Preliminary Communications

Indirect Fluorescent Antibody Test in Diagnosis of Hepatic Amoebiasis

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Attention has been drawn (Jeanes, 1964) to the potential value of the indirect fluorescent antibody test in the serological diagnosis of amoebiasis. In this laboratory the test has now been adapted to give results on a quantitative basis, and current trials are confirming the early promise of the method as an aid to the diagnosis of this disease. An account is given here of the method used, together with a preliminary report of the results obtained, in a small series of cases of hepatic amoebiasis.

METHODS AND MATERIALS

A virulent dixenic strain of *Entamoeba histolytica* was maintained in Dobell's HSre medium (Dobell and Laidlaw, 1926) with repeated subcultivation on every second or third day. To prepare smears, amoebae were withdrawn from the bottom of the culture tube with a Pasteur pipette, washed twice in modified Ringer's solution (Dobell and Laidlaw, 1926), and resuspended in sufficient of the solution to contain 30–50 amoebae per c.mm. Drops of this suspension were placed on alcohol-cleaned slides to cover an area of approximately 5 mm. diameter. The slides were then dried rapidly on a hot-plate at 56° C. and stored at –20° C. until required for use.

Fluorescein-labelled anti-human globulin was used at the highest dilution which would give brilliant (+ + +) fluorescent staining of amoebae in a trial test with a 1:128 dilution of a standard positive control serum.

The optical system used was a Reichert Zetopan fluorescence microscope fitted with a dark-ground condenser, a UV-passing primary filter (Schott UG1/1.5 mm.), and a colourless UV-absorbing secondary filter (Wratten 2B).

Titration of the patients' sera was performed by the following method, based on the techniques developed by Coons *et al.* (1942) and Weller and Coons (1954). The sera were diluted in phosphate-buffered saline (Chadwick and Fothergill, 1962) to give a range of twofold dilutions from 1:8. A single drop of each dilution was applied to a separate smear of *E. histolytica*, and the slides were incubated in a moist chamber at 37° C. After 30 minutes the slides were washed in buffered saline for 10 minutes and then allowed to dry. A single drop of fluorescein-labelled anti-human globulin was next applied to each smear and the slides were incubated for a further 30 minutes at 37° C. in the moist chamber. The slides were again washed in buffered saline for 10 minutes. Finally, each smear was counter-stained with a 0.05% solution of Evan's blue (Nichols and McComb, 1962) for 10 minutes, washed again, and mounted under a cover-slip in a mixture of nine parts of glycerin with one part of buffered saline.

The smears were examined under the fluorescence microscope and the intensity of fluorescence was recorded according to the scale adopted by Wilkinson (1961). The highest dilution of serum giving definite (+ +) fluorescent staining of the amoebae was taken as the end-point of the titration. Positive and negative controls were included in every series of tests.

The samples of serum tested were obtained from eight patients with hepatic amoebiasis, the diagnosis being based either on strong clinical evidence or on the results of other investigations. Sera from 196 patients attending this hospital

with miscellaneous disorders but with no known evidence of amoebiasis were also tested.

RESULTS

The results obtained with the sera of the eight patients believed to be suffering from hepatic amoebiasis are shown in the accompanying Table. The titres ranged from 1:64 to 1:16,384. Of the 196 sera from patients in whom there was no reason to suspect amoebic infection a titre of 1:16 was obtained in seven and in the remaining 189 it was 1:8 or less.

Serum Antibody Levels Against E. histolytica as Shown by the Indirect Fluorescent Antibody Test in Eight Cases of Hepatic Amoebiasis

Case 1 ..	1:1,024	Case 4 ..	1:64	Case 7 ..	1:16,384
„ 2 ..	1:128	„ 5 ..	1:512	„ 8 ..	1:2,048
„ 3 ..	1:128	„ 6 ..	1:128		

COMMENT

The preliminary results described here are in keeping with the observation of Goldman (1953, 1954, 1959) that the immunological reactions of various species of amoeba may be studied by immunofluorescence techniques. Other tests used in the serological study of amoebiasis include the complement-fixation test (Izar, 1914; Craig, 1939), and, more recently, the haemagglutination technique (Kessel *et al.*, 1965) and the gel-diffusion precipitin technique (Powell *et al.*, 1965). It seems likely that the indirect fluorescent antibody test will prove to be a useful additional or alternative aid to the elucidation of an often difficult clinical problem.

SUMMARY

The indirect fluorescent antibody test was used in an attempt to determine the level of antibody against *Entamoeba histolytica* in eight patients with hepatic amoebiasis and in 196 patients with various other disorders. There was a clear distinction between the results obtained in the two groups of cases.

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