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Experimental Acute Amebic Colitis in Dogs.

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Cases of spontaneous dysentery in dogs, in which an endamoeba similar in form and habits to *E. histolytica* was abundant in the exudate, have been reported by Darling,¹ Ware,² Fischer³ and Bauche and Motais.⁴ Three of these reports were on single infections; Ware's series comprised 7 dogs. On at least 5 different occasions the dog has been utilized experimentally to study strains of the dysentery ameba from human sources. The first of these was Löscher,⁵ who was successful in one of 4 attempts in injecting into his experimental animals the cellular exudate from his classical case of the infection. This series, as well as the later cases (Kruse and Pasquale,⁶ Harris,⁷ Kartulis,⁸ and Dale and Dobell,⁹) indicate that infection was successful only from rectal injection, that it was believed to be dependent in part on the age of the dog, and that some infections resulted in death, while others cleared up spontaneously. On the whole, the unsuccessful attempts to employ dogs for a study of human strains of *E. histolytica* have resulted in more recent years in utilizing the kitten and rhesus monkey for this purpose.

The present study was the result of the discovery of amebic dysentery in 2 dogs picked up on the streets of New Orleans. One of the animals died before the material could be utilized, but the other was estimated to have been first observed one or 2 days after the onset of the dysentery. From this animal the strain has been transferred through 6 successive sub-plants, in which 14 dogs have been utilized. Thirteen of these dogs have become positive for the infection. Only enough dogs have been used to provide for the continuance of the strain and to study the effect of the organism on the host. The ameba has also been successfully implanted into one of 2 rhesus

¹ Darling, S. T., *Proc. Med. Assoc. Isth. Canal Zone*, 1915, vi, i, 60.

² Ware, F., *J. Comp. Path. and Therap.*, 1916, xxix, 126.

³ Fischer, Walther, *China Med. J.*, 1918, xxxii, 13.

⁴ Bauche, J., and Motais, F., *Bull. Soc. Path. Exot.*, 1920, xiii, 161.

⁵ Löscher, F., *Arch. Path. Anat.*, 1875, lxv, 196.

⁶ Kruse, W., and Pasquale, A., *Z. Hyg. u. Infekt.*, 1894, xvi, 1.

⁷ Harris, H. F., *Arch. Path. Anat.*, 1901, clxvi, 67.

⁸ Kartulis, S., *Handb. d. path. Mikroorg.*, 1913, 2nd edition, vii, 651.

⁹ Dale, H. H., and Dobell, C., *J. Phar. and Exp. Therap.*, 1917, x, 399.

monkeys, although attempts to infect 4 laboratory rats and 2 cats were unsuccessful.

The technic employed has consisted in the transfer of 1 to 2 cc. of bloody mucus exudate containing the active amebae from the cecum of an acute case of the disease through the large bowel into the distal 5 cc. of the ileum of the animal to be infected. This has been made possible by utilizing a pipette of 30 cm. length and 7 mm. outer diameter to which a 25 cc. rubber bulb is attached. The tube is gently inserted into the rectum of the first animal and with a careful pressure is advanced through the colon and cecum until the ileo-cecal valve is encountered. The bulb is then compressed and the air which is expelled is usually sufficient to cause the relaxation of the ileo-cecal valve. The tube is then slowly withdrawn as the pressure on the bulb is gradually released, thus allowing the exudate from the cecum and upper levels of the colon to be aspirated into the pipette. The material without leaving the pipette is then inserted up the large bowel and injected into the ileum of the second dog, which has been prepared by having all solid fecal matter removed by enema from the large bowel. In this way the amebae are placed in a location from which they will enter the large bowel in a similar manner to that in which natural amebic colitis is acquired. The same technic is employed on each successive day to determine the conditions under which the infection actually comes to involve the tissues.

The incubation period. Dale and Dobell (l. c.) found that the incubation period of human strains of *E. histolytica* in kittens was 1 to 6 days, with an average of 2 days. In this series of dogs one animal became infected in 24 hours, 6 in 2 days, 2 in 3 days, one in 4 days, one in 5 days and in one dog (an old animal) the infection was not observed until the twenty-third day. Infection in this series was defined as the recovery of abundant mucus with or without red blood cells, but in which, on microscopic examination, motile *E. histolytica* were readily found.

The age of the host. Dogs from 2 months of age up to 4 years have been employed. The only animal in which infection has not been developed was a 3-months-old puppy.

Course of the infection. The onset has usually been sudden, with the copious evacuation of blood and mucus a day or two after the animal was first observed to be "infected". In most animals the infection has been acute, the host dying in 2 to 3 weeks after inoculation. In one case the condition, now of several months' standing, has become chronic; in another, the 4-year-old dog, spontaneous

recovery has apparently developed. In 2 members of the series with acute amebic colitis the animals have been completely relieved of symptoms and the freshly withdrawn bowel contents have become microscopically negative after administration of di-hydranol.

The amebae and their relationship to the tissues. In no case have cysts of the amebae been recovered from the bowel evacuations, but motile *E. histolytica* have frequently been observed in freshly passed exudate. In the earliest stage following injection the amebae occur free in the mucus of the cecum or loosely adherent to the cecal epithelium, without ingested red blood cells. Twenty-four to 48 hours later the presence of ingested red blood cells and the abundance of host tissue elements from the cecal contents indicates that the organisms have probably invaded the tissues. Their recovery as tissue parasites from the colon and rectum occurs from one to several days later. These amebae are easily cultivated *in vitro* in a modified egg-Ringer's medium. Autopsy records show the largest number of lesions with their accompanying amebae in the cecum. Lesser foci have been found in order of frequency in the several levels of the colon, the rectum, the appendix and the distal 10 cm. of the ileum. This corresponds for the most part to the infection in man.

On the whole, however, the lesions in the dog are less discrete and more superficial in character than in the human host. In some animals the leukocyte count apparently indicates a bacteremia, the nature of which has not yet been determined. It is not improbable that in acute infections with fatal termination the bacterial invaders may actually play the primary rôle in producing death.

The significance of natural amebiasis in dogs. All the evidence in the literature, as well as that in this series of dogs preponderates in favor of the belief that the ameba in canine amebic colitis is the same organism as *Endamoeba histolytica* of man. Since cysts are apparently not formed in any type of the infection in dogs, the dog is not likely to be a menace to man or to other dogs. The inference is, therefore, that the dog receives its infection from man.

Summary and Conclusions. 1. By utilizing a technic whereby muco-sanguinous exudate from active cases of amebic colitis in dogs is injected *per anum* into the distal portion of the ileum, infection corresponding to spontaneous amebiasis in this host has been produced in 13 out of 14 dogs (93%), ranging in age from 2 months to 4 years. 2. The incubation period, as determined by the presence of active *Endamoeba histolytica* in mucus withdrawn directly from the cecum, has ranged from one to 23 days, with the largest number of findings on the second day. 3. Acute cases, prob-

ably complicated by bacteremia, terminate with death in about 2 weeks. Chronic cases and one spontaneous recovery have also been observed. Carrier cases, with the passing of cysts in the stools, apparently do not develop in dogs. 4. The cecum is the primary seat of infection with *E. histolytica* in the dog. 5. The dog apparently receives its amebic infection from man but due to the failure to produce cysts the infection apparently cannot be transferred in nature from dog to dog or from dog to man.

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**Observations on the Significance of the Agglutinins and Lysins
Produced by Typhoid Inoculation.**

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In a previous paper the results of agglutination reactions performed upon the sera of students inoculated with typhoid vaccine were reported. Two sets of tests were carried out, one with serum taken before inoculation, the other with serum taken 10 days after inoculation.

This paper deals with a third set of agglutination reactions, together with parallel complement fixation reactions and bacteriolytic tests, performed upon sera from the same group of 90 students 4 months after inoculation. The antigen used in the complement fixation reactions was a saline suspension of typhoid bacilli from which lipoids had been extracted with alcohol and ether. The bacteriolytic tests were carried out by diluting one capillary drop (about 0.025 cc.) of serum to 0.5 cc. with saline, and adding to this about 500 to 1000 typhoid bacilli. This mixture was incubated for one hour and then plated in plain agar. In negative control sera there was practically always a marked multiplication of the bacilli.

Because of the marked difference in the agglutinin titer between students who had received previous typhoid vaccine and those who had not, a second inoculation was given to a number of volunteers from the latter group. Each of the tests mentioned above were performed upon sera drawn from these students 10 days after inoculation.

Analysis of the data collected in this study reveals the following observations: 1. Agglutinins are demonstrable more universally 4