nor diarrhea has been produced, and at autopsy the wall of the large intestine of each animal was completely free of any inflammatory areas. We conclude, therefore, that the bacteria associated with the amebæ in our inocula have not been responsible for the reduction in the incubation period, for the increased ease with which we have transferred our strain of ameba, or for the increase in the number and degree of severity of the amebic lesions produced.

7923 C

Observations on Antigens for Complement Fixation in Amebiasis.*

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Since the publication of the technique of the complement fixation test for amebiasis devised by the senior author (Craig)[1], in which absolute alcoholic extracts of cultures of *Endamoeba histolytica* were employed as antigens, several modifications of the test have been published and other antigenic extracts have been described.

Menendez[2] and Spector[3] employed simple alcoholic extracts of cultures of *E. histolytica* as antigens with satisfactory results, and Menendez stated that a simple suspension of cultures of *E. histolytica* in formalinized saline also possessed good antigenic properties. Sherwood and Heathman[4] and Heathman[5] used antigens prepared by extracting the dried sediment of cultures of *E. histolytica,* rich in the amebæ, with ether, 96 % alcohol and acetone, after which cholesterin was added in different amounts, and found that such antigens were efficient. Tsuchiya[6] and Weiss and Arnold[7] successfully employed absolute alcohol extracts of cultures of *E. histolytica* in their modifications of this complement fixation test. With all of these antigenic extracts these observers obtained a high percentage of positive results in infections with *E. histolytica* and

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

2 Menendez, P. E., *Am. J. Hyg.*, 1932, **15**, 785.

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

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

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

6 Tsuchiya, H., *J. Lab. and Clin. Med.*, 1934, **19**, 495.

7 Weiss, W., and Arnold, L., *Am. J. Digest. Dis. and Nutrition*, 1934, **1**, 231.

negative results in other infections or diseases and in healthy individuals, thus amply confirming the specificity of the test.

We have recently endeavored to obtain more easily prepared antigens by extracting the mucoid material which may be readily obtained from the intestine of dogs suffering from acute amebic dysentery experimentally produced. Such material is usually very rich in amebæ and contains very few bacteria as compared with cultures of this parasite. The utilization of this material as a source of antigen for the complement fixation test was first suggested by our assistant, Dr. Edwin S. Kagy, and it has been found that extracts prepared from it are suitable for use as antigen and are sometimes stronger in antigenic properties than the absolute alcohol extracts of cultures of *E. histolytica.*

The method of infecting dogs with this ameba has been described by Faust[8] and the exact technique of the complement fixation test by Craig.[9–10] The mucoid material from the intestine of the infected dogs used in the preparation of the antigenic extracts was obtained by aspiration from the cecum and upper portion of the colon, utilizing a glass pipette to which a 25 cc. bulb was attached. The material, so obtained, consisting of bloody mucus, rich in amebæ, has been extracted and fractionated in various ways and the extracts and fractions thereof have been titrated for their antigenic value.

Antigens prepared by extracting the dried residue of the mucoid material with ether and the lipoids precipitated from this extract with acetone, the resulting residue being added to alcoholic extracts of the same material, gave excellent reactions when used in proper amounts but we found that, with one exception, such antigens could not be used diluted. This particular antigen, when diluted with 4 parts of normal saline, gave satisfactory results. Attempts to fractionate this antigen, and other antigens prepared in the same manner, by extracting the ether insoluble portion with water or the insoluble portion of such an extraction with absolute alcohol, resulted in no improvement in antigenic qualities and such fractions were usually either hemolytic or anticomplementary, and of much less practical value than the original antigenic extracts.

More recently simple alcoholic extracts of the mucoid material obtained from the infected intestine of the dog have been investigated and it has been found that the extraction of this material, when it is rich in amebæ, with absolute alcohol, in the proportion of

[8] Faust, E. C., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 908.

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

[10] Craig, C. F., *Amebiasis and Amebic Dysentery*, Springfield, Ill., and Baltimore, Md., 1934, page 231.

one part of the material to 7 parts of absolute alcohol, yields an excellent antigen for complement fixation in amebiasis. The extraction is conducted in an incubator at 45° C. for 15 days, the flask containing the material being thoroughly shaken several times a day during that time. After extraction, the mixture is filtered, diluted with from 3 to 5 parts of normal saline and tested for its hemolytic, anticomplementary and antigenic properties.

Antigenic extracts prepared in this simple manner appear to be more active than when the acetone insoluble lipoids are either used alone or added to alcoholic extracts of cultures of *Endamoeba histolytica* or of the mucoid material. It has been found that the dilution mentioned can generally be used with excellent results, whereas in our experience most of the lipoid antigens gave poor results when diluted.

Alcoholic extracts of the mucoid material obtained from the intestine of dogs suffering from amebic dysentery gave fully as good results as extracts made from cultures of *E. histolytica.* Owing to the difficulty experienced by many in the cultivation of this organism, the preparation of antigens from cultures has been abandoned by many laboratories. It has been shown by Faust that dogs are comparatively easily infected with *E. histolytica* and that the infection can be maintained in the laboratory by transmission from animal to animal. Sufficient mucoid material for extraction may easily be obtained from the intestine, in an acutely infected dog, and as this material is usually much richer in the amebæ than are cultures, and much more free from bacteria, it is believed that it furnishes an excellent source of antigen for use in complement fixation in the diagnosis of amebiasis.

7924 C

Effect of Temperature of Storage on Bacteria in Water Samples.

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Bacteriological control of water supplies frequently involves examination of samples which have been shipped considerable distances. All editions of Standard Methods for the Examination of Water and Sewage provide for icing samples during shipment. Berry,[1] Jordan and Irons,[2], Hale and Melia,[3] Albert, Hinman and