2.2% with post-operative total deafness. These results are comparable with the few published so far.

Though it is early to assess permanent cure, the indications are encouraging, and it is considered that stapedectomy is at present the operation of choice for suitable cases of otosclerosis.

I wish to express my thanks to Dr. W. K. Slack for his comments on the anaesthetic technique and to the staff of the anaesthetic department of Whipps Cross Hospital for their co-operation. I am also indebted to Dr. D. J. Durcan for his assistance and support throughout the series.

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CHRONIC AMOEBIC HEPATITIS

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[WITH SPECIAL PLATE]

Chronic amoebic hepatitis is the subject of much controversy: many doubt its existence, while others ascribe to it only little clinical significance, and compare it with the post-hepatitic syndrome which occasionally follows viral hepatitis. The predominant conception is that amoebic hepatitis, in general, is a rather rare complication of amoebiasis and constitutes a pre-suppurative stage.

On the basis of 25 years' experience as a clinician I consider that chronic amoebic hepatitis exists as a distinct entity. It occurs more frequently than is supposed, and is closely connected with the whole problem of amoebiasis.

For the past four years I have collected experimental data and the results of clinical observations which strengthen my concepts on this subject. Since, as most doctors believe (Sherlock, 1958), the pathological basis of these features is not certain, and there are no good reports of hepatic histology, I consider it my duty to describe the results of our observations.

The cases which I shall describe have been taken from material which we have observed and followed in Greece. The reason I emphasize this is because it is known that amoebiasis presents a clinical picture which varies with the geographic location of the countries in which it is found. It will be interesting to determine whether this entity, with or without variations, occurs in other countries. These cases are reported, first, because they are very characteristic, and, secondly, because they will help towards a better understanding of both the clinical features of the condition and the conclusions I have drawn (Doxiades and Candreviotis, 1961).

Case 1

Four years ago a 32-year-old woman was admitted to the Evangelismos Hospital with generalized oedema and free fluid in the right chest and peritoneal cavity. In the ascitic fluid typical features of *Entamoeba histolytica* were found on repeated examinations. The ascitic fluid was injected intrahepatically into one guinea-pig and into the portal vein of four others. The guinea-pigs were killed 17 days later. Histological examination of the liver of the animal which had been inoculated intrahepatically revealed an abscess containing an abundance of *E. histolytica*.

Repeated needle biopsies of the patient's liver, also a biopsy specimen of the liver taken at exploratory laparotomy, showed the presence of *E. histolytica* among the liver cells without any evidence of suppuration. Granulomas containing amoebae were also found.

The patient was given antiamoebic therapy and her condition improved greatly. Several stool examinations for parasites during the above period were negative (Doxiades *et al.*, 1961).

Case 2

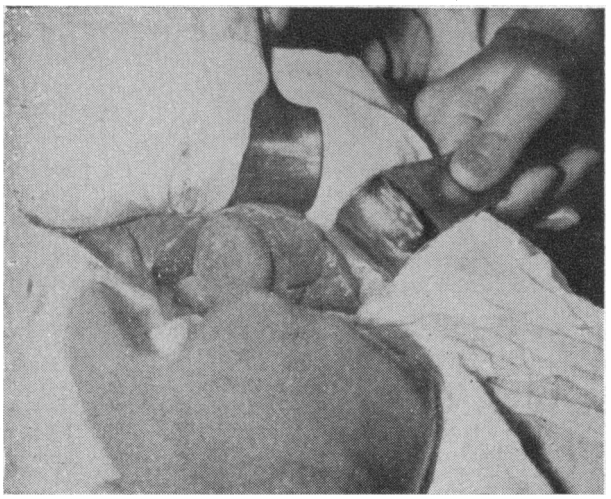
A 48-year-old man was admitted to the hospital on May 14, 1961, having suffered for the past year from loss of appetite, nausea, headaches, fatigue, and weakness accompanied by tenderness and a feeling of fullness in the region of the right hypochondrium. It is worth mentioning that the patient had previously been admitted to another hospital with a diagnosis of carcinoma of the liver.

On clinical examination the liver was found to be enlarged 6 cm. below the right costal margin and firm. Laboratory findings were as follows: Thymol turbidity 3 units; cephalin flocculation ++; $ZnSO_4$ turbidity 4 units; serum bilirubin 2 mg. per 100 ml.; alkaline phosphatase 4.5 units; "bromsulphalein" retention 8%; serum glutamic oxalacetic transaminase (S.G.O.T.) 36 units; serum glutamic pyruvic-transaminase (S.G.P.T.) 60 units. On stool examination *E. histolyticae* were found on two occasions. Proctoscopy did not reveal any pathological lesion. Total protein level and other laboratory findings were within normal limits.

On June 13 needle biopsy of the liver was performed; the specimen presented a histological picture of chronic hepatitis of moderate intensity, characterized by slight enlargement of the portal triads with inflammatory cellular infiltration, mainly by lymphocytes. In some sections small spheroid formations with the morphological features of *E. histolytica* were found, either at the portal triads or in the portal capillaries, among the liver cells (Special Plate, Fig. 1). The liver cells showed moderate degenerative changes and fatty degeneration. No tumour cells were found.

On June 27 an exploratory laparotomy was carried out, in which no tumour was seen. The surface of the liver was strikingly granular, as shown in the accompanying photograph. A small specimen of liver was excised, half of which was examined histologically. This presented the same picture of chronic hepatitis characterized by marked enlargement of the portal areas and showing excessive proliferation of connective tissue, forming bands which divided the liver

parenchyma into lobules of varying size and shape, with lymphocytic infiltration. In one portal area a spheroid cellular formation was found showing the morphological features of *E. histolytica* (Special Plate, Fig. 2). Again malignancy was not found microscopically in the sections examined. The remainder of the specimen was homogenized with normal saline and the material injected into experimental animals as follows: (*a*) 0.2 ml. directly into the liver of a hamster (No. 1) and 0.8 ml. into the peritoneal cavity; (*b*) 1 ml. directly into the liver of another hamster (No. 2); (*c*) 1 ml. into the liver of a guinea-pig; (*d*) 2 ml. into the liver and 2 ml. into the peritoneal cavity of a rabbit (No. 1); and (*e*) 3 ml. into the liver of another rabbit (No. 2). Twenty-one days later the animals were killed and their livers examined. The liver of hamster No. 2 showed macroscopically a strikingly granular surface with some sparsely



Case 2. Exploratory laparotomy showing granular surface of liver.

dispersed greyish-white miliary nodules. Histologically it presented the picture of a marked chronic hepatitis identical with that seen in the patient's liver. The portal triads were excessively enlarged and infiltrated by lymphocytes; there was also excessive connective-tissue proliferation, and the liver parenchyma was divided into pseudo-lobules of varying size and shape. In some places small spheroid cellular formations with the morphological features of *E. histolytica*, such as those found in the specimen of the patient's liver, were evident (Special Plate, Fig. 3). In the liver of rabbit No. 2 a pea-sized abscess was found. Histologically, this abscess showed a central necrotic area surrounded by cellular inflammatory tissue with many amoebae among the inflammatory cells.

The patient was treated with antiamoebic drugs. The last two stool examinations for parasites (July 11 and 17) were negative. On July 19 the patient left the hospital in good health and was advised to continue antiamoebic therapy.

On October 17, 1961, the patient was readmitted for follow-up examination. The liver at this time was found to be smaller and not so hard, and the patient's general condition much improved. Results of liver-function tests and other laboratory examinations were within normal limits. A liver biopsy showed microscopical findings similar to the last one, but with a less inflammatory process. Amoebae were not found.

Case 3

A 41-year-old woman was admitted to the hospital on March 29, 1961, with a feeling of fullness in the region of the right hypochondrium. She had suffered from slight jaundice for four years. Of significance is the fact that she had had persistent diarrhoea in her early childhood. In 1957 she felt a slight pain on the right side of the abdomen. A stool examination at this time revealed *E. histolytica* of *minuta* type. She was therefore treated with antiamoebic drugs, but treatment was interrupted because of allergic manifestations and fever. Some time later a slight icteric tinge of conjunctivae and skin developed, and this was attributed to the amoebiasis. The jaundice during its long course showed periods of intensification as well as intervals of complete recession. Treatment with emetine or other drugs was not tried because of the patient's refusal. *X*-ray examination revealed atony of the gall-bladder, and the patient was treated for a cholecystopathy with the corresponding diet, but the jaundice persisted. Results of liver-function tests in 1959 were as follows: thymol turbidity 8 units; cephalin flocculation +. In this year the patient's urine was for the first time of a dark colour, but no discoloration of the stools was noted. On physical examination an enlarged liver (5 cm. below the costal margin) was found, and the spleen was palpable 4 cm. below the costal margin.

The present admission was decided on as there were no signs of a constant recession of the jaundice. Clinical examination revealed a slightly icteric colour of skin and conjunctivae as well as enlargement of the liver, which was palpable 5 cm. below the right costal margin and slightly tender. The spleen was also palpable 4 cm. below the left costal margin. Laboratory findings were as follows: Serum bilirubin 5.25 mg. per 100 ml. (direct 3.45 mg. and indirect 1.80 mg. per 100 ml.); thymol turbidity 16 units; cephalin flocculation +++; $ZnSO_4$ turbidity 20.8 units; alkaline phosphatase 13.6 units; S.G.O.T. 250 and S.G.P.T. 325 units. Total proteins 8.75 mg. per 100 ml. (albumin 6 mg., globulin 2.75 mg.); serum cholesterol 227 mg. per 100 ml. Other haematological findings were within normal limits. A urine test for urobilinogen was positive, with a trace of bile. Stool examination for parasites was negative; proctoscopy did not reveal any pathological lesion.

On May 30, 1961, an exploratory laparotomy was performed, when the liver was found to be enlarged, firm in consistency, and granular in appearance. A specimen was taken for histological examination. Microscopically, a chronic hepatitis was detected, characterized by dense inflammatory leucocytic infiltration by lymphocytes and some polymorphonuclears, mainly in the portal areas. There was also an increase in portal connective tissue extending into the liver parenchyma. In some areas the liver cells showed degenerative changes characterized by a decrease in size and in the density of the nuclei. The cytoplasm was cloudy and granular or dark-stained and the cytoplasmic margins were ill defined. A few cells showed fatty degeneration or were fragmented. The most characteristic lesions, however, were some granulomatous formations, consisting of epithelioid cells among which a spheroid formation with the features of amoeba (amoebic granuloma) was found (Special Plate, Fig. 4).

On June 21 a needle biopsy of the liver was performed, and the specimen was diluted in normal saline. Of this solution 0.1 ml. was injected into the liver of a hamster (No. 1) and 0.2 ml. into the liver of another hamster (No. 2); 3 ml. of the solution was injected into the liver of a guinea-pig and 1 ml. into a rabbit intrahepatically; 1 ml. was also injected into the portal vein of another rabbit, and 1 ml. into the same rabbit intrahepatically. The animals were killed 21 days later. The liver of hamster No. 1 had a marked granular appearance, which presented the same histological picture as that of the patient's liver. An excessive sclerotic hepatitis was seen, characterized by dense inflammatory infiltration of the liver, associated with an exaggerated connective-tissue proliferation, leading to a cirrhotic condition. The connective-tissue bands divided the liver parenchyma into lobules of varying size and shape. In the inflamed area typical spheroid formations with the characteristics of amoeba were detected (Special Plate, Fig. 5).

The patient was treated with antiamoebic drugs. No amoebae have been found on repeated examination of the stools. Liver-function tests gave the following results: serum bilirubin 5 mg. per 100 ml.; thymol turbidity 1.8

units; cephalin flocculation ++; $ZnSO_4$ turbidity 14.8 units; alkaline phosphatase 8.5 units; S.G.O.T. 92 and S.G.P.T. 100 units.

The patient left the hospital on July 15, 1961, much improved. In the following October she was seen again, and her general condition and the results of liver-function tests were practically the same, with S.G.O.T. 44 and S.G.P.T. 82 units.

Case 4

A 56-year-old woman was admitted to the Evangelismos Hospital on January 27, 1961, because of upper gastro-intestinal haemorrhage and chronic pyelonephritis. Since 1947 she had suffered from hypertension. The gastro-intestinal bleeding persisted, and the patient was given repeated blood transfusions. The blood-urea nitrogen level rose progressively to 500 mg. per 100 ml. The patient died on March 4, 1961.

Necropsy revealed a bleeding duodenal ulcer and shrunken kidneys. There was extensive ulceration in the rectum, microscopical examination of which showed absence of the mucosa and infiltration of the submucosa by inflammatory cells. A focus containing *E. histolytica* was seen.

The liver was slightly enlarged, weighing 1,600 g., and greyish in colour. Microscopical examination showed the presence of diffuse hepatitis characterized by inflammatory-cell proliferation, mainly in the vicinity of the portal areas, but without abscess formation. Among the liver cells a spheroid formation with the morphological features of *E. histolytica* was found.

Discussion

The four cases described above are similar to the many which I have had the opportunity to observe. The combined findings in the cases I have seen have led me to the following conclusions: (1) The possibility of amoebic infection should be considered even in the absence of the dysentery syndrome. In fact, dysentery is seldom present, but may be found in the patient's past history. (2) It is not necessary to find amoebae in the stools in order to suspect the presence of amoebiasis. Nobody, however, doubts the importance of finding amoebae in the stools as a means of direct diagnosis. Nevertheless, a negative finding no more excludes the presence of amoebiasis than the negative result of a sputum test excludes tuberculosis. (3) Ulceration of the colon, which is commonly found in amoebic dysentery, is rarely present in the chronic forms of amoebiasis. (4) The experiment on kittens is seldom successful, and is of value only when the result is positive.

Only when we free our minds from the old prejudices and accept the fact that amoebiasis may be diagnosed from the therapeutic result—which also, incidentally, applies to many other diseases—shall we be able, in my opinion, to face the problem of amoebiasis as it presents itself to-day (Doxiades, 1958, 1961).

What I would most like to emphasize is the importance of experience on the part of those who examine the stools for amoebae. I am sure that in most hospitals the necessary experience is lacking. Though specialists exist in the institutes of tropical medicine, neither the doctors nor the hospitals are likely to consult these specialized institutes unless they have patients who come from tropical areas. The ideal solution would be to find a serodiagnostic method which, like those of Wassermann and Widal, would distinguish between patients who are infected and those who are not. Lacking this assistance, and because so far no existing method has proved statistically satisfactory, our aim must be to facilitate diagnosis either by a better knowledge of the clinical picture or by the improved use of direct methods, such as radiological examination of the liver, needle biopsy of the liver to search for amoebae, or inoculation of pathological material into the liver of an experimental animal. This last method has proved effective on many occasions.

Recently we have obtained better demonstration of amoebae in cultures by a fluorescent method. We are now attempting to apply this method to our biopsy sections, and we hope that accurate demonstration of the amoeba by fluorescent microscopy will before long be possible.

As things now stand, it is, in my opinion, necessary that medical men should be acquainted with the symptoms of chronic amoebiasis, which may well prove to be less rare than is generally held.

Conclusions and Summary

On the basis of clinical observations and experimental data it is considered that chronic non-suppurative amoebic hepatitis exists as a distinct clinico-pathological entity. Four characteristic cases are described in support of this view. In the liver of such cases granuloma formations are occasionally seen. Spheroid formations morphologically similar to amoebae were found in the granuloma formations and among the liver cells.

The amoeba can be transmitted from the patient's liver to the liver of an experimental animal by inoculation. In this way pathological changes similar to those seen in the patient's liver can develop.

A fluorescent method is being developed by which it is hoped to obtain better demonstration of the amoeba in these cases.

I would like to express my thanks to N. Candreviotis, Assistant Professor of Pathology, for his kind help and co-operation.

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Samples were submitted by private individuals to the Birmingham Analytical Laboratories for foreign matter to be identified. "Of 19 specimens received in this category, 8 consisted of bread and the 'foreign bodies' identified included insects, a fragment of wood, brown paper, machinery grease, and black sticky adhesive. Complaints regarding 4 specimens of milk were due to the presence of an American cockroach in one instance, a mat of mould in each of two other cases, and markings on the outside of a well-worn bottle in another. Stout was found to contain harmless yeast, lemonade a mat of mould, and bananas a tropical spider's egg which, disappointingly, did not hatch out when incubated. Green matter in a pig's pudding was rightly suspect but was identified as part of a leek; and, happily, egg-like objects in a can of baked beans and pork were found to be the detached radicles of the beans. Insect pests were confirmed in a sample of sugar. Perhaps the most unexpected foreign matter encountered was a small piece of dried fish in an opened packet of self-raising flour. It was diffidently suggested that the contamination might have occurred in the complainant's own kitchen on a previous occasion. Where relevant, all complaints were passed on to the Food Inspection Department." (Report for the First Quarter, 1962. Birmingham City Analyst.)

T. DOXIADES: CHRONIC AMOEBIC HEPATITIS

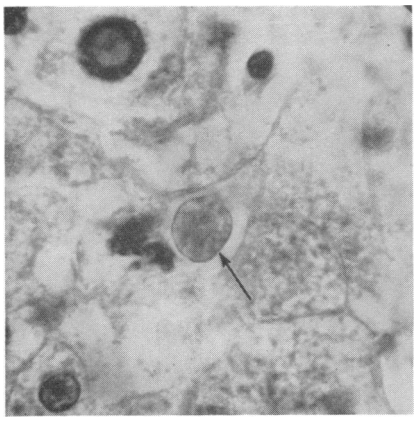

FIG. 1.—Case 2. Needle liver biopsy. *Entamoeba histolytica* among liver cells.

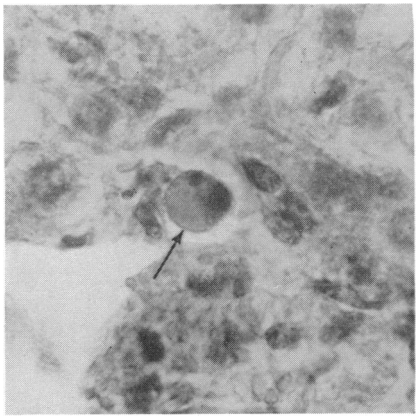

FIG. 2.—Case 2. Surgical biopsy. *Entamoeba histolytica* in portal area.

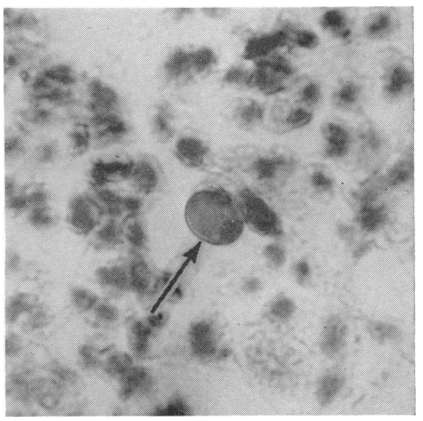

FIG. 3.—Case 2. Amoeba, with same features as in Fig. 2, from liver of hamster No. 2.

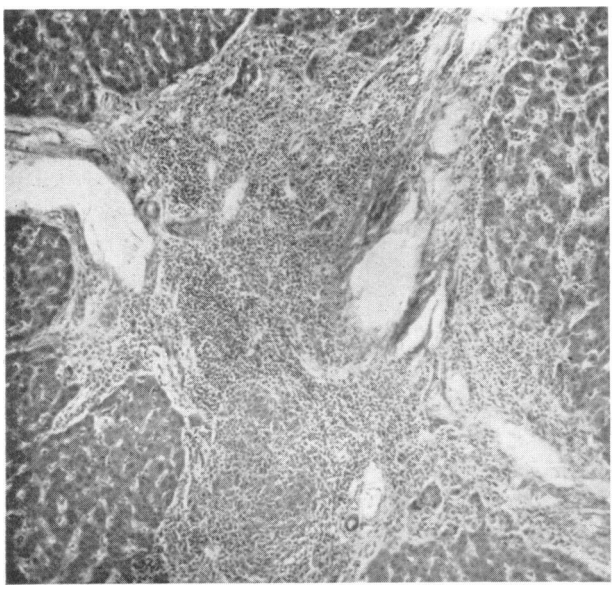

FIG. 4.—Case 3. Amoebic granuloma in liver.

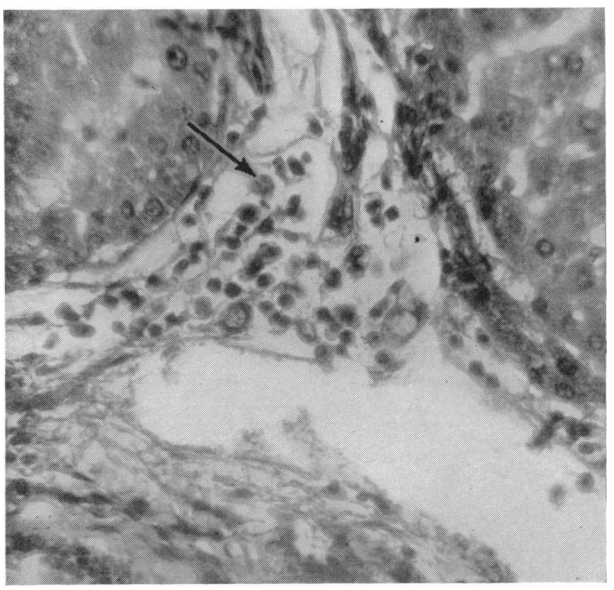

FIG. 5.—Case 3. Amoebae in liver of hamster No. 1.

T. DOXIADES AND N. CANDREVIOTIS: DETECTION OF ENTAMOEBA HISTOLYTICA

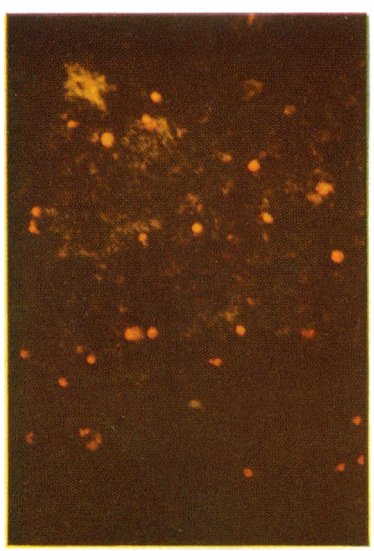

FIG. 1.—Numerous amoebae are visible in low magnification.

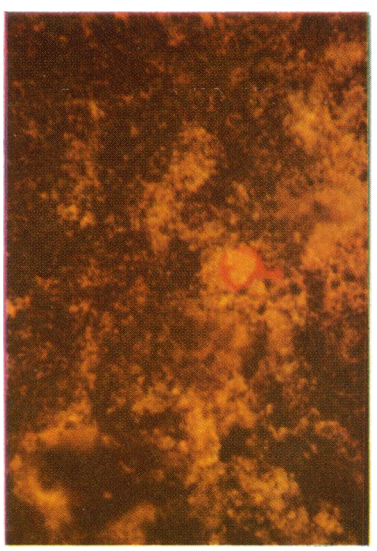

FIG. 2.—An *Entamoeba histolytica* with vacuoles in the cytoplasm.

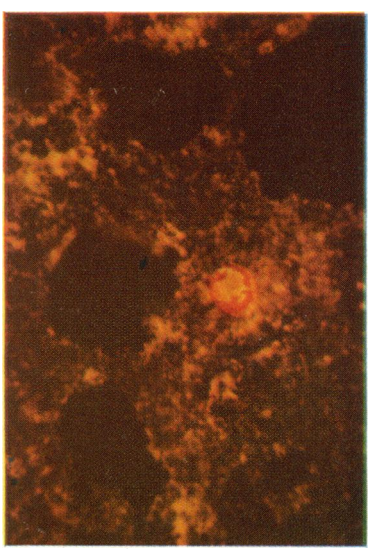

FIG. 3.—An *Entamoeba histolytica* with a well-recognized pseudopodium and vacuoles in the cytoplasm.