

## OBSERVATIONS ON NATURAL INFECTIONS OF ENDAMOEBIA HISTOLYTICA IN ATELES AND RHESUS MONKEYS<sup>1</sup>

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Two years ago the opportunity was presented for the study of a number of monkeys that were carriers of an amoeba indistinguishable from *Endamoeba histolytica* of man. Since amoebic infections in monkeys are in many respects similar to those in man, the observations to be recorded in the following pages may help to clear up some of the problems yet to be solved on host-parasite relations in human amoebiasis.

Although the pathological anatomy of amoebiasis, both experimental and spontaneous, has been thoroughly investigated in the many hosts which are susceptible, there are still a number of phases in which opinions differ. One of these is whether or not the presence of *E. histolytica* in the gut is necessarily associated with tissue invasion. Views in favor of the hypothesis that infection is always accompanied by tissue invasion have been expressed by Bartlett (1), Hiyeda and Suzuki (2), Craig (3), and many others. These investigators have demonstrated that serious lesions of the large intestine may exist without causing symptoms noticeable either to the patient or the physician, lesions which may only be discovered at autopsy. Craig (4), therefore, takes the stand that, "neither the evidence of animal experimentation or the post-mortem findings on carriers of *Endamoeba histolytica* lends any support to the theory that this organism can live in the intestine indefinitely without producing lesions."

<sup>1</sup> Read at the Thirty-Sixth Annual Meeting of the American Society of Tropical Medicine, Louisville, Kentucky, March 12-15, 1940.

On the other hand experimental proof that *E. histolytica* is not an obligatory parasite has been presented by a number of workers who believe that invasion of the gut wall does not always accompany the presence of this organism. Thus the fact that the amoeba can be cultivated in a medium free from blood cells or serum indicates that the organism in vitro, at least, is not dependent on tissue elements for food. Dobell (5), and Hegner (6) have shown that *E. histolytica* can survive in the gut of monkeys without producing lesions which can be detected by our present day methods. Andrews and Atchley (7) concluded that invasion of the tissue by amoebae was unlikely in carriers of *histolytica*, since they showed that in a high proportion of these carriers the stools gave negative occult blood reactions.

Just how *E. histolytica* is able to affect an entrance into the tissue is another controversial subject and opinions differ considerably as to the mechanism of production of lesions. There are some who believe that a cytolyisin is secreted by the amoebae and that the lesions are a result of its dissolving action, while others maintain that the amoebae are able to move about and injure the tissue with the sole aid of their pseudopodia. A third group feels that there is sufficient evidence to show that probably both methods are used. Craig (3) states, "that *Endamoeba histolytica* secretes a substance which dissolves intestinal epithelial cells and red blood corpuscles has been demonstrated by the writer (1927) who was able to extract such substances from cultures of the parasite. . . ." However, an examination of Craig's report of 1927 does not support this statement, for he says in this report that, "the fact that the extracts prepared from young cultures (twenty-four to forty-eight hours old) of *Endamoeba histolytica* are hemolytic and that the supernatant fluid of such cultures is not so, indicates that the hemolysin is not an exotoxin but is present only in the living amoebae . . .," and further, "the absence of a cytolytic substance in the culture medium lends support to the theory that the amoebae penetrate the tissues by means of pseudopodia rather than by dissolving it. . . ." No sound experimental proof for either of these views has been presented and they are based entirely upon the interpretation of the microscopic pathology of the amoebic lesions.

## MATERIAL AND METHODS

The group of eleven monkeys used in this study were composed of three species, *Ateles geoffroyi* Kuhl, *Ateles dariensis* Goldman, both native species and *Macacus rhesus* an old world form. For a tabular summary of these see table 1.

The native spider monkeys came from different sections of Panama and were represented by both young and old specimens. The *rhesus* monkeys came originally from the United States and were obtained by us from a research institution on the Canal Zone; these were all of adult age.

TABLE 1  
*Summary of infected monkeys*

MONKEY NUMBER	SPECIES	ORIGIN	DATE OF ARRIVAL	DATE KILLED	LENGTH OF EXPERIMENT	GROSS APPEARANCE OF GUT	MICROSCOPIC FINDINGS
					days		
199-B	Red spider	Puerto Armuellas	6/ 9/38	8/27/38	48	Negative	Amoebic invasion
200-B	Red spider	Puerto Armuellas	6/ 9/38	8/19/38	40	Granular appearing mucosa	Amoebic invasion
216-B	Red spider	Darien	11/26/38	Died 3/ 4/39	98	Granular necrotic mucosa	Amoebic invasion
220-B	Bl. spider	Darien	12/12/38	3/ 8/39	86	Negative	Amoebic invasion
221-B	Bl. spider	Darien	12/12/38	3/ 8/39	86	Negative	Amoebic invasion
224-B	Bl. spider	Darien	12/21/38	3/ 4/39	73	Negative	Amoebic invasion
239-B	<i>M. rhesus</i>	A.M.R.B.*	3/28/39	3/19/40	356	Negative	Amoebic invasion
241-B	<i>M. rhesus</i>	A.M.R.B.*	3/28/39	7/25/40	484	Negative	Negative
242-B	<i>M. rhesus</i>	A.M.R.B.*	3/28/39	3/19/40	356	Negative	Amoebic invasion
243-B	<i>M. rhesus</i>	A.M.R.B.*	3/28/39	7/25/40	484	Negative	Negative
177-B	Red spider	Bayano	4/ 1/38	3/21/40	623	Negative	Negative

\* Army Medical Research Board.

All the monkeys, both native and old world species, were infected with an amoeba identical with *E. histolytica* of man when they arrived at the laboratory. We say identical with *E. histolytica* of man only in point of morphology since no infection or culture experiments were carried out to determine its physiological characteristics. Dobell (5) concludes from a long series of experiments that the *histolytica* like *Endamoeba* living naturally in *Macacus rhesus* is identical with the one infecting man.

During the interval between their arrival at the laboratory and death all of the monkeys were carefully observed for symptoms; frequent stool examinations were done from time to time, both

by saline and stained preparations, to check the presence of the organisms and to study their morphology.

When the animals were sacrificed, stained smears were made immediately from the contents of the large bowel. The mucosa was then examined for gross evidences of pathology and finally the gut was fixed in Zenkers fluid to which had been added enough acetic acid to make a five per cent concentration. For microscopic study sections were made from numerous blocks of all the regions of the gut and stained, either by the iron-hematoxylin or Delafield hematoxylin technic.

The occult blood tests were done in the usual routine manner by noting the color change when the fecal material was added to a fresh benzidine-acetic acid-hydrogen peroxide mixture. If no color change occurred within three to five minutes the reaction was considered negative. The four monkeys used in the occult blood experiment were not placed on any special diet since ordinarily they eat no meat or fish.

#### RESULTS

*Monkey 199-B.* This animal, an adult male weighing 10 pounds and 5 ounces arrived at the laboratory from Puerto Armuelles on June 9, 1938. During the routine fecal examination it was discovered that it was passing cysts and trophozoites of *Endamoeba histolytica*. It was observed for a period of 48 days during which time no dysenteric symptoms were noted and cysts and trophozoites of the amoeba continued to be passed. On August 27, 1938, the animal was sacrificed. Amoebae were found in the contents of the large bowel and a careful examination of the mucosa did not reveal any gross lesions. However, in microscopic study of sections from blocks of the cecal region it was found that actual invasion of the mucosa by the amoebae had occurred. The lesions were small and involved only the mucosal portions of the gut. In some instances only the superficial portions were invaded and in others the invasion had extended down to the muscularis mucosa. No amoebae were found in the sub-mucosa.

*Monkey 200-B.* An adult female, weighing 9 pounds and 5

ounces on arrival at the laboratory. It also came from the region of Puerto Armuelles. Cysts and trophozoites of *E. histolytica* were found during routine fecal examinations and were present off and on for a period of 40 days when the animal was sacrificed. During this period no dysentery was noted and the animal enjoyed good health. On August 19, 1938 when the animal was killed, *E. histolytica* was present in the contents of the large bowel and gross examination revealed a roughened or granular mucosa in some areas. This was most noticeable in the region of the caecum. Microscopic examination of these granular areas showed amoebic invasion similar to that found in no. 199-B, the only difference seemed to be in the amount of mucosal surface involved, which in this case was much greater.

*Monkey 216-B.* This animal was the youngest of the group and the only one to develop dysentery. It arrived at the laboratory from the Province of Darien on November 26, 1938, weighing 2½ pounds. *E. histolytica* trophozoites were present in stool specimens on its arrival and during the 98 days of its captive life they were never absent. Cysts were never found. The stools from this animal were never formed and most of the time diarrhea was present. Two weeks before death it developed a frank dysentery and died on March 4, 1939. An autopsy showed severe anemia, emaciation, rickets and dysentery. Microscopic examination of the gut revealed almost complete destruction of the mucosa and diffuse invasion of the submucosa with involvement of the lymph follicles.

*Monkey 220-B.* A female juvenile, weighing 4½ pounds when it arrived at the laboratory on December 12, 1938, from Darien. *E. histolytica* in both the cystic and trophic stages were found in the stools during routine fecal examinations. As far as we were able to determine, during the 86 days that the monkey was under observation, no diarrhea or dysentery was present. On March 8, 1939, the animal was sacrificed. Cysts and trophozoites of *histolytica* were found in abundance in the lumen contents of the large bowel and careful examination of the mucosa with the lower powers of a dissecting microscope revealed no gross lesions. In the microscopic study of the tissues, blocks from two or three

areas in the caecum showed small foci of amoebic invasion. The lesions were small and were limited to the mucosa, extending in some instances to the muscularis mucosa.

*Monkey 221-B.* A juvenile male, weighing 5 pounds, arrived at the laboratory on December 12, 1938, from Darien. Routine fecal examinations revealed the presence of *E. histolytica*. Clinical symptoms were never noted during an observation period of 86 days and the animal was chloroformed on March 8, 1939. Essentially, the same gut findings were encountered as in monkeys 199-B and 220-B. There were no gross lesions and the microscopic pathology was the same.

*Monkey 224-B.* A juvenile male weighing  $3\frac{1}{2}$  pounds, arrived from Darien on December 21, 1938, and was found to be passing cysts and trophozoites of *E. histolytica* in the stools. During the 73 days before it was sacrificed it remained healthy and no outward signs of amoebiasis developed. At autopsy amoebae were present in the large bowel contents and no gross lesions were observed. Microscopic examination of the gut tissues showed the usual mucosal invasion in the caecum.

*Monkey 239-B.* An adult male *rhesus* monkey, weighing 11 pounds, was received at the laboratory on March 28, 1939, from the Army Medical Research Board Laboratory. While at that laboratory the animal had been used in experimental work on malaria. During the 356 days that the animal was under our observation, cysts of *E. histolytica* were present almost constantly in the stool specimens, while trophozoites were present only occasionally. A number of occult blood examinations were done on the stool specimens and all were negative. This monkey remained in excellent health and when it was killed on March 19, 1940, it weighed  $24\frac{1}{2}$  pounds, a gain of  $13\frac{1}{2}$  pounds in a little less than a year. Occult blood test made on a stool specimen on the day it was sacrificed were negative. The lumen contents of the bowel contained many trophozoites of *E. histolytica* and some cysts. No gross lesions were observed. In sections from two blocks of tissue taken from the upper portion of the large gut, a number of amoebae were seen invading the mucosa.

*Monkey 241-B.* An adult female *rhesus* monkey weighing 9

pounds, arrived on March 28, 1939, from the Army Medical Research Board Laboratory. From the time that it was received until it was sacrificed 484 days later, cysts and trophozoites of *E. histolytica* were to be found in the stools. On September 5, 1939, this female gave birth to a normal infant. The adult was killed on July 25, 1940, and although amoebae were found in the lumen contents of the large bowel neither gross nor microscopic lesions were found. Occult blood tests done from time to time were negative except for a short period following the birth of the infant.

*Monkey 242-B.* An adult female *rhesus* monkey which weighed 8 pounds was received from the Army Medical Research Board Laboratory on March 28, 1939. The monkey was infected with *E. histolytica* when it arrived and continued to pass cysts and trophozoites of the organism until it was killed 356 days later. On December 23, 1939, it gave birth to a normal infant. Numerous occult blood tests done at various intervals were negative except for a period immediately following the birth of the infant. The animal was sacrificed on March 19, 1940. Occult blood reactions on this date were negative. At autopsy examination of the lumen contents of the large bowel showed many trophozoites of *histolytica* and no gross lesions were observed. A few amoebae were found in the mucosa when the tissues were examined microscopically.

*Monkey 243-B.* An adult female *rhesus* monkey of 9 pounds. It came from the Army Medical Research Board Laboratory with the other three *rhesus* monkeys on March 28, 1939. It was observed for a period of 484 days and cysts and trophozoites were constantly present in the fecal specimens. On October 23, 1939 it gave birth to a normal infant. As with the other two females, occult blood reactions were positive for only a short period following parturition. When sacrificed on July 25, 1940, amoebae were present in the lumen contents. Examination revealed no gross lesions and microscopically the gut appeared normal.

*Monkey 177-B.* A juvenile female monkey which weighed 4 pounds and 11 ounces when it was received at the laboratory on April 1, 1938. This animal was observed for a period of 623

days and amoebae were present in the stool at various periods during the entire time. It gained weight, never appeared to experience any ill effect from the infections and never developed clinical symptoms. When the animal was chloroformed on March 21, 1940, amoebae were found in the lumen contents, but no gut pathology was noted either in the gross or microscopic examinations.

#### DISCUSSION

The data presented in the foregoing section, based on observations extending over a period of two years, indicate that in monkeys, as well as in man, infection with *E. histolytica* can occur without the production of symptoms or noticeable ill effects. These monkey infections, naturally acquired, were in every respects comparable with the carrier state in man, in whom the organisms are found in the feces for weeks, months and even years without any show of symptoms. The infections in our eleven monkeys ranged from a minimum of 40 days to a maximum of 623 days and with the exception of monkey 216-B which died of the infection, neither diarrhea nor dysentery was noted.

The autopsies on the ten animals that were sacrificed presented some interesting data. The large gut in nine was negative by macroscopic examination and in the gut of 199-B the only abnormality was a slight granulation of the mucosa of the caecum. In the microscopic examination of these same guts seven out of the ten showed lesions which contained *E. histolytica*. In the spider monkeys the amoebae had broken through the epithelial layer of cells and had penetrated the mucosa along the fibers of the membrana propia or between the basement membrane and the cells of the glandular epithelium. In many instances the invasion involved only the upper portion of the mucosa, while in others the invasion had extended down to the muscularis mucosa, beyond which the amoebae never seem to pass. The microscopic pathology was identical with that described by Hegner, Johnson and Stabler (9) in experimentally infected spider monkeys. In one *Ateles* monkey, 177-B, the one in which the infection had been observed for a period of 623 days, no gut pathology was



demonstrable either in the gross or microscopic examinations. In the *rhesus* monkeys the epithelium of the large gut is ciliated and apparently offers greater resistance to the penetration of the amoebae than the epithelium of the gut in the spider monkeys. As a result only a very few organisms succeeded in passing this barrier. Amoebae were seen, either singly or in small groups of four or five, in the mucosa of *rhesus* no. 239-B and no. 242-B. The amoebae were usually situated in the interstitial tissue or underneath the cells of the glandular epithelium. No great amount of damage accompanied this invasion and only a slight cellular reaction resulted. These lesions were so small that in most instances they were found only when the oil immersion lens was used in examining the stained sections. In *rhesus* monkeys 241-B and 243-B no amoebae were found in the tissue.

In a previous section of the report it was pointed out that Andrews and Atchley (7) concluded that invasion of the tissue by amoebae was unlikely in carriers of *E. histolytica* whose stools gave negative occult blood reactions. The information gained from the study of the occult blood test in our four *rhesus* monkeys demonstrates conclusively that amoebic lesions can occur without producing a positive reaction. Rhesus monkeys 239-B and 242-B gave negative occult blood reactions during the period of the experiment and yet at autopsy amoebae were found in the mucosa. As stated previously these lesions were small and the hemorrhage, if occurring at all, was too small to show up in the test. Accordingly, it would appear from our data that the occult blood reaction is of little value in determining whether or not amoebic lesions occur in the large bowel of carriers.

These studies show that in the absence of symptoms and positive occult blood reactions amoebic invasion of the gut does occur and that the lesions produced are sometimes so minute as to escape notice during macroscopic examination. Further, these observations recorded here lend additional support to the belief that few if any of the carriers of *E. histolytica* escape without damage to the tissue by the amoebae, and that in the best interests of the individuals involved, treatment should be carried out in every case.

## CONCLUSIONS

1. Eleven monkeys, four *Ateles geoffroyi*, three *Ateles dariensis* and four *Macacus rhesus* were found to be naturally infected with *Endamoeba histolytica*.

2. The infections in ten of these monkeys were unaccompanied by observable symptoms and were comparable in every respect with the carrier infections in man.

3. Pathological studies have shown that in the absence of symptoms and macroscopic lesions, microscopic examination of the infected guts will often demonstrate invasion of the tissue on the part of the amoebae.

4. A study of the occult blood reaction in four *Macacus rhesus* infected with *histolytica* indicated that this test cannot be used to determine the presence or absence of lesions.

5. The information gained lends support to the belief that every carrier of *Endamoeba histolytica* should be treated, not only from a public health view point but also from the standpoint of the individual, since it is unlikely that any of them escape without some damage to the tissue.

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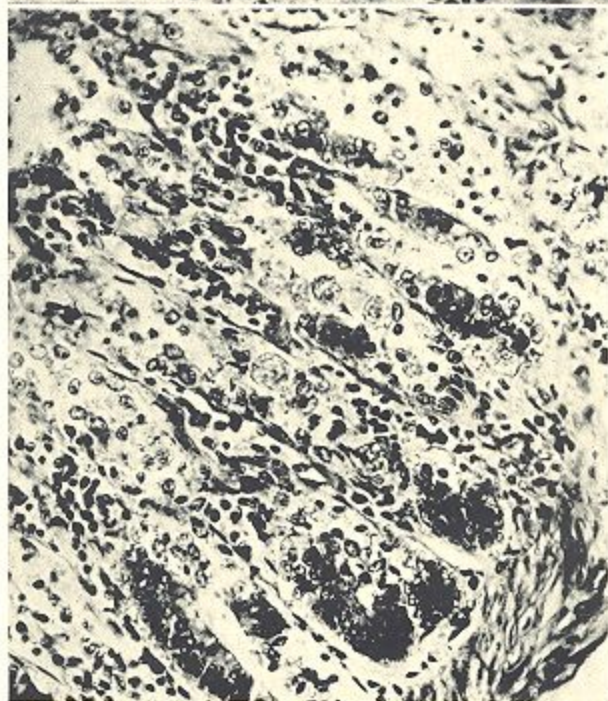


FIG. 1. Section of gut of black spider monkey 224-B. Amoebae are seen penetrating the mucosa between the cells of the glandular epithelium and the basement membrane. Iron-hematoxylin stain. Apochromate 4 mm. Periplan 8. Camera length 20 cm.

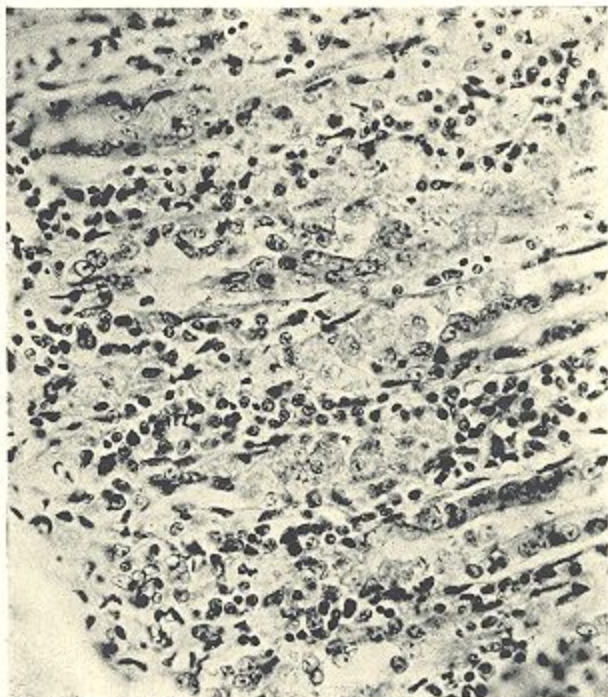


FIG. 2. Section of gut of red spider monkey 200-B showing the same type of lesion as in 224-B. A confluence of areolas as pictured gave a granular appearance to the mucosa. Iron-hematoxylin stain. Apochromate 4 mm. Periplan 8. Camera length 20 cm.

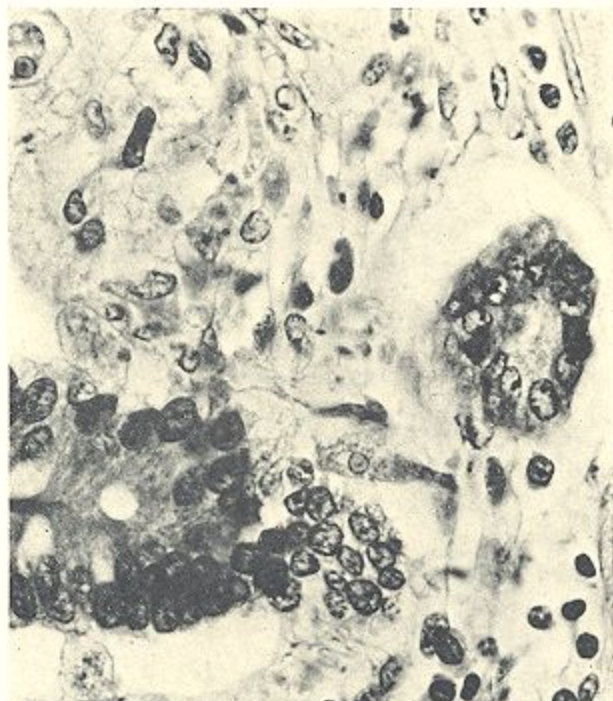


FIG. 4. Portion of the mucosa of rhesus 242-B showing amoebae in the interglandular tissue. Iron-hematoxylin stain. Apochromate 1.8 mm. Periplan 8. Camera length 20 cm.

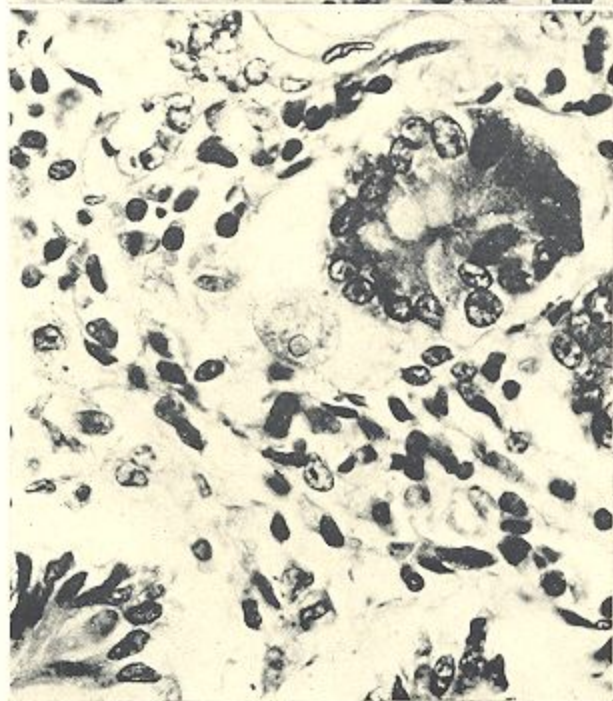


FIG. 3. A portion of the mucosa of rhesus monkey 239-B, showing an amoeba lying between the cells of the glandular epithelium and the basement membrane. Iron-hematoxylin stain. Apochromate 1.8 mm. Periplan 8. Camera length 20 cm.