Preliminary Communications

Detection of Entamoeba histolytica by Fluorescent Microscopy

[WITH SPECIAL PLATE]

Detection of *Entamoeba histolytica* in the stools and tissues is sometimes very difficult, and in some cases the amoeba may be confused with other cellular elements. In previous papers we described cases of chronic amoebic hepatitis with the presence of *E. histolytica* in the liver tissue (Doxiades *et al.*, 1961; Doxiades and Candreviotis, 1961; Doxiades, 1962).

Though experience on the part of the investigator plays an important part, the amoeba cannot always be recognized. In an effort to facilitate its detection a number of new staining methods have been introduced, but none has proved satisfactory (Luthringer and Glenner, 1961; Salfelder, 1961). Since fluorescent microscopy has given good results in the detection of cellular elements, we thought that this method might be helpful in the recognition of the amoeba. Goldman (1953) has already described a method for the cytochemical differentiation of *E. histolytica* and *E. coli* by means of fluorescent antibody.

In the present paper are reported the results of experiments in the detection of *E. histolytica* by means of fluorescent microscopy. Smears of *E. histolytica* taken from cultures were stained with haematoxylin–eosin, while other smears were treated by the method of von Bertalanffy (1960) and its modifications after Schümmelfeder and his colleagues (1925). Figs. 1–3 on the Special Plate were taken by the Leitz "ortholux" fluorescent microscope, blue light; staining was with acridine orange in McIlvaine buffer at *p*H 3.0–5.0.

It is our intention to extend our efforts to the detection of *E. histolytica* in the stools and in the liver tissue of animals and man, as well as to establish the development of antibodies, by means of fluorescent microscopy.

We should like to express our thanks to Professor Schümmelfeder, of the University of Bonn, for his kind help, and to the Wellcome Foundation Ltd. for a financial contribution which enabled the photomicrographs to be reproduced in colour.

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During 1961 59,306 people in the United Kingdom passed Red Cross first-aid examinations, an increase of approximately 9,000 on 1960. In addition, 29,687 passed nursing examinations and 10,759 maternal and child welfare examinations.

Medical Memoranda

Eosinophilic Lung Abscess—A New Entity

Though tropical eosinophilia was recognized as a "new disease entity" about two decades ago (Weingarten, 1943) its aetiology remained a mystery until the experiment of Buckley (1958) on a human volunteer. The idea of a filarial origin, however, started from a positive filarial complement-fixation test (F.C.F.T.) in high dilution and its subsequent decline after diethylcarbamazine therapy (Danaraj, 1958). The successful demonstration of segments of microfilariae in lung, liver, and lymph nodes by Webb *et al.* (1960) undoubtedly established filarial infection as one of the causal factors (if not the factor) of tropical eosinophilia. Clinical and radiological aspects of the disease have been fully described (Viswanathan, 1954; Islam *et al.*, 1960). A case of tropical eosinophilia with pulmonary cavitation or presenting clinically as lung abscess has never been described before.

CASE HISTORY

A Moslem businessman aged 41 was admitted to the Dacca Medical College Hospital on March 4, 1961, with remittent fever (100–103° F. (37.8–39.4° C.)), cough with moderate expectoration, and dyspnoea for seven days. He had had three similar attacks of milder nature in the past—in June 1948, June 1952, and September 1959. Only antispasmodics had to be used during these attacks. He had suffered from kala-azar at the age of 25. There was no history of bronchial asthma or eosinophilia (tropical) in the family.

On admission he was found to be of average build and nutrition. He had to be propped up because of dyspnoea. There was no cyanosis, clubbing, or enlargement of lymph nodes. Expiration was wheezy and prolonged. Rales and rhonchi were heard over both sides. The liver was enlarged two fingerbreadths below the right costal margin, and was smooth, soft, and moderately tender. Symptomatic treatment with aminophylline and ephedrine was given until the results of the investigations were known.

Investigations.—Blood count: leucocytes, 11,000/c.mm. (polymorphs 35%, lymphocytes 10%, eosinophils 55%); R.B.C., 4.000.000/c.mm.; Hb, 12 g./100 ml.; E.S.R., 50 mm. in the first hour (Westergren); blood urea, 28 mg./100 ml.; plasma protein, 6.4 g./100 ml. Sputum showed a large number of eosinophils and eosinophilic fragments, stained by the method described by Khaleque *et al.* (1960). No acid-fast bacilli were found. F.C.F.T. was positive. A radiograph (Fig. 1) showed a cavity in the right and the left midzone with fluid level.

A diagnosis of tropical eosinophilia was made, and the possibility that the cavities could be of eosinophilic origin was kept in mind. All antibiotics and chemotherapeutic agents were strictly withheld. On March 15 the leucocyte count was 14,500/c.mm., with eosinophils 30%. Diethylcarbamazine, 300 mg. daily in three divided doses, was started on March 15 and the patient steadily improved. A radiograph on March 24 (Fig. 2) showed complete disappearance of the lesion on the right, whereas the cavity on the left was somewhat increased in size. On March 25 the leucocytes numbered 9,000/c.mm., with eosinophils 14%. A radiograph on April 4 (Fig. 3) showed a remarkable clearance of the lesion on the left side as well. The cavity with fluid level was replaced by an opacity much smaller. On April 5 the leucocytes numbered 6,400/c.mm., with eosinophils 6%.

The patient now had practically no symptoms or signs except a few scattered rhonchi on both sides. Diethylcarbamazine was discontinued on April 8. On the 17th

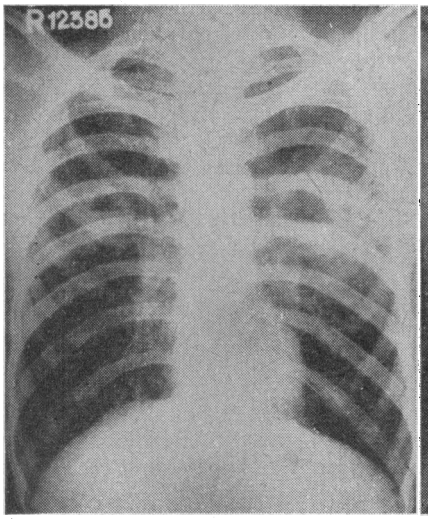

FIG. 1

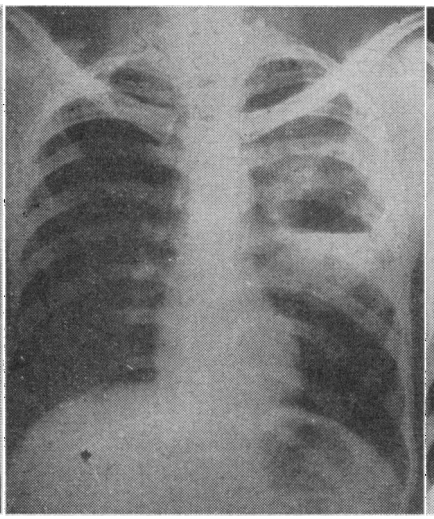

FIG. 2

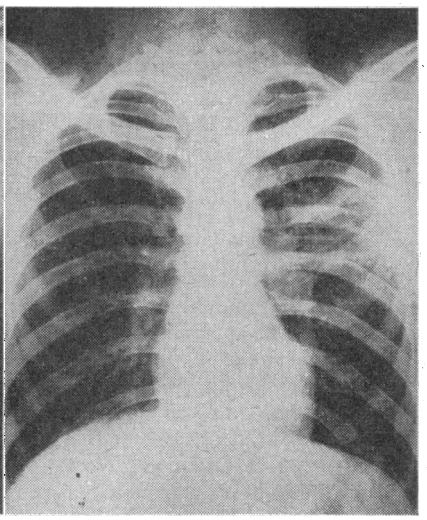

FIG. 3

FIG. 1.—Radiograph taken on March 6, showing cavity in right and left midzone. FIG. 2.—Radiograph taken on March 24, showing disappearance of lesion on right and increase in size of cavity in left midzone. FIG. 3.—Radiograph taken on April 4, showing clearance of lesions.

he developed mild dyspnoea with a temperature of 100° F. (37.8° C.). On that day the leucocytes were 10,500/c.mm., with 20% eosinophils. Radiologically, however, there was no deterioration of the lesion. Diethylcarbamazine was again given—400 mg. daily in four divided doses. Subsequent *x*-ray films and blood counts showed steady improvement. The patient was discharged on May 3 symptom-free. On June 6 he was keeping well. *X*-ray examination showed a small opacity in the cavity area on the left side. A tomogram did not reveal any evidence of cavity. A bronchogram showed nothing abnormal. Needle biopsy of the lung from the site of the lesion showed normal lung tissue with a moderate amount of fibrosis. There was no evidence of necrosis or cellular infiltration. F.C.F.T. was persistently negative. The opacity remained stationary on subsequent examinations at intervals of three weeks.

COMMENT

In cases of tropical eosinophilia Webb *et al.* (1960) demonstrated beyond question (1) the presence of segments of microfilariae in the centre of the granulomatous reaction, and (2) destruction of the alveolar wall with the formation of "eosinophil abscess" in the centre of the nodules. On this basis, therefore, pulmonary cavitation is a definite possibility in tropical eosinophilia, though it has not yet been reported. J. W. Crofton (1961, personal communication) could not recall hearing of a case of tropical eosinophilia presenting with pulmonary cavitation, but believed that this was a possibility because of the necrosis which the disease can produce. Webb (1961, personal communication) mentioned a case of tropical eosinophilia with cystic changes in the upper lobe of the right lung which, from a chest radiograph, was originally thought to be cystic bronchiectasis. Treatment with diethylcarbamazine resulted in resolution of the lesion. This, according to Webb, could be due to tissue breakdown. The case presented here might be confused with (1) a congenital cyst with a superimposed eosinophilic condition; (2) a tuberculous cavity with eosinophilia due to hypersensitivity reactions to antituberculous drugs, notably streptomycin and P.A.S.; and (3) a case of tropical eosinophilia developing lung abscess.

In this particular case, however, the high eosinophil count, the large number of eosinophils in the sputum, the positive F.C.F.T., and the dramatic response to diethylcarbamazine establish the diagnosis of tropical eosinophilia beyond doubt. Disappearance of the ring shadow and a normal bronchogram after treatment exclude the possibility of a congenital cyst. Repeated examination of sputum for acid-fast bacilli and the fact that no antituberculous drugs were given rule out the second possibility. We do not know of any antibacterial activity of diethylcarbamazine. If it were a case of tropical eosinophilia with an independent lung abscess the eosinophil count should not have been so high and there should have been an increase rather than a decrease of neutrophil leucocytes (3,600/c.mm. in this case). Moreover, it is far-fetched to think of spontaneous resolution of a lung abscess so well developed radiologically. We therefore believe that this is a case of tropical eosinophilia with clinical and radiological evidence of lung abscess and that it should be called "eosinophilic lung abscess"—an entity distinct from other varieties of lung abscess and never described before.

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The British Red Cross sent over £165,000 towards the cost of the relief work which was done in the Congo during the fighting and the resulting emergency there in 1960 to 1961.