There is little, however, to recommend AFP determinations over ordinary clinical and biochemical parameters in assessing minor response to therapy. The AFP level (together with the size of the liver and pulmonary metastases) may prove to be a useful objective indication of response and should be of considerable value if complete remission is achieved.

**DISCUSSION**

Since the AFP level always fell when a clinical response to treatment occurred, albeit of short duration, it appeared that the AFP-secreting parts of the tumour were at least as sensitive as other regions of the tumour and probably also underwent regression when the liver size decreased. The opposite situation has not yet occurred, viz. that AFP secretion has continued despite tumour regression related to therapy. The problem of treating a tumour that is often multifocal is obviously a difficult one. We have not yet been able to show that AFP levels are higher in one part of the tumour than another. In fact, at autopsy the serum is usually found to have the highest level of AFP: the various nodules in the liver have almost identical lower values, the residual non-tumorous liver slightly lower and lung metastases and other organs the lowest. Obviously contamination with blood and imbibition of serum are the factors determining these results and real differences will only be shown by demonstrating actual AFP synthesis in the tumour. This has been attempted by Hull using diethylnitrosamine-induced tumours in monkeys.

Our experience to date in treating primary liver cancer is that no treatment has produced true remission. The administration of methotrexate (but not 5-fluorouracil) directly into the hepatic artery shows the greatest promise. The next best regimen is probably radiotherapy, which is, of course, much easier technically and could possibly be improved with homogeneous, high-energy irradiation techniques.

It is fairly obvious, however, that the lack of response to treatment is determined largely by the late stage at which the disease is seen. There are few symptoms which might cause the patient to present early in the disease, since Bantu patients are often insensitive to their own hepatomegaly if it is not painful.

The only course of action remaining then, if cases are to be detected early, is to look for the disease in the healthy but vulnerable population. A project is currently under way in which all new recruits to a large mine are being examined for hepatomegaly and screened by a sensitive radio-immunnoassay for AFP. It is difficult at present to envisage the correct clinical management of a patient in whom the diagnosis is made early.

**SUMMARY**

Serum AFP levels in primary cancer of the liver are occasionally affected by therapy and have always fallen when a clear-cut clinical response was obtained. It might therefore be of value to use the AFP level for determining comparative effectiveness of various therapeutic agents and for gauging remission or relapse of the tumour.

We wish to thank Prof. J. H. S. Gear, Director of the South African Institute for Medical Research; Prof. J. F. Murray, Deputy Director; Dr A. M. Coetzee, Director of Medical Services of the Rand Mines Group; and the remaining members of the South African Primary Liver Cancer Research Group. This project was supported by the Council for Scientific and Industrial Research, the South African Medical Research Council and the Atomic Energy Board.

**REFERENCES**


**A RAPID LATEX-AGGLUTINATION TEST FOR INVASIVE AMOEBIASIS**

M. N. Morris, Ph.D., S. J. Powell, M.D., M.R.C.P. (Edin.) and R. Elsdon-Dew, M.D., F.R.S.S.A.F.,

Amoebiasis Research Unit, Institute for Parasitology, Durban

In this paper we present a brief preliminary report on a simple amoebic latex-agglutination slide test. Excellent correlation was observed with results obtained from the well-established gel-diffusion test applied to 250 consecutive sera sent to this laboratory for the diagnosis of invasive amoebiasis. A more extensive study involving detailed clinical evaluation is in progress and will be reported later. It is hoped that this test will meet the demand for a simple and reliable means of diagnosing invasive amoebiasis.

Since the introduction and evaluation of the amoebic gel-diffusion precipitin test, this technique has been extensively used in Durban. In the year 1969, for example, it was applied to 4 800 sera at the Institute for Parasitology as a routine diagnostic service to local hospitals. It has proved a reliable and sensitive index of past or present tissue invasion by *Entamoeba histolytica*. Clinical correlation has shown that 98% of proved cases of amoebic liver abscess and 96% of proved amoebic dysentery gave a positive reaction with this test, against a background of 16% in Bantu general hospital cases with no clinical evidence of amoebiasis and less than 1% in White blood donors. At King Edward VIII Hospital, Durban, where invasive amoebiasis is common and still carries a not insignificant fatality rate, the gel-diffusion test has proved to be a most valuable serological method for the differential diagnosis of diseases encountered in the wards.

The amoebic gel-diffusion test is unfortunately a specialized technique beyond the scope of most hospital or clinical laboratories for a number of reasons—the main drawback being the difficulty of antigen production.
Another factor detracting from the value of this test is the time taken for the diffusion process. In most cases results are only available after 48 hours. In a few cases, however, a positive result may be given after 24 hours; occasionally the test has to be repeated, which means that results are only available 96 hours after receipt of the specimen. Consequently in recent years attempts have been made to develop a rapid, simple and reliable diagnostic aid suitable for widespread use.

It is hoped that the amoebic latex-agglutination test presented here will satisfy these requirements.

MATERIALS AND METHODS

Antigenic extract was prepared from *Entamoeba histolytica* grown in axenic culture by the method of Diamond. The amoebae were harvested and washed by gentle centrifugation in 0·1 M-mercaptosuccinic acid. Approximately 1 ml of packed cells was suspended in 10 ml distilled water and gently disrupted in a Dounce homogenizer. The slurry was centrifuged at 35 000 × g to give a clear amber-coloured supernatant which was dispersed into 1-ml portions and freeze-dried for future distribution and storage.

The sera tested were taken from a series of 250 consecutive specimens sent to this laboratory from King Edward VIII Hospital, Durban, for the amoebic gel-diffusion test. A clear glass slide was ruled with a wax pencil to give squares of approximately 2 cm sides. To perform the test, antigenic extract was added to a commercially available latex suspension to give a volume suitable for one day's use. One drop of this sensitized latex was placed in each square on the glass slide from a Pasteur pipette. One drop of serum was then placed alongside each latex drop. The two drops in each square were then thoroughly mixed by means of a piece of orange stick and the slide was rocked gently by hand for 5 minutes. The state of agglutination was then observed with the aid of a low-power microscope.

A negative reaction was recorded where the latex remained as an opaque homogeneous suspension. A positive reaction was recorded where the latex had agglutinated, however small the aggregates. Amoebic gel-diffusion tests were performed on the sera in the routine manner.

RESULTS

The appearance of the amoebic latex-slide test for two extreme cases is shown in Fig. 1. The reactions with 250 consecutive sera tested are recorded in Table I. Serological diagnosis appears to differ in only 8 of the 250 consecutive cases tested which presented with symptoms compatible with amoebiasis on admission. In 3 cases the latex test was negative where gel diffusion was positive and in 5 cases latex was positive with gel diffusion negative.

### TABLE I. COMPARISON OF RESULTS OF AMOEBIC LATEX-AGGLUTINATION AND GEL-DIFFUSION TESTS WITH 250 CONSECUTIVE CASES RECEIVED FOR DIAGNOSIS OF INVASIVE AMOEBIASIS

<table>
<thead>
<tr>
<th>Classification of cases</th>
<th>Total</th>
<th>Latex</th>
<th>Gel</th>
<th>Positive Presentations encountered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dysentery</td>
<td>66</td>
<td>24</td>
<td>25</td>
<td>Bloody stool, diarrhoea</td>
</tr>
<tr>
<td>Liver specifically</td>
<td>88</td>
<td>44</td>
<td>43</td>
<td>Tender hepatomegaly</td>
</tr>
<tr>
<td>Abdominal generally</td>
<td>42</td>
<td>10</td>
<td>11</td>
<td>Peritonitis, palpable mass</td>
</tr>
<tr>
<td>Thoracic generally</td>
<td>16</td>
<td>5</td>
<td>4</td>
<td>Pleural effusion, pericarditis</td>
</tr>
<tr>
<td>Other conditions</td>
<td>38</td>
<td>7</td>
<td>8</td>
<td>Headache, pyrexia, anaemia</td>
</tr>
</tbody>
</table>

### DISCUSSION

It is apparent that the correlation between the results of the amoebic latex-agglutination test and the amoebic gel-diffusion test is excellent when it is considered that the 250 sera examined in this brief preliminary study represent an extremely heterogenous sample drawn from all types of cases presenting histories compatible with a diagnosis of invasive amoebiasis. Possibly the most valuable application of these serological tests in making a differential diagnosis is the preliminary exclusion of invasive amoebiasis which is implied by a negative result. It must be remembered that a serological test is an aid to diagnosis; it is not an end in itself. A detailed clinical evaluation of the amoebic latex-agglutination test confined to well-documented cases is in progress.

The new test carries the advantage of being an exceedingly simple and rapid procedure. If necessary a result could be reported within 10 minutes of a specimen being received in the laboratory. It is felt that this test is suitable for widespread use, even where laboratory facilities are minimal.

Amoebae are being cultured in this laboratory at present. The amoebiasis Research Unit is sponsored by the South African Medical Research Council, the Natal Provincial Administration, the University of Natal, and the US Public Health Service (Grant AI 09654-01).

REFERENCES


Fig. 1. Amoebic latex-agglutination test on sera from a case of typhoid (on left) and proved amoebic liver abscess (right).