$HgCl_2$ are not viable. The difference in viability constitutes an important difference between the large bodies produced by the interaction of the 2 strains and by refrigeration on one hand, and by toxic influences on the other hand. The meeting of the 2 strains induces a complex reproductive process consisting of several consecutive steps. After a short delay vigorous multiplication is resumed. It is not likely that this process is simply degenerative.

No union was observed between cells at the meeting of 2 strains and the present observations do not support the supposition that the interaction between the strains is sexual.

15571

Differences in Culturability, Infectivity and Pathogenicity of Human Strains of *Endamoeba histolytica.**

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Introduction. Clinical and experimental data have provided a wealth of divergent views concerning the ability of *Endamoeba histolytica* to produce disease. In man and experimental animals there is a wide range of clinical states in amebiasis, from a fulminating infection to an essentially symptomless condition. Both the intrinsic pathogenicity of the particular "strain" of the organism and the host's general threshold of resistance to infection contribute to infectability and the degree of pathology produced.

Since the accumulated knowledge fails to explain many theoretical and practical questions concerning *E. histolytica* and its host, a renewed attack has been made on the problem. The particular objective of the present study consists in an evaluation of culturability, infectivity and pathogenicity of different "strains" of the parasite.

Material and Technics. Eighteen persons have served as sources of the parasite. The youngest was 2 years and the oldest 59 years old. Twelve were males, 5 females and the record on one was not available. Six were white, 12 were Negroes and one was an Indian. All but one, a Honduran, were American-born and the majority were natives of Louisiana who had never left the state. Twelve persons sought medical help because of colitis, including diarrhea and chronic dysentery. Two individuals in the series were suffering from bronchopneumonia. One patient complained of rheumatism and one was suffering from oxyuriasis. A food handler undergoing routine coprological diagnosis had no complaint. The clinical diagnosis of one was not obtained. Of the entire group 7 passed formed stools from which cysts of *E. histolytica* were obtained and 4 submitted unformed stools containing motile trophozoites of the organism. In 5 instances proctoscopic material was obtained for inoculation. In one case a purgative specimen was used. In another, a dysenteric stool was cultured in the hospital laboratory. In 11 instances cysts of trophozoites in freshly passed stools were utilized.

The basic culture medium consisted of an egg-slant base, made of a coagulated emulsion of whole egg and Ringer's solution, with liquid overlay of physiological salt solution containing 0.5% crude liver extract (Lilly). After tubing, a small amount of specially milled rice starch, furnished by Doctor Charles W. Rees of the National Institute of Health, Bethesda, Md., was sterilized and planted in the overlay. Formed stool specimens provided cysts, which were concentrated by the zinc sulfate centrifugal floatation technic, thoroughly washed in distilled water and then seeded in the tubes. Small amounts of mucus

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or feces and mucus from the unformed or liquid stools, proctoscopic material and the purged sample were planted directly in the medium. Culture tubes were incubated at 37°C and transfers were usually made every 48 hours, although some "strains" required a different interval for subculturing and others varied considerably in their growth periods from week to week. The optimum transfer period was determined only after prolonged observation with each "strain." Routinely at the appropriate time for transfer the tube was opened and under aseptic conditions a small measured amount of the liquid at the base of the overlay was drawn up into a sterile pipette and transferred to a fresh culture tube. A second measured amount was then removed from the first tube and placed on a microscopic slide provided with a 22 mm square coverglass, for observation and for count of the amebae. When fewer than 10 amebae were found in the entire preparation, growth was designated as very poor; when an average of one to 5 appeared in each low-power field, it was designated as poor, 5 to 20 as fair, 20 to 50 as good, and more than 50 per low-power field, as excellent. At times no amebae were seen in the cover-glass preparation but subcultures proved positive. Moreover, under conditions which did not vary, all cultures sometimes developed better than average, while at other times great difficulty was experienced in maintaining even those which usually exhibited luxuriant growth. During the period of this study encystation occurred only sporadically and in scant amount.

The experimental animals consisted of young kittens, mature cats, puppies from one to 5 months old and dogs about 6 to 9 months of age or older. Although puppies proved to be as infectable as kittens, they were not readily obtained. Cats were tested only incidentally. Older dogs were used to maintain "strains" over a period of several months. Confirming the experience of previous investigators, the present workers found kittens to be relatively satisfactory for acute experiments, both because of the ease of infectability and because of the rapid development of lesions. Early in the study it was decided to employ a minimum of 5 kittens to test the infectivity and pathogenicity of each "strain." Except for 3 "strains" (Nos. 2, 9 and 12), the minimum goal was reached and in several instances considerably exceeded.

The senior author[1] has described a practical technic for inoculating dogs *per anum* by means of a long glass tube to which a rubber bulb is attached, so that an inoculum with amebic trophozoites can be deposited in the cecum and usually into the posterior segment of the ileum, the anteriormost level of the bowel where amebic lesions develop. The technic referred to provides an opportunity for the amebae to make contact with the intestinal mucosa and to penetrate the tissues without trauma to the bowel and without the hazard of shock and lowered resistance attendant on the implantation of the inoculum by needle through the wall of the posterior segment of the ileum following laparotomy.[2] Although the nonflexible glass inoculation tube can be used in the kitten without danger of injury to the delicate bowel wall, a very satisfactory substitute consists in a No. 10 or 12 flexible rubber catheter, to which a hypodermic syringe is attached.

In 5 instances ("strains" No. 6, 7, 8, 10, 15) cysts were concentrated, washed, and were fed to kittens, cats and puppies to initiate infection. In 4 instances ("strains" No. 11, 13, 17 and 18) trophozoites obtained directly from human sources were inoculated intracecally. In these 9 "strains," as well as in all others except No. 9 and 12 where material was too scant, coincident or subsequent inoculations were made intracecally in other hosts with cultured trophozoites.

Pathogenicity has been evaluated by 2 types of data, clinical and postmortem. An estimate of the effect of the amebae on their experimental hosts during life was based on the behavior of the animal compared with the preinoculated condition, loss of appetite, loss of weight, condition of the fur, number and type of bowel evacuations and the relative prevalence of amebae in the stools. On

[1] Faust, E. C., *Porto Rico J. Pub. Health and Trop. Med.*, 1931, **6**, 391.

[2] Meleney, H. E., and Frye, W. W., *Am. J. Hyg.*, 1933, **17**, 637.

this basis an attempt was made to estimate the damage to the host in categories of +, ++, +++ and ++++.

Only kittens will be considered in the postmortem study, since they alone were employed in numbers sufficient for statistical use. A total of 93 kittens came to necropsy or were sacrificed. Two of these confirmed clinical and antemortem laboratory evidence that the particular animals were refractory to infection. In one which had been positive some weeks earlier there was no indication of infection, suggesting that spontaneous recovery had occurred. No autopsy was performed in 11 instances and in 3 kittens advanced postmortem changes made careful pathological examination useless. The 76 remaining in the series were available for full study. The workers were from time to time assisted by Doctor Guillermo M. Carrera, of the Department of Pathology and Bacteriology, Tulane University.

Eight levels of the bowel potentially involved in amebic ulceration were inspected separately in each kitten, *e.g.*, posterior segment of the ileum, cecum, appendix, ascending colon, transverse colon, descending colon, sigmoid colon and rectum. The degree of involvement of each segment was then recorded on the basis of naked eye and dissecting-microscope inspection. In each autopsy the presence of amebae recovered from the lesion or free in the bowel was also recorded. The following symbols were employed to designate the degrees of pathogenicity: one to 10 small shallow amebic ulcers per segment, +; 10 to 25 distinct shallow ulcers or a smaller number of deeper ones, ++; deep ulceration with some evidence of confluence, +++; extensive confluent lesions over essentially the entire segment of the bowel, ++++. These estimates were made for each of the 8 segments of the 76 autopsied animals. "Strain" No. 5, with 10 kittens in the series, will illustrate how the amount of tissue damage was recorded and evaluated (Table I). Each + was assigned a numerical value, with + as one, ++ as 2, +++ as 3 and ++++ as 4. Thus the maximum total units for an entire length of intestine with severest involvement would be 32. Then, the

TABLE I.

Evaluation of Tissue Damage (with Percentage Involvement) in 10 Kittens Experimentally Inoculated with "Strain" No. 5, *Endamoeba histolytica*, as Studied at Autopsy.

	Ileum	Cecum	App.	Asc. colon	Trans. colon	Desc. colon	Sigmoid colon	Rectum	Total unit value	Pathogenic index
	—	+(20)	+(20)	+(20)	+(30)	+(20)	+(30)	+(40)		
		++(30)			++(20)	++(10)	++(20)	++(30)		
		+++(20)		+++(10)		+++(10)	+++(10)	+++(10)		
Total % involvement		Σ = 70	Σ = 20	Σ = 30	Σ = 50	Σ = 40	Σ = 60	Σ = 60		
Unit value		1.4	0.2	0.5	0.7	0.7	1.0	1.3	5.8	5.8 ÷ 32 = 0.18

TABLE II.
Summary of Data on Culturability, Infectivity and Pathogenicity of 18 "Strains" of *E. histolytica* From Human Sources.

Source and type of inoculum	Culturability	Infectivity	Pathogenicity	
			Clinical estimate	Pathogenic index
Proctoscopic;	Very poor			
trophozoites	No. 2	Low ?	+ ?	
Idem.	" 9	" ?	?	
"	" 12	" ?	?	
	Poor			
Stool; cysts	No. 7	"	?	
Purge; trophozoites	" 11	High	++++	0.08
Proctoscopic; "	" 14	"	+++	0.23
	Fair			
Stool; "	No. 3	"	++	0.17
Proctoscopic; "	" 13	"	++++	0.11
Stool → culture;				
trophozoites	" 16	Moderate	+ to ++++	0.15
	Good			
Stool; cysts	No. 1	"	+	0.11
" ; trophozoites	" 4	"	+	0.02
" ; cysts	" 6	Low	+++	0.13
" ; "	" 8	"	+	0.08
" ; "	" 15	Moderate	++	0.11
" ; trophozoites	" 17	"	++++	0.12
" ; "	" 18	"	++++	0.28
	Excellent			
Stool; cysts	No. 5	High	++	0.18
" ; "	" 10	Moderate	++	0.12

total number of units for all members of the series divided by the number of animals in the series equals the average total unit value per animal (*i.e.*, for "strain" No. 5 = 5.8). However, since it is more convenient to evaluate and compare pathogenic indexes as percentages of 1.0, the average total unit values per animal have been divided by 32. Thus, for "strain" No. 5 the pathogenic index was found to be 0.18.

Presentation of Results. The most important data on culturability, infectivity and pathogenicity of the 18 "strains" studied have been assembled in Table II.

There is no evidence of a consistent correlation between the *in vitro* and *in vivo* experiments, yet the following relations have been found to exist. (1) On the whole, cysts and trophozoites of *E. histolytica* obtained from stools provided better material for culture than did trophozoites from proctoscopic material. (2) The majority of "strains" which developed poorly in cultures were not satisfactory for infecting kittens and with one notable exception (No. 14) failed to produce significant lesions in these animals; conversely, the majority of "strains" which developed fairly well to abundantly in culture proved successful for infecting kittens, although these strains did not necessarily produce extensive tissue damage in the test animals. (3) Clinical evidence is at times misleading for evaluating the extent and degree of amebic lesions produced in the kitten.

In "strains" No. 6, 7, 8, 10, 11, 13, 15, 17 and 18, in which the ameba was inoculated both from original source material (cysts by mouth and trophozoites intracecally) and following growth in the test tube, there was no detectable difference in the rate of infection or the lesions produced from the 2 sources of inocula. In some tests, months after the original material ceased to be available, the cultured organisms proved as satisfactory for inoculation as earlier in the study. In other words, growth in the test tube did not reduce or enhance infectivity and pathogenicity. "Strain" No. 13, which from the start was highly infective, failed to grow in culture from human source material. Later it became established from amebae obtained postmortem from kittens, although it has never

TABLE III.
Summary of Consolidated Postmortem Data on Amebic Involvement of Intestine in 76 Experimental Kittens.

Degree of involvement	Number and % of lesions at different levels							
	Ileum	Cecum	App.	Asc. colon	Transv. colon	Desc. colon	Sigmoid colon	Rectum
Negative	76 (100.0)	33 (43.4)	62 (81.6)	56 (73.7)	57 (75.0)	54 (71.0)	39 (51.3)	41 (53.9)
+		19 (25.0)	11 (14.5)	9 (11.7)	12 (15.8)	8 (10.6)	16 (21.2)	12 (15.8)
++		14 (18.4)	3 (3.9)	4 (5.3)	5 (6.6)	10 (13.2)	14 (18.4)	15 (19.8)
+++		6 (7.9)		6 (7.9)	1 (1.3)	3 (3.9)	5 (6.6)	5 (6.6)
++++		4 (5.3)		1 (1.3)	1 (1.3)	1 (1.3)	2 (2.6)	3 (3.9)
Total	0	43 (56.6)	14 (18.4)	20 (26.3)	19 (25.0)	22 (29.0)	37 (48.7)	35 (46.1)

until recently exhibited vigorous culture growth. "Strains" No. 17 and 18 were obtained from a sister and brother and quite possibly may be identical. Experience with them has been parallel except that "strain" No. 18 provided a much higher average pathogenic index in the experimental animals.

In the series of kittens for which complete autopsies were performed 54 (71.1%) showed typical amebic lesions, which varied in degree from a solitary pinpoint ulcer to extensive involvement of the large bowel. The regional distribution of the lesions, together with the number and the estimated degrees of pathogenicity for each of the 8 segments examined, are summarized in Table III.

Except for the absence of lesions in the ileum and on the ileocecal valve, the levels of greatest frequency and intensity of amebic ulceration are those which previous investigators have discovered at autopsy of human beings and experimental animals, namely that the cecum is the region of greatest involvement, followed by the rectum. This is particularly true of the early acute infection, whereas in prolonged chronic amebic colitis there is a tendency for secondary amebic lesions to develop in the rectal area following invasion of amebae which have previously propagated at the cecal level.

In the 2 "strains" of this series with the lowest pathogenic indexes (No. 4 and 8) there were no lesions discovered in the ileum, ascending or transverse colon. With "strain" No. 4 patent lesions were developed in only one of 6 animals. These consisted of first degree damage in the appendix and second degree in the rectum, while no other levels were invaded. In the 7 kittens employed in a study of "strain" No. 8, 2 had first degree lesions in the cecum, one had first degree lesions in the ascending colon and descending colon, 3 had first degree and one had second degree lesions in the sigmoid colon, and 2 each had first and second degree lesions in the rectum. The observations for "strains" No. 14 and 18, both with high pathogenic indexes, indicate much more extensive involvement. In "strain" No. 14, with 7 animals in the series, every level of the bowel from cecum to rectum was invaded; except for the appendix there were lesions of third or fourth degree in one or more kittens. This "strain" was also highly pathogenic for the cat, which is relatively refractive to amebiasis. In "strain" No. 18, with 5 kittens in the series, there was no evident pathology in the appendix but third or fourth degree lesions were demonstrated at the other levels in 2 members of the series.

In all kittens of the autopsy series antemortem-aspirated material had been obtained *per anum* from the intestine at most from one to 2 days before death or sacrifice of the

TABLE IV.
Summary of Records on *E. histolytica* and on Typical Amebic Lesions in the Intestine of Experimental Kittens.

E. histolytica		Coincidence of amebic lesions			
	No.	%		No.	%
Antemortem +, postmortem +	27	35.5	Present	26	34.2
			Not found	1	1.3
" +, " —	15	19.7	Present	13	17.1
			Not found	2	2.6
" —, " +	4	5.3	Present	4	5.2
			Not found	0	0
" —, " —	30	39.5	Present	11	14.5
			Not found	19	25.0
Total antemortem +	42	55.3	Total present	54	
" " —	34	44.7	" not found	22	
" postmortem +	31	40.8			
" " —	45	59.2			
Grand total	76	100.0	Grand total	76	100.0

animal, to provide microscopic evidence of infection. In 47 cases (61.8%) trophozoites were demonstrated. Postmortem search for the organism was successful in 31 cases (40.8%). In 4 of this latter group amebae had not been demonstrated from antemortem stools or aspirates. Moreover, no lesions were found in one puppy and one adult dog inoculated with "strain" No. 18, but motile amebae were demonstrated both antemortem and postmortem. The relationship of amebae recovered antemortem and postmortem and of amebic lesions is summarized in Table IV.

An examination of Table IV indicates that in 27 kittens (35.5%) there was consistent microscopic demonstration of the amebae and of amebic lesions. In an additional 15 animals (19.7%) the organisms were found shortly before death; typical lesions were found in 13 of these but amebae were not demonstrated postmortem from bowel contents or superficial scrapings. On the other hand, in 4 cases no amebae were recovered antemortem, although they were demonstrated postmortem in association with typical lesions. A fourth category consisted of 30 kittens (39.5%) in which amebae were not demonstrated immediately before death or at autopsy; there were typical lesions in 11 of these but there was no gross evidence of damage in 19 animals. Among these 30 animals some had earlier provided specific microscopic evidence of infection. The data favor the view that in 25.0% of the 76 kittens studied postmortem an infection (*i.e.*, tissue invasion) had never developed, or, if it had existed earlier, the amebae had been expelled with spontaneous recovery and tissue repair. In the single instance in which amebae were demonstrated from the bowel contents both antemortem and postmortem but in which lesions were not found at autopsy there is the possibility that one or more minute inapparent cryptic ulcers were present. In the 2 animals in which there was antemortem but not postmortem demonstration of the amebae and no lesions were discovered at autopsy the infection had probably been spontaneously cleared.

Discussion. This study has provided considerable evidence that culturability, infectivity, and pathogenicity constitute 3 separate attributes of *Endamoeba histolytica,* and that they are not necessarily correlated with one another. A "strain" which grows well in culture (Table II) may be established in the intestine of a highly susceptible animal only with difficulty and following persistent trial. Conversely, a highly pathogenic "strain" which produces infection readily in the test animal, may be established and maintained in culture only after considerable effort.

A factor which will require careful experimental inquiry is the presence or absence of certain species of bacteria associated with the amebae in the inoculum utilized to initiate cultures. Until such information is available in rather complete form it will not be possible to rule this out as an extrinsic influence on initial and subsequent growth.

The present study has provided data indicating that the infectivity of a particular "strain" of *E. histolytica* and the associated infectability of a susceptible host are not always correlated with symptoms and lesions. In 2 "strains" which had a low rating for infectivity the degree of pathogenicity was high. Conversely, in certain "strains" with a high estimate of infection only incidental lesions developed in the series. Nevertheless, on the whole, "strains" which were established with difficulty in the experimental host produced only a few low-grade lesions, while those with medium to high infectivity caused moderate to severe pathology.

One of the most interesting developments in the present investigation has been the rather conclusive evidence (Table III) that the clinical estimate of the extent of the disease in the experimental host frequently fails to coincide with the data obtained at autopsy. At times the antemortem estimate is in excess of the tissue damage, while at times it is too conservative. This latter type of prognostication corresponds frequently with that of human cases of amebiasis, in which the pathologist at necropsy finds extensive involvement of the large bowel not indicated by antemortem records.[3]

Summary. Investigations have been undertaken in an attempt to reconcile some of the many conflicting opinions which have developed regarding the pathogenicity of *E. histolytica*. The problem undertaken and presented in this communication concerns the culturability, infectivity and pathogenicity of 18 different "strains" of the organism, obtained from persons in New Orleans. The hosts varied in age from 2 to 59 years. All but one were hospital or clinic patients, the majority of whom complained of some type of colitis. In 11 cases stools containing cysts or trophozoites were obtained; in 5 cases proctoscopic material was utilized, in one case a purgative specimen was secured and in one instance the inoculum consisted of material previously cultured in a hospital laboratory.

For culture work a solid egg-slant base was employed, with a liquid overlay of physiological salt solution containing 0.5% crude liver extract and special rice starch. Cysts were concentrated and washed before seeding the cultures; trophozoites in fecal or mucous samples were placed directly in the culture tubes. Kittens served as the principal animals to test infectivity and pathogenicity of each strain. A minimum of 5 kittens was employed. Pathogenicity was first estimated on the basis of clinical evidence, then carefully studied at autopsy. The length of intestine from the posterior level of the ileum through the rectum was considered as 8 separate segments and amebic invasion of the bowel separately assessed for each segment. Four degrees of pathogenicity were recognized and were designated as +, ++, +++ and ++++. Based on these symbols a pathogenic index was developed to afford comparisons between different series.

Of the 18 "strains" 6 proved difficult to grow in culture; 4 of these were unsatisfactory for animal work and were discarded. All other "strains" immediately or eventually provided fair to luxuriant cultures and with 2 exceptions were very satisfactory for infection experiments. Seventy-six kittens infected with 14 "strains" of *E. histolytica* provided autopsy material for evaluation of tissue damage produced by the amebae. The clinical estimates of pathogenicity at times failed to correspond with the postmortem findings.

It was not possible to predict the culturability of *E. histolytica* on the history of the patient from whom the inoculum was obtained, on the type of inoculum utilized or the stage of the parasite. Nor was culturability a necessary index of infectivity or pathogenicity. It is suggested that culturability, infectivity and pathogenicity are 3 separate characteristics of *E. histolytica*. A considerable amount of investigation is required to determine what extrinsic factors may influence the development of the parasite *in vitro* and in the susceptible host.

[3] Clark, H. C., *Am. J. Trop. Med.*, 1925, **5**, 157.