An evaluation of an agar gel diffusion test with crude and purified antigens in the diagnosis of hydatid disease *

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The sensitivity of the indirect haemagglutination test for the diagnosis of hydatid disease, although high, is insufficient. Agar gel diffusion tests for the diagnosis of this disease have not received much attention in the past and have been considered unsatisfactory. The authors propose such a test and evaluate its results in comparison with those of the indirect haemagglutination test.

Besides the intradermal skin test, the serological methods commonly employed for the diagnosis of hydatid disease are the complement fixation test, the indirect haemagglutination test, the latex-agglutination test, and the indirect fluorescent antibody test (1).

The test of choice appears to be the haemagglutination test introduced by Garabedian et al. (2). Its sensitivity, as reported by various authors (1), varies from 66% to 100%, with an average of approximately 83%. Furthermore, nonspecific reactions have been reported for this test, together with lower sensitivity for the sera of patients with hydatid lung cyst (1).

On the other hand, tests based on the detection of precipitating antibodies are not commonly used. The older precipitin test and the agar gel diffusion test have been judged to be inadequate (1, 3). Immunoelectrophoresis performed with concentrated serum has given better results, with a positivity rate of 57–92% (4–7).

The present paper reports on the evaluation of a simple agar gel diffusion (AGD) test with crude and purified hydatid antigens for the diagnosis of hydatid disease in comparison with the indirect haemagglutination (IHA) test.

MATERIALS AND METHODS

Sera

Blood samples were obtained from 58 persons with clinically diagnosed hydatidosis, confirmed in most cases by surgery. Furthermore, the serum of 13 patients with hepatic cirrhosis, 12 with lung tumours, 4 with pulmonary tuberculosis, 4 with chronic bronchitis, and 5 with silicosis was tested. In addition, serum from 32 normal persons was tested by the AGD test against crude concentrated hydatid fluid.

Antigens

Four different antigen preparations were used: concentrated crude hydatid fluid, a purified hydatid fluid fraction containing antigens A and B (8), isolated antigen B, and a polysaccharide antigen from the hydatid membrane (9).

Concentrated hydatid fluid. Sheep hydatid fluid was dialysed for 24 h against distilled water and concentrated 5-fold by ultrafiltration (Hollow Fibers, Bio-Rad Laboratories, Richmond, CA 94804, USA). An average nitrogen content of 700 mg/litre was found

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by a modified ninhydrin method (10). The concentrated hydatid fluid was used as such for the AGD test and was diluted to a concentration of approximately 10 mg/litre for the IHA test.

Hydatid fluid fraction (antigens A and B). This fraction was obtained according to the procedure described by Oriol et al. (8), with minor modifications. Hydatid fluid extracted from cysts was dialysed against acetate buffer, pH 5.0, 0.005 mol/litre, the precipitate was redissolved in phosphate buffer, pH 7.2, 0.15 mol/litre and the host globulins were removed by DEAE–Sephadex chromatography in Tris-HCl buffer, pH 6.5, 0.05 mol/litre, with a molarity range of potassium chloride 0–1 mol/litre. The final purified fraction contains two major parasite antigens designated as A and B according to Oriol et al. (8). A third parasite component may be detected in the serum of only a few patients (6). The preparation used in the immunoelectrophoresis test had an average nitrogen content of 500 mg/litre.

Antigen B. Antigen B, which is thermostable and insoluble in water, was separated from the previous fraction (antigens A and B) by extraction with 90% phenol. The antigen is found in the phenol phase, from which it can be precipitated by dialysis against distilled water. The precipitate was redissolved in phosphate buffer, pH 7.2, 0.15 mol/litre. The preparation used for the immunoelectrophoresis test had an average nitrogen content of 100 mg/litre.

Hydatid membrane polysaccharide antigen. The isolation and characterization of this antigen has been described by Russi et al. (9). The preparation used in the present study had the following composition: nitrogen, 5.6%; galactose, 51.8%; *N*-acetylglucosamine, 4.7%; *N*-acetylgalactosamine, 13.7%. A 200-mg/litre solution was used for the immunoelectrophoresis test.

Immunodiffusion and immunoelectrophoresis

Agar gel diffusion was performed in Petri dishes 10 cm in diameter. Each plate was filled with 25 ml of a solution containing 7 g of agarose per litre in barbital–glycine-buffered saline, pH 7.6. Wells 7.5 mm in diameter, with centres 11 mm apart, were cut. Immunoelectrophoresis was performed by the micromethod of Scheidegger (11), with agarose at a concentration of 10 g/litre in 0.05 mol/litre barbital buffer, pH 8.6. Antiserum against sheep hydatid fluid was prepared in rabbits according to Kagan et al. (12).

Indirect haemagglutination test

Indirect haemagglutination tests were performed with a Takatsy microtitrator (Cooke Engineering Co., Alexandria, VA, USA). Sheep blood cells were treated with a 1 : 20 000 dilution of tannic acid for 10 min. at 37°C and subsequently coated with a 1 : 30 to 1 : 100 dilution of concentrated hydatid fluid. All the serum specimens were absorbed with sheep blood cells and 2-fold dilutions were performed in phosphate saline with 1% rabbit serum, preabsorbed with sheep blood cells. Titres below 1 : 200 were considered to be negative (1).

RESULTS

When the serum of patients with hydatid disease was tested by the AGD test with crude concentrated hydatid fluid, different patterns of precipitation were obtained. Fig. 1 (A) shows the result of one such test. It is evident that with the same hydatid fluid preparation some serum specimens produce several well-defined lines of precipitation, whereas others produce only one or a few faint lines, or do not react at all. The specimens that showed the highest reactivity were chosen as reference sera for the standardization of hydatid fluid preparations and one reference serum was always included in the test whenever serum of unknown source had to be tested. Fig. 1 (B) shows the patterns of precipitation of 5 hydatid fluid preparations with one reference serum. Only the preparations with the highest reactivity were used in all subsequent tests.

Fig. 2 shows immunoelectrophoresis patterns of the purified fractions with whole hydatid fluid rabbit antiserum and with human serum in comparison with the original concentrated crude hydatid fluid. Several lines of precipitation, of both host and parasitic origin, are detected by rabbit antiserum in the crude hydatid fluid, whereas the purified fraction obtained according to Oriol et al. (8) contains antigens A and B only and is devoid of host components. A third component of parasitic origin with greater mobility was also detected with the serum of a few patients, as was found by Williams et al. (6). Moreover, we found antigen B to be pure, as shown by immunoelectrophoresis with rabbit antiserum. The hydatid membrane polysaccharide antigen gave also only one line of precipitation, slightly to the cathode side of the origin, with whole hydatid fluid rabbit antiserum and with the sera of some patients, as described by Russi et al. (9).

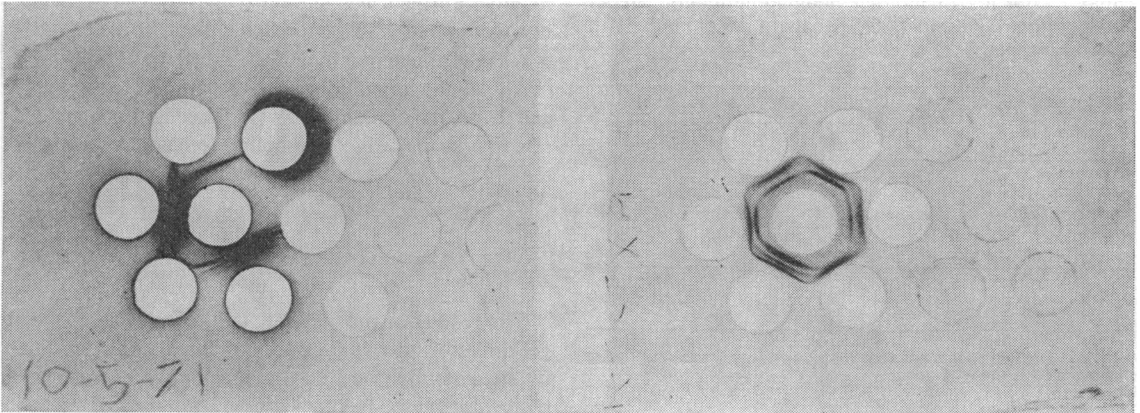

Fig. 1. Agar gel diffusion for the diagnosis of hydatid disease.

A. Centre well, standardized hydatid fluid preparation; peripheral wells, different human hydatid sera.

B. Centre well, reference human hydatid serum; peripheral wells, different hydatid fluid preparations.

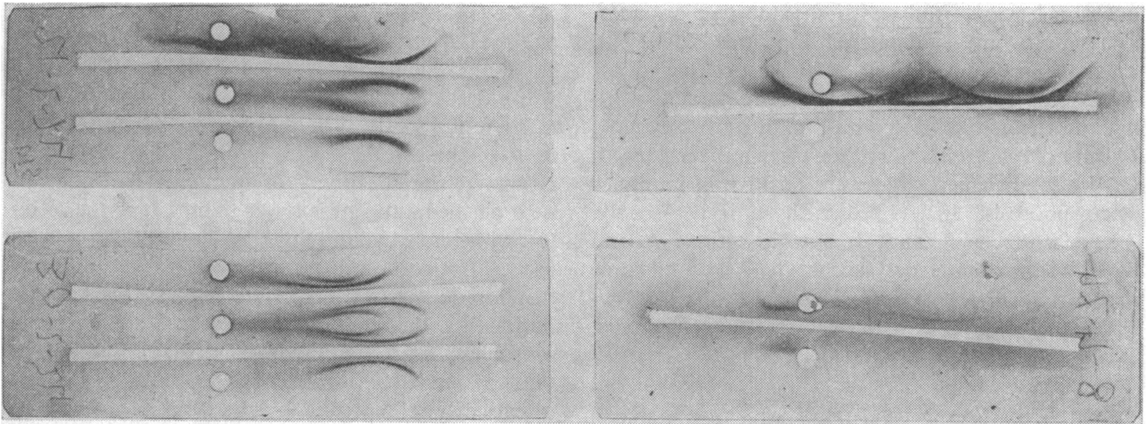

Fig. 2. Immunoelectrophoresis of hydatid fractions.

A. Antigens: Upper well, concentrated hydatid fluid; centre well, antigens A and B; lower well, antigen B.
Sera: Top slide, whole hydatid fluid rabbit antiserum; bottom slide, human serum (PDG).

B. Antigens: Upper well, concentrated hydatid fluid; lower well, membrane polysaccharide.
Sera: Top slide, whole hydatid fluid rabbit antiserum; bottom slide, human serum (RS).

Table 1 compares the results of the AGD and IHA tests in 58 patients with hydatid disease. Of the 29 patients with hydatid disease of the liver, 21 (72%) gave positive results in the IHA test and 24 (83%) in the AGD test. Of the 20 patients with hydatid disease of the lung, 13 (65%) gave positive results in the IHA test and 17 (85%) in the AGD test. Of the 9 patients with hydatid disease with multiple localizations, 7 (78%) gave positive results in the IHA test and 8 (89%) in the AGD test. Altogether 41 patients (71%) gave positive results in the IHA test, whereas 49 (85%) were positive in the AGD test.

Of the 38 patients suffering from illnesses other than hydatid disease, none gave positive results in the IHA test, whereas the serum of one patient with pulmonary tuberculosis produced a faint line of

Table 1. Results of indirect haemagglutination (IHA) and agar gel diffusion (AGD) tests in 58 patients with hydatid disease [a]

Cyst localization	No. of cases	IHA		AGD	
		No.	%	No.	%
hepatic	29	21	72.4	24	82.8
pulmonary	20	13	65.0	17	85.0
multiple	9	7	77.7	8	88.9
Total	58	41	70.7	49	84.5

[a] The 41 serum specimens that were positive in the IHA test were positive also in the AGD test.

precipitation in the AGD test. The serum of all the 32 normal persons gave negative results in the AGD test.

The 49 serum specimens that had produced positive results in the AGD test were further analysed by immunoelectrophoresis with the purified antigens. The results are summarized in Table 2. In all, 35 serum specimens (71% of those positive in the AGD test and 60% of those from patients with hydatid disease) reacted with one or more antigens; 14 did not react with any of the three antigens tested. Of the 35 specimens that were positive in immunoelectrophoresis, 18 reacted with antigen A only, 7 with antigens A and B, 6 with antigen A and with the membrane polysaccharide, 2 with all three antigens, one with antigen B and with the polysaccharide, and one with antigen B only. The most reactive antigen was shown to be antigen A, which was detected by 33 serum specimens, corresponding to 67% of those positive in the AGD test and to 57% of those from patients with hydatid disease. Antigen B and the membrane polysaccharide were less reactive than antigen A, being detected by approximately 20% of the serum specimens positive in the AGD test.

Table 2. Results of electrophoresis with purified hydatid antigens for 49 serum specimens that were positive in the AGD test

Serum specimens		Positive to antigen(s) : [a]
No.	%	
18	36.7	A only
7	14.3	A and B
6	12.2	A and MP
2	4.1	A, B, and MP
1	2.0	B and MP
1	2.0	B only
14	28.6	Neither A, nor B, nor MP

[a] In all, 33 serum specimens (67.3%) were positive to antigen A; 11 (22.5%) were positive to antigen B; and 9 (18.4%) were positive to the membrane polysaccharide antigen (MP).

DISCUSSION

The results of the present study show that the agar gel diffusion test with concentrated hydatid fluid can be successfully used in the serological diagnosis of hydatid disease. In our series of 58 cases of hydatid disease, the test gave a positive result in 85%, whereas the IHA test was positive in 71% of cases. It would appear to be useful, therefore, to perform the AGD test together with the IHA test.

The rate of nonspecific reactions to the AGD test is low (2.6%) compared with rates reported by others (1) for the IHA test. We found the specificity of the IHA test to be absolute, since all titres below 1 : 200 were considered as negative (1).

Immunoelectrophoretic analysis of serum specimens found positive in the AGD test showed that most of these reacted with hydatid fluid antigen A. However, the sensitivity of immunoelectrophoresis with purified antigens is lower than that of the AGD test with crude hydatid fluid. Williams et al. (6), who used thrice-concentrated serum in an immunoelectrophoretic test against crude hydatid fluid and a purified fraction containing antigens A and B, found a decrease in positivity from 57 to 50%. The finding that the sensitivity of serodiagnostic tests in hydatid disease is not improved by the isolation of specific parasite antigens is not unexpected. Indeed, parasitic infections cannot be compared to microbial infections, in which the same antigenic components are generally present throughout the course of the disease. In the case of hydatidosis and other parasitic diseases, the parasite penetrates into the host as an egg and undergoes a whole process of differentiation into a cyst form with sequential developmental stages. It should be investigated whether a correlation exists between the hydatid cyst stage and the reactivity pattern of the serum against different purified antigens. Moreover, each antigen should be tested by different serological tests in order to find out the most suitable test for obtaining higher positivity. Indeed, the positivity rate of 9 out of 58 (16%) found in this study for the membrane polysaccharide antigen in the immuno-

electrophoretic test increases to 50% and 70%, respectively, when the same antigen is tested by quantitative precipitin analysis (9) and by the indirect haemagglutination technique (A. Siracusano et al., unpublished data, 1974). In the light of these observations, the exclusive use of purified hydatid antigens cannot be advocated for routine use in diagnostic procedures (6).

On the basis of our findings, it appears that the AGD test, performed with a properly standardized hydatid fluid antigen, is a highly specific and sensitive test for the diagnosis of hydatid disease.

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RÉSUMÉ

ÉVALUATION D'UNE ÉPREUVE DE DIFFUSION EN GEL DE GÉLOSE À L'AIDE D'ANTIGÈNES SIMPLES OU PURIFIÉS DANS LE DIAGNOSTIC DE L'HYDATIDOSE

On a fait une étude comparative de l'épreuve classique d'hémagglutination indirecte et d'une épreuve de diffusion en gel de gélose chez 58 malades atteints d'hydatidose et 38 malades souffrant d'autres affections.

Chez les porteurs de kystes hydatiques, le pourcentage moyen de sérums positifs a été de 71% par l'épreuve d'hémagglutination et de 85% par l'épreuve de diffusion. Les sérums des 32 sujets témoins se sont tous révélés négatifs à l'épreuve de diffusion.

Les 49 sérums positifs en épreuve de diffusion ont été examinés en outre par immunoélectrophorèse: 18 (36,7%) ont réagi avec l'antigène A seul; 7 (14,3%) avec les antigènes A et B; 6 (12,2%) avec l'antigène A et l'antigène MP (antigène polysaccharidique extrait de la membrane hydatique); 2 (4,1%) avec les trois antigènes; 1 (2%) avec les antigènes B et MP; 1 (2%) avec l'antigène B seul. Quatorze sérums (28,6%) n'ont réagi avec aucun antigène.

De ces résultats on peut conclure que l'épreuve de diffusion en gel de gélose, pratiquée avec un antigène convenablement normalisé, est une épreuve sensible pour le diagnostic de l'hydatidose humaine.

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