table 31). Thus hog feces and manure heaps may act as the spreading sources of the protozoa. It is noteworthy that drinking water which was kept in jars contained cysts in three samples out of 76 examined. In the same community two direct fecal examinations were carried out and 24.4% of 865 samples were positive for *E. histolytica* cysts. Almost 49.0% of the positive cases complained of liver enlargement and abdominal discomfort. The high rate of cyst carriers may induce dysentery followed by complications such as hepatic amebiasis and some other forms of extra-intestinal amebiasis.

Group living: Brooke *et al.* (1956) reported 87% of *E. histolytica* carrier in the prisoner-of-war camp of Koje-Island where sanitary conditions were poor. Cho and Soh

Table 31. *E. histolytica* cysts in various samples from Jeju Do (Kim, 1967)

Materials	No. of examined	Positive(%)	Other parasites
Hog feces	177	*44(24.8)	0
Manure heap	112	7(6.2)	*Taenia* spp.-1
Garden soil	126	0	0
House fly	5,442	1(0.02)	*E. coli*-2 *Ascaris*-2 *Trichocephalus*-1 *Enterobius*-1 *Taenia* spp.-3
Dust	137	0	*E. coli*-2 *Endolimax nana*-1 *Ascaris*-2, *Enterobius*-27
Fingernail dirt	128	0	*Taenia* spp.-12, *Enterobius*-2
Drinking water	76	3(3.9)	*Taenia* spp.-2

*No differentiation from *E.historytica* was done.

(1970) also reported 32.4% positives among 105 children in an orphanage in Gaejong, Jeonra-bug-Do, but among 149 inhabitants of the surrounding villages only 7.4% were positive. This endorses the theory that poor sanitation or crowding is directly related to the horizontal spread of the infection.

Family infection: In households where sanitation is poorly practiced the family infection cannot be overlooked. Cho *et al.* (1967) examined *E. histolytica* cyst carriers by household in Jeju-Do(Table 32). Of 65 households 51(78.5%) had positive. Amog them, only 12 households(23.5) had single positive case among their families, and 39(76.5%) had 2-5 persons infected in the same family. Kim(1971) visited 62 houses in which infants were positive for *E. histolytica* infection. Most of them were low-income and undernourished, and drinking water and food matters were handled in poor manner. By examining the respective family of the infants, the protozoa positives were 46.2% among mothers, 34.1% among fathers and 38.3% among brethren. *E. histolytica* cyst positives were; mothers 17.3%, fathers 7.4% and siblings 9.0%. The figures suggest that mother may play an important role as source of amebic infection among families. It again emphasizes that family infection is likely to be common in poorly controlled environment.

Table 32. *Entamoeba histolytica* infection by household in Jeju-Do (Cho *et al.*, 1967)

Village	Sinwon	Yong-Heung	Jochun	Total
No. exam. household	18	32	15	65
Average member/household	7	5	6	6
Positive household(%)	16(88.9)	26(81.3)	9(60.0)	51(78.5)
No. person infected/household positive(%)				
1	3(18.7)	7(26.9)	2(22.2)	12(23.5)
2	4(25.0)	10(38.5)	7(77.8)	21(41.2)
3	6(37.5)	7(26.9)	—	13(25.5)
4	2(12.5)	2(7.7)	—	4(7.8)
5	1(6.3)	—	—	1(2.0)

DIAGNOSIS

Unless the symptoms and signs are pathognomonic accurate diagnosis requires demonstration of *Entamoeba histolytica* in its trophic or cystic stage. In case the pathogenic agent cannot be isolated, scanning and ultrasonograph are the helpful methods(Fig. 25, 26, 27) and immunologic diagnosis is the alternative and promising method for this purpose, particularly in extra-intestinal amebiasis. In this chapter laboratory methods which the author and his associates have utilized shall be reviewed.

Direct method

In laboratory the direct smear method and concentration techniques such as the for-

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5	1(6.3)	—	—	1(2.0)

DIAGNOSIS

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Direct method

In laboratory the direct smear method and concentration techniques such as the for-

malin-ether concentration method are routinely employed except in special occasions. Recently, Cho *et al.* (1972d) developed the "Rolling Method" aiming at accomplishing more effective results in laboratory or field. The steps of the method are as follows:

1) Fecal specimen(0.5-1.0gm) is thoroughly comminuted in 10-30 ml of tap water and strained through wet cheese-cloth into a watch glass or petri dish.

2) Hold the container(watch glass or petri dish), and turn it to right and left, then leave the suspension until the objects concentrate in the center of the bottom due to their own gravity.

3) Leave container horizontally until the suspension is fully precipitated.

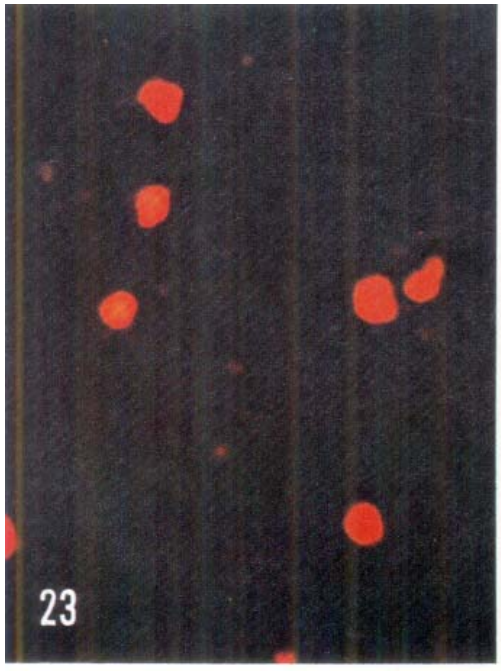
4) Pipette the sediment without disturbing the rest and transfer to the slide, then mount with cover glass.

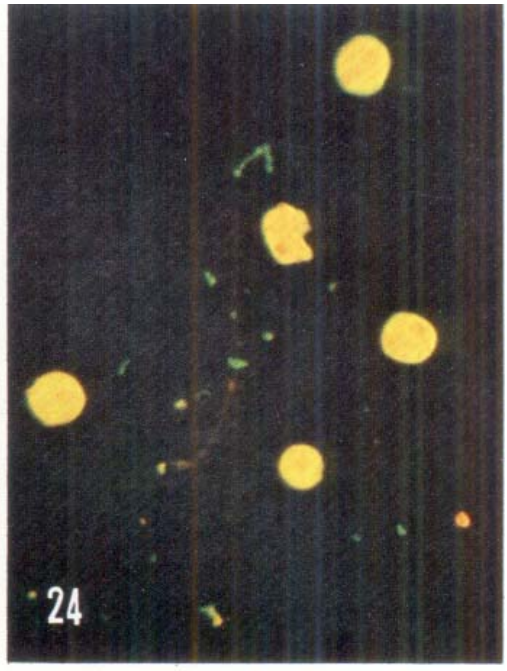
For examination of protozoan cysts, a drop of 2% iodine solution is mixed and the mixture mount with a coverglass.

To evaluate the method, comparison with direct fecal smear method and formalin-ether concentration method(MGL method) were made. The protozoa from 80 specimens were *Entamoeba histolytica* in 29 cases, *Entamoeba coli* in 56 cases, *Endolimax nana* in 39 cases, *Giardia lamblia* in 27 cases, *Iodamoeba bütschlii* in three cases, and *Chilomastix mesnili* in five cases. Of 29 *Entamoeba histolytica* positives, the cyst detection rates of each method were 37.9% with the direct method, 72.4% with the MGL and 34.5% with the rolling method respectively. Identical recovery of the cysts between each method were 2 cases from direct, MGL and rolling, 6 cases from direct and MGL, 6 cases from MGL and rolling, and 3 cases from direct and rolling(Table 33). Of 56 *Entamoeba coli* cases, the cyst detection rates were 60.7% by direct method, 75.0% by MGL and 82.2% by rolling method. Identical recovery rates were 48.2% from direct, MGL and rolling, 50.0% from direct and MGL, 60.7% from MGL and rolling, and 55.4% from direct and rolling. Of 39 *Endolimax nana* positives, the cyst detection rates were 33.3% by direct method, 64.1% by MGL and 25.6% by rolling method. Identical recovery of the cysts were 1 case from direct, MGL and rolling, 4 cases from direct and MGL, and 8 cases from MGL and rolling method of 39 *Iodamoeba bütschlii* cases, direct and MGL proved 2 cases each, and rolling

Table 33. Direct versus MGL versus rolling method in the detection of intestinal protozoa(Cho *et al.*, 1972 d)

Species	Combined total infection	Identified by		
		Direct(%)	MGL(%)	Rolling(%)
Entamoeba histolytica	29	11(37.9)	21(72.4)	10(34.5)
Entamoeba coli	56	34(60.7)	42(75.0)	46(82.2)
Endolimax nana	39	13(33.3)	25(64.1)	10(25.6)
Giardia lamblia	27	21(77.8)	19(70.4)	11(40.7)
Iodamoeba bütschlii	3	2(66.7)	2(66.7)	1(33.3)
Chilomastix mesnili	5	2(40.0)	5(100.0)	





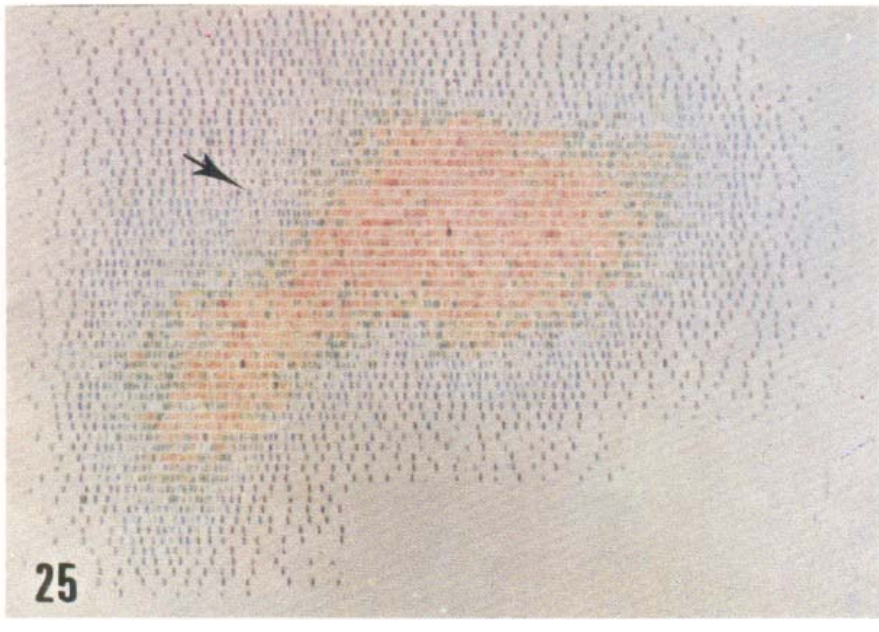
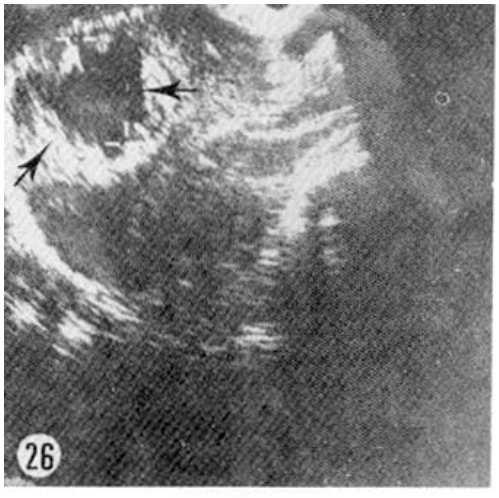
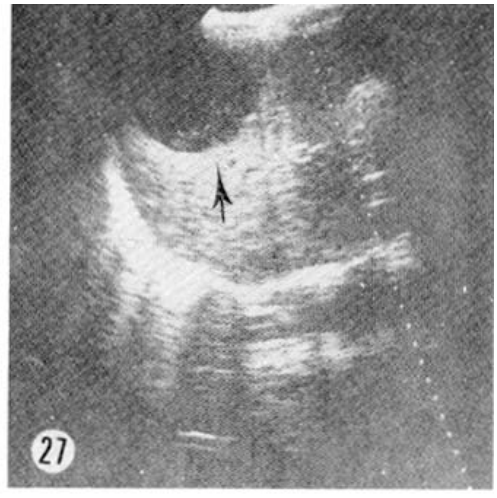
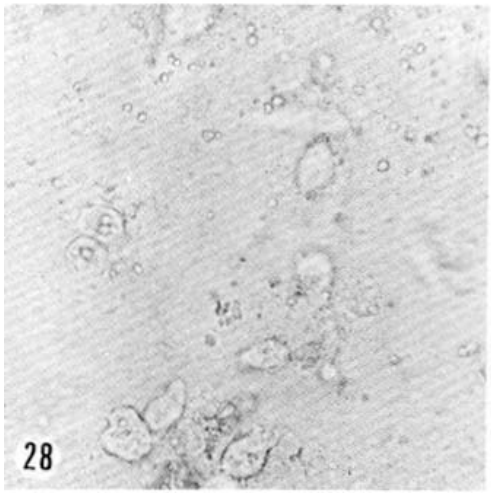


Fig. 23, 24. Negative(left) and positive(right) reactions of indirect fluorescent antibody (IFA) test for diagnosis of amebiasis.

Fig. 25. Liver scan. Showing the cold area(arrow) in right upper portion.







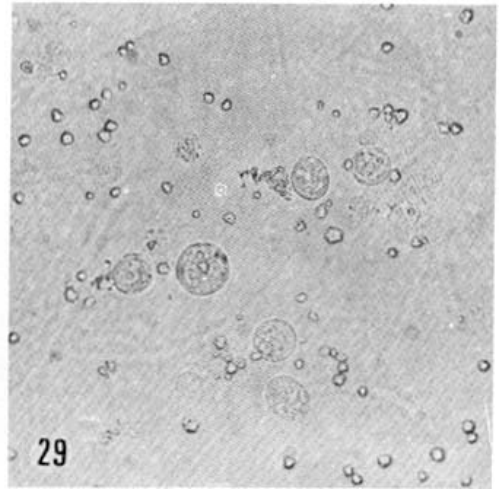


Fig. 26, 27. Ultrasonogram of amebic liver abscess. Showing cold areas before (Fig. 26) and after treatment (Fig. 27).

Fig. 28, 29. Ameba immobilization test. Normal (Fig. 28) and immobilized trophozoites (Fig. 29).

method proved 1 case. In the detection of protozoan cysts, MGL revealed the best result among 3 methods. Direct and rolling method showed similar results. As a conclusion, the rolling method showed lower cyst detection rates in protozoan infections than formalin-ether concentration method, though the results between the rolling and direct fecal smear method revealed no difference. It suggest that concentration method still holds superiority than direct fecal smear method and rolling method in detection of *Entamoeba histolytica* cyst.

Serological method

The most reliable diagnosis of *Entamoeba histolytica* infection is made by demonstration of the parasite in excreta or tissues of the host. However, laboratorians often face negative findings even in clinically proven cases particularly in extra-intestinal amebiasis. Several immunodiagnostic methods have been developed in order to supplement this insufficiency. These are; complement fixing antibody test(CFT), ameba immobilization test (AI), indirect fluorescent antibody test(IFA), soluble antigen fluorescent antibody test (SAFA), gel-diffusion precipitation test, immuno-electrophoresis(IEF), indirect hemagglutination test(IHA), intradermal test, bentonite flocculation test, latex agglutination test, *etc* (Table 35). But from the viewpoint of reproducibility, each of tests has its own merits (Table 34). As a routine procedure in laboratory, however, IHA, IFA, and AI are commonly used because the actual assessment time requires only one to three hours compared to CFT and precipitin tests which need a longer time. For this reason, the author and associates tried to evaluate various methods, specially IHA, IFA, AI, and agglutination test in the diagnosis of amebiasis.

Table 34. Reproducibility of serological tests for diagnosis of amebiasis

Serological tests	Time requirement(appoximately)	Sensitivity	Specificity
Complement fixation test	1~2 hours	++	+
Ameba immobilization test	1 hour	+	+
Indirect fluorescent antibody test	5 hours	++	+++
Soluble antigen fluorescent antibody test	5 hours	++	+++
Double diffusion test	3 days	++	+++
Immunoelectrophoresis	3 days	+	+++
Countercurrent immuno-electrophoresis test	24 hours	++	+++
Indirect hemagglutination test	18 hours	+++	++
Intradermal skin test	30 minutes	+	+
Bentonite flocculation test	30 minutes	++	+
Latex agglutination test	30 minutes	+	+

Note: +: weak ++: moderate +++: strong

1) Indirect fluorescent antibody test(IFA), ameba immobilization test(AI) and indirect hemagglutination test(IHA)

Along with the rapid development of new serologic techniques for the study of antibody response in *E. histolytica* infection, comparison of the techniques has been performed by several investigators(Maddison, 1965; Krupp, 1969). Since IFA was employed first by Goldman(1966) for the epidemiological study of amebiasis, it has also been evaluated from the point of view of specificity, sensitivity and reproducibility by different workers (Maddison *et al.*, 1968), and regarded as of value for routine diagnosis(Fig. 23, 24). The ameba immobilization test was used in *E. histolytica* infection by Biagi-F and Buentello (1961) and other workers. Hong *et al.*(1968) also evaluated this method experimentally and clinically. First, they prepared immune rabbit sera with a 48-hour-culture of *E. histolytica*. The highest immobilization reaction occurred after 45~105 minutes and the remobilization of the parasite took place gradually. Immobilization appeared in higher rates in the inactivated rabbit sera group, but the difference among ameba strains was not remarkable. In human amebiasis sera, the highest peak of immobilization reaction occurred for 45 to 90 minutes(Fig. 28, 29). Positive rates for the AI according to clinical features were; 83.3%~100% in liver abscess cases, 83.3~90.7% in hepatomegaly cases, 45.4% in asymptomatic cyst passers and 31.5% in healthy controls. It was found that the high rate of immobilization decreased during two to three months after treatment. To elucidate the reproducibility of IFA and AI, Cho and Soh(1969) carried out a comparative study using sera of 84 Jeju islanders, a highly endemic area of amebiasis in Korea(Table 35, 36, 37). The sera were divided into seven groups; 1) liver abscess(*E. histolytica* found in

Table 35. Reactivity of indirect fluorescent antibody(IFA) and ameba immobilization(AI) tests on *Entamoeba histolytica*(YS-9) with the sera of amebiasis cases in Cheju-Island(Cho and Soh, 1969)

Group	Clinical status	No. of tested	IFA						AI	
			Neg.	Positive titers						
			(%)	1:16	1:64	1:256	1:1024	Total (%)	<50 (%)	>51 (%)
1	Liver abscess *E. histolytica* present in liver	5		1	3	1		5 (100)		5 (100)
2	Liver abscess *E. histolytica* present in stool	6		2	4			6 (100)		6 (100)
3	Liver abscess *E. histolytica* absent	15	9 (60)	2		3	1	6 (40)		15 (100)
4	Hepatomegaly *E. histolytica* present in stool	10	6 (60)	3	1			4 (40)	2 (20)	8 (80)
5	Hepatomegaly *E. histolytica* absent	21	14 (66.7)	5	2			7 (33.3)	2 (9.5)	19 (90.5)
6	Asymptomatic *E. histolytica* present in stool	10	5 (50)	5				5 (50)	6 (60)	4 (40)
7	Control	17	16 (94.1)	1				1 (5.9)	13 (76.5)	4 (23.5)
	Total	84	50 (59.5)	19	10	4	1	34 (40.5)	23 (27.4)	61 (72.6)

Table 36. Comparisons of indirect fluorescent antibody(IFA) and ameba immobilization(AI) tests in sera of amebiasis cases in Cheju-Island(Cho and Soh, 1969)

Diagnosis	No. of tested	IFA and AI tests			
		F+I+	F−I−	F+I−	F−I+
Liver abscess					
1) *E. histolytica* present in liver	5	5			
2) *E. histolytica* present in stool	6	6			
3) *E. histolytica* absent	15	6			9
Hepatomegaly					
1) *E. histolytica* present in stool	10	4	2		4
2) *E. histolytica* absent	21	7	2		12
Asymptomatic					
E. histolytica present in stool	10	2	3	3	2
Control	17	1	13		3
Total	84	31	20	3	30

liver), 2) liver abscess(*E. histolytica* found only in stool), 3) liver abscess(*E. histolytica* not demonstrated by examinations of abscess and stool), 4) hepatomegaly(*E. histolytica* found in stool), 5) hepatomegaly(*E. histolytica* not found in stool), 6) cyst carrier, asymptomatic healthy individuals and 7) healthy control group.

The results showed that in the indirect fluorescent antibody test, 100% of sera in group 1 and group 2, 40% in group 3 and group 4, 33.3% in group 5, 50% in group 6 and 5.9% in control group were found positive at 1 : 16 or higher. Higher titers appeared in proven liver abscess, although titers were lower in cyst carrier and in control group. In the immobilization test, 100% of sera in group 1, 2 and 3, 80% in group 4, 90.5% in group 5, 40% in cyst carrier and 23.5% in the control group were positive. Both tests were positive in all sera of group 1 and 2, six sera out of 15 in group 3, four out of 10 in group 4, seven out of 21 group 5, two out of 10 in cyst carriers and one out of 17 in controls. IFA titers were not correlated with the rate of immobilization in this study.

These results suggest that the two methods are preferable for the diagnosis of suspicious amebiasis.

In order to find a more reliable and reproducible way to diagnose the infection Min (1975) conducted comparison studies with several commonly accepted immunological tests. Among the methods IHA, IFA and AI were chosen because of their sensitivity and the level of antibody response.

Serum samples were collected from 157 persons; 52 patients with proven amebic infections, 22 apparently healthy persons, and 83 patients with miscellaneous diseases in whom *E. histolytica* had not been found. The cases were grouped into six groups;

Table 37. Distribution of antibody reactivity by immunological tests in *Entamoeba histolytica* infected cases(Min, 1975)

Group Clinical type	Test	Reactivity				Total	
		Negative	Low	Medium	High	No.positive/ No.exam.	% positive
1. Amebic liver abscess	IHA		1	3	8	12/12	100.0
	IFA		3	2	7	12/12	100.0
	AI	1		6	5	11/12	91.6
2. Amebic hepatitis	IHA				14	14/14	100.0
	IFA	1	5	3	5	13/14	92.8
	AI	2	1	6	5	12/14	85.9
3. Amebic lung abscess	IHA				1	1/1	100.0
	IFA			1		1/1	100.0
	AI				1	1/1	100.0
4. Acute intestinal amebiasis	IHA			1	3	4/4	100.0
	IFA		1	3		4/4	100.0
	AI		1	3		4/4	100.0
5. Mild symptomatic *E. histolytica*	IHA				11	11/11	100.0
cyst passer	IFA	1	1	5	4	10/11	90.9
	AI	2		2	7	9/11	81.8
6. Asymptomatic *E. histolytica*	IHA	1	1	1	7	9/10	90.0
cyst carrier	IFA	2	1	6	1	8/10	80.0
	AI	3	1	3	3	7/10	70.0
7. Healthy control	IHA	15	1	6		7/22	31.8
	IFA	21	1			1/22	4.5
	AI	15	1	6		7/22	31.8

Note: IHA.. Negative: ≤32, Low: 128, Medium: 512, High: ≥2,048
IFA.. Negative: ≤8, Low: 16, Medium: 32-64, High: ≥128
AI.. Negative: ≤50, Low: 51-65, Medium: 66-80, High: ≥81

amebic liver abscess, amebic hepatitis, amebic lung abscess, acute intestinal amebiasis, mild symptomatic cyst passers, and asymptomatic cyst carriers. The positive results by the diagnostic methods were: 100, 100, 100, 100, 100 and 90.0% by IHA test; 100, 92.8, 100, 100, 90.9 and 80.0% by IFA test; and 91.6, 85.9, 100, 100, 81.8, and 70.0% by AI test respectively in order of the groups above. The IHA test was performed with *E. histolytica* HK-9 antigen(ICN-Chemicals & Radioisotope Div., U.S.A.) and IFA and AI tests were done with *E. histolytica* YS-27 strain according to the methods described by Cho and Soh (1969).

Healthy controls showed positive rates of 31.8% by IHA, 4.5% by IFA and 31.8% by AI tests showing a good specificity in IFA.

In general, concomitant positivity of three methods such as IHA, IFA and AI would support that the disease was of amebic origin; one test positive only or concomitant negativity of the three tests is clinically non-significant, and two tests positives would need supplementary stool examination for reliable diagnosis.

2) Latex agglutination test(LA) and Gel-diffusion precipitation tests(GDP)

The use of the latex agglutination test was reported by Morris *et al.*(1970) in

the diagnosis of invasive amebiasis, and the test kit is now marketed under the trade name of Serameba. The gel-diffusion was introduced by Krupp and Powell(1971). Cho (1974) evaluated the tests such as LA and GDP with the same serum samples which were assayed for IHA, IFA and AI(Table 38, 39, 40, 41). According to his report the percentages of positive LA test were 40.0 in amebic liver abscess, 23.1 in amebic hepatitis, 14.3 in mild symptomatic *E. histolytica* cyst passers and 12.5 in asymptomatic *E. histolytica* cyst carriers. Acute intestinal amebiasis and healthy controls showed a negative reaction. In comparison with other serological tests the positive rates of LA reaction were 22.9% in IHA, IFA and AI positive sera, and 16.7% in IHA and IFA positive sera. Other serum groups were negative for the reaction(Table 39). The data reveal a very low sensitivity of the LA test of the serum samples compared to the results reported by Morris *et al.*(1970).

The percentage of positive GDP test was 75.0 in acute intestinal amebiasis, 22.2% in mild symptomatic *E. histolytica* cyst passers, and healthy controls showed a negative reaction (Table 40). Of the sera from patients with miscellaneous non-amebic diseases, only the group

Table 38. Results of latex agglutination(LA) test on serum from 24 patients with extraintestinal amebiasis, 19 cases with intestinal amebiasis and 20 healthy controls(Cho, 1974)

Clinical type	No. tested	Positive	
		No.	%
Extra-intestinal amebiasis			
Amebic liver abscess	10	4	40.0
Amebic hepatitis	13	3	23.1
Amebic lung abscess	1	0	0.0
Total	24	7	29.2
Intestinal amebiasis			
Acute intestinal amebiasis	4	0	0.0
Mild symptomatic *E. histolytica* cyst passer	7	1	14.3
Asymptomatic *E. histolytica* cyst carrier	8	1	12.5
Total	19	2	10.5
Healthy controls	20	0	0.0

Table 39. Comparison of latex agglutination(LA) test with the results of indirect hemagglutination (IHA), indirect fluorescent antibody(IFA), and ameba immobilization(AI) tests for the diagnosis of *E. histolytica* infections(Cho, 1974)

IHA, IFA and AI tests	LA test		Total
	Positive(%)	Negative(%)	
IHA, IFA, AI positive	8(22.9)	27(77.1)	35
IHA and IFA positive	1(16.7)	5(83.3)	6
IHA and AI positive	0	3	3
IHA positive only	0	5	5
AI positive only	0	5	5
IHA, IFA, AI negative	0	9	9
Total	9	54	63

Table 40. Results of gel-diffusion precipitation(GDP) test on serum from 22 patients with extra-intestinal amebiasis, 22 cases with intestinal amebiasis, 21 healthy controls and 74 patients with miscellaneous non-amebic diseases(Cho, 1974)

Clinical type	No. tested	Positive	
		No.	%
Extra-intestinal amebiasis			
Amebic liver abscess	8	6	75.0
Amebic hepatitis	13	9	69.2
Amebic lung abscess	1	0	0.0
Total	22	15	68.2
Intestinal amebiasis			
Acute intestinal amebiasis	4	2	50.0
Mild symptomatic *E. histolytica* cyst passer	9	2	22.2
Asymptomatic *E. histolytica* cyst carrier	9	4	44.4
Total	22	8	36.4
Healthy controls	21	0	0.0
Miscellaneous non-amebic disease			
Liver disease	15	0	0.0
Gastrointestinal disease	43	2	4.7
Other disease	16	0	0.0
Total	74	2	2.7

Table 41. Comparison of gel-diffusion precipitation(GDP) test with indirect hemagglutination(IHA), indirect fluorescent antibody(IFA), and ameba immobilization(AI) tests for the diagnosis of *E. histolytica* infections (Cho, 1974)

IHA, IFA and AI tests	GDP Test		Total
	Positive(%)	Negative(%)	
IHA, IFA, AI positive	22(51.2)	21(48.8)	43
IHA and IFA positive	3(60.0)	2(40.0)	5
IHA and AI positive		8	8
IFA and AI positive		5	5
IHA positive only		8	8
IFA positive only		4	4
AI positive only		27	27
IHA, IFA, AI negative		39	39
Total	25	114	139

of gastrointestinal diseases showed a positive reaction of 4.7%. In comparison with other serological tests the positive rates of GDP test were 51.2% in IHA, IFA and AI positive sera, and 60.0% in IHA and IFA positive sera. Other serum groups showed negative reactions(Table 41).

The results indicate that the GDP test is less sensitive than IHA, IFA and AI tests in detecting active infection, although the test is highly specific. No cross reaction was demonstrated when the test was tried against sera from healthy controls and from patients with liver diseases other than amebiasis, and the test did not give as many false-positive

reactions against sera from patients with gastro-intestinal diseases as did with other tests. Therefore, while a negative GDP test may not be significant, a positive reaction certainly is indicative of existing or past infection.

TREATMENT

Of the number of drugs that had been used in the treatment of *Entamoeba histolytica* infection, none was completely satisfactory against the trophozoites and cysts both in tissues and intestinal cavity at the same time. But several synthetics and antibiotics which are effective both against *E. histolytica* in intestine and liver tissues, have been reported recently (Kradolfer and Jarumilinta, 1965; Powell, 1969).

Antiamebic drug: As a method to assess the efficacy of drugs, Cho *et al.* (1969) undertook an *in vitro* study examining the amebicidal activities of all the conventional and newly developed antiamebic drugs. Those were emetine, carbarsone, diodoquin, chloroquine, atabrine, chloramphenicol, tetracycline, niridazole (Ambilhar) and metronidazole (Flasinyl).

Niridazole is a nitro-thiazole derivative, 1-(5-nitro-2-thiazolyl)-2-imidazolidinone (Fig. 30). 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 Doshi *et al.* (1968).

Metronidazole is a nitro-imidazole preparation, 1-6-hydroxyethyl-2-methyl-5-nitroimidazole (Fig. 30). It was reported to be potent as an intestinal and systemic amebicide (Powell, 1969; Khambatta, 1969).

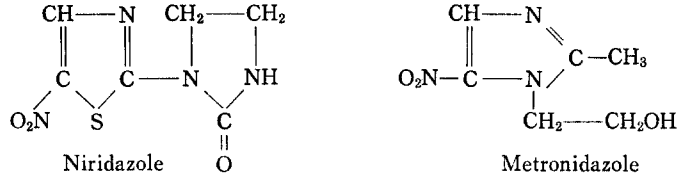


Fig. 30. Chemical structures of niridazole and metronidazole.

The amebicidal assay was carried out against five strains of *E. histolytica* which had been kept in the laboratory. The modified Boeck and Drbohlav's diphasic medium (Faust and Russell, 1964), which consisted of coagulated egg slant 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 4.5ml of the sample solution, and a control tube contained only the vehicle. The tubes were immediately seeded with 0.5ml of a rich inoculum containing approximately 10,000 trophozoites of 48-hour culture of the different strains of amebae, and was added with a small amount of rice starch. After 48

reactions against sera from patients with gastro-intestinal diseases as did with other tests. Therefore, while a negative GDP test may not be significant, a positive reaction certainly is indicative of existing or past infection.

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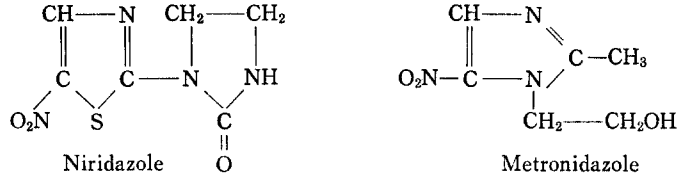

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hours of incubation at 37°C growth of the amebae was checked microscopically. As the amebae proliferated at the bottom of the culture tube, the supernatant fluid was decanted prior to examination, leaving approximately 0.5ml of sediment. One or more preparations were made from each concentration by placing one drop of the sediment on a microscope slide covered with a 22-mm square cover slip. The entire area of the cover slip was carefully examined for trophozoites confirming the motility of amebae under high power magnification. To determine the criteria of amebicidal action of each drug, complete absence of living amebae in each drug-culture mixture was ascertained by examining the ameba 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 titer (Table 42, 43). These findings coincide with those of previous reports on traditional anti-

Table 42. Amebicidal activity of conventional antiamebic drugs *in vitro*(Cho *et al.*, 1969)

Drug	Ameba strain	No. of experiment	Amebicidal titer	Effective drug conc. in μg 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

Table 43. Amebicidal activity of antibiotics and newly appeared synthetics(Cho *et al.*, 1969)

Drug	Ameba strain	No. of experiment	Amebicidal titer	Effective drug conc. in μg 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

amebic drugs. Amebicidal concentrations were found to be as follows: 1 : 5,000 to 1 : 20,000 for carbarsone, 1 : 8,000 to 1 : 16,000 for diodoquin, 1 : 50,000 for chloroquine, 1 : 1,000 to 1 : 4,000 for atabrine, 1 : 1,000 to 1 : 2,000 for chloramphenicol and 1 : 5,000 to 1 : 8,000 for tetracycline. The newly appeared chemicals showed higher amebicidal titer at the concentrations of 1 : 500,000 to 1 : 5,000,000 for niridazole, 1 : 50,000 to 1 : 100,000 for metronidazole. Emetine and chloramphenicol were more amebicidal at lower concentration to intestine originated amebae(YS-14, YS-15 and NAMRU-II strains) than to liver originated amebae(YS-24 and YS-25 strains), while carbarsone, chloroquine and metronidazole were more amebicidal at higher concentrations. Diodoquin had lower amebicidal titer for amebae originating from trophozoites(NAMRU-II, YS-24 and YS-25 strains) than those originating from cysts(YS-14 and YS-15 strains), but niridazole showed controverse results. The amebicidal concentration of atabrine was not constant varying according to amebic strain, but tetracycline, on the other hand, showed almost unchanged amebicidal titers.

Metronidazole: Since Powell *et al.*(1966) used metronidazole, a nitroimidazole derivative, for the treatment of amebiasis, a number of clinical trials have confirmed its satisfactory amebicidal effects in acute and chronic forms of intestinal amebiasis and in amebic

liver abscess(Powell, 1969), although the optimal curative regimens varied somewhat from trial to trial.

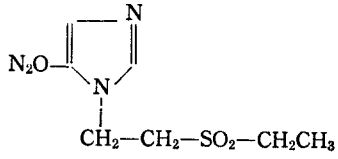
Cho *et al.*(1971) tested the drug in nine cases of extra-intestinal amebiasis; three cases of hepatitis, one case of lung abscess and five cases of liver abscess. Flasinyl(Han-Il Pharmaceutic Co., Seoul) which contains metronidazole 200mg/tablet, was used in this study. It was given orally in four treatment regimens;

1) six patients received at dose of 300~400mg thrice daily for three to eight days,
2) a seven year-old child received at dose of 200mg twice daily for 10 days,
3) one severe case was given 600mg thrice daily for seven days followed by a single dose of 2,200mg one month later, and
4) another case who was given 1,200mg twice daily for two days.

In liver abscess cases, pus was aspirated as many as 10 times during the course of treatment, and tetracycline, 250mg, was administered intravenously in order to prevent contamination during aspiration. The diagnosis and prognosis were determined by tenderness and enlargement of liver, hematological findings, urine and stool examination, X-ray or liver scan demonstration of abscess in liver, and ameba immobilization test(AI). Clinical improvement was observed in all cases. Liver enlargement was reduced to normal and no relapse was observed until five months after the treatment. Laboratory findings were parallel with clinical recovery. Clinical symptoms in amebic hepatitis were completely alleviated by the treatment.

X-ray findings in the amebic lung abscess case after three days of treatment with the daily dose of 1.2gm showed a remarkable improvement. An additional administration of the drug for six days resulted in a complete resolution of the pneumonic changes. No remarkable side effect due to the drug administration was noticed, and no relapse occurred until five months after treatment.

Tinidazole(***Fasigyn***): Tinidazole, an active trichomonacide, was produced by Pfizer Co. (Fig. 31), and several investigators(Ahmad, 1973; Mistry, 1973) reported the satisfactory results in treatment of amebiasis with the drug. Soh and Min(1974) carried out an efficacy study of Tinidazole in short term treatment. A total of 50 cases(18 males, 32 females) with various forms of amebic infection; 20 cyst carriers, 15 chronic and 13 acute infective cases and two liver abscesses, was treated with Tinidazole. Out of 13 acute cases seven excreted trophozoites and cysts in stool. Physical examination, laboratory examination of blood and urinalysis were done before and after medication. Each tablet contained 400 mg of Tinidazole base. It was given orally for two or three days according to the treatment



Ethyl 2—(2-methyl-5-nitro-1-imidazolyl) ethyl sulphone

Fig. 31. Chemical structure of Tinidazole(Fasigyn)

schedule. Twenty carriers and 10 chronic patients ranging from 16 to 75 years of age were given at dose of 2,000mg once daily for two days; five chronic, 11 acute and two liver abscess patients were given 2000mg once daily. A 12 year-old girl was given 1200mg daily and 10 year-old boy 800mg once daily for three consecutive days. Of 20 cyst carriers 15(75.0%) were categorized as having obtained a good result in the parasitological sense(Table 44).

Table 44. Regimen of Fasigyn to various group of *E. histolytica* infection and amebicidal activity based on the rate of negative conversion after the treatment(Soh and Min, 1974)

Group	Stool exam. before treatment			Dose			No. of negative conversion			
	No. of patient	Cyst	Tropho-zoite	mg/day	Dura-tion	No. of patient	*1	*30	*45	%
Cyst carrier	20	20	—	2,000	2days	20	12	15	15	75.0
Chronic	15	15	—	2,000	2 〃	10	0	3	3	30.0
				2,000	3 〃	5	2	5	**4	80.0
Acute	13	13		2,000	3 〃	11	4	10	**9	81.8
				1,200	3 〃	1	1	1	1	84.6
				800	3 〃	1	1	1	1	100.0
			(7)	2,000	3 〃	(7)	(7)	(7)	(7)	100.0
Liver abscess	2	2	—	2,000	3 〃	2	2	2	2	100.0
Total	50	50	(7)			50(7)				

( ): Trophozoite form from 7 out of 13 acute cases.
*: Day after the last treatment.
**: One relapsed: converted to cyst positive at the 45th day.

In the chronic cases, only 3 out of 10 cases showed a conversion to cyst-negative after two-day regimen, but after three-day regimen the parasitological improvements were; 4 (80.0%) in 13 of acute cases. The trophozoites disappeared in all cases within 24 hours after the first administration. All of the liver abscess cases also showed satisfactory results. With the three-day regimen the symptoms in acute intestinal amebiasis and liver abscess improved within one week after the treatment. Chronic cases with 2 days of treatment showed unsatisfactory results. In the amebic liver abscess patients, the combined therapy with other antibiotics was performed to prevent secondary infection during the surgical aspiration. During the follow-up examination parasitological relapse was found in two cases, one each in the chronic and acute group, on the 45th day. Out of 50 cases, nine showed mild and transient side effects, but none of them interfered the completion of the regimen. The doses administered in this study were well tolerated and no toxicity to hematopoietic organs, liver and kidneys was recognized. Leucocytosis and neutrophilia returned to normal after the therapy. No abnormal change was noted in the urine. The overall results indicate that the continuous administration of a lesser dose following the initial massive dose regimen is desirable in various forms of amebiasis.

Tiberal: Tiberal(α-chloromethyl-2-methyl-5-nitro-1-imidazole ethanol), synthetic of F. Hoffmann La Roche & Co., is a new effective antiprotozoal agent. With this pharmaceutic agent two sets of double blind trials were carried out by Cho *et al.*(1972c). Twenty intestinal amebiasis patients were given of tiberal versus metronidazole. And thirty *E. histolytica* cyst carriers were administered with tiberal versus metronidazole versus placebo. All patients were administered the drug according to the following regimen; 62.5mg(a quarter of one capsule) twice daily for seven days to two to six years old, 125mg twice daily for seven days to 7 to 12 years old and 500mg twice daily for five days to those above 13 years of age(Tiberal=Ornidazole).

The therapeutic effect was assessed according to the changes of clinical symptoms and stool examinations. Stool examinations were performed before treatment, on the fifth day and the last day of medication. Follow-up examinations were done at weekly intervals for four weeks. When *E. histolytica* was negative by direct fecal smear method the result was reconfirmed by concentration techniques. The dosages of the drug corresponded to about 10-20mg/kg/day. Laboratory data showed no notable changes or differences in the tiberal and metronidazole administered group, which could be attributed to the administration of those drugs. Clinical signs of dysentery improved within two to five days after medication. Results of stool examination in intestinal amebiasis cases resulted similar effectiveness by both chemicals. However, in cyst carriers, cure rate with metronidazole was 70% (seven of 10 cases), whereas that of tiberal was 100%. The examination for *E. histolytica* in cyst carriers converted to negative by the fifth day of treatment. However, in the metronidazole group two cases relapsed one week after the treatment, and positives increased to 3 cases at the time of final follow-up examination. Tiberal showed similar effectiveness against *Entamoeba coli*, *Endolimax nana*, *Giardia lamblia*, *Chilomastix mesnili* and *Iodamoeba bütschlii*, but metronidazole group showed less effective results. Placebo group revealed no change before and after the treatment. In general, metronidazole acts less satisfactorily to *E. histolytica* cyst passer than to amebic dysentery. The efficacy of tiberal was furtherly assessed in 60 asymptomatic cyst carriers, 40 oligosymptomatic, 20 hepatic amebiasis patients, 30 *Giardia lamblia* infections and 20 trichomonal vaginitis patients using double blind trial method(tiberal versus metronidazole versus placebo, or tiberal versus metronidazole: Cho *et al.*, 1976) (Table 45). The regimen of drugs were; for those below 6 years of age 250mg, between 7 and 12 years 500mg, and over 12 years 1gm, daily, dividing into 2 doses for 7 consecutive days. In asymptomatic cyst carriers, a cure was obtained in 18 out of 20 cases in the group of tiberal, and in 14 out of 20 cases in the group of metronidazole; 2 cases relapsed in the tiberal group, and 3 cases relapsed and 1 case failed to respond to treatment in the metronidazole group. In oligosymptomatic cyst passers the cyst became negative from the 5th day of treatment in both groups, but relapse occurred in 2 out of 20 cases in the tiberal group, and 3 out of 20 cases in the metronidazole group during 1~4 weeks after the completion of medication.

In hepatic amebiasis, the clinical and parasitological effects of both tiberal and metro-

Table 45. Results of double blind trial in cyst carriers by tiberal and metronidazole versus placebo(Cho *et al.*, 1976)

No. of positive cases	Tiberal(10cases)						Metronidazole(10 cases)						Placebo(10 cases)					
	*E.h.	E.c.	E.n.	G.l.	C.m.	I.b.	E.h.	E.c.	E.n.	G.l.	C.m.	I.b.	E.h.	E.c.	E.n.	G.l.	C.m.	I.b.
Before	10	6	2	1	11	1	10	4	3	2			10	7	2	3		
Day 5													4	4	1	3		
After													7	4	1	3		
one week							2	1	1				8	4	1	2		
two weeks							2	2	1				2	2	1	3		
three weeks							2	1	1				8	3		3		
four weeks				1			3	2	2				10	4	1	3		1

*E.h.: *Entamoeba histolytica* E.c.: *Entamoeba coli*
E.n.: *Endolimax nana* G.l.: *Giardia lamblia*
C.m.: *Chilomastix mesnili* I.b.: *Iodamoeba bütschlii*

nidazole were similarly satisfactory; the stool became free of *E. histolytica* and none of the patients resulted to have relapse during the follow-up period of 6 months. In *Giardia lamblia* infection, all became negative after treatment and no relapse was encountered in the tiberal group, but the parasite again appeared in 1 out 10 cases of metronidazole group. In the trial of trichomonal vaginitis, both drugs gave excellent therapeutic results with rapid clinical improvement and disappearance of the parasite.

Parenteral use of "Tiberal" (Ornidazole) in hepatic amebiasis: Recently F. Hoffmann-La Roche produced a parenteral form of tiberal(Ro7-0207/616). Soh *et al.*(1978) performed a study in order to find the optimal regimen. Before use the contents of each ampule containing 500mg in 3ml water were again diluted with 7.5ml of distilled water, and the two ampules were injected intravenously in the morning; another 2 ampules 12 hours later. The oral form of tiberal was given to another group of patients. Five tablets (each tablet contains 0.5gm) were given in a single dose. In principle, no further antiamebic drug administration was allowed until the final check, except in inevitable cases. For assessment, stool examination and subjective and objective symptoms were checked until the patients were discharged. Follow-up observation was continued as a rule until for six months after treatment. When the findings were within normal range, the case was regarded as cured. Symptoms such as fever, abdominal pain and liver tenderness were alleviated in all patients of the two groups within a few days after the treatment (Table 46, 47). A reduction in size of the liver was also noticed within several days. However, one-day regimen without aspiration seemed insufficient to clear the clinical signs. Aspiration and repeated administration of the same antiamebic or antibiotics seemed helpful to ensure the high effectivity of "Tiberal" in treating hepatic amebiasis. Follow-up examination or interview for three to six months after the hospital treatment showed no case of relapse. This strongly suggests that "Tiberal" may act as a very effective amebi-

Table 46. Treatment of hepatic amebiasis by intravenous administration of 2gm of Ornidazole in two doses (Soh *et al.*, 1978)

Case No.	Sex	Age	Admission day	Temp.(°C)		WBC/mm³ (unit, 100)		Liver(finger breadth)		E.h. cyst in feces		Assessment
				***B	A	B	A	B	A	B	A	
1	M	23	6	36.5	36.5	125	92	1	0	+	—	cured
2	M	52	5	37.9	36.5	113	92	2	1**	+	—	cured
3	M	44	2	38.8	36.5	123	86	3	0	++	+	cured
4	M	40	30	38.8	36.5	112	74	2	0	+	—	cured
5	M	23	5	39.0	36.5	135	70	1	0	+	—	cured

* One ampule contains Ornidazole (Tiberal) 500mg in 3ml. Before use it was diluted with distilled water 7.5ml.

** Not palpable after 6 months

*** A: After treatment, B: before treatment

Table 47. Treatment of hepatic amebiasis by administration of 2.5gm Ornidazole in single dose(Soh *et al.*, 1978)

Case No.	Sex	Age	Admission day	Temp.(°C)		WBC/mm³ (unit, 100)		Liver(finger breadth)		E.h. cyst in feces		Assessment
				B	A	B	A	B	A	B	A	
1	M	53	11	38.0	36.8	144	99	3	0	++	—	cured
2	M	30	3	37.5	36.6	94	76	3	0	+	—	cured
3	F	52	25	37.8	36.8	126	65	3	0	++	—	improved
4	F	10	4	39.5	36.8	130	90	2	0	+	—	cured
5	M	43	12	38.8	36.6	127	110	4	0	+	+	cured
6	F	22	5	38.5	36.7	121	80	4	0	+	—	cured
7	M	42	**o.p.	37.8	36.5	121	?	3	1	+	—	improved

* Repeated administration of Ornidazole(Tiberal)

** o.p.: Treated at out-patient clinic

cide for the *E. histolytica* in tissues also. No notable untoward side effects were encountered either with oral or with parenteral administration.

OTHER ENDAMOEBIDAE

In this chapter human commensals such as *Entamoeba gingivalis*, *Entamoeba coli*, *Endolimax nana*, *Iodamoeba bütschlii*, and *Dientamoeba fragilis* are reviewed in aspect with the prevalences in Korea.

ENTAMOEBA GINGIVALIS

Entamoeba gingivalis inhabits the gingival tissues of man, and has been generally recognized as non-pathogenic(Fig. 32-A). But the frequent presence in pyorrheic lesions suggests that the protozoon might reveal pathogenicity in certain environment. Jhee(1962) undertook a survey of oral protozoa at the Dental Clinic, Severance Hospital during 1959 and 1960. Among 80 out-patients examined, 38.1% of 48 periodontal cases were found *E. gingivalis* and 12.5% *Trichomonas tenax* to harbor, but the findings were negative among dental caries cases. Cha(1972) did another survey at the same clinic in 1971. Among 262 examined patients 97 of 212 cases of periodontal disease were found to be *E. gingivalis* positive and no ameba was detected from caries and other dental diseases but 8(20%) of 39 healthy individuals were *E. gingivalis* positive. In order to gain further information on the relation between oral protozoa and dental diseases Kim and Cho(1973) examined the prevalence among personnels of the Korean Air Force, a total of 254 subjects, in 1973 and found 54.3% harbored oral protozoa. Of the 139 subjects with periodontal disease 60.4% were positive for *E. gingivalis* and 20.9% positive for *T. tenax*. Of the nine cases of pericoronitis 30.3% harbored *T. tenax* and 22.2% *E. gingivalis*. Of 90 normal subjects 36.7% harbored *E. gingivalis* and 8.9% *T. tenax*. Caries, periapical abscess and other disease groups were negative for both oral protozoa. By site, *E. gingivalis* was present in 29.5% and *T. tenax* in 8.9% of 78 samples collected from periodontal sulcus; *E. gingivalis* in 62.2% and *T. tenax* in 21.1% of 151 samples from calculus, and *E. gingivalis* in 20.0% and *T. tenax* in 13.3% of 15 samples from other sites. Both protozoa were not detected in 10 specimens collected from caries cavities. Through the results, they suggested that positive rates of both oral protozoa increased proportionally to the periodontal index and the simplified calculus index.

Although oral protozoa have been thought to be non-pathogenic scavengers in oral cavity, some possibilities still exist for a causal relationsphip of these parasites in inducing oral diseases. Further studies might be required to answer the following critical questions about the pathogenicity of oral protozoa; Do the protozoa cause any mechanical damage to gingival tissues which may facilitate secondary bacterial infections, and do the parasites contain or produce some enzymes to facilitate tissue invasion? What are the morphological,

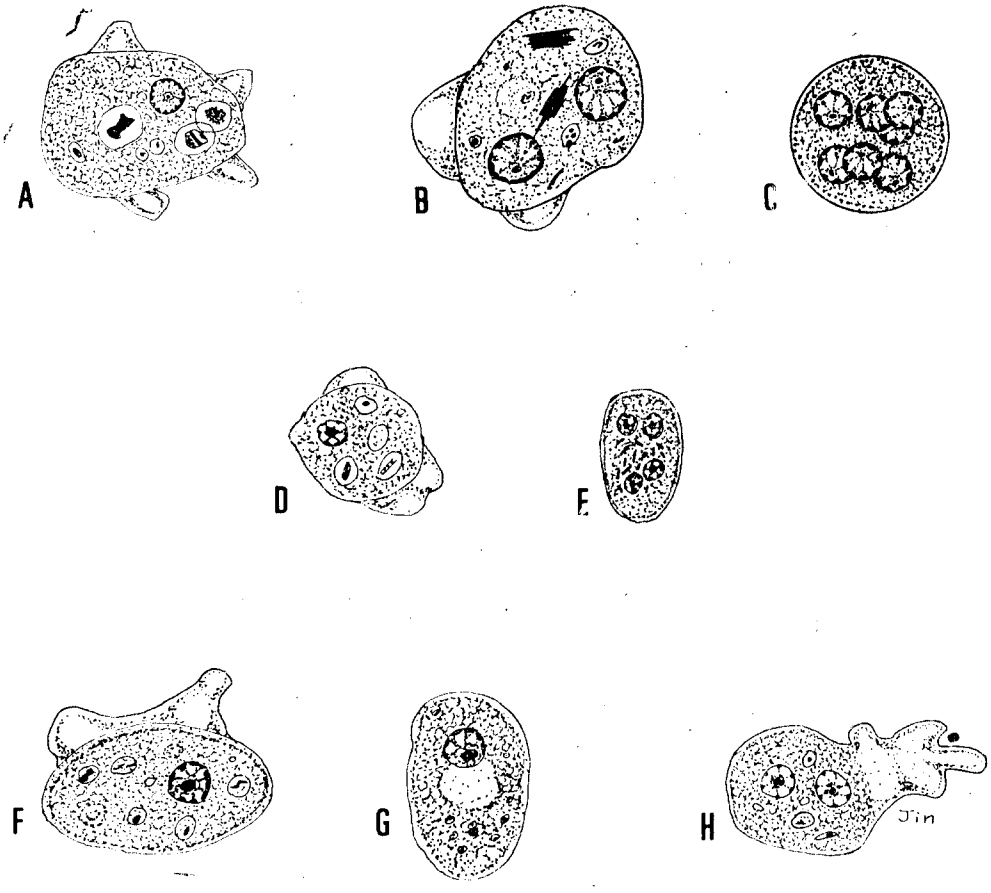


Fig. 32. Endamoebidae.

A, Trophozoite of *Entamoeba gingivalis*
B, C, Trophozoite and cyst of *Entamoeba coli*
D, E, Trophozoite and cyst of *Endolimax nana*
F, G, Trophozoite and cyst of *Iodamoeba bütschlii*
H, Trophozoite of *Dientamoeba fragilis*

physiological and cytochemical differences between these organisms in normal mouth and in diseased tissues? Could any environmental factors(bacterial or viral) vest these protozoa with virulence?

To confirm the enzyme activity of *E. gingivalis*, Cho *et al.* (1973) examined the distribution of acid phosphatase in the cytoplasm. The report describes that the plasma membrane was found to be negative for acid phosphatase reaction in general. But in some ameba dense deposits of lead phosphate appeared at the limited portion of plasma membrane, and it was identified as a uroid-like structure as was suggested by Yang and Cho (1972). In the cytoplasm the same five phases of vacuoles as shown in *E. histolytica* were recognized. Some vacuoles contained newly ingested bacteria. Although the vacuole itself was devoid of enzyme activity the bacteria showed coarse lead phosphate deposits. Some vacuoles contained strong enzyme active precipitates, whereas vacuole membranes showed almost negative. Small enzyme positive granules, 0.4~0.7μm in diameter, were occasionally observed surrounding the nucleus. In the nucleus, highly active enzyme granules, 0.05~0.1μm in diameter, were scattered or assembled in the nucleoplasm. The organisms incubated in the substrate-free medium showed no localized reaction product. Only non-specific deposits of lead precipitates were seen occasionally. Some vacuoles revealed strong enzyme activity in the ingested bacteria or the contents in vacuolar lumen, whereas limiting membranes of vacuole revealed negative or weak reactions, which was contrary to the findings in the vacuole phases of *E. histolytica*.

Granular enzyme precipitates were scattered or assembled in the nucleoplasm of *E. gingivalis*, but Kim and Cho(1973) reported that the nucleus of *E. histolytica* was devoid of enzyme activity. Although it is still uncertain whether the intranuclear acid phosphatase active granules found in *E. gingivalis* have any functional significance, it is presumed that they may be a part of the lysosomal system of this protozoan. On the function of acid phosphatase in the nucleus, Love *et al.*(1969) assumed that nuclear or nucleolar ribonuclease degrades RNA to nucleotides which, in turn, could be hydrolysed by acid phosphatase to contribute to the large amounts of orthophosphate found in the nucleolus.

ENTAMOEBA COLI

E. coli is a non-pathogenic commensal in the large intestine of man. There has been no evidence that it ever produces pathological lesions, although the ingestion of red blood cells is occasionally observed. It is sometimes misdiagnosed to *E. histolytica* by an unexperienced technician (Fig. 32-B,C). A number of researchers published reports on the incidence of this and other protozoa. Among these the following figures may represent the overall situation; Choi(1926), 26.4% of 334 examined: Brooke *et al.*(1956), 27.1% in 919 civilians; Soh *et al.*(1961), 11.1% in 10,320 specimens; Kim *et al.*(1971), 20.5% in 2,250 specimens.

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ENDOLIMAX NANA

The protozoon is recognized as a harmless parasite of the large intestine of man (Fig. 32-D,E). The incidence has been variable, depending on methods and reporters: Brooke *et al.*(1956), 8.3% in 919 civilians; Soh *et al.*(1961), 7.7% in 10,320 specimens; Kim *et al.*(1971), 10% in 2,250 specimes. But upon repeated fecal examination the rates usually increase. Choi(1926) detected 62(3.1%) out of 2,000 examined in Severance Hospital but the positive rate increased to 41.6% when the examination of fecal specimens was repeated six times in the same subject. A similar rate of increase was observed in other intestinal protozoa such as *E. histolytica*, *E. coli*, and *Iodamoeba bütschlii*, *etc.* Thus it is conjectured that the numbers of actual positives are far greater than reflected by the figures in reports which were obtained on the basis of a single examination of feces.

IODAMOEBA BÜTSCHLII

I. bütschlii is generally recognized as a harmless parasite of the large intestine of man, even though clinical cases were reported elsewhere(Andrews,. 1947; Derrick, 1948) (Fig. 32-F,G). The prevalence in Korea was reported by a number of researchers: Soh *et al.*(1961), 0.5%; Brooke *et al.*(1956), 0.8%; Kim *et al.*(1971), 0.6%. But the number of positive findings increased significantly when the examination was repeated with same specimen. Choi (1926) detected 0.85% of fecal specimens to be positive by a single examination, but the rate increased to 16.4% when the examination by the same person was repeated six times.

DIENTAMOEBA FRAGILIS

Dientamoeba fragilis is a parasite of the large intestine which, unlike most other amebae, has a trophozoite but no cyst stage in its life cycle (Fig. 32-H). How this parasite is transmitted from one person to another is still an unsolved problem, and whether the parasite is potentially pathogenic or not is still a matter of debate. But several reports emphasize that this protozoon should be considered a pathogenic species rather than a commensal. Burrow *et al.* (1954) found a marked fibrosis in the wall of appendix which might be due to the continuous irritation by *D. fragilis*. The patient complained of lower right quadrant pain, nausea and vomiting. Yang and Schalten (1977) found *D. fragilis* in 4.2% of approximately 43,000 individuals who submitted stools for parasitological examination during 1970 to 1974 at the Parasitology Laboratory in Ontario, Canada. The symptoms in 255 of patients, in whom *D. fragilis* was the only parasite found and for whom detailed symptoms had been supplied, included; diarrhea, abdominal pain, anal pruritus, and loose stools. The incidence in Korea is rather low as compared to reports

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ENDOLIMAX NANA

The protozoon is recognized as a harmless parasite of the large intestine of man (Fig. 32-D,E). The incidence has been variable, depending on methods and reporters: Brooke *et al.*(1956), 8.3% in 919 civilians; Soh *et al.*(1961), 7.7% in 10,320 specimens; Kim *et al.*(1971), 10% in 2,250 specimes. But upon repeated fecal examination the rates usually increase. Choi(1926) detected 62(3.1%) out of 2,000 examined in Severance Hospital but the positive rate increased to 41.6% when the examination of fecal specimens was repeated six times in the same subject. A similar rate of increase was observed in other intestinal protozoa such as *E. histolytica*, *E. coli*, and *Iodamoeba bütschlii*, *etc.* Thus it is conjectured that the numbers of actual positives are far greater than reflected by the figures in reports which were obtained on the basis of a single examination of feces.

IODAMOEBA BÜTSCHLII

I. bütschlii is generally recognized as a harmless parasite of the large intestine of man, even though clinical cases were reported elsewhere(Andrews,. 1947; Derrick, 1948) (Fig. 32-F,G). The prevalence in Korea was reported by a number of researchers: Soh *et al.*(1961), 0.5%; Brooke *et al.*(1956), 0.8%; Kim *et al.*(1971), 0.6%. But the number of positive findings increased significantly when the examination was repeated with same specimen. Choi (1926) detected 0.85% of fecal specimens to be positive by a single examination, but the rate increased to 16.4% when the examination by the same person was repeated six times.

DIENTAMOEBA FRAGILIS

Dientamoeba fragilis is a parasite of the large intestine which, unlike most other amebae, has a trophozoite but no cyst stage in its life cycle (Fig. 32-H). How this parasite is transmitted from one person to another is still an unsolved problem, and whether the parasite is potentially pathogenic or not is still a matter of debate. But several reports emphasize that this protozoon should be considered a pathogenic species rather than a commensal. Burrow *et al.* (1954) found a marked fibrosis in the wall of appendix which might be due to the continuous irritation by *D. fragilis*. The patient complained of lower right quadrant pain, nausea and vomiting. Yang and Schalten (1977) found *D. fragilis* in 4.2% of approximately 43,000 individuals who submitted stools for parasitological examination during 1970 to 1974 at the Parasitology Laboratory in Ontario, Canada. The symptoms in 255 of patients, in whom *D. fragilis* was the only parasite found and for whom detailed symptoms had been supplied, included; diarrhea, abdominal pain, anal pruritus, and loose stools. The incidence in Korea is rather low as compared to reports

from Canada: Choi (1926) one case out of 2,000 specimens: Chiba (1931) 0.5% in 200 cases: Brooke *et al.* (1956) four positives (0.2%) out of 1,682: Kim *et al.* (1971) 0.1% in 2,250 cases. Nevertheless, a greater number of positive findings is to be expected when a well-trained technician pays more attention during examination, and it may well be assumed that many cases of abdominal distress of unknown etiology might be due to *D. fragilis* infection.

FREE-LIVING AMEBAE ISOLATED IN KOREA

Strain

Recently, free-living amebae (*Naegleria* sp. or *Acanthamoeba* sp.) have been proved to be causative agent of primary amebic meningo-encephalitis (PAME) in man (Fig. 33). Cotter (1973) reported there had been about 100 cases of fatal primary amebic meningo-encephalitis due to limax amebae. The laboratory isolation of pathogenic free-living amebae may be made from nasal discharge, spinal fluid, or sectioned brain tissues. Willaert (1973) pointed out that cases of PAME occurred generally during summer season, and that infection source of PAME may be swimming pools, lakes, muddy puddles, sea water pools, warm springs, and others. On the assumption that the free-living amebae isolated from waters of swimming pools, sewage and ponds are pathogenic, Hwang *et al.* (1976) isolated pathogenic free-living amebae from Seoul area. Those were *Naegleria* sp., YM-1, from sewage in Seoul and *Acanthamoeba* spp., YM-2, YM-3 from puddles in periphery of Seoul.

Virulence

The virulence of isolated amebae was examined by infecting white mice, weighing about 20gm and kept on an ordinary diet. Each mouse was infected intranasally with 2×10^3 amebae of three different strains under ether anesthesia.

Clinical manifestation in the infected mice were observed. Autopsies were performed immediately after death, and those mice which survived 20 days were etherized and autopsied. The brain, spinal cord, lung and nasal mucosa were placed on the non-nutrient agar plate with killed *Escherichia coli* for detection of free-living amebae. Pathological observation was carried out and brain tissues for histopathologic examinations were fixed in 10% formaldehyde and stained with hematoxylin and eosin.

Naegleria sp., YM-1, was detected in the tissues of brain, spinal cord, nasal mucosa and lungs of the mice infected intranasally at 20th days. *Acanthamoeba* sp., YM-2, was detected from brain tissues, but YM-3 only in the nasal mucosa.

One of seven mice infected with *Naegleria* sp., YM-1, died of PAME and *Acanthamoeba*

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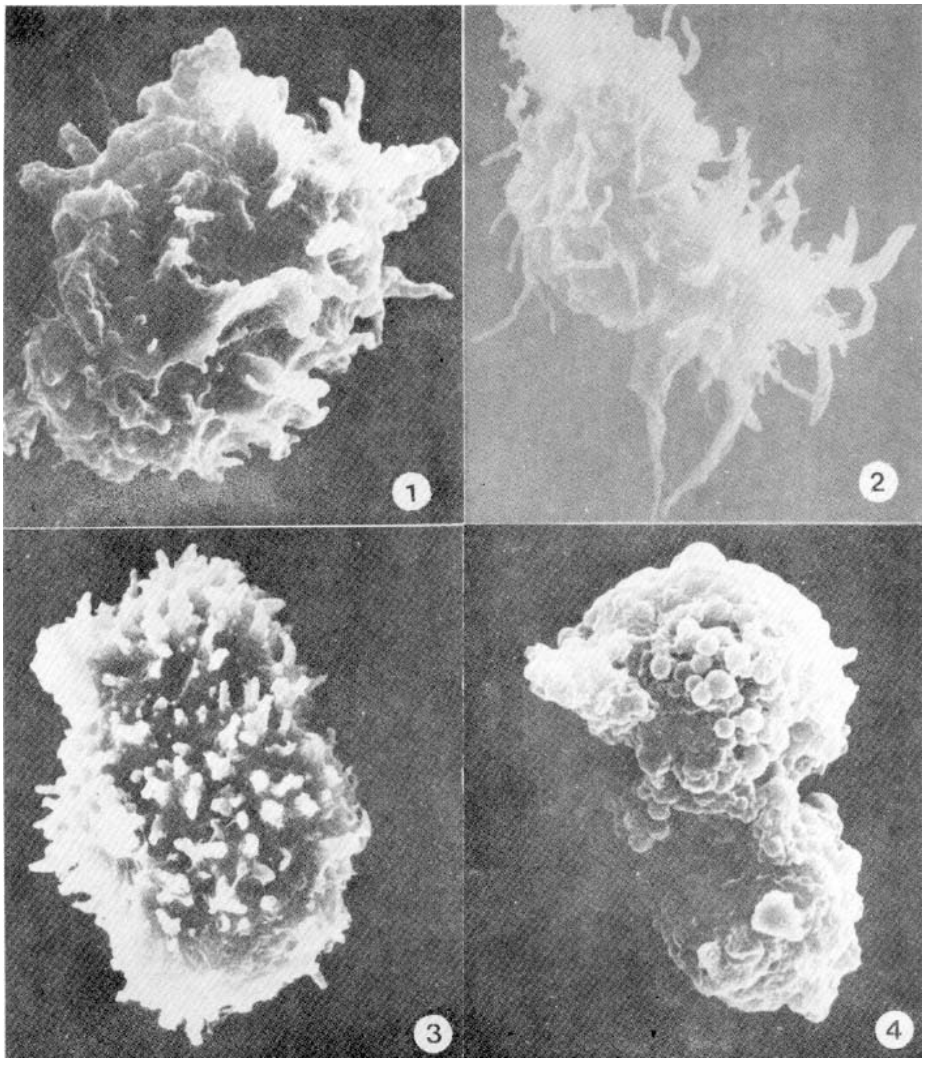
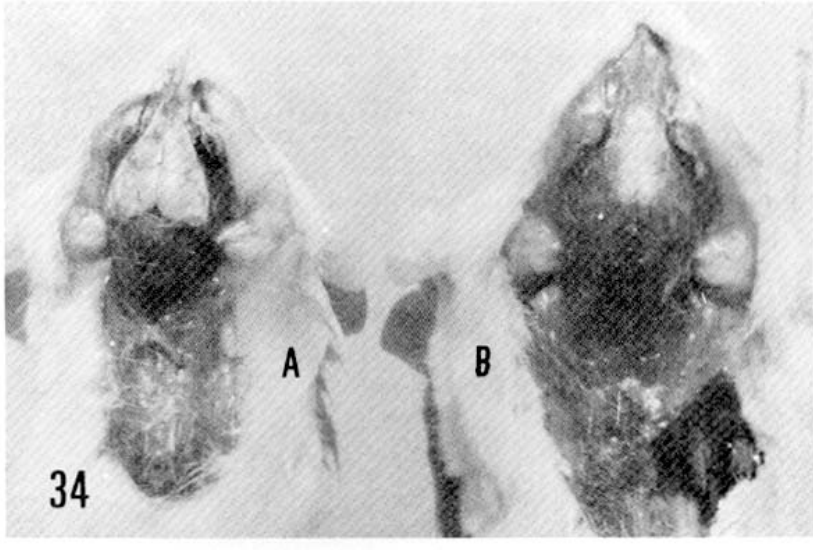
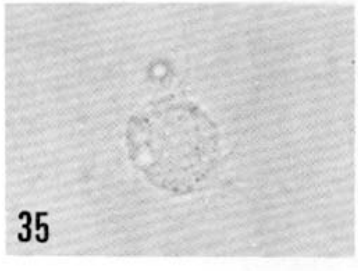
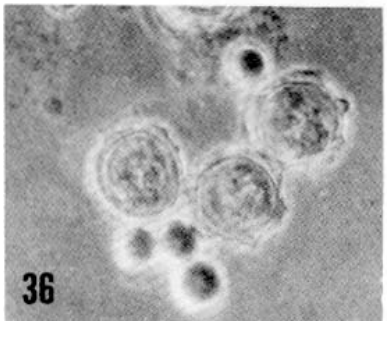


Fig. 33. Scanning electron micrographs of free-living amebae.

1. *Acanthamoeba royreba* trophozoite.
2. *Acanthamoeba* sp., YM-4 trophozoite.
3. *Acanthamoeba lenticulata* trophozoite.
4. *Naegleria fowleri* trophozoite.







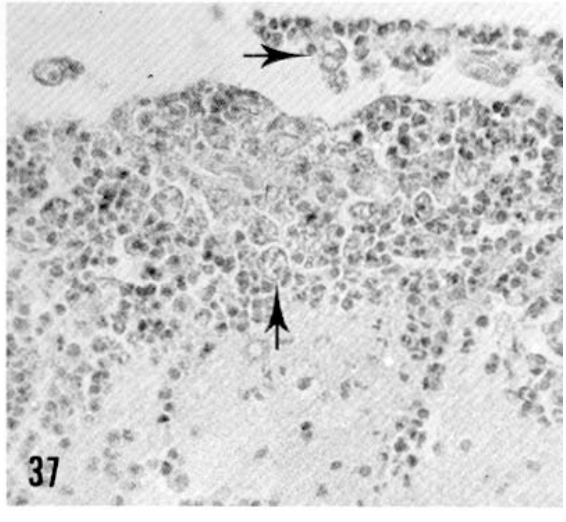


Fig. 34. Brain of mouse infected with *Acanthamoeba* sp.(YM-2). Showing extensively edematous and hemorrhagic changes(B) comparing with the normal(A).
Fig. 35, 36. Trophozoite and cyst of *Acanthamoeba* sp. (YM-2)
Fig. 37. Mouse brain infected with *Acanthamoeba* sp. (YM-2). Acute inflammatory change and the amebae (arrow) are shown.

sp., YM-2, 2 out of 5 mice. None of five mice infected with *Acanthamoeba* sp., YM-3, died.

Examination or necropsy revealed typical lesions of acute PAME with brain edema. The most extensive lesions occurred in the olfactory lobe and prefrontal area with hemorrhage and necrosis (Fig. 34). Nasal cavity was filled with purulent and hemorrhagic exudate and lungs were engorged with blood.

Brain section stained with hematoxylin and eosin demonstrated many invading amebae in the infiltrated sites (Fig. 35, 36, 37).

Clinically the condition of mice was normal up to the 3rd day after infection with free-living amebae. At fourth day, visible signs of distress and dysfunction of central nervous system appeared. Anorexia and altered behavioral patterns (less mobile and less scratching of the head) occurred followed by one or more of the following symptoms: hunched head, fasting, loss of coordination and paralysis. Mice in whom the infection assumed an acute course usually progressed to acute meningo-encephalitis with death by ninth day.

The report by Hwang *et al.* (1976) alarms that pathogenic strains of free-living amebae are distributed in surrounding of communities in Seoul area, and may occasionally cause primary amebic meningo-encephalitis in the residents.

In previous Ringsted *et al.* (1976) reviewed specimens originated from a Korean child who died at 15 years age and found *Acanthamoeba* sp. in various tissues.

Fate in Environment and in Host

Ahn *et al.* (1979) examined *Acanthamoeba culbertsoni* and *Naegleria fowleri* to ensure the possibility of resistance or adaptation in gastro-intestinal tract of the host and the various environmental circumstances as well. White mice weighing about 20gm which are known to be susceptible to free-living amebae were used in this study. Each mouse was infected orally or intranasally with *Acanthamoeba culbertsoni* or *Naegleria fowleri* under anesthesia with secobarbital by intraperitoneal injection. Effect of components of gastro-intestinal juice (mucin, pepsin, HCl, glucose and trypsin) on survival of free-living amebae was examined.

For the viability test trophozoites of free-living ameba were put in a culture medium containing natural food-preservatives(salt, soybean sauce, garlic, vinegar and red pepper) at various concentrations. The survival of free-living ameba in tap water, sewage and in pesticides was also examined *in vitro*. In the results *Acanthamoeba culbertsoni* and *Naegleria fowleri* were detected from brain tissues of mice inoculated orally, but not from the content of gastro-intestinal tract of the same mice. Trophozoites of *Acanthamoeba culbertsoni* and *Naegleria fowleri* survived and propagated well in the culture medium containing components of gastro-intestinal juices such as mucin, pepsin, glucose and trypsin, but could not survive in the medium containing hydrochloric acid. *Naegleri fowleri* survived well in sewage or tap water, and persisted more than 72 hours in natural food preservatives: salt(1∼20%), soybean sauce(1∼15%), garlic(1∼20%) and red pepper(1∼

10gm%), but the amebae were killed in vinegar within 24 hours at the concentrations of 0.1~1.0%. The results indicate that free-living amebae are unable to resist gastric juice which contains hydrochloric acid and food preservative such as vinegar. *Acanthamoeba culbertsoni* tolerated well pesticides such as Kasugamin, 2,4-D, Bla-S and Hosbel at practically applicable concentration, except in Diazinon and Cartrap. They were destroyed within 24 hours at 1:1000 of Diazinon and 48 hours at 1:1000 of Cartrap. The overall results by Ahn *et al.*(1979) suggest that the two species of free-living amebae can resist various environmental factors but are easily destroyed by gastric juice. It explains the reason why the free-living amebae have been exclusively limited to brain, and not to digestive tract and its annexed organs.

Pathogenicity

The relationship between the pathogenicity of free-living amebae and agglutinability with phytoagglutinin was studied by Kim *et al.* (1979). The free-living amebae tested in the study were *Naegleria fowleri*, *Acanthamoeba culbertsoni*, *A. hatchotti*, *A. lenticulata*, *A. royreba*, *A. triangularis* and *Acanthamoeba* sp. (YM-4). For reference, *Entamoeba histolytica* strains isolated from amebic hepatic abscess cases were also tested.

Free-living amebae were cultured on a non-nutrient agar plate(Kasprzak and Mazur, 1972), then CGV medium for *Acanthamoeba* sp. and CGVS medium for *Naegleria* sp. (Willaert and LeRay, 1973) were used. White mice weighing 15~20gm were used for the pathogenicity test. Free-living ameba trophozoites were inoculated into the nasal cavity of mice under anesthesia with intraperitioneal injection of secobarbital, and pathologic behaviour and brain tissue pathology were observed during the experiment.

Free-living ameba trophozoites were washed three times with 0.01M phosphate buffer saline (pH 7.0~7.2) and then suspended carefully. One drop of the cell suspension was mixed with the same amount of concanavalin A(con. A) of various concentrations on the Boerner slide and incubated at 36°C for a given period of time. Microscopic observation to determine the occurrence of agglutination was performed. Agglutination was also measured using spectrophotometer by transmittance of free-living amebae suspension in the presence of con. A.

Cell suspension in 0.01M phosphate buffer saline (pH 7.0~7.2) was added to the same volume of con. A at various concentration establishing a final volume of 2ml, and then agitated well. Experimental readings were done with the spectrophotometer (Bausch and Lomb) at 660nm at 30°C. Any increase in transmittance observed in each experimental readings was corrected from the zero time reading. Percent agglutination was calculated from the expression $(Y-X/X)\times 100$, where X is the transmittance at zero time and Y is the transmittance at any given incubation time.

Acanthamoeba sp., YM-4 trophozoite was agglutinated easily by more than 25μg/ml of con. A. Pathogenic *Acanthamoeba culbertsoni*, which was subcultured for a long time, was agglutinated by 25μg/ml of con. A.

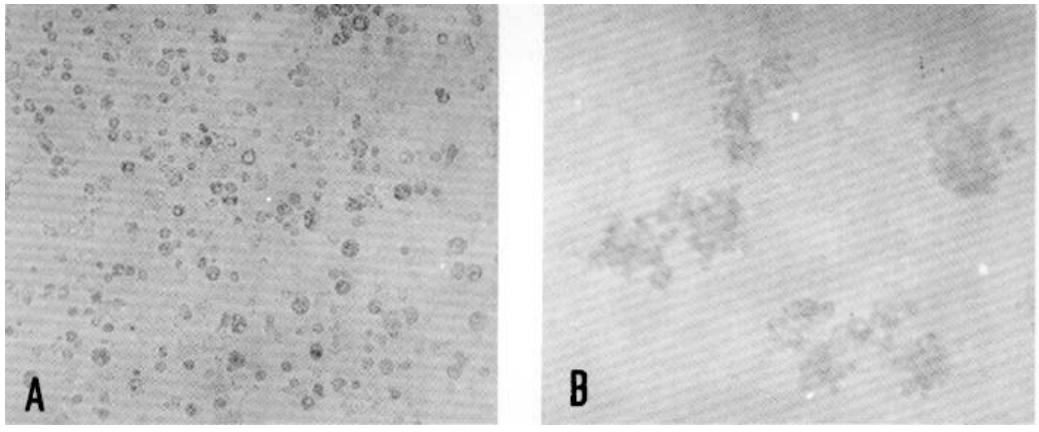


Fig. 38. Agglutination of *Acanthamoeba* sp. (YM-4) before (A) and 45 minutes (B) after treatment with concanavalin A.

Pathogenic *Acanthamoeba lenticulata* was also agglutinated by 25μg/ml of con. A, non-pathogenic *Acanthamoeba royreba* by 100μg/ml, non-pathogenic *Acanthamoeba hatchotti* by 25μg/ml and *Acanthamoeba triangularis* by 100μg/ml. But no agglutination was observed with *Naegleria fowleri* and *Entamoeba histolytica* at any concentration of the tested.

The best result in agglutination was achieved using a cell density of 150×10^4/ml of *Acanthamoeba* sp., YM-4. Agglutination reached the highest rate after 45~60 minutes at a concentration of 100μg/ml. In this way con. A induced agglutination seemed to relate with the pathogenicity in the *Naegleria* sp. but not in the *Acanthamoeba* sp. (Fig. 38).

Cytochemical Ultrastructure

In order to elucidate the difference in pathogenicity of free-living amebae in more detail, Chung *et al.* (1980) observed the ultrastructure of free-living amebae which were treated with con. A, horseradish peroxidase type II(Sigma) or con. A—horseradish peroxidase respectively, utilizing the electron microscope (Hitachi-H 500). In pathogenic *Acanthamoeba lenticulata* and *Acanthamoeba* sp. (YM-4) which were treated with con. A, the electron density of the surface of the cell membrane was slightly increased, but there was no obvious difference in the electron density of the cell surface of non-pathogenic *Acanthamoeba royreba* and pathogenic *Naegleria fowleri* (Fig. 39, 40). In all free-living amebae which were treated with horseradish peroxidase, the electron-dense reaction product was found exclusively on the mitochondria and lysosomes, but there was no difference in electron density of the surface of the cell membrane or other ultrastructure (Fig. 41). Ultrastructural demonstration of con. A-horseradish peroxidase activity of the surface of the cell membrane was obvious only in pathogenic *Acanthamoeba lenticulata*, *Acanthamoeba* sp. (YM-4) and *Naegleria fowleri*, but not in non-pathogenic *Acanthamoeba royreba* (Fig. 42). The electron density of mitochondria and lysosomes was similar with that observed in the group treated with horseradish peroxidase. No obvious difference was observed with regard

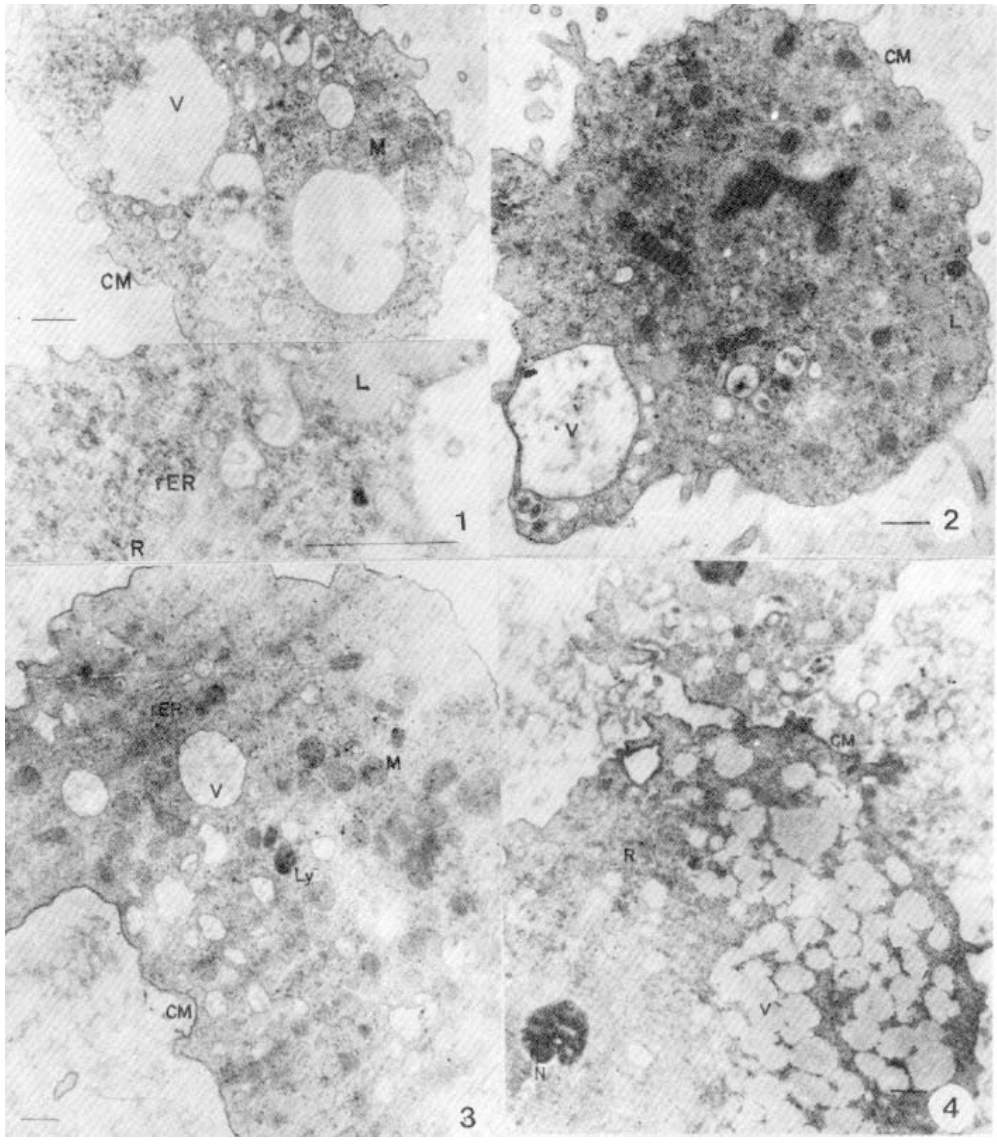


Fig. 39. Electron microscopic findings in the control group.

1. *A. royreba;* Irregular cell membrane(CM), vacuoles(V) of different size, mitochondria(M). lipid droplet(L), rought endoplasmic reticulum(rER) and ribosomes(R) are observed.
2. *Acanthamoeba* sp., YM-4; Cell membrane, vacuoles, mitochondria and lipid droplet are shown.
3. *A. lenticulata*; Rough endoplasmic reticulum, mitochondria and vacuoles are demonstrated.
4. *N fowleri*; Many small vacuoles and nucleus with nucleolus are observed.

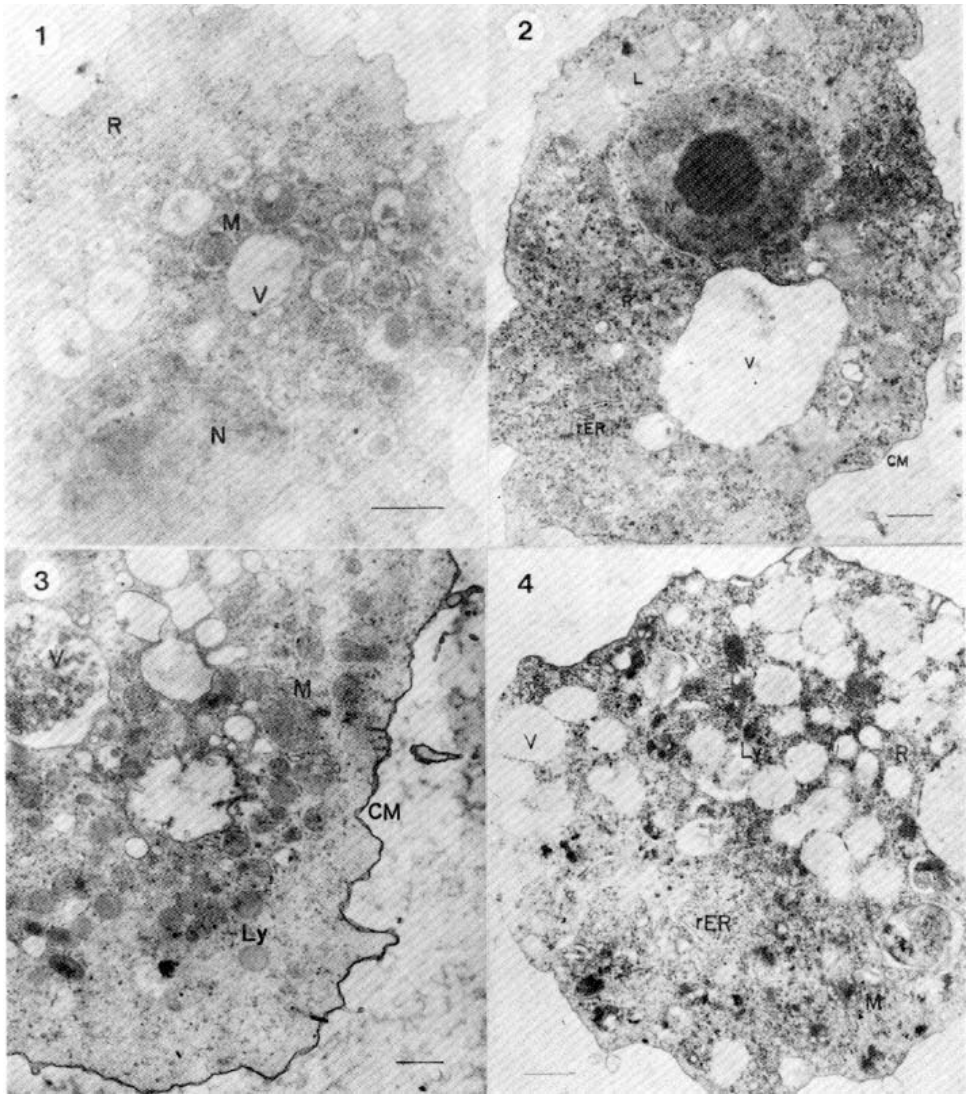


Fig. 40. Electron microscopic findings in the concanavalin A treated group.

1. *A. royreba;* No difference in the ultrastructure compared with the control.
2. *Acanthamoeba* sp., YM-4; Electron density along cell membrane increased slightly.
3. *A. lenticulata*; Electron density of cell membrane increased slightly, and lysosomes (LY) are observed.
4. *N. fowleri*; No remarkable finding was shown compared with control.

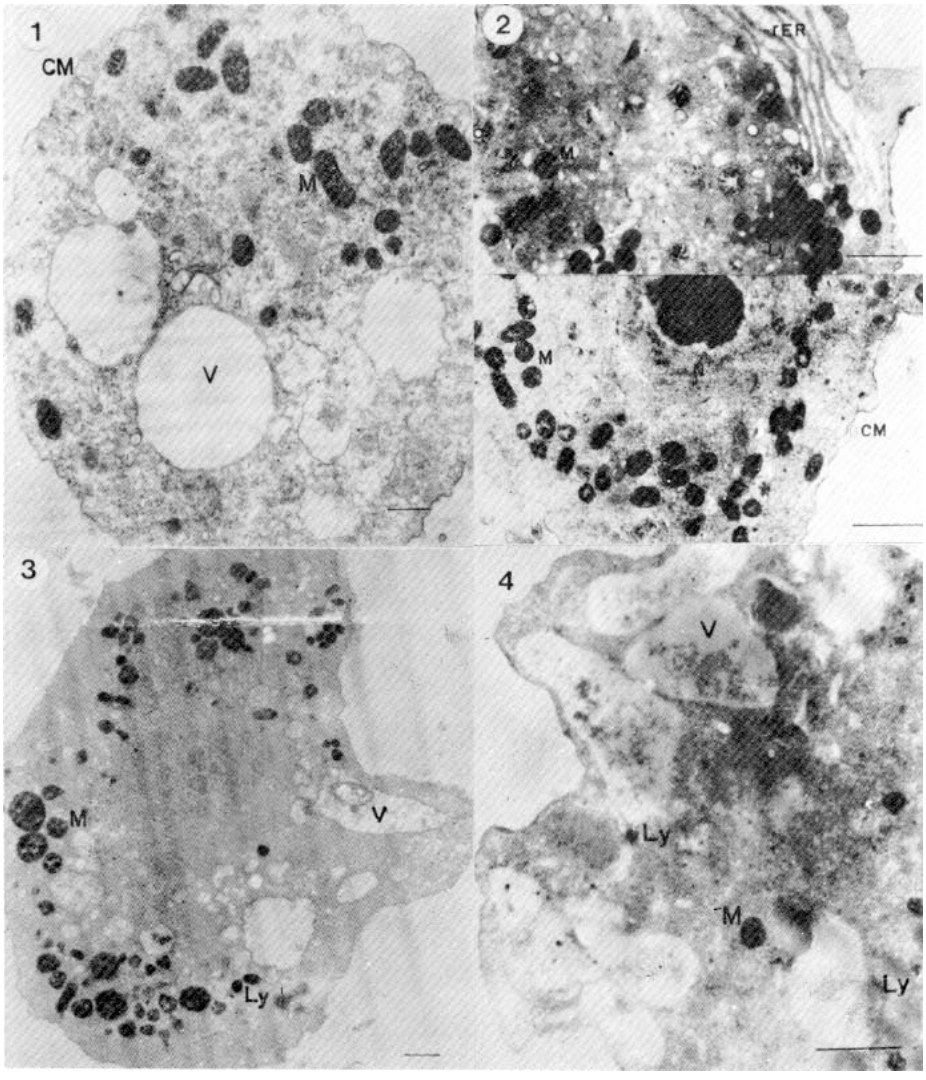


Fig. 41. Electron microscopic findings in the horseradish peroxidase treated group.

1. *A. royreba;* Mitochondria were demonstrated remarkably.
2. *Acanthamoeba* sp., YM-4; Electron-dense reaction product was shown exclusively on the mitochondria and lysosome, and rough endoplasmic reticulum was observed.
3. *A. lenticulata;* Electron-dense mitochondria and lysosome are shown.
4. *N. fowleri;* Mitochondria and lipid droplet are shown.

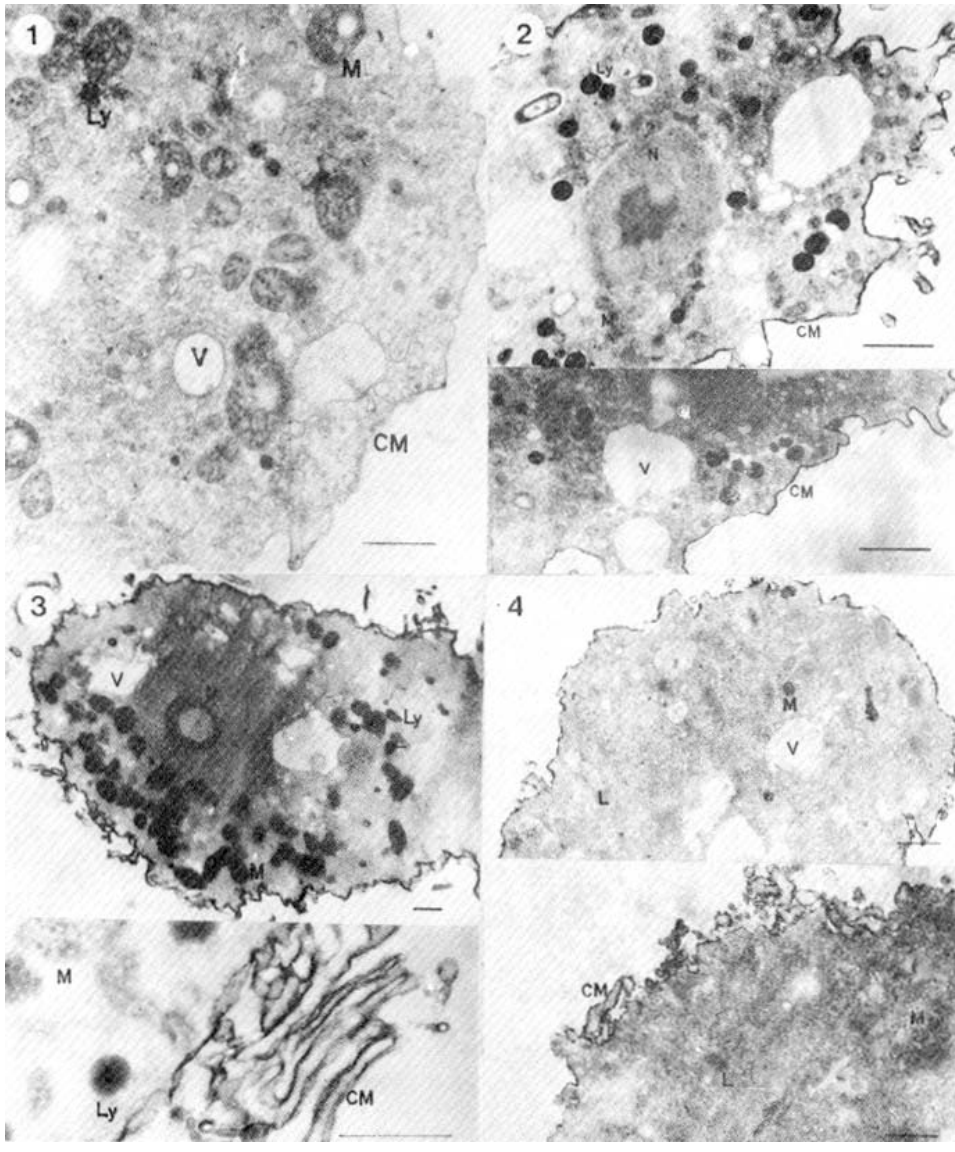


Fig. 42. Electron microscopic findings in the group treated with concanavalin A and horseradish peroxidase.

1. *A. royreba;* There was no obvious difference with regard to the electron density of the cell membrane compared with control.
2. *Acanthamoeba* sp., YM-4; Electron-dense reaction product was demonstrated remarkably on the cell membrane, mitochondria and lysosome.
3. *A. lenticulata*; Electron-dense reaction product was observed exclusively on the cell membrane, mitochondria and lysosome.
4. *N. fowleri;* Increased electron density on the form of intermittent aggregation.

to electron density of other ultrastructures between control and the group treated with con. A or horseradish peroxidase respectively. The overall results suggest that con. A-horseradish peroxidase activity is clearly demonstrable on the cell membranes of the pathogenic strains of free-living amebae.

Diagnosis

Naegleria is a small (10-35μm) limax-like ameba with a transient flagellate stage in its life cycle. In altered environmental condition, it converts to a smooth and double-walled cyst. *Acanthamoeba* is a slightly larger ameba (15-45μm) and is characterized by fine, tapering, hyaline, and often filamentous surface projections called "Acanthopodia". It has no flagellate stage, but produces a double-walled cyst with a wrinkled ectocyst and a stellate, polyhedral, or even round endocyst. Both organisms are uninucleate, and the nucleus is characterized by a large dense, centrally located endosome.

Hitherto, the diagnosis of primary amebic meningo-encephalitis was established only by post-mortem isolation of trophozoites and cysts in the brain. It is imperative to find the causal agent before treatment, although the detection of parasites from infected sites of the brain and from cerebrospinal fluid is not always easy. To overcome these difficulties, Im and Oh (1978) studied immobilization tests on several free-living amebae. To prepare antiserum rabbits received trophozoites of *Acanthamoeba culbertsoni*, 10^6, or *Naegleria fowleri*, 10^5, respectively every other day three doses and finally one booster dose one week later. Antiserum was collected the day following the booster injection or two months, and stored at $-30°C$ until use. In the test one drop of ameba suspension was mixed with the antiserum on a slide, then observed the mobile state under microscope. Peak immobilization occurred in 30 minutes, then gradual recovery to normal motility took place. The immobilization rates decreased according to the serial dilution of antiserum. At dilutions higher than 1 : 8, the rates were the same as in the normal serum. The antibodies in anti-*Acanthamoeba culbertsoni* rabbit serum reached highest titer on the third day after booster immunization, and from first to sixth week in anti-*Naegleria fowleri* rabbit serum. The antigenic difference between the two amebae was shown in the cross matching test. The report suggests that the immobilization reaction may be of value as a supplementary test in the diagnosis of primary amebic meningo-encephalitis, especially in *Acanthamoeba* infection. In reported cases of meningo-encephalitis probably due to *Acanthamoeba*, survival after onset of neurologic symptoms is usually within severval weeks to a month, whereas in cases due to *Naegleria fowleri*, survival rarely exceeds one week after onset of clinical symptoms arising from brain damage, a period which may not be sufficiently long to allow production of humoral antibodies.

Immuno-prophylaxis

Several reports indicated that infection with free-living amebae induced production of

immune bodies in the host. John *et al.* (1977) reported that mice immunized by intraperitoneal injection of live *Naegleria fowleri* acquired resistance to challenge infections by the same amebae. Thong *et al.* (1973) reported that protective immunity to *Naegleria* meningo-encephalitis could be transferred by immune serum.

Hwang *et al.* (1980) undertook a comparative study of immunologic response of immunized and immunosuppressed mice to *Acanthamoeba* sp.(YM-4) in order to examine the possibility of acquiring protective immunity. White male mice, weighing 8 to 20gm, were used for experimental purposes. *Acanthamoeba* sp. YM-4 strains were cultured in CGV medium (Willaert and LeRay, 1973). Mice were divided into four groups; untreated control group, immunized group, prednisolone treated group and gamma-ray irradiated group. Immunized group was divided into 3 subgroups; intraperitoneal injection of 5×10^5 and intraperitoneal injection of two doses of 5×10^5 live amebae(YM-4) and intradermal injection of 0.2mg of YM-4 antigen once weekly for two weeks. In the prednisolone treated group the hormone was injected intramuscularly in five doses of 10mg/kg every other day. In the gamma-ray irradiated group mice were treated with a total dose of 300 rad. As challenge infection, *Acanthamoeba* sp. (YM-4), 1×10^4 was injected under ether anesthesia into the brain through occipital region of the mouse by the following time schedule; five weeks after immunization, two days after the last prednisolone administration and two days after gamma-ray irradiation. On the 30th day the survived animals were sacrificed and brain tissues were cultured in the non-nutrient agar (Kasprzak & Mazur, 1972), or stained with hematoxylin-eosin in order to examine the pathological change of the tissues. Changes of antibody titer in the sera of the experimental animals were checked by indirect fluorescent antibody technique.

Immunized group: Control animals died within 7 to 21 days in 64% of cases; one dose immunized animals died within 5 to 21 days in 40.7% of cases, two doses immunized animals died in 3 to 12 days in 8.3% of cases and intradermal injection group died in 11 to 23 days in 25% of cases (Table 48). Thus, significant difference in mortality was confirmed between control group and immunized group, suggesting that mice can acquire protective immunity to *Acanthamoeba* infection (Fig. 43).

Table 48. Death rate of mice after challenge infection with *Acanthamoeba* sp., YM-4

Immunization		*No. of mouse	No. of death(%)	Average survival period day(range)
Route	Amount			
Intraperitoneal				
1	500,000 once	27	11 (40.7)**	13 (5~21)
2	500,000 twice	36	3 (8.3)**	6 (3~12)
Intradermal	0.2mg, ameba extract	8	2 (25.0)**	17(11~23)
Control		25	16 (64.0)	14 (7~21)

Note : *No. of intracranial challenge: 10^4 amebae.
**Statistically significant, $p<0.05$

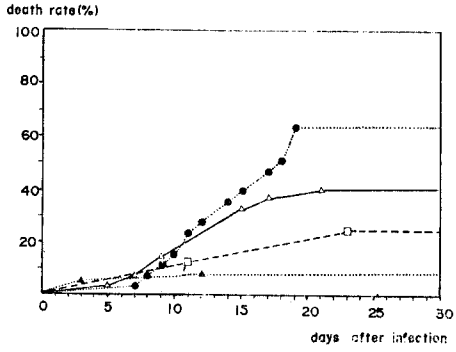


Fig. 43. Cummulative death curve after intracranial infection of amebae in immunized mice.
△——△ : intraperitoneally immunized with 5×10^5 amebae once.
▲·····▲ : intraperitoneally immunized with 5×10^5 amebae twice.
□- - -□ : intradermally immunized.
●·····● : control.

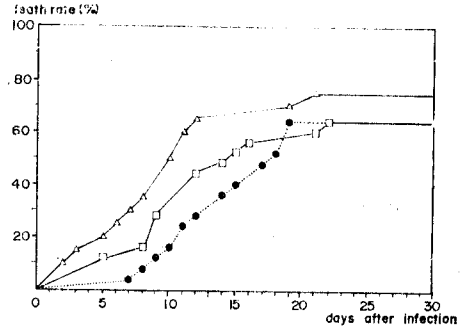


Fig. 44. Cummulative death curve after intracranial infection of amebae in immunosuppressed mice.
△——△ : prednisolone treated.
□——□ : gamma-ray irradiated.
●·····● : control.

Table 49. Death rate after intracranial infection of 1×10^4 *Acanthamoeba* sp. (YM-4) in prednisolone and gamma-ray treated mice

Method of treatment	*No. of mouse	No. of death(%)	Average survival period day (range)
Prednisolone	20	15 (75.0)**	9 (2~21)
Gamma ray	25	16 (64.0)**	9 (5~22)
Control	25	16 (64.0)	14 (7~21)

Note : *No. intracranial inoculation: 10^4 amebae.
**Statistically not significant

Immunosuppression group: Mice of prednisolone administered group died within 2 to 21 days in 75% of cases and mice of gamma-ray irradiation group died within 5 to 22 days in 64% of cases (Table 49). But compared to untreated control group, mice belonging to the prednisolone pretreated group and those belonging to the gamma-ray pretreated group showed no significant difference in mortality (Fig. 44).

Indirect fluorescent antibody titer using *Acanthamoeba* sp., YM-4 strain as antigen showed a significant increase in mice which had been intraperitoneally or intradermally immunized, but a lesser increase in mice treated with prednisolone or irradiated with gamma-ray: 1 : 16~1 : 32 in immunized group but 1 : 4 or less in control group and immuno-suppressed group. These results emphasize that artificial immunization may reduce the mortality of hosts infected with pathogenic free-living amebae, in terms of immuno-prophylaxis.

SYNOPSIS

Entamoeba histolytica, E. coli, E. gingivalis, Iodamoeba bütschlii, Endolimax nana and *Dientamoeba fragilis* are the known protozoa which are prevalent among Koreans. However, *Entamoeba histolytica* is the only species of the utmost importance in the sense of clinical implication, even though some of the remaining species have been proclaimed to cause pathogenicity. Beside, the free-living pathogenic amebae have recently become the center of interest among clinicians and parasitologists, because they cause fatal primary meningo-encephalitis. On the occasion, the author tried to review the important points among the results which have been accomplished in the laboratory, heading with those protozoa.

ENTAMOEBA HISTOLYTICA

Biology

Morphology—Several strains, YS-1, 5, 9, 10, 14, 15, 16, 24, 25 and 27 were subjected for the study. In immune serum, active form of *E. histolytica* was immobilized after about 30 minutes of the treatment. At the beginning stage of the immobilization helical aggregates which asscciated with vacuoles appeared abundantly in the cytoplasm but gradually tended to aggregate along peripheral region of the cell, specially in the intact immobilized state. When the cells remobilized in about 90 minutes, pseudopodia appeared again, then the helical aggregates in the cytoplasm disappeared.

Cultivation—In the egg slant medium overlayed with modified diphasic medium using calf, cow, rabbit, dog, pig and human serum, propagation of the amebae was most luxuriant on the third day of culture in the group to which two drops(=2/15ml) of calf serum were added. The amebae which grew in the calf serum added media produced lesions in the cecum of rabbits.

Virulence—Virulences of the strains were examined with the weaned Sprague-Dawley rats under various conditions, and found that the strains which originated from clinical amebiasis showed more invasiveness than the strains from cyst-passers in general. Some strain, YS-14, showed attenuation by long period of *in vitro* culture but recovered by animal passages.

Temperature adaptation—Under three different temperature conditions, 30°C, 32°C and 37°C, strains originated from non-clinical cases were likely more adaptable to lower temperatures, but the strains from pathogenic state the amebae propagated more at 37°C.

Hemolytic ability—It was different according to the strains of *E. histolytica* and the difference of red blood cells of animal. Each strain showed selective ability to hemolyse the red blood cells. It was also found that normal red blood corpuscles inhibited the multiplication of *E. histolytica* but when the cells were hemolysed, then the propagation

was accelerated.

Host-parasite relationship

Mast cell—*E. histolytica* infection provoked the degranulation of mast cells accompanying with the increase of the cell. Eosinophilia was also noticed as secondary reaction due to degranulation of mast cells.

Nutrition—Nutritional condition correlated with the intensity of *E. histolytica* infection. Young rats were fed with the diets of depleted protein, low protein, moderate protein and high protein. The higher cecal ulceration rate and higher infectivity were occurred in the depleted or low protein diet groups, whereas the high protein diet fed group showed very mild pathological changes. Thus the decreased dose of protein diet retarded the growth of host and decreased the resistance to amebic infection.

Stress and Hormone—Castrated and testosterone injected rats showed more susceptibility to the ameba infection. Shaking stress resulted more severe pathological changes than in the non-shaking group. In another experiment, the exposed ceca of laparotomized rats were compressed with surgical forceps to produce congestion followed by the amebae inoculation, and found the direct physical damage enhanced the infectivity. Previous infection with *Shigella dysenteriae* also enhanced the pathogenicity.

Symptomatology

In intestinal amebiasis, diarrhea, tenesmus, abdominal pain and fever were the common subjective symptoms, and tenderness was uncountered as main objective symptoms in clinics. Bloody-mucous stool and leucocytosis were the physical findings. In Korea, complication such as liver abscess was relatively common.

Hepatic amebiasis—Prevalence of hepatomegaly in Jeju-Do where the amebiasis is highly prevalent was examined. Hepatomegaly cases were 37.1% among the 213 villagers in Yongheung-Ri, and *E. histolytica* cysts were found in 59.7% among the hepatomegaly cases. Amebic liver abscess was more common in age groups of 30~39. For the diagnosis ameba immobilization test, indirect fluorescent antibody test and indirect hemagglutination test were tried and concluded that the tests were highly valued to diagnose amebic abscess in spite of the negative finding of the protozoa from the aspirated pus. In an establishment of amebic liver abscess, it was proved that chemical hepatic injuries with hepatotoxic agents such as thioacetamide and carbon tetrachloride disposed and quickened the production of amebic liver abscess even in the less susceptible animal such as rat, and concomitant micro-organisms enhanced the virulence in establishment of the abscess in liver.

Epidemiology

Prevalence—The overall prevalence of the cyst carriers at national level was about

6.4%, even though it differed by locality even in the same province. In Jeju-Do, prevalence went up to 34.8%. Higher infection rates were in age groups of 50 years and above in either cyst carriers or clinical cases. By sex, prevalence in female was generally higher than the male group. But it was not true in all occasions. Among 102 amebic liver abscess cases in Jeju-Do, 83.3% were in males and only 16.7% in females. Although no logical explanation can be established, environmental factors, habits and physical conditions may contribute to such diversity. By occupation, laborers showed higher incidence than the economically fixed groups. Seasonally, no clear-cut difference between warmer months and colder months was noticed in incidence of the cyst carriers, but the clinical cases were more number during warmer months.

Environmental factor—Korean still utilize human excrement as fertilizer for vegetable growing. In addition no adequate sewage system is provided in rural and many of the urban areas yet. In such environments and living behaviours, a high incidence of amebiasis is to be natural. The important roles in the transmission were by means of drinking water, insects, rodents, hog feces and poorly controlled individual hygiene. In houses in Jeju-Do where the sanitary environment was not appreciable, mother played an important role as source of amebic infection among families; infections in mothers 17.3%, fathers 7.4% and siblings 9.0%. Of 65 households, 51 had positive cases of *E. histolytica* cyst. Among them, only 23.5% had single positive case and 66.5% had 2~5 persons infected in the same family.

Diagnosis

No further effective method was experienced than the conventional direct smear method and concentration method in detecting the protozoa. But serological methods such as indirect fluorescent antibody test(IFA), ameba immobilization test(AI) and indirect hemagglutination test(IHA) were proved as reproducible indirect methods in routine examination specially in extra-intestinal amebiasis. Among the tests, IHA showed the most sensitive. At any rate, concomitant positives of the three methods seemed to be valued to put diagnosis definitely in acute intestinal amebiasis and amebic liver abscess. However, there were still positives among the cyst-negative healthy people. The low specificity was found more in groups of IHA and AI. Application of latex agglutination test and gel-diffusion precipitation test were also found to be useful as supplementary methods to attain more confirmatory diagnosis.

Treatment

Among a number of amebicides, nitroimidazole derivatives such as metronidazole, ornidazole and tinidazole were proved to be the choice of drugs in both of cyst carriers and clinical cases involving extra-intestinal amebiasis. But in cyst carriers, relapse was experienced by administration of metronidazole. Ornidazole and tinidazole showed similar effectiveness specially in treatment of hepatic amebiasis in a single day dose of 2gm in two

divided doses. In any case, pus aspiration should be repeated following the drug administration. Parenteral use of ornidazole in hepatic amebiasis proved also very effective by injection two times in every 12 hours. The total amount of the ornidazole was 2.0gm, and no notable untoward side effects were encountered either by oral or parenteral administration.

OTHER ENDAMOEBIDAE

The positive rate of oral protozoa according to the site of oral cavity revealed that *E. gingivalis* was 29.5% and *Trichomonas tenax* was 8.9% among 78 samples collected from periodontal sulcus; *E. gingivalis* 62.2% and *T. tenax* 21.1% among 51 samples from calculus; and *E. gingivalis* 20.0% and *T. tenax* 13.3% among 15 samples from other sites. In general, positive rates of the both protozoan increased proportionally to the periodontal index and the simplified calculus index. Althouth oral protozoa have been recognized originally to be non-pathogenic scavengers in the oral cavity, several problems are left to be solved in possible causal relationships of these parasites to be etiology of oral diseases. To elucidate the virulence, enzyme activity of the protozoa was examined with electron microscope, but plasma membrane showed negative reaction for acid phosphatase in general. In vacuoles, some showed strong enzyme activity in the ingested contents, whereas limiting membranes of vacuole revealed negative or weak reactions which were the contrary to the findings in the vacuole phases of *E. histolytica*. Further studies will be needed to clarify the pathogenicity in oral cavity, specially on mechanical damage, enzymes and symbiotic actions with oral micro-organisms, *etc. Entamoeba coli, Endolimax nana, Iodamoeba bütschlii and Dientamoeba fragilis* have been recognized as commensal in Korea, although pathogenicity of the last one was reported elsewhere in western countries.

FREE-LIVING AMEBAE

Among the free-living amebae, *Naegleria* sp. and *Acanthamoeba* sp. have been proved to be causative agents of primary amebic meningo-encephalitis in human. In Korea, we have collected a number of amebae which are belonged to the above two genera. Among them, *Acanthamoeba* species, YM-2 and 4 showed virulence in laboratory mice. Anticipating or presuming human infection in the past or future, the author and the associates performed series of *in vivo* or *in vitro* studies with the isolated limax amebae. In sewage, paddy and pond the protozoa distributed relatively common even though many of them were non-invasive in laboratory mice. The protozoa resisted in food-preservatives, but were weak in acid *e.g.*, gastric juice, but persisted in intestinal juice. *Acanthamoeba* were well tolerated in ordinary concentration of pesticides.

To visualize the relationship between the pathogenicity of free-living amebae, agglutinability with phytoagglutinin was examined. The pathogenic *Naegleria fowleri* showed no agglutination, but the agglutinability was not unique in *Acanthamoeba* species regardless

of the virulent or non-virulent.

To determine more clearly the pathogenic strain from the non-pathogenic, cytochemical method was applied. For the purpose concanavalin A(phytoagglutinin) and horseradish peroxidase system was applied, and found that ultrastructural demonstration of concanavalin A-horseradish peroxidase activity on the surface of the cell membrane was obvious only in pathogenic *Acanthamoeba lenticulata*, *Acanthamoeba* sp. YM-4, and *Naegleria fowleri*. In diagnosis of the infections of the pathogenic free-living amebae, ameba immobilization test was applied. The validity was realized and immunization study was also performed. For the purpose the immunized groups by intraperitoneal injection of live *Acanthamoeba* YM-4 once or twice, or intradermal injection of YM-4 antigen, and immunosuppression groups by treatment with prednisolone or gamma-ray irradiation were subjected. In the study, the immunized group survived much longer period and less mortality, but contrarywise results in the immunosuppressed groups suggest that the acquired protective immunity is available.

圖 文 要 約

韓國의 人體寄生아메바

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蘇 鎭 琯

韓國에 있어서 아메바症은 오래 전부터 중요한 感染病의 하나로 널리 알려져 왔다. 특히 夏節에는 全國的으로 쉽게 만연됨은 常識化되어 있는 實情이다.

아메바目, order Amoebina에 속한 네 科, Naegleriidae, Amoebidae, Endamoebidae, Paramoebidae 중 韓國人 感染이 보고된 것은 Family Endamoebidae중의 6가지이나, 現今 感染 가능성이 認定되고 있는 自由生活아메바를 포함하여 고찰하고자 한다.

이질아메바(*Entamoeba histolytica*)
대장아메바(*Entamoeba coli*)
치은아메바(*Entamoeba gingivalis*)
옥도아메바(*Iodamoeba bütschlii*)
왜소아메바(*Endolimax nana*)
쌍핵아메바(*Dientamoeba fragilis*)
자유생활아메바(*Naegleria* spp., *Acanthamoeba* spp.)

이 중 이질아메바는 病源性이 뚜렷한 것으로 國民保健에 至大한 영향을 미치고 있다.

著者는 이에 관심을 가지고 韓國에 있어서의 아메바感染에 관한 實態와 그 問題點 및 解決方案등을 實驗的, 臨床的 또는 疫學的 見地에서 模索해왔다. 그러나 제한된 人員, 장비로 불과 20년 동안에 마무리한다는 것이 얼마나 어려운 일인가를 本 論文을 정리하면서 더욱 절실히 느꼈다. 다만 寄生蟲學會의 배려에 따라 부족을 느끼면서도 그간 얻어진 所見들을 정리하였고, 또 國內外 先輩同僚들의 귀한 文獻들을 인용하면서 主題에 副應될 內容이 될 수 있도록 成文의 補完에 最善을 다 하였다.

이질아메바

1. 生物學

形態: 光學顯微鏡상 아메바의 生活史, 즉 trophozoite, precyst, cyst 등에 따라 그 形態나 크기가 一定치 않다. 電子顯微鏡상 이질아메바의 trophozoite는 그 形態가 매우 불규칙하다. 偽足으로 생각되는 plasmalemma가 관찰되며 cytoplasm內에는 食胞(food vacuole), 탐식된 박테리아, 녹말분, helix體 등이 관찰되고 核은 圓形이고 核膜에는 규칙적으로 nuclear pore들이 보였다. Trophozoite는 면역혈청내에서 非動化되며 이때 電子顯微鏡所見상 chromatoid體로 보이는 helix體들이 세포질내에 응집되나 시간이 경과함에 따라 아메바의 운동상태가 회복되면서 helix體의 응집 역시 없어지면서 正常化되었다. 그러나 글리코겐粒子, acid phosphatase活性은 면역혈청내에서 변동이 없었다.

培養: 成牛, 개, 家豚, 토끼, 사람 등의 혈청이 포함된 二相培地에서 번식이 되나 仔牛血淸을 追加했을 때 더욱 잘 되었으며 3日後에는 번식이 절정에 이르렀고 感染動物에서의 病變 역시 더욱 뚜렷하였다.

毒性: 急性痢疾 또는 肝膿瘍환자로부터 분리된 株는 無症狀씨스트感染者에서 분리된 株보다도 病原性이 강하게 나타나나 장기간 계대배양할 경우 그 毒性은 弱化되었다. 그러나 動物接種을 계속할 경우 그 毒性은 다시 회복되는 것을 관찰하였다.

溫度에 대한 適應: 培地의 溫度가 30°C 以下일 경우 번식이 안되며 病原性이 강한 株일수록 37°C의 溫度에서 適應이 잘 되며 번식이 잘 되었다.

赤血球에 대한 溶血能 : 분리된 株에 따라 家豚, 羊, 牛, 토끼, 개 및 사람의 赤血球에 대한 溶血能이 서로 다르나 培地에서의 溶血은 아메바의 번식에 매우 중요한 요소가 되었다. 正常 赤血球는 이질아메바의 번식을 억제하는 반면 赤血球가 일단 溶血되면 오히려 번식을 促進시켰다.

2. 宿主—寄生蟲의 相互關係

肥滿細胞 : 이질아메바感染은 肥滿細胞의 局所的 증가를 초래하고 脫顆粒을 조장하며 알러지반응에 관여하는 것으로 생각되었으며 이차적으로 好酸球의 증가가 관찰되었다.

榮養 : 蛋白缺乏 또는 低榮養食은 아메바感染에 대한 宿主의 저항력을 低下시켰으며 大腸內 아메바성 潰瘍의 數와 크기가 正常 食餌群에 비하여 많았고 더 크게 나타났다.

스트레쓰 및 홀몬 : 雄性홀몬인 testosterone투여는 아메바의 感染을 조장시키나 雌性홀몬인 estradiol은 그 반대 경향을 보였다. 한편 振動刺戟은 아메바의 感染을 促進시키고 病變의 深化를 초래하였으며 大腸部位에 대한 직접적인 挫傷 또는 病原菌의 同時感染도 感染力과 病變을 더 深化시켰다.

3. 症勢, 病理

腸아메바症 : 腸아메바症의 併發症으로는 肝肥大 또는 肝膿瘍이 제일 많았으며, 腸穿孔, 肺膿瘍, 關節炎, 子宮頸部炎, 股關節炎 등도 韓國에서 報告된 바 있다.

아메바性 肝膿瘍 : 腸아메바症의 併發症으로 오며 특히 이질아메바感染者가 많은 濟州道에서는 部落民의 11~37%가 肝肥大를 보였으며 肝肥大患者의 59.7%에서 이질아메바의 씨스트를 검출한 바 있다. thioacetamide, carbon tetrachloride 등 肝毒物質은 肝膿瘍의 成立을 促進하였다.

4. 疫 學

이질아메바의 씨스트保有者는 全國的인 조사에서 6.4%로 나타났으며 成人層에 感染率이 높았고 씨스트保有者 또는 이질아메바症 患者는 女性이 男性보다 높은 比率을 보였다. 그러나 濟州道에서 조사한 아메바性 肝膿瘍 患者는 性比에 있어서 男性이 83.3%이고 女性이 16.7%이었다. 職業別로는 勞動者, 木工 등 低所得層에서 더 많았고, 씨스트保有者도 季節的으로 그 陽性者數에 差를 볼 수 없었으나 臨床的인 아메바症은 夏節에 더 많았다. 특히 糞尿처리가 非衛生的인 경우 즉 豚舍를 便所와 같이 사용하는 濟州道, 集團收容所, 孤兒院 등에서는 더 높은 感染率을 보였다. 일부 가정에 비치된 물독에서도 아메바씨스트가 檢出되었고, 한 家族단위로 볼 때 단독감염(23.5%)보다 가족 여러 사람의 감염예(76.5%)가 더 많다.

5. 診斷 및 治療

아메바씨스트의 檢出을 위하여 시도한 Rolling method는 직접도말검사법과 같은 檢出率을 보였으나 集蟲法에는 미치지 못하였으며, 間接螢光抗體法(IFA), 間接血球凝集反應法(IHA), 아메바非動化反應法(AI) 등은 매우 實用的인 血淸學的 診斷法으로 三者의 同時陽性反應은 肝膿瘍, 急性痢疾을 진단함에 거의 100%의 信憑度를 나타냄으로써 이질아메바症의 診斷에 커다란 도움을 주었다.

治療劑로 nitroimidazole系 藥品이 殺아메바劑로 有效하였으며 특히 ornidazole은 씨스트에도 강력한 殺蟲效果를 보였다. ornidazole은 經口用과 아울러 靜注用도 副作用없이 肝膿瘍 治療에 著效를 보였다.

其他 아메바類

齒齦아메바는 齒齦炎, 齒周炎, 齒牙카리에스患者로부터 더 高率로 검출되나 그 病原性은 분명치 않았으며 건강인에서도 20~30%의 양성율을 나타내고 있다.

大腸아메바는 11~20%, 矮小아메바는 3~8%, 옥도아메바는 1%미만, 二核아메바는 0.1~0.5%의

양성율을 보이나 그 병원성은 증명된 바 없다.

自由生活아메바

서울시내 下水溝, 웅덩이에서, 또 市場에서 사 온 붕어의 아가미에서 *Naegleria* sp.와 *Acanthamoeba* sp.를 1966년이래 10여종 분리하였으며, 그중 *Naegleria* sp.(YM-1株), *Acanthamoeba* sp.(YM-2, YM-4株)는 실험마우스의 腦에 病巢를 발생시켰다. 이들은 鼻腔에서 嗅覺神經을 따라 腦에 침입하나 胃液에는 抵抗力이 약하였으며 일반 臟器에서의 感染은 증명할 수 없었다.

自由生活아메바의 phytoagglutinin(con. A)에 대한 凝集反應은 病原性이 인정되는 *N. fowleri*에는 반응을 일으키지 않았으나 *Acanthamoeba* sp.는 病原性의 有無와 관계없이 凝集태도에 차를 인정치 못하였다. 아메바에 concanavalin A와 horseradish peroxidase를 작용시킨 후 電子顯微鏡으로 관찰한 바 病原性인 *Acanthamoeba* sp.와 *Naegleria* sp.에서는 細胞膜 周邊에 電子密度가 높았으나 非病原性인 아메바들에서는 이러한 반응이 관찰되지 않았다. 그러므로 이 방법의 응용은 病原性인 自由生活아메바를 識別하는데 중요한 길잡이가 될 줄 믿는다.

自由生活아메바에 감염되었을 경우 숙주 체내에 면역체가 형성됨이 인정되어 다음 感染에 대한 抵抗力을 얻을 수 있는 지를 알기 위하여 마우스에 *Acanthamoeba* sp. YM-4株 抗原을 피내주사하거나 복강내에 1차 또는 2차 면역접종한 경우와 면역억제제로 알려진 프레드니소론을 注射후 또는 감마線 照射후 *Acanthamoeba* sp. YM-4株를 腦內感染시켰을 때 水溶性 抗原을 接種하거나 살아있는 아메바를 복강내에 1차 또는 2차 접종한 경우 사망율이 25.0%, 40.7% 및 8.3%로 무처치 대조군(64.0%)에 비해 낮았으며, 반면에 프레드니소론 처치群이나 감마線 照射群은 사망율이 75.0% 및 64.0%로 대조群보다 높아 人工的으로 免疫을 부여할 경우 사망율이 低下함을 관찰하였다. 螢光抗體價도 免疫群에서 높음이 관찰되었다. 이와 같은 所見은 自由生活아메바에 대한 人工的 免疫接種의 可能性을 뒷받침하는 것으로 생각되었다.

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