CELLULOSE ACETATE MEMBRANE PRECIPITIN (CAP) TEST IN AMOEBIASIS USING ANTIGENS FROM FOUR DIFFERENT STRAINS OF *ENTAMOEBA HISTOLYTICA*

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INTRODUCTION

Many serological tests such as immunoelectrophoresis, latex agglutination and skin tests had been developed, and their value in the diagnosis of amoebiasis assessed (Savanat and Chaicumpa, 1969; Savanat *et al.*, 1973 a, 1974). However, there are certain inherent disadvantages in each of the tests that cannot be applied in the field surveys or in poorly equipped laboratories. The cellulose acetate membrane precipitin (CAP) test reported by Stamm and Phillips (1977) has been used in field work so the present study was undertaken to assess the diagnostic value of the CAP test using antigens from 4 different strains of *Entamoeba histolytica*, and the results evaluated in comparison with the immunoelectrophoresis (IEP) test.

MATERIALS AND METHODS

Entamoeba histolytica antigens were prepared from each strain of axenically grown *E. histolytica* namely, HK-9, HT-10, HT-12 and HT-31 according to the method described by Diamond (1968).

Eighty-one sera samples were obtained from patients with amoebic liver abscess admitted to the Hospital for Tropical Diseases, Bangkok, and 100 blood samples from donors from the National Blood Bank were used as control.

Immunoelectrophoresis (IEP) was performed as previously described (Savanat *et al.*, 1973 b).

Cellulose acetate membrane precipitin (CAP) test described by Stamm and Phillips (1977) was used with some modification. A humid chamber was formed by inserting a layer of foam at the bottom of a plastic container. The foam consists of 3 rows with 6 square depressions measuring $2 \times 2 \times 0.2$ cm. Two layers of filter papers with corresponding holes were placed on top of the foam (Fig. 1). A persplex template was constructed

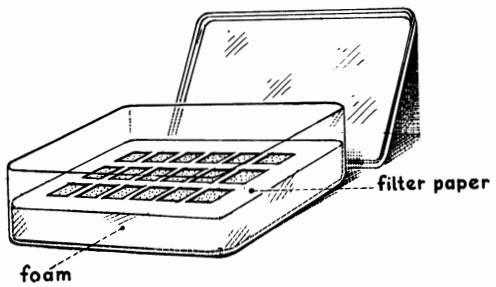

Fig. 1—Moist chamber for incubation of cellulose acetate membrane.

having 6 patterns of 5 holes arranged as described by Stamm and Phillips. Sepharose III cellulose polyacetate membrane strip (Gelman) $1'' \times 6''$ with phosphate buffered saline (PBS) pH 7.2 was placed over the template and depressions were made thereon by gently pressing the membrane above the square holes with a glass rod.

The membrane was lifted from the template and placed over the filter paper on the foam support so that the 5 wells pattern was precisely above the square holes of the filter paper. The centre depression in the cellulose acetate membrane was filled with 3 µl of the

antigen extract from each strain of *E. histolytica* and the peripheral depressions with 5µl of test or control sera using a Finnpipette. The membranes were incubated for four hours at room temperature (28° - 30°C) and the reaction visualised by Nigrosine staining.

RESULTS

A typical result with CAP test is shown in Fig. 2. As many as 3 precipitating bands were demonstrated. Results of CAP and IEP tests against sera from patients with amoebic liver abscess using antigens from 4 different strains of *E. histolytica* are shown in Table 1. The percentage CAP positivity of sera from 81 patients with amoebic liver abscess against the strains HK-9, HT-10, HT-12 and HT-31 were 97.5, 95, 97.5 and 87.7 respectively. Chi square analysis showed that the antigen from the strain HT-31 gave significantly less positive result than that of strain HT-12, and HK-9 ($p < 0.02$). With the IEP test, all these sera were positive against all 4 strains. All sera from 100 blood donors were negative by both CAP and IEP tests. The number of precipitating bands visualised in the CAP test is shown in Table 2. Analysis by paired 't'test showed that the antigen from the strain HT-12 produced significantly higher number of precipitating bands than the antigens from strain HK-9, HT-10 and HT-31. With the

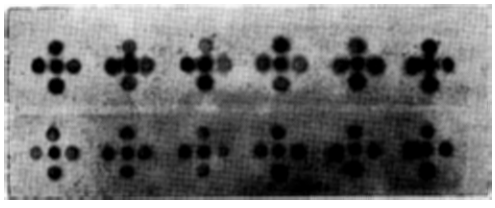

Fig. 2—Typical result with cellulose acetate membrane precipitin (CAP) test.
Upper : patients with amoebic liver abscess.
Lower : normal controls.

Table 1

Comparative CAP and IEP tests in amoebic liver abscess cases with antigens from 4 strains of *E. histolytica*.

Source of the antigen from strains	No. tested	CAP	IEP
		No. positive	No. positive
HK-9	81	79 (97.5)	81 (100)
HT-10	81	77 (95.1)	81 (100)
HT-12	81	79 (97.5)	81 (100)
HT-31	81	71 (87.7)	81 (100)

Percentage positive shown in parenthesis.

Table 2

Number of patients showing precipitating bands in the CAP and IEP tests with antigens from 4 strains of *E. histolytica*.

Source of the antigen from strains	CAP : No. of Bands				IEP : No. of Bands			
	0	1	2	3	0	1-3	4-6	> 6
HK-9	2	41	35	3	0	35	40	6
HT-10	4	34	39	4	0	38	38	8
HT-12	2	29	15	15	0	27	41	13
HT-31	10	50	20	1	0	45	30	6

antigens from strain HK-9 and HT-10, no significant difference in the number of precipitating band was found ($p > 0.5$). The antigens from strain HT-10 but not from the HK-9 strain produced significantly more number of precipitating bands than that of strain HT-31.

In the IEP test, it was found that the number of precipitating bands produced ranged from 1-13 (Table 2). The antigen from strain HT-12 gave significantly higher number of precipitating bands than those from strains HT-10, HK-9 and HT-31. The antigen from strain HT-10 gave higher number of precipitating bands than those from strains HK-9 and HT-31.

DISCUSSION

In a search for an appropriate method for the diagnosis of amoebiasis in the field, CAP as well as latex agglutination tests could be considered as the most suitable technique. The CAP test is technically simple and non expensive and can be used as a substitute for the counterimmunoelectrophoresis test routinely used in many laboratories. Furthermore, the membrane can be impregnated with the antigen and still show precipitin arcs when tested with positive sera after storage at 37°C or at room temperature for 4 months or at 4°C for 16 months (Stamm and Phillips, 1977). This CAP test was shown by Stamm and Phillips to be more sensitive than the gel diffusion test since as many as 34 sera from patients with amoebiasis were positive but negative in the gel diffusion test, and the reverse was not observed. In the present study, the percentage positivity in the CAP test was between 87.7 to 97.5 depending on the strains used. The test was slightly inferior to the IEP test in which all 81 sera tested were found to be positive.

Comparative study of different strains of *E. histolytica* to be used as antigen in the CAP test revealed that strains HT-12, HT-10 and HK-9 were equally suitable for the diagnosis of amoebiasis ($p > 0.5$), but the antigen from strains HT-12 and HK-9 gave significantly higher positive result than that of HT-31. The strain HT-12 gave a significantly larger number of precipitating bands than other strains. Thus, on the basis of the sensitivity of the CAP test and the number of precipitating bands produced, the HT-12 appears to be most suitable and should be selected for use in future studies.

In the IEP test, all 4 strains were equally sensitive as evident by equal percentage of positivity upon testing with sera from patients with amoebic liver abscess. However, there was a difference in the number of precipitating arcs produced, highest with strain HT-12 followed by strain HT-10 and HK-9 which was indistinguishable from strain HT-31. This result was at variance from that reported by Chang *et al.*, (1979) who showed in the two-dimensional immunoelectrophoresis test against homologous rabbit antisera 32 and 20 precipitating arcs against the antigens from strains HT-31 and HK-9 respectively. This discrepancy could be attributable to the difference in the antisera used. It is possible that the patients equally showed antigenic components of these 2 strains even though the numbers vary. For the routine diagnosis test, the antigen from strain HT-12 is recommended.

SUMMARY

A simple cellulose acetate membrane precipitin (CAP) test was evaluated against immunoelectrophoresis (IEP) test using saline extract from 4 different strains (HK-9, HT-10, HT-12 and HT-31) of axenically grown *Entamoeba histolytica* as the antigens. All 81 sera from patients with amoebic liver abscess were positive in the IEP test against the antigens from all 4 strains.

With the CAP test the number positive against antigens from HK-9, HT-10, HT-12 and HT-31 were 79, 77, 79 and 71 respectively Sera from 100 blood donors were negative by both IEP and CAP tests against antigens from all 4 strains. Comparison between the number of precipitating bands demonstrated by either IEP or CAP test showed that strain HT-12 was the best source of antigen in exhibiting significantly more number of precipitating bands, strain HT-31 was the poorest. The strain HT-10 was comparatively superior to strain HT-31 in the CAP test whereas in the IEP test strain HK-9 and HT-31 were both inferior to strain HT-10.

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