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Immunoelectrophoresis Test for Amoebiasis*

T. SAVANAT, M.B., Ph.D. & WANPEN CHAICUMPA, D.V.M.

Haemagglutination and immunoelectrophoresis tests were investigated to find which was more suitable for the immunodiagnosis of amoebiasis. Both tests were positive in more than 90 % of sera from patients with amoebic liver abscess. With serum from blood donors and patients with other diseases a much lower percentage of positives was given by the immunoelectrophoresis test, showing that this test had a closer correlation with clinically important disease.

The immunoelectrophoretic patterns were of several varieties, but a single prominent band located near the well was considered as characteristic of amoebiasis.

Follow-up studies showed that both haemagglutinating and precipitating antibodies persisted for several months, accompanied in certain patients by changes in the immunoelectrophoretic pattern. Antibody activities were shown by means of column chromatography and "reversed" immunoelectrophoresis to be associated with serum IgG.

Although the parasitological diagnosis of amoebiasis is satisfactory in intestinal forms of the disease, it is not satisfactory in cases of liver abscess. Amoebae are often not found in the aspirated pus, and the diagnosis of amoebiasis is usually made presumptively on the finding of sterile pus of characteristic appearance. In patients with abscesses that are deep, small or multiple, the diagnosis is often delayed owing to difficulty in obtaining pus, sometimes with unhappy consequences. The need for a routine immunodiagnostic test is, therefore, great, especially in places with a high incidence of amoebiasis such as Thailand.

In recent years, many immunological tests for amoebiasis have become available (Kessel et al., 1965; Maddison, Powell & Elsdon-Dew, 1965a, 1965b; Milgram, Healy & Kagan, 1966; Jeanes, 1966; Goldman, 1966; Boonpucknavig & Nairn, 1967). Among these tests, haemagglutination, complement-fixation and gel-diffusion seem to be promising in terms of sensitivity, specificity and reproducibility. There are, however, some inherent disadvantages which may render them unsatisfactory for routine use. In haemagglutination and complement-fixation, for instance, a fresh preparation of sensitized red blood cells is required for each performance of the test. It is obviously uneconomical when only a few serum samples are tested at a time. Furthermore, the persistence of the haemagglutinating antibody long after the actual infection has subsided makes it difficult to differentiate between present and past infection.

By virtue of its better resolution, immunoelectrophoresis (IEP) is theoretically considered to be superior to the gel-diffusion test. The IEP was used by Maddison (1965) to demonstrate precipitating antibodies against *Entamoeba histolytica* in the serum of patients with amoebic liver abscess. It was also used by Goldman & Siddiqui (1965) and Krupp (1966) in the antigenic analysis of *E. histolytica*. Unfortunately, no extensive study of this technique has been made to assess its value in diagnosis. It was therefore decided to use this test in our patients and to compare results with those obtained with the conventional haemagglutination test.

MATERIALS AND METHODS

The amoebae (*E. histolytica*, strain HB 301) used in this study were kindly provided by Dr David Weinman, Parasitology Division, SEATO Medical Research Laboratories, Bangkok, Thailand. The protozoa were grown in a modified serum-free Boeck & Drbohlav medium (Brooke, 1958) together with sterile rice powder (Difco) and *Clostridium perfringens* as a sole concomitant organism.

* From the Department of Microbiology and Immunology, Faculty of Tropical Medicine, University of Medical Sciences, Bangkok, Thailand. This work was supported in part by the World Health Organization and in part by a grant-in-aid from the University of Medical Sciences, Bangkok.

Antigen preparation

Amoebae were harvested from 48-hour cultures and washed 4 times at room temperature (28°C–32°C) by centrifugation at 180 *g* for 3 minutes; the first 2 washings were done in Locke solution and the last 2 washings in physiological saline. The cells were then counted in a haemocytometer and the cell concentration adjusted to give 10^6 cells per ml. Complete disruption of cells was accomplished by 4 cycles of repeated freezing and thawing in dry-ice and alcohol. The cell extract was then separated from the cell debris and rice powder by means of centrifugation at 8000 *g* for 20 minutes at 4°C. Batches of such extract were pooled and concentrated 10 times by dialysis against Carbowax M20 followed by further dialysis against distilled water overnight at 4°C. The concentrated cell extract was again centrifuged at 8000 *g* for 20 minutes at 4°C in order to remove any remaining cell debris, starch granules or any small precipitates. The opalescent supernatant was dispensed in 0.5-ml volumes and then lyophilized. For use in the gel-diffusion and immunoelectrophoresis tests, sterile distilled water was added so that the protein content as measured by the biuret method was 11 mg per ml.

Cl. perfringens *antigen*

Cl. perfringens was grown in Boeck & Drbohlav medium with added rice starch for 48 hours at 37°C. The bacterial cells were harvested and washed 4 times in physiological saline by centrifugation at 1600 *g* for 10 minutes. The organisms were concentrated to approximately 5×10^9 organisms per ml, and subsequently subjected to freezing at −70°C in a deep-freeze unit and then thawed in running water. After 25–30 cycles of this process, most of the cells were disrupted. The extract was then separated from the bacterial residue by centrifugation at 1600 *g* for 10 minutes at 4°C. The supernatant was dialysed against water at 4°C overnight, concentrated against Carbowax, followed by centrifugation again at 8000 *g* for 30 minutes at 4°C, and finally preserved by lyophilization. Before use, water was added to give a protein concentration of 11 mg per ml, as measured by the biuret method.

Sera

Sera were obtained from the following sources and were maintained in the freezing compartment of an ordinary refrigerator (at a temperature of −8°C).

(1) Sera from 93 patients with amoebic liver abscess, in 25 of whom the diagnosis was proved by finding *E. histolytica* in the pus either by microscopic examination or by culture. In the remaining 68 cases, examination for *E. histolytica* was not done or was negative, but liver aspirations all yielded bacteria-free " anchovy sauce " pus.

(2) Sera from 6 patients who had suffered from amoebic liver abscess in the past, the infection having subsided for more than 1 year.

(3) Sera from 8 patients with intestinal amoebiasis; 6 had dysentery and 2 others were asymptomatic and were admitted to the hospital for other reasons. *E. histolytica* trophozoites were found in the stools in all cases.

(4) Sera from 95 patients with unrelated diseases (14 carcinoma of the liver, 6 portal cirrhosis, 9 cardiac cirrhosis, 12 opisthorchiasis, 3 pyogenic liver abscess, 6 cholecystitis, 11 hepatomegaly of undetermined nature, 2 leptospirosis, 2 hookworm infestations, 1 volvulus of the gall bladder, 2 malaria, 1 liver cyst, 2 colitis (non-amoebic), 1 pneumonitis, 1 typhoid fever, 1 scrub typhus, 1 carcinoma of pancreas, 1 polycystic kidney, 1 infectious hepatitis, 1 carcinoma of the stomach, 7 eosinophilic meningitis, 6 pyrexia of unknown etiology, 1 schistosomiasis, 1 *Entamoeba gingivalis* infection of the gum, 1 abdominal pain of unknown cause and 1 haemolytic jaundice.

(5) Sera from 348 blood-donors from the Red Cross Institute.

Immunological tests

Haemagglutination test. This test was used only in a limited number of cases in the early part of this study. Owing to a high percentage of positive reactions in persons without clinical amoebiasis (16%–17%), the test was subsequently discontinued.

The technique used was essentially similar to that described by Kessel et al. (1965) but with the following modifications:

(1) Sheep red blood cells were used in place of human group-O red blood cells.

(2) The concentration of sheep red blood cells at various stages of manipulation (at the time of tannic acid treatment or at the time of sensitization with *E. histolytica* antigen) was constantly maintained at 2.5%.

(3) 1% of normal rabbit serum was used as a stabilizing agent instead of normal human group-O serum. This serum was incorporated into the phosphate-buffered saline (pH 7.2) in the final suspension of sensitized red cells and as diluent for

serum to be tested. Prior to use, the rabbit serum was inactivated by heating at 56°C for ½ hour, followed by absorption with an equal volume of packed sheep red blood cells twice at 4°C for 10 minutes.

(4) Twofold serial dilutions of serum were used instead of the fourfold dilutions originally described. To achieve this in the micro-titre system, in which only fourfold dilutions are possible, 2 series of starting dilutions of serum were used, i.e., 1 : 40 and 1 : 80, followed by the conventional fourfold dilution procedure.

Precipitin test and immunoelectrophoresis. For the precipitin test, gel-diffusion in 2 dimensions (Ouchterlony technique) was done on microscopic slides covered with 1% agar (Crowle, 1961). Holes of 3-mm diameter were cut in a hexagonal pattern around a central well of the same size, the distance between the central and peripheral wells being 5 mm. *E. histolytica* antigen and serum samples were filled twice in appropriate wells at an interval of 15 minutes, and reading was done within 72 hours.

For immunoelectrophoresis, Scheidegger's (1955) slide method was used. The antigen was filled in the well 3 times at 15-minute intervals and then subjected to electrophoresis in 0.05-μ sodium barbital (Veronal) buffer, pH 8.4, at 4°C for 1½ hours at 6 volts per cm. After electrophoresis, 0.15 ml of serum was added to the trough and the reaction was allowed to develop for 3 days at 4°C. The slides were washed in physiological saline and stained with amido black 10 B.

In most of the tests, *Cl. perfringens*, as well as *E. histolytica*, antigens were placed in separate wells prior to electrophoresis. The serum subsequently added to the trough would react with both types of antigen and thus render possible differentiation between specific and non-specific bands against *E. histolytica* antigen.

In certain "reversed" immunoelectrophoresis experiments, serum from the patient was placed in the well at the centre of the slide, and then subjected to electrophoresis. Two troughs were cut on each side of the well, one was filled with *E. histolytica* extract, and the other with anti-normal human serum or anti-human immunoglobulin serum.

Column chromatography

Chromatographic separation of serum through a Sephadex G-200 column (2×80 cm) was done at room temperature (28°C–32°C) according to the technique described by the manufacturer,[1] using 0.1 M tris-HCl buffer containing 1 M NaCl, pH 8.0, and a flow rate of 15 ml–20 ml per hour. Three distinct peaks were obtained which correspond to fractions 1–3 (Fig. 1 and 2). Fractions were cut, pooled, dialysed against distilled water at 4°C and then concentrated by Carbowax to give the original volume. Each fraction was then used in the test.

[1] Pharmacia Fine Chemicals.

RESULTS

Haemagglutination test

Results of the haemagglutination test are presented in Table 1. Titres below 1 : 40 are considered negative, titres of 1 : 40–1 : 60 are regarded as low, 1 : 320–1 : 1280 medium, and more than 1 : 1280 high.

Altogether, 43 out of 47 cases of amoebic liver abscess were positive (91.5%). Among 4 patients with a negative HA test, 3 were also negative to the IEP test. The remaining 1 was positive to the IEP test, giving a pattern 4.1 (see below). Second samples from 2 of these patients were available for retesting, and they too were negative.

Out of 5 cases of old amoebic liver abscess, 3 were negative, and the other 2 were positive in a lower titre range. Of 29 patients with unrelated diseases, 5 were positive (17.2%). Among these, only 1 was positive by the IEP test. Among 313 samples from blood donors, 51 were positive (16.3%), and most of them were in the lower titre range.

FIG. 1

CHROMATOGRAPHIC SEPARATION OF THE SERUM OF A PATIENT WITH AMOEBIC LIVER ABSCESS (S.R.) INTO 3 DISTINCT PEAKS

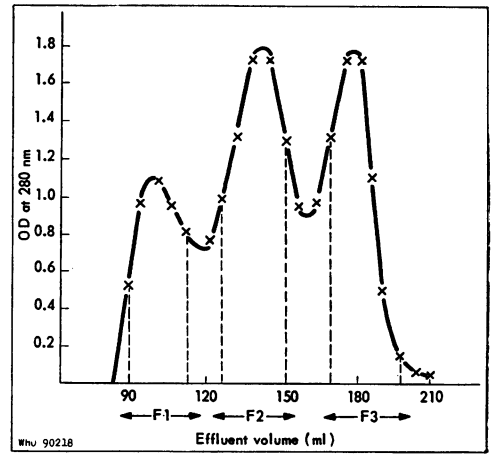
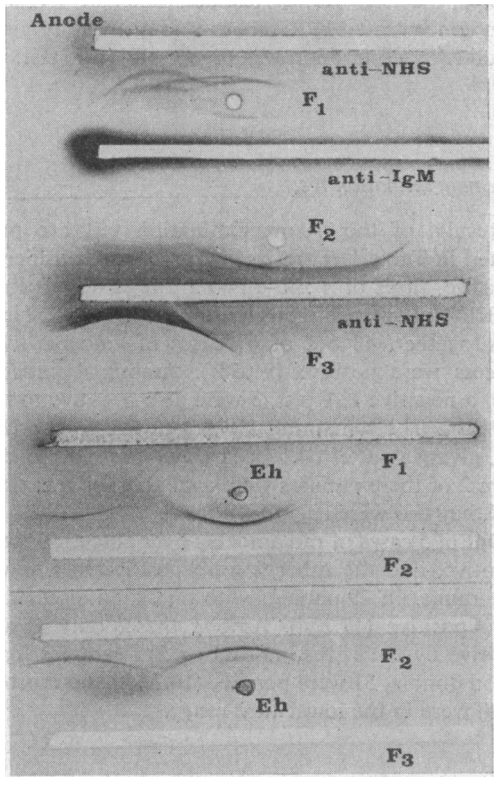


FIG. 2

FROM THE SAME PATIENT (S.R.) AS IN FIG. 1, IT IS SHOWN THAT THE PRECIPITATING ANTIBODY RESIDES IN THE IgG-RICH FRACTION 2[a]



[a] anti-NHS = anti-normal human serum; F_1, F_2, F_3 = fractions 1, 2, 3, respectively. Eh = *E. histolytica* extract.

Precipitin test and immunoelectrophoresis

Sera from blood donors and from patients with unrelated diseases were first screened by means of the precipitin test. Those positive were subsequently tested by means of immunoelectrophoresis. Sera from the patients with amoebic liver abscess were tested by immunoelectrophoresis without a prior precipitin test.

The immunoelectrophoretic patterns obtained were of several varieties. For the sake of convenience, however, the patterns are loosely classified into 4 types:

Pattern 1 (Fig. 3). At least 1 prominent band located near to the well. This band would correspond to band 4 of Krupp (1966).

Pattern 2 (Fig. 4). As pattern 1, but having at least 1 more prominent additional band moving towards the anode. This band is long and sharp, and would correspond to band 2 of Krupp (1966).

Pattern 3. A single band of slow mobility toward the cathode. Its position corresponded to that produced by the interaction of the antibody in the serum with the extract from *Cl. perfringens*, and this band could be absorbed with alcohol-killed *Cl. perfringens* cells. It appears that this band is the same as the " Welchii " band, first described by Maddison (1965). (The binding between the antigen and antibody giving rise to this band was not firm, since the band became dissociated after washing in physiological saline.)

Pattern 4; miscellaneous. This type includes all other patterns which cannot be grouped in the 3 previous groups. Subsequent analysis showed that

TABLE 1

HAEMAGGLUTINATION ANTIBODY TITRES IN THE SERA OF PATIENTS WITH AMOEBIC LIVER ABSCESS, WITH OTHER DISEASES AND IN BLOOD DONORS

Reactivity	Amoebic liver abscess				Other diseases	Blood donor
	Acute case			Old case		
	Confirmed	Un-confirmed	Total			
Negative (≤ 1 : 40)	—	4	4	3	24	262
Low (1 : 40–160)	2	1	3	2	2	43
Medium (1 : 320–1280)	3	14	17	—	2	8
High (> 1 : 1280)	7	16	23	—	1	—
No. positive/No. negative	12/12	31/35	43/37	2/5	5/29	51/313
Percentage	100	88.6	91.5	40	17.2	16.3

FIG. 3
EXAMPLES OF IMMUNOELECTROPHORETIC PATTERN 1

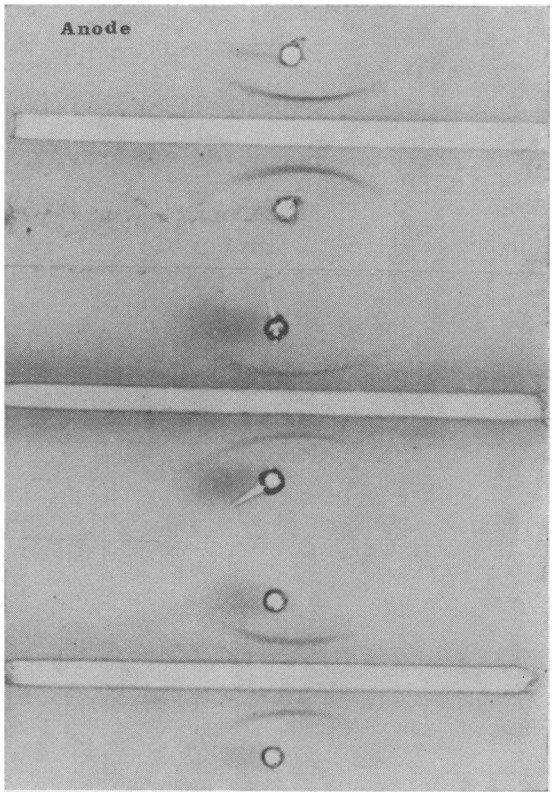
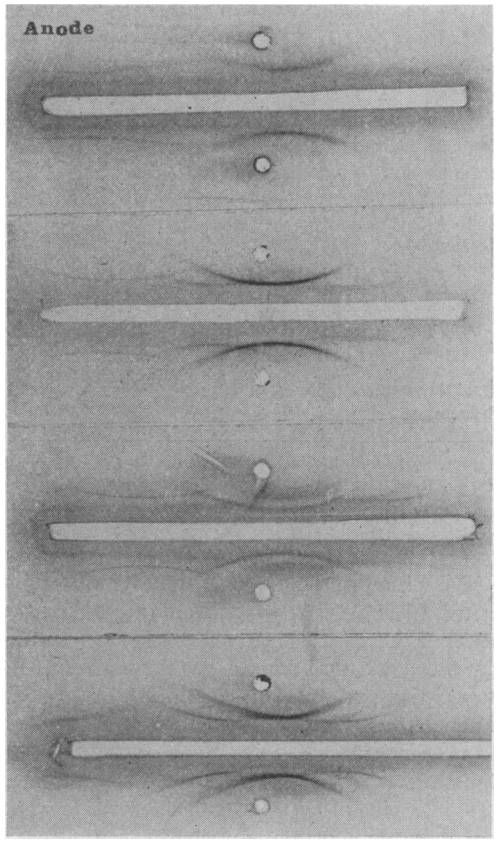


FIG. 4
VARIANTS OF IMMUNOELECTROPHORETIC PATTERN 2[a]



[a] The band characteristic of this pattern is long and sharp with migration towards the anode.

the reaction of this type could be subdivided into 3 subpatterns, namely:

(1) *Subpattern 4.1.* A single band of α-2 electrophoretic mobility. This band is located in a position analogous to band 3 of Krupp (1966).

(2) *Subpattern 4.2.* A single band moving towards the anode with a migration rate comparable to albumin. In position, it is analogous to band 1 of Krupp (1966). Like pattern 3 reactivity, this band is reactive for *Cl. perfringens* antigen.

(3) *Subpattern 4.3.* Like subpattern 4.2, but with the additional presence of a band characteristic of pattern 3.

Only the band of subpattern 4.1 is reactive to *E. histolytica* antigen.

Immunoelectrophoresis test

The results are presented in Table 2.

Acute amoebic liver abscess

All 25 sera from " confirmed " cases of amoebic liver abscess were positive by this test (9 were of pattern 1 and 16 were of pattern 2). Out of 68 sera from " unconfirmed " amoebic liver abscess, 63 were definitely positive, 12 of them showing pattern 1 and the remaining 51 pattern 2. Two other sera showed reaction of subpattern 4.1, and would be considered as positive. Out of the remaining 3 sera, 2 showed pattern 3 and 1 was virtually non-reactive.

Old amoebic liver abscess

Of 6 sera from patients recovered from amoebic liver abscess for more than 1 year, only 1 was reactive, showing subpattern 4.1. The remaining 5 were negative, 2 of which showed a pattern 3.

TABLE 2
RESULTS OF PRECIPITIN AND IMMUNOELECTROPHORESIS TESTS IN BLOOD DONORS, IN PATIENTS WITH HEPATIC AND INTESTINAL AMOEBIASIS AND IN PATIENTS WITH OTHER DISEASES

Reactivity	Amoebic liver abscess				Intestinal amoebiasis		Other diseases	Blood donor
	Acute case			Old case	Amoebic dysentery	Asymptomatic		
	Confirmed	Unconfirmed	Total					
Negative	—	1	1	3	1	2	61	335
Pattern 1	9	12	21	—	2	—	4	2
Pattern 2	16	51	67	—	2	—	3	—
Pattern 3	—	2	2	2	—	—	19	3
Subpattern 4.1	—	2	2	1	—	—	—	—
Subpattern 4.2	—	—	—	—	—	—	5	8
Subpattern 4.3	—	—	—	—	1	—	3	—
No. positive/No. examined	25/25	67/68	92/93	3/6	5/6	0/2	34/95	16/348 [a]
Percentage	100	98.5	98.9	50	83.3	0	35.8	4.6
Actual No. positive [b]/No. examined	25/25	65/68	90/93	1/6	4/6	0/2	7/95	2/348
Percentage	100	95.6	96.8	16.7	66.7	0	7.4	0.6

[a] Only 13 out of 16 sera with positive precipitin test were sufficient for the IEP test.
[b] Only IEP patterns 1.2 and 4.1 are considered as positive.

Intestinal amoebiasis

Of 6 cases of amoebic dysentery, only 4 were positive (66.7%), 3 of which were of pattern 1 and 1 pattern 2. Of the 2 negatives, 1 showed reaction of subpattern 4.2, and the other was non-reactive. The latter patient, without any past history of dysentery, had acute mucous and bloody diarrhoea for 4 days, and prompt treatment was given on the first day of admission.

Two cases of opisthorchiasis with associated asymptomatic intestinal amoebiasis were negative.

Other diseases

Out of 95 sera tested, 7 were positive (4 gave reactions of pattern 1, and the other 3 of pattern 2). These sera were taken from cases of carcinoma of the liver (2), pyrexia of unknown etiology with pain in the right hypochondrium (4) and hepatomegaly (1). Among the remaining 88 negative sera, 19 showed reactions of pattern 3, 5 of subpattern 4.2 and 3 of subpattern 4.3.

Blood donors

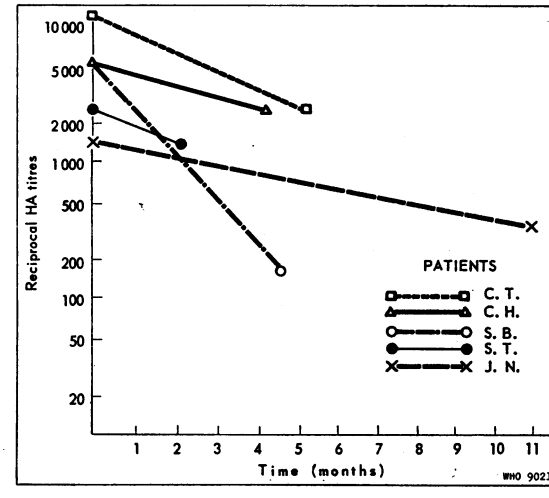
Out of 348 sera tested, 16 were reactive. However, only 2 sera were really positive showing pattern 1 (0.6%). Of the remaining sera, 3 and 8 showed patterns 3 and 4.2, respectively. (The actual number would probably be higher, because 3 sera which were found positive by gel-diffusion were not available for the immunoelectrophoresis test.)

FOLLOW-UP STUDY AFTER TREATMENT

Haemagglutination test

In 5 patients in which follow-up HA antibody titres were determined over a period of more than 2½ months, the test was consistently positive, but there was some decline in antibody titres (Fig. 5).

FIG. 5
FOLLOW-UP HA TITRES IN 5 PATIENTS WITH AMOEBIC LIVER ABSCESS

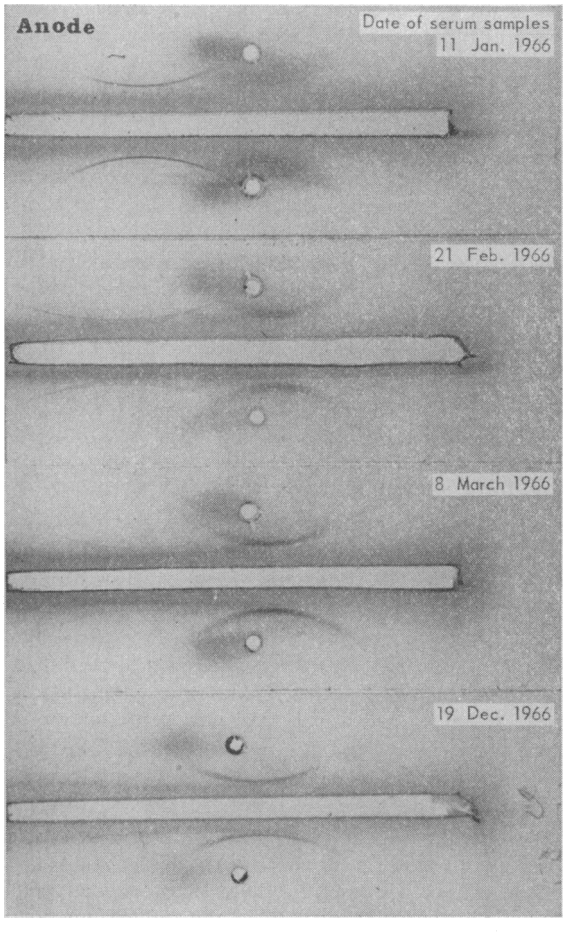


Immunoelectrophoresis

In 6 out of 12 patients on whose sera the test was repeated over a period of more than 2 months, there was no significant change over that period. In 1 case (J.N., Fig. 6), there was a change from pattern 2 to pattern 1, the reason for which is obscure but it could be due to the antibody responsible for band 2 of Krupp having a relatively short life compared with that of the antibodies relating to the other bands. Alternatively, this antibody may be present in relatively smaller quantity, and the over-all decrease of serum antibodies after successful chemotherapy

FIG. 6

CHANGES IN THE IMMUNOELECTROPHORETIC PATTERN IN A FOLLOW-UP CASE OF AMOEBIC LIVER ABSCESS: PATIENT J.N.[a]



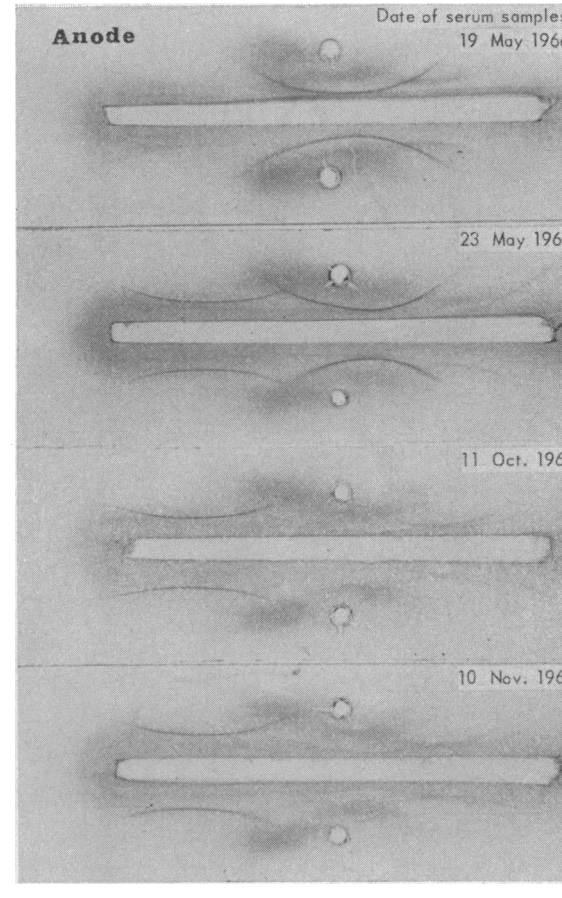
[a] Note the disappearance of the band at the anodal end.

would result in the early disappearance of this band.

In the 5 remaining cases in this series, there was a change from pattern 1 to pattern 2 on immunoelectrophoresis (Fig. 7, 8 and 9).

FIG. 7

CHANGES IN THE IMMUNOELECTROPHORETIC PATTERN IN A FOLLOW-UP CASE OF AMOEBIC LIVER ABSCESS: PATIENT C.T.[a]

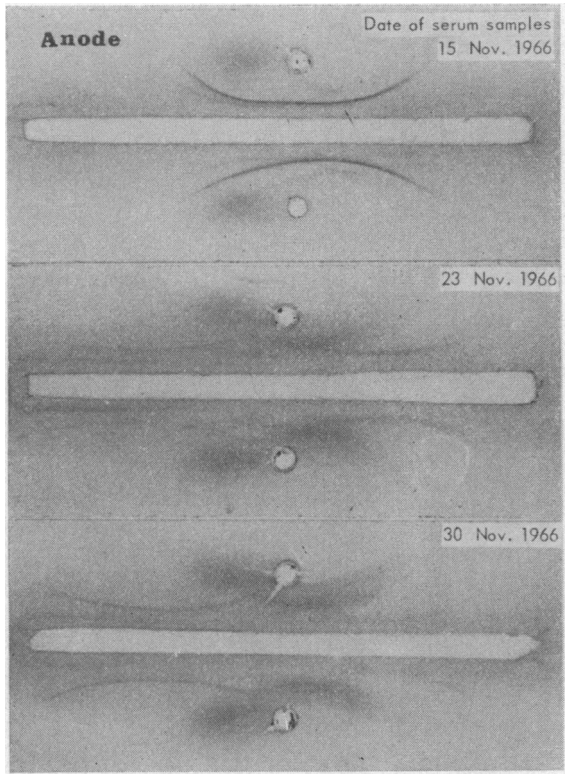


[a] Note the accentuation of the band at the anodal end.

DISCUSSION

Maddison (1965) tested a few serum samples from cases of amoebiasis by means of the immunoelectrophoresis (IEP) test, and compared the results with the conventional gel-diffusion test on agar plates. She found that the number of precipitating bands was smaller in the IEP, leading her to believe that the

FIG. 8
CHANGES IN THE IMMUNOELECTROPHORETIC PATTERN IN A FOLLOW-UP CASE OF AMOEBIC LIVER ABSCESS: PATIENT T.S.[a]



[a] Note the emergence of the band at the anodal end in the second sample.

IEP test was relatively insensitive. In the present study, however, the IEP test was found to be more sensitive than the micro gel-diffusion test, as shown by larger numbers of precipitating bands. The relative insensitivity of Maddison's IEP test could be due to the relatively smaller quantities of either the antigen or the serum used.

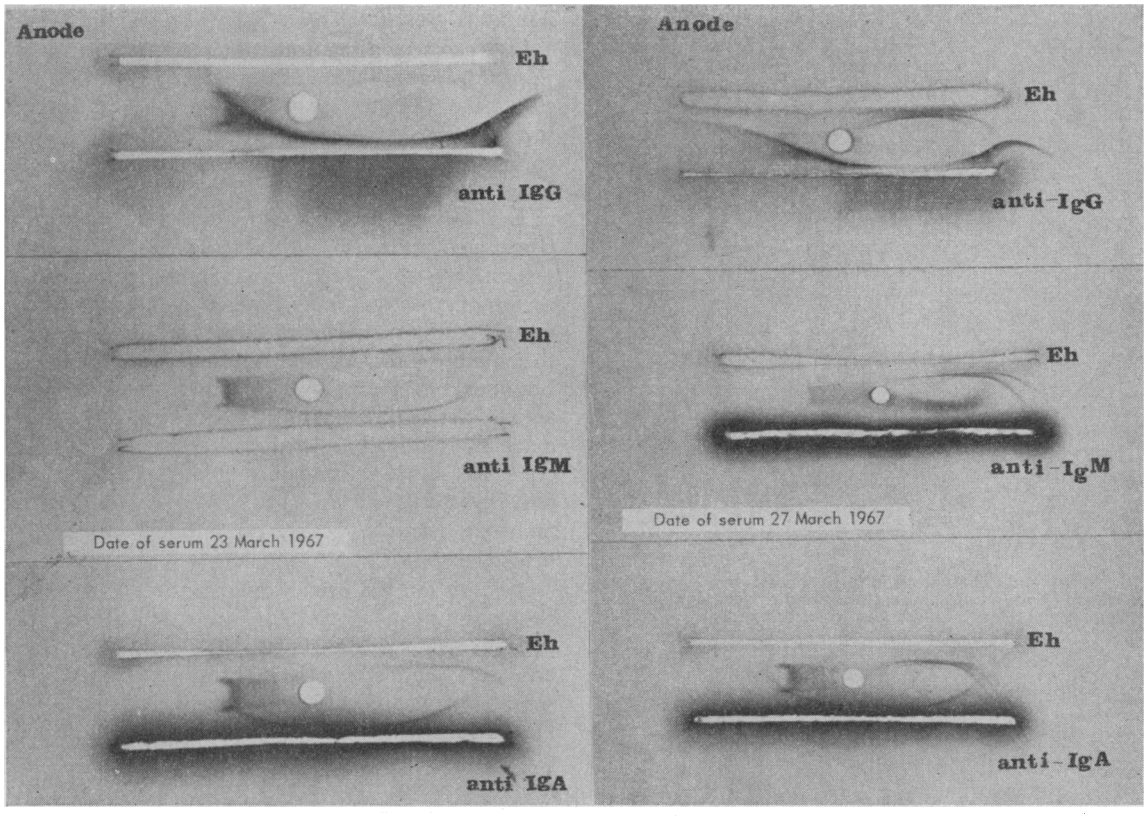
Though the antigen used in the IEP test is inherently contaminated with the bacterial antigen, this does not interfere appreciably with reading and interpretation of the results, since there occurs in general only 1 band, and in rare instance 2 bands, which is reactive against the contaminating *Cl. perfringens* antigen and it occupies a unique position in the immunoelectrophoretic pattern and can be easily differentiated from the bands reactive against *E. histolytica* antigen. Immunoelectrophoresis of *Cl. perfringens* extract concurrently with that of the *E. histolytica* facilitates this differentiation in case of doubt. The use of antigen prepared from axenically grown amoebae (Parke, Davis) has in practice very little diagnostic advantage over the crude antigen prepared from the monoxenic culture, since in a few experiments in which these 2 types of antigen were allowed to react on the same slide with the serum from a patient with amoebic liver abscess, the immunoelectrophoretic patterns produced were on a whole comparable, except for the quantitative difference of certain bands and the additional presence of the *Cl. perfringens* band when the latter was used (Fig. 10).

The inherent advantage of the IEP test is its ability to differentiate between non-specific and specific bands in the IEP pattern, making it more applicable to the diagnosis of amoebiasis than the HA test using the same type of antigen, although the HA test is in general more sensitive, since it can detect quantitative differences in circulating antibody following treatment. The test, however, is not suitable for diagnostic application, as judged by the high percentage of positivity in the blood-donor group (16.3%). It is of course possible that some of these positive results represented, even in the absence of clinical amoebiasis, true positives in the sense that these subjects may well have had amoebiasis in the past. But this does not alter the contention that the HA test is unsuitable for diagnostic use; the ability to detect minute amounts of antibody may result in sensitivity too high to be of practical use. We are concerned not with the sensitivity alone, nor even with specificity, but with the ability to discriminate between patients with clinically important diseases and those without.

In the confirmed cases of amoebic liver abscess (those from which amoebae were actually recovered from the pus), there were no false negative results. Altogether, 4.4% (3/68) of the unconfirmed cases (in which amoebae were not recovered) gave negative results; these may have been false negatives. But in some at least, it is possible that the amoebae had been killed by prior drug treatment (chloroquine is freely used by the public), resulting in the failure to recover amoebae and possibly also allowing the test to become negative. However, there is another possible explanation for what appear to be the false negative results. During the course of this study, we came across a patient with pus of a character indistinguishable from that of the typical amoebic liver abscess, and a provisional diagnosis of amoebic

FIG. 9

REVERSED IMMUNOELECTROPHORESIS STUDY SHOWING THAT THE PRECIPITATING BAND DEVELOPED AS A RESULT OF INTERACTION OF *E. HISTOLYTICA* EXTRACT AND SERA FROM PATIENT H.N. BEARS A RESEMBLANCE TO THE IgG BAND IRRESPECTIVE OF THE TIME WHEN THE SERA WERE TAKEN [a]



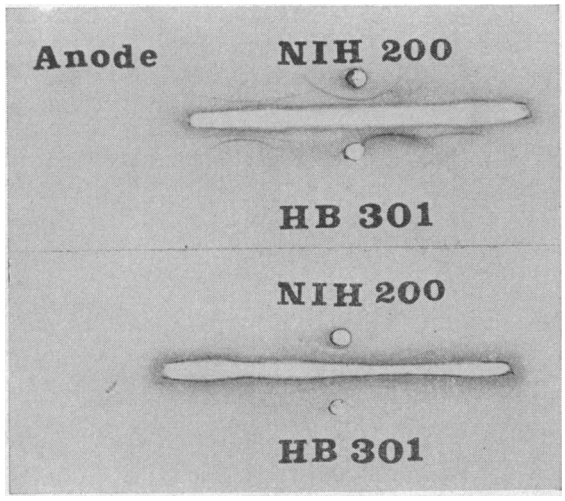
[a] In the usual IEP test, these 2 serum samples taken 4 days apart showed reactions of patterns 1 and 2, respectively. Eh = *E. histolytica* extract.

liver abscess was made. However, the IEP test was consistently negative on several occasions. Finally the patient died and autopsy was performed. The liver showed an extensive hepatoma containing diffluent necrotic areas, and the pus previously obtained must have resulted from the liquefaction of necrotic tumour. Since information on the follow-up of these 3 patients was not available, it is not known whether any of them subsequently succumbed with carcinoma of the liver.

The apparently high percentage of positivity of the IEP test in patients with other diseases (7 out of 95 = 7.4%) may not be due to wholly false positives, since 2 of these patients had histories of mucous and bloody diarrhoea within a year prior to admission. Another patient had *E. histolytica* trophozoites in the stools at the time serum was collected for examination. Among these 3 patients, 2 had fever and pain in the liver region, so much so as to lead the attending physician to make a tentative diagnosis of hepatic amoebiasis. However, these 2 patients recovered though neither anti-amoebic drugs nor antibiotics were given. This raises the question of the correct diagnosis of hepatic amoebiasis. To many investigators, response to amoebicidal drugs and a past history of dysentery are considered sufficient. It is not generally accepted that spontaneous resolution of hepatic amoebiasis occurs; but spontaneous cure *is* known to occur in many diseases, parasitic or otherwise, and may perhaps occasionally do so even in amoebic infection of the liver. These 2 patients may be examples of this occurrence.

FIG. 10

IMMUNOELECTROPHORETIC PATTERNS OF 2 SERA FROM PATIENTS WITH AMOEBIC LIVER ABSCESS [a] USING ANTIGENS PREPARED FROM AN AXENIC CULTURE [b] AND A MONOXENIC CULTURE [c]



[a] The bands characteristic of patterns 1 and 2 are demonstrated in both preparations; there are, however, some qualitative and quantitative differences in other bands.

[b] NIH 200; Parke, Davis.

[c] HB 301.

In the remaining 4 patients without apparent liver abscess but with a positive IEP test, 2 had *E. coli* trophozoites in the bowel, and thus may be regarded as " true " false positive. No explanation offers itself to account for the results in the other 2 patients.

The high percentage of negativity in old cases of amoebic liver abscess suggests that the IEP test may be of some help in differentiating between recent and past infections. In the light of the current evidence, it may be tentatively assumed that a positive IEP test in a case of amoebic liver abscess a year after successful chemotherapy would be an indication of recent infection. Observation on changes in the immunoelectrophoretic pattern might also be used in the manner analogous to a rising antibody titre in support of recent infection.

For the diagnosis of intestinal amoebiasis, however, the IEP test was not very sensitive, since only 4 out of 6 cases (66.7%) were positive. Among the 2 negatives, one (a boy of 13 years of age) had a history of illness of only 4 days prior to admission. It is probable that during this period, antibody against *E. histolytica* may not be formed to a sufficiently high level to be detected by this method. The HA test of the serum of this patient was also negative. In all 4 positive sera, the patients had histories of intermittent mucous and bloody diarrhoea of more than 2 months' duration.

In 2 cases of asymptomatic amoebiasis in which *E. histolytica* trophozoites were demonstrated in the stool, the IEP test was consistently negative on several occasions. This finding is in effect not contradictory to the results of Kessel et al. (1965) and Milgram, Healy & Kagan (1966) who showed by means of a haemagglutination test that the percentage positivity in such cases was 28 and 9, respectively. The lack of antibody response in asymptomatic cases may be due to the commensal nature of the amoebae in these circumstances and their failure to invade the tissues. A recent report of Boonpucknavig et al. (1967) showed that the most active antigenic determinant of amoebae was associated with the microsomal fractions, and it is conceivable that antibody response would be evoked only by disrupted amoebae. If no destruction of amoebae were to occur, no antibody would be produced.

Analysis of the immunoelectrophoretic patterns shows that there is 1 predominant band in the region of the well (Krupp band 4) which should be considered as characteristic, and which is observed repeatedly when sera from patients with amoebiasis are tested. Furthermore, we have observed that this particular band became very faint when the antigen prepared from the Laredo strain was employed, whereas all other bands remained mostly unchanged. The quantitative deficiency of the antigen responsible for this band in the non-pathogenic strain may be related to its non-pathogenic character, and this suggestion awaits further supportive evidence.

Most cases of amoebic liver abscess showed the reaction characterized as pattern 2. The number of bands in this group ranged from 2 to 8. This variation may simply be a reflection of quantitative differences of precipitating antibodies in these patients, or alternatively may reflect infections by different strains of *E. histolytica*. Antigenic analysis by Krupp (1966) showed that 8 strains of " true " *E. histolytica* under study could be classified into at least 3 groups. Strain NIH-200 and DKB belonged to 1 group, strains F_{22} and BH belonged to the second group and strains JH, K_9 and JS to the third group. The eighth strain (NRS) resembled in most respects strains F_{22} and BH, except for the possession of 1 additional antigen.

By means of column chromatography, it was shown that antibody activity was associated with fraction 2, which contained IgG and some IgA. When the serum was subjected to a reversed immunoelectrophoresis, the precipitating band produced as a result of interaction of serum antibody and *E. histolytica* antigen was shown to bear resemblance to the IgG band, although the band of the former was much thinner. This is not surprising in view of the probability that the anti-IgG used would react with all the IgG in the serum, whereas *E. histolytica* antigen would react only with a proportion of the IgG, i.e., the portion which possesses antibody activity towards itself.

It appears, then, that the antibody activity in amoebiasis is associated with the IgG fraction of the serum, and this conclusion is in agreement with that of Boonpucknavig & Nairn (1967).

RÉSUMÉ

LE DIAGNOSTIC DE L'AMIBIASE PAR L'IMMUNOÉLECTROPHORÈSE

On a procédé à une étude comparative de la valeur de l'immunoélectrophorèse et de l'épreuve d'hémagglutination en tant que méthodes de diagnostic de l'amibiase. L'antigène a été obtenu à partir d'*Entamoeba histolytica* cultivées sur un milieu contenant également *Clostridium perfringens*.

En cas d'abcès amibiens du foie, 90 sérums sur 93 (96,8%) ont été reconnus positifs à l'immunoélectrophorèse, tandis que 43 sérums sur 47 (91,5%) ont réagi à l'épreuve d'hémagglutination. L'immunoélectrophorèse pratiquée sur des sérums de donneurs de sang a montré un très faible taux de positivité (2 sur 348, soit 0,6%); avec des sérums prélevés chez des sujets souffrant d'affections autres que l'amibiase, des résultats positifs ont été enregistrés dans 7 cas sur 95 (7,4%). Effectuée sur des sérums de sujets appartenant aux mêmes catégories, l'épreuve d'hémagglutination a été positive dans 51 cas sur 313, soit 16,3% (donneurs de sang) et 5 cas sur 29, soit 17,2% (autres maladies). Il semble donc qu'en présence d'un antigène hétérogène, la valeur diagnostique de l'immunoélectrophorèse soit supérieure à celle de l'épreuve d'hémagglutination. Le principal de ses avantages réside dans le fait qu'elle permet de distinguer les arcs de précipitation dus respectivement à l'antigène *E. histolytica* et à l'antigène *Cl. perfringens*.

Chez des malades guéris depuis plus d'un an d'un abcès hépatique amibien, la proportion des résultats positifs à l'immunoélectrophorèse a été très faible (1 sur 6) ce qui montre l'intérêt de la méthode pour le diagnostic différentiel des cas anciens et des cas récents. Dans 6 cas d'amibiase intestinale et dans 2 cas d'amibiase asymptomatique, l'immunoélectrophorèse n'a fourni respectivement que 4 et 0 résultats positifs.

En cas de positivité des sérums, les images immunoélectrophorétiques ont revêtu divers aspects, un arc de précipitation principal localisé près du puits contenant l'antigène étant considéré comme caractéristique de l'amibiase.

L'étude de l'évolution sérologique après traitement montre que les anticorps hémagglutinants et précipitants persistent pendant plusieurs mois, les titres des premiers diminuant cependant légèrement. Chez 5 malades traités pour abcès hépatique, on a noté des modifications des images immunoélectrophorétiques sans variations concomitantes des titres d'anticorps hémagglutinants.

La chromatographie sur colonne permet d'obtenir différentes fractions. L'activité d'anticorps précipitant est concentrée dans la fraction du sérum riche en IgG. En inversant le sens de l'immunoélectrophorèse, on peut prouver que cette activité est attribuable à l'IgG.

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