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Observations Upon Complement Fixation in Experimental Amebiasis in Dogs.*

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The writer[1] demonstrated the presence of complement fixing bodies in the blood serum of individuals infected with *Endamoeba histolytica* and subsequently[2, 3, 4, 5, 6] reported the results obtained with a complement fixation test in cases of human amebiasis and in normal individuals, together with the technique of the test. These observations have been confirmed by Spector,[7] Fernandez,[8] Heathman,[9] and Sherwood and Heathman.[10]

The observations here reported were undertaken to throw further light upon the phenomenon of complement fixation in amebiasis, using the dog, an animal that Faust[11] has shown is easily infected with *Endamoeba histolytica* and in which the lesions produced by this parasite are comparable to those occurring in intestinal amebiasis in man. Alcoholic extracts of cultures of *Endamoeba histolytica* were used as antigens.

Tests were made upon 29 dogs infected with *Endamoeba histo-*

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1 Craig, C. F., *Am. J. Trop. Med.*, 1927, **7**, 225.

2 Craig, C. F., *Proc. Nat. Acad. Sci.*, 1928, **14**, 520.

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4 Craig, C. F., *Am. J. Trop. Med.*, 1929, **9**, 277.

5 Craig, C. F., *J. Am. Med. Assn.*, 1930, **95**, 10.

6 Craig, C. F., *Ann. Int. Med.*, 1931, **5**, 170.

7 Spector, B. K., *J. Prev. Med.*, 1932, **6**, 117.

8 Fernandez, P. E., *Am. J. Hyg.*, 1932, **15**, 785.

9 Heathman, L., *Am. J. Hyg.*, 1932, **16**, 97.

10 Sherwood, N. P., and Heathman, L., *Am. J. Hyg.*, 1932, **16**, 124.

11 Faust, E. C., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 908.

lytica and in all but one (96.5%) a positive complement fixation reaction developed in the blood serum. In the dog giving a negative reaction death occurred 7 days after colonic inoculation from a very severe amebic colitis. Of the dogs giving a positive reaction, 21 (75%) gave a 4+ reaction; 3 (10.7%) gave a 3+ reaction; 2 (7.1%) gave a 2+ reaction; and 2 (7.1%) gave a 1+ reaction.

Of the 28 dogs giving a positive reaction 22 died and 5 were sacrificed, 3 while still suffering from intestinal amebiasis while 2 had recovered from the infection. Of the 22 dogs that died, all showed typical amebic ulcerations in the colon and in all motile forms of *Endamoeba histolytica* were found in the lesions. The 3 dogs killed while still suffering from the infection showed both healed and active amebic lesions in the colon and motile *Endamoeba histolytica* were present in the active lesions. In the 2 dogs sacrificed after recovery, healed amebic ulcerations were found in the colon but the gut was negative for *Endamoeba histolytica*. Thus, the positive results obtained with the test in these dogs were confirmed in 100% of the cases by the pathological lesions found at autopsy and in all but the 2 recovered cases, by the presence of motile *Endamoeba histolytica* in the lesions. In addition, this parasite was found in the feces of every dog giving a positive complement fixation test.

The *time of appearance of the reaction* in the infected dogs varied between 3 days and 14 days after inoculation in those animals tested before inoculation. In one dog a 2+ reaction was obtained 3 days after inoculation; in 3, a 4+ reaction 4 days after inoculation; in 1, a 4+ reaction in 5 days; in 1, a 3+ reaction in 5 days; in 1, a 4+ reaction in 9 days; in 5, a 4+ reaction in 10 days; in 3, a 3+ reaction in 12 days; in 1, a 1+ reaction in 13 days; in 1, a 2+ reaction in 14 days; and in 1, a 4+ reaction in 14 days after inoculation.

The very early appearance of the reaction in some dogs was remarkable but no more so than the extent and character of the amebic lesions observed in such animals at autopsy. In Dog 125, giving a double-plus reaction 3 days after inoculation death occurred upon the same day and, at autopsy, wide-spread superficial amebic ulcerations were found in the appendix and colon and motile trophozoites of *Endamoeba histolytica* were present in the lesions. In Dog 126, a 3+ reaction appeared upon the 5th day after inoculation and the animal died upon the same day. At autopsy, amebic ulcerations were numerous throughout the colon and motile amebae were found in the lesions; while in Dog 143, giving a 4+ reaction

4 days after inoculation, and dying upon the 8th day after inoculation, amebic ulcers were numerous throughout the colon, especially in the rectum, and motile trophozoites of *Endamoeba histolytica* were present in the lesions. In these very susceptible dogs the reaction appears very promptly and the invasion of the tissues by the amebae, with the production of very severe lesions, is surprisingly rapid and extensive.

The gradual disappearance of the positive reaction was noted in the dogs that recovered spontaneously or after being placed upon a liver diet which resulted in the disappearance of the symptoms of infection.

The results of the complement fixation test upon 42 dogs free from infection with *Endamoeba histolytica* used as controls were uniformly negative and at autopsy these animals did not show any evidence of amebic infection.

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Studies Upon the Filterable and Non-Filterable State in the Tunnicliff Coccus of Measles.

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This communication reports the results of tests upon the differences in filtrability of the Tunnicliff coccus of measles under *in vivo* and *in vitro* growth conditions. The question whether microorganisms ordinarily regarded as non-filtrable can become filtrable and vice versa, under special conditions of growth, is of more than academic interest. Kendall,[1] with the use of his "K" medium, claims that it is relatively easy to cause non-filtrable organisms to become filtrable and filtrable to become microscopically visible and non-filtrable.

Different environmental conditions cause some microorganisms to undergo rather marked changes in size and shape of the individual cell. Particularly is this true for certain parasitic varieties capable of cultivation *in vitro,* where they are distinctly larger and often differently shaped than they are *in vivo.* Adaptation to the artificial environment is the accepted explanation for the morphological variation. The reaction of the culture medium, quality of nutritives,

[1] Kendall, A. I., *Northwestern Univ. Bull.*, 1931, **32**, No. 8.