PRELIMINARY NOTE ON THE MORPHOLOGY AND PATHOGENICITY OF E. *histolytica* IN P. R.

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In a previous paper(1) attention was called to the apparent inconsistency between the high incidence of infection with E. *histolytica* in Porto Rico and the extreme rarity of recognized clinical amoebic dysentery or diarrhoea in the Island. Possible explanations were presented and were briefly discusssd. Among these was the possibility of the predominating native strains of E. *histolytica* being non-pathogenic. Many investigators, notably Brumpt(2), have for a long time believed in the existence of non-pathogenic amoeba indistinguishable morphologically from E. *histolytica.*

The present paper deals with an attempt during a limited period of time to test the pathogenicity of native strains of amoeba by experimental inoculation of cysts and trophozoites into kittens. A few notes on the morphology of native strains of E. *histolytica* have been included.

We should like to emphasize that amoebic dysentery, although very rare, is unquestionably present in Porto Rico. We have seen a case of a white male from the rural district of Arecibo who was a patient in the Municipal Hospital of Arecibo suffering from acute diarrhoea with abdominal pain and tenesmus. Examination of the fresh stool revealed actively motile amoebae with ingested red blood corpuscles. On about one-fourth of an ordinary fecal smear fixed in Schaudinn's and stained with iron hematoxylin 126 amoebae were counted. These amoebae ranged in diameter from 11.1 to 23.1 micra, a few of them measuring below 14 micra. Of the 126 organisms 43, or 33 per cent, contained from one to nine red blood corpuscles each.

In the present study a total of 331 stools were examined for cysts of E. *histolytica.* Of these a large majority were routine specimens from the University Hospital of the School of Tropical Medicine and the Presbyterian Hospital in Santurce. The remainder

* Bailey K. Ashford fellow during summer of 1930.

were requested and selected at random from the Boys' Charity School and from a small rural community near the town of Dorado. The specimens therefore may be said to be representative of both the urban and the rural population of Porto Rico. Since only stools, with numerous cysts were sought, very little time was spent in the search for cysts. When a carrier was found the cysts were studied morphologically in fresh preparations, in iodine solution and in permanent preparations fixed in Schaudinn's and stained with iron hematoxylin. From each of thirteen of these carriers the diameter of some twenty or more cysts (a total of 305 cysts) was measured with an ocular micrometer. To the measurements obtained from the permanent preparations, following Dobell and Jepps(3), ten per cent was added to reach the natural sizes.

Morphology.—The cysts were in most cases round or ovoid. In one carrier the shape was very irregular making the round or ovoid forms the exception. The cyst wall was thin and colorless, measuring about 0.5 micron in thickness. Chromatoid bodies could be seen in many cysts as highly refringent rods or masses in fresh preparations and as black or blue-black rods or masses in preparations stained with iron hematoxylin. In some carriers the chromatoid bodies were present mostly as several long, slender rods in each cyst, but this feature was not limited to any particular strain. Many cysts showed indistinct glycogen vacuoles which stained reddish brown with iodine solution. The diameter of the cysts varied from 5.9 to 15.7 micra. The relative frequency of certain sizes and the apparent existence of two main races of cysts is best shown by means of a curve (Fig. 1). Although the number of cysts measured was small, it may be said that the tendency to bimodality in the curve suggests the existence of two main races of cysts the diameters of which do not seem to be very far from the diameters of the two main races described by Dobell and Jepps in England and by Scott (7) in Jamaica. Our experience with trophozoites, particularly the large tissue-invading amoeba, is limited to three cases and to cultures from three carriers. The amoeba, expelled by the patients show a round nucleus with a peripheral layer of chromatin granules and a central deeply staining karyosome, a granular endoplasm free from bacteria and containing red blood corpuscles, and a hyaline extoplasm which goes to form the pseudopodia. Alive, these amoebae displayed the characteristic throwing out with explosive rapidity of thin, blade-like ectoplasmic pseudopodia. The organisms as a rule measured from 15 to 24 micra.

The amoebae obtained in culture showed, while very fresh, a rapid slug-like locomotion with little or no differentiation between endoplasm and ectoplasm, but after half an hour or so on the slide they tended to slow down to a hit-and-miss, non-progressive movement characterized by the shooting forth and retraction of typical hyaline bladelike pseudopodia. These amoebae from cultures ingested starch granules but seemed to be consistently free from bacteria. Under favorable conditions their nuclei often showed as refractile finely beaded rings with small round central karyosomes. In general the amoebae from cultures were smaller in size, from 13 to 21 micra in diameter, with very few of them reaching the upper limit.

Experimental Infection.—Eight carriers were utilized as sources of amoebae for the inoculation of kittens. To insure the viability of the cysts care was taken to keep the stools moist at all times and to place them as soon as possible in the ice box at a temperature of 2 to 10 degrees C. The stools were often diluted with water and passed through a fine wire sieve to remove coarser particles. When trophozoites were employed they were inoculated only if they were very fresh and showed some movement at the time.

The cysts inoculated were never lsss than two nor more than six days old. These ages were chosen because it was with cysts of this age that Sellards and Theiler(4) obtained their best results and because Dobell and Laidlaw(5) discovered that cysts will not encyst in culture until they have been held outside the body of the host at a lower temperature for at least two days. The assumption was made that cysts less than two days old would not encyst in the large intestine of the cat.

The day before inoculation the kittens received no food but were given 20 to 30 c. c. of water twice by stomach tube. This procedure was intended to insure a relative freedom of the large intestine from fecal material without initiating dehydration. In the morning of the day of inoculation the kittens were given a meal of meat and milk and late in the afternoon the inoculation was performed. This was done under ether anaesthesia by the introduction per rectum, as far as it would go, of a flexible rubber catheter through which the inoculum was forced with a glass syringe. The anal opening was then sealed by means of a cotton plug soaked in thick collodion. Four or five hours after inoculation the kittens were given 20 to 30 c. c. of water by stomach tube. On the three or four subsequent days they received no food but about an ounce of water

was given twice daily. After that the cotton plug was removed and ordinary feeding with meat and milk was resumed. Water was given twice daily by stomach tube to insure the intake of fluid apparently necessary for the development of E. *histolytica* infection in kittens.

The number of cysts in a given inoculum was estimated by counting the cysts in one fourth of the area covered by a drop of the inoculum spread under a coverslip. By surveying small areas at different points the attempt was made to take care of any differences in the distribution of the cysts. The number of cysts used were usually in excess of 1,200 per cubic centimeter. The new medium recommended by Misao and Eiichi(6) was employed for the culture work.

In all, twenty-five young kittens were inoculated with amoebic cysts in the manner above described. The animals were inoculated between July 29, 1930, and September 13, 1930. Of the twenty-five kittens so treated twenty-four died from within a few hours to several days following inoculation. In none of these animals were cysts of E. *histolytica* found at autopsy nor were there any gross or microscopic lesions demonstrated. One kitten was sacrificed five hours following inoculation and a large number of cysts were found on examination of the intestinal contents.

From this experimental attempt to infect kittens with strains of amoebae found in Porto Rico we have only to report failure. One cat was inoculated with a culture of E. *histolytica* but the results were also negative.

DISCUSSION

The observations noted above on the morphology of E. *histolytica* in Porto Rico may be summed up by saying that the native amoebae are indistinguishable morphologically from E. *histolytica* as this has been described in most other places. The negative outcome of our attempt to infect kittens cannot, of course, be accepted as final. The number of kittens inoculated was too small and death took place much too soon following inoculation. It appears that any further attempt to study the pathogenicity of this parasite in Porto Rico should include as well the study of control strains from other localities. By the same token the methods of infection should be carefully worked out and perhaps other experimental animals should be used as well as cats in attempting to produce amoebic dysentery experimentally.

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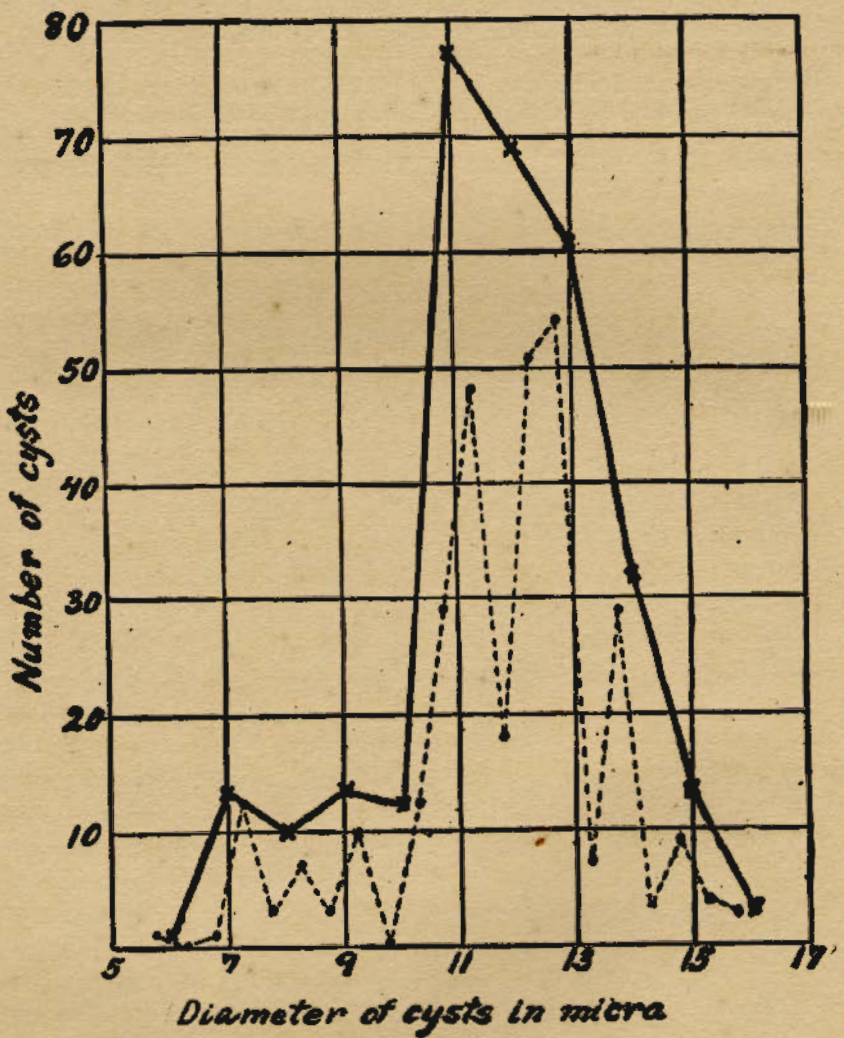
FIGURE I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Number of cysts
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17
Diameter of cysts in micra