

## Recent Advances in the Diagnosis of Hydatidosis (1970-1976)\*

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ABSTRACT. In this extensive review experiences reported from 1970 to 1976 on the diagnosis of infection with Echinococcus granulosus and E. multilocularis in humans by immunodiagnostic procedures (complement fixation test, indirect hemagglutination test, latex test, bentonite flocculation test, immunofluorescence test, enzyme-linked immunosorbent assay, agar gel methods, radioimmunoassay, intradermal test, etc.) are evaluated. There are 144 references.

The cosmopolitan distribution of Echinococcus granulosus, the circumpolar distribution of E. multilocularis, the discovery of at least two other species of Echinococcus (E. oligarthrus and E. vogeli) in Central and South America, and the recognition of at least nine biological strains of E. granulosus have resulted in a continued and vigorous effort in many parts of the world to develop and evaluate more efficient antigens and more sensitive and specific immunologic procedures (KAGAN, 1973a) for the diagnosis of human diseases caused by these organisms. The literature on immunodiagnosis has been reviewed a number of times (KAGAN, 1968, 1970, 1973a, 1973b, 1976; APT, 1975). Although the last review appeared in 1976, it covered the scientific literature on immunodiagnosis from 1968 to 1972. This review covers the literature from 1970 to 1976.

### Complement-fixation test (CFT)

The CFT is still used in various parts of the world. The test, however, has a number of serious deficiencies. The sensitivity of the test appears to be highly correlated with the quality of the antigen used. The test also yields very high levels of nonspecific reactions with sera from patients with various types of cancer. For increased sensitivity and specificity, more than one antigen should be employed and more than one dilution of antigen. The recent literature on the CFT is presented in Table 1.

### Indirect hemagglutination test (IHAT)

The IHAT continues to be used both for diagnosis and for epidemiologic studies. The technique has been stabilized by use of various fixing agents, and stable sensitized cells can be lyophilized or stored frozen for long periods. The sensitivity of all serologic techniques varies from country to country and is probably influenced by the location of the cyst, the duration of infection, and the type of Echinococcus endemic in the area (KAGAN, 1973, 1973b). VARELA-DIAZ et al. (1975a) have carefully evaluated a number of variables that influence the test. Its specificity must be controlled very carefully because of the high level of nonspecific reactivity reported. This has led some workers to recommend the latex test (LAT)

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over the IHAT for seroepidemiologic studies (VARELA-DIAZ et al., 1976). For maximum sensitivity and specificity, the use of a companion test is recommended. The recent literature is reviewed in Table 2.

#### Latex test (LAT)

Because of the simplicity, stability, and specificity of the LAT, the technique has been used both for diagnosis and epidemiologic studies. GUI SANTES and VARELA-DIAZ (1975c) recommend the LAT as a screening procedure and recommend that the positive specimens be confirmed by a more specific technique such as immunoelectrophoresis (IEP) or double diffusion in gel (DDG). There are, however, inherent difficulties in the use of the LAT. The latex particles must be standardized to size and chemical composition. The specificity of the test also appears to vary from country to country. Thus in Argentina, the specificity is satisfactory and the test has been recommended for seroepidemiologic screening, but in Egypt, high levels of nonspecific cross reactivity with the sera of patients with schistosomiasis have been reported (BOTROS et al., 1973). Russian workers have used the technique extensively. The recent literature is reviewed in Table 3.

#### Bentonite flocculation test (BFT)

The BFT, like the LAT, is technically uncomplicated once one has become experienced in preparing the particles and reading the test. It is a test of low reactivity (KAGAN, 1974) and is therefore excellent as a companion test with a technique of high reactivity such as IHAT. For this reason, the IHAT and BFT are used for diagnosis of hydatid infection in the writer's laboratory.

Relatively little has been published on the test in recent years. KNIERIM et al. (1971) compared the BFT with the IHAT and found the former to be less sensitive. In Egypt, BOTROS et al. (1975) found the BFT to be more specific than the IHAT. MAHAJAN and CHITKARA (1975) evaluated the BFT, IHAT, and IDT in 50 normal individuals and 44 persons with hydatid disease. The nonspecific serologic response with the normal sera was 14% and 8% and 16% for the BFT, IHAT and IDT, respectively. With persons with hydatid disease, the sensitivity of the BFT was 91%; of the IHAT, 86%; and of the IDT, 73%.

#### Immunofluorescence test (IFT)

Because the IFT is technically difficult to perform and because other equally sensitive and specific tests are available, the IFT has not been widely used in the diagnosis of hydatid disease. The technique, however, offers certain advantages that make it attractive for diagnosis and postoperative evaluation. The use of class-specific immunoglobulin conjugates for the detection of IgM and IgG is a plus for this method. The rapid decline in antibody titer within a year after a cyst is removed was carefully evaluated by AMBROISE-THOMAS (1976). The recent literature is reviewed in Table 4.

#### Enzyme-linked immunosorbent assay (ELISA)

A new technique employing enzymes conjugated to antibody or antigen to detect and measure antigens or antibodies, respectively, is finding wide application in the diagnosis of parasitic diseases (VOLLER et al., 1976). FARAG et al. (1975), working in France, employed whole and purified hydatid antigens and evaluated the test with sera from 43 patients with proven hydatid disease. The purified antigen gave both more sensitive and specific results than whole hydatid fluid antigen. Whole hydatid fluid antigen gave nonspecific results with sera from patients with taeniasis, schistosomiasis, and fascioliosis. The purified "Band 5" antigen of BOUT et al. (1974) gave no nonspecific results. CAPRON et al. (1975) reviewed the use of ELISA in parasitic diseases.

The ELISA technique combines the sensitivity of radioimmunoassay with the technical simplicity of the IHAT. With purified or stage-specific antigens, the technique is most sensitive and specific. The technique will certainly play an important role in the immunodiagnosis of parasitic diseases and warrants further evaluation for the diagnosis of hydatid infection.

## Agar gel methods

Among the agar gel methods employed, double diffusion in gel (DDG), immunoelectrophoresis (IEP), and countercurrent electrophoresis (CEP) are the three most popular. GUI SANTES and YARZABEL (1971) evaluated the sensitivity of DDG, and found the technique to be more sensitive than IEP but had a higher rate of nonspecific results. CONTRERAS and KNIERIM (1974) obtained the opposite results. BOMBARDIERI et al. (1974), employing agarose in a barbital-glycine-buffered saline, tested 4 antigens and 58 sera in an Ouchterlony plate method. The sensitivity of the DDG was compared to that of the IHAT. The DDG test was reactive with 49 (85%) of the sera, whereas the IHA reacted with 41 (71%). In the immunoelectrophoresis (IEP) test with 49 sera positive by DDG, 14 (28.6%) did not show "Band 5." The antigens used were concentrated hydatid cyst fluid, a purified lipoprotein antigen A and B (ORIOLE et al., 1971), isolated antigen B, and a polysaccharide antigen (RUSSI et al., 1974). The most reactive antigen was antigen A. GUI SANTES and VARELA-DIAZ (1975) evaluated 25 sera by DDG. They reported 100% sensitivity and specificity. GUI SANTES and YARZABEL (1975) evaluated DDG against IEP and found a higher sensitivity for the former (88.7%) against 82.2% for the latter. The nonspecific response was 20.9%.

Immunoelectrophoresis (IEP) is the test of choice for demonstrating the specific band 5 of *E. granulosus* infections. Since publication of the classic paper on diagnosis by IEP by CAPRON et al. (1967), the test has been evaluated in numerous studies (Table 5). Several points have emerged. Band 5 is not found in all sera from patients with *E. granulosus* infection. Band 5 may be a complex of bands (VARELA-DIAZ et al., 1975c). The test has been carefully standardized (GUI SANTES et al., 1975). Because of the specificity of IEP, when band 5 can be identified, many workers recommend this method as the diagnostic method of choice. The recent literature on IEP is outlined in Table 5.

Countercurrent electrophoresis (CEP), which has been called cross-over electrophoresis, counter immunoelectrophoresis, electrosineresis, and immunoelectrodiffusion, is essentially diffusion in agar in an electrical field. This technique has all the advantages of DDG with the added advantage that the reaction can be completed in from 15 minutes to several hours, depending on the antigen used. Some workers find the method slightly more sensitive than IEP. One of the drawbacks of CEP is its inability to readily recognize Band 5. The technique can be performed on plates coated with agarose or with cellulose acetate membranes (GENTILINI and PINON, 1972). Using CEP, PINON (1976) claims he can detect from the characteristic "glove finger" pattern of the bands whether or not the cyst is ready to rupture. Another major advantage of CEP is that, compared to IEP, it requires less concentrated antigens. CEP has been used routinely in France (PINON, 1976), with excellent results. The recent literature is listed in Table 6.

## New immunologic methods

A solid phase radioimmunoassay (RIA) for the diagnosis of hydatidosis was reported by MUSIANA et al. (1974). This technique employed  $^{125}\text{I}$  radioidinated human anti-EA binding reaction. The sensitivity of RIA is well recognized, but the technique requires purified antigen (which is available for hydatid disease) and rather expensive specialized equipment for handling labelled materials. The ELISA method, however, combines the principle of RIA and IFT and does not require specialized equipment. For this reason, the RIA procedure will probably not be widely used.

A host lymphocyte transformation test with echinococcus antigens (EA) was reported by YUSUF et al. (1975). Lymphocytes from patients with hydatidosis showed statistically significant lymphocyte transformation with human and sheep hydatid fluid or scolex antigens when compared to lymphocytes from normal individuals. Patients with high results on lymphocyte transformation tests had low serologic titers by IHAT's. *In vitro* tests for the detection of cell mediated immunity (CMI) in parasitology have not been widely used. Blast transformation is just one of many techniques that may become part of the diagnostic armamentarium.

A possible extension of the IFT may be the adaptation of the use of defined antigen substrate spheres (DASS) to the diagnosis of hydatid disease. This technique was initially developed for the diagnosis of schistosomiasis (DEELDER and PLOEM, 1974) and is based on the

principle of coupling antigen to sepharose beads which serve as the antigen substrate. In 1975, DEELDER and STREEFKERK extended the usefulness of the test by combining the principle of the ELISA test with the use of beads, which eliminates the need for a fluorometer and substitutes the development of a color which can be read by the eye. The DASS method merits evaluation for the diagnosis of hydatid infections.

### Antigens

ORIOLE et al. (1971) and PAULUZZI and DOTTORINI (1972) have made great strides in purifying and characterizing antigens. These two groups purified lipoprotein antigens (ORIOLE et al., 1971) from hydatid fluid which reacted preferentially in diagnostic tests (WILLIAMS et al., 1971) but did not have the same sensitivity as the crude antigens. The major breakthrough, however, is the isolation of Band 5 antigen from hydatid fluid by BOUT et al. (1974). This antigen turned out to be similar to antigen A, which ORIOLE et al. (1971) isolated and BOMBARDIERI et al. (1974) found to be the most reactive antigen in agar gel diffusion (AGD). The specific Band 5 antigen is a lipoprotein of molecular weight (MW) 60 000. POZZUOLI et al. (1974) isolated a protein of 400 000 MW and one of 150 000 MW. The 400 000 MW was more immunoreactive than the 150 000 MW protein. With the isolation of the specific Band 5, a specific antiserum has been made. We have used this antiserum, kindly supplied by Dr. CAPRON, and have identified the specific band in our control serum used in DDG. PINON (1976) has used this serum to identify the specific band in CEP. The recent literature is listed on Table 7.

### Immunoglobulins

Using the radioallergosorbent test (RAST), a number of workers have measured significant increases in IgG and IgE. IgE antibody was detectable in some instances in the absence of an IgG response (HULDT et al., 1973). With the availability of specific hydatid antigens, the usefulness of the RAST will increase. In spite of the six papers published since 1975 on the use of RAST in hydatid disease, the test has not been fully evaluated. The recent literature is listed in Table 8.

### The intradermal test (IDT)

The IDT is used extensively in the immunological diagnosis of hydatid disease. When compared to other diagnostic procedures, however, the test has a high level of nonspecific results but compares favorably in sensitivity. The ease and comparative sensitivity of the procedure make the technique very attractive, and it is used extensively for epidemiology and diagnosis all over the world. I doubt whether this technique will ever become obsolete.

Despite the shortcomings of the test, the IDT can yield more useful data if the antigen is standardized to relatively low nitrogen content (KAGAN et al., 1966; TODOROV et al., 1970; WILLIAMS, 1972). In spite of the purity of the IDT antigen produced by boiling hydatid fluid (WILLIAMS, 1972), SCHANTZ et al. (1975) obtained relatively high levels of nonspecific responses in a study in Peru.

Perusal of the reports on the IDT show that most workers inject 0.1 ml of antigen into the skin. In all of our work (KAGAN et al., 1974), we have used 0.05 ml and have not observed the high level of nonspecific results reported in the literature. This point should be evaluated in a special study. The literature on the IDT is listed in Table 9.

### Conclusion

Great strides have been made in the serologic diagnosis of hydatid disease. It is well documented that hydatid disease is caused by several species and physiologically distinct strains of *E. granulosus* (SMYTH and DAVIES, 1974) and that it differs markedly in clinical manifestations and host responses to infection. This is reflected by the diversity of serologic and immunologic results obtained by workers in different endemic areas of the world. Species-specific components for *E. granulosus* and *E. multilocularis* have been identified, and

at least one species-specific component for *E. granulosus* has been isolated (BOUT et al., 1974). Of all the serologic procedures used, all but the ELISA test have been evaluated by several groups in different endemic areas. The sensitivity of diagnostic tests increases when more than one procedure is used. The tests currently recommended for the diagnosis of hydatid disease are either IHAT or LAT as the primary test and IEP, DDG, or CEP as the confirming test. For evaluating the efficiency of operation in the removal of hydatid cysts, the CFT and IFT are best, and if sequential sera are available, the IEP can be used. The IDT, when carefully used with adjusted antigens in a standardized test, may also serve as an effective epidemiologic tool. For best results, however, both skin and serologic tests should be done.

Table 1 Complement Fixation Test (CFT)

BRADSTREET (1969) Reviewed 10 years of experience in England with the CFT and the intradermal test (IDT) between 1957 and 1967. Evaluating 101 proven cases of hydatid disease, the CFT was 93% sensitive at a titer of 1:2 or greater; the specificity of the test, however, was only 84% since 14 of 90 normal individuals had titers of 1:2 or greater. The CFT diminished to negative after 5 years in most of the 37 patients followed. On some patients, titers in the CFT persisted for 8 to 10 years.
MOCHMANN, HERING (1970) Employed the CFT for diagnosis between 1951 and 1962. Among 2 000 specimens tested 18 of 40 from known cases were positive. A number of cases are described in detail.
GARABEDIAN (1971) Evaluated whole scolex antigen initially developed for CFT in the IHAT. The antigen was more sensitive in the IHAT (83%) than in the CFT (66-74%).
LITTLE (1976) In a review of cases in Australia over a ten year period the sensitivity of the CFT was 52% and the IDT, 67%.
HESS, ECKERT, FROHLICH (1974) Compared the CFT, IHAT, and IFT in 36 proven cases of <i>E. granulosus</i> (Eg) and 16 proven cases of <i>E. multilocularis</i> (Em) infections. The sensitivity of the CFT for Eg was 77% and for Em 36%; for IHAT, 94% and 100%; and for IFT, 89% and 100%. Nonspecific reaction in normal individuals was 2% in the CFT, 1% in the IHAT, and 6% in the IFT.

Table 2 Indirect hemagglutination Test (IHA)

de ROSA, PUCCINI, SIMONE (1970) Testing sheep sera, found a sensitivity of 70% and a specificity of 88%.
APT, KNIERIM (1970) Evaluated the IHAT and IDT on 208 patients with hydatid infection. The IHAT had the higher sensitivity and specificity.
WILLIAMS, PREZIOSO (1971) Employed gluteraldehyde treated cells.
PAULUZZI, de ROSA, DOTTORINI (1971) Correlated 120 samples of human and animal cyst fluid for reactivity in the IHAT with protein content. In general, the higher the protein content in the cyst fluid, the more antigenic in the test.
MATOSSIAN, KANE (1971) After stabilizing human or sheep erythrocytes with pyruvic aldehyde and sensitizing them with hydatid antigen, found the cells could be stored by lyophilization.
KNIERIM, ESKUCH (1971) Evaluated a rapid slide hemagglutination procedure.
KNIERIM, MUNOZ, ESCUCHE, SANDOVAL, RAMIREZ (1971) Compared a rapid slide IHAT, a tube IHAT, and a bentonite flocculation test. Specificity ranged from 64 to 72% and nonspecificity from 3 to 8%.
STEPANKOVSKAIA (1972) Compared IHAT with the LAT. The IHAT was superior.

Table 2 (continued)

GIUNCHI, PAULUZZI, de ROSA (1972) Screened 1,502 individuals for sensitivity in the IHAT. Recommended test for epidemiologic investigations.
MAHAJAN, GANGULY, CHITKARA, AGARWAL (1973) Compared the IHAT, the intradermal test (IDT), and the CFT in India and found the IHA test to be superior (sensitivity 87% versus 72 and 66%, respectively). Specificity of the IDT was better than that of the IHA test. Investigators recommend IHAT and IDT for diagnosis.
BOTROS, MOCH, BARSOUM (1973) Compared IHA test and LAT in Egypt; report high non-specific cross-sensitivity with schistosomiasis patients.
DUBRISAY (1973) Over a 2-year period, observed 7 hydatid cysts of the liver; IHAT, IEP, and DDG tests for 4 of them were negative.
SCHWATZ (1973b) In an evaluation of sheep infected with <i>E. granulosus</i> no significant differences were noted in IHAT and IDT between infected animals and animals negative for cysts at autopsy.
MAMO, DAKROUB (1974) Bound hydatid antigen to human O cells with chronic chloride.
MOCH, CORNELIUS, BOTROS, BARSOUM, MAHMOUD (1974) Evaluated the IHAT and LAT for diagnosis of echinococcus in camels in Egypt. Ninety-four (32.6%) of 288 camels harbored cysts; 74% of the sera reacted in the IHAT, the LAT was less sensitive. For the animals with proven cysts, the IHAT was 88% sensitive.
ALI-KHAN (1974) Reported gluteraldehyde-fixed cells for the diagnosis of hydatid disease to be both stable and sensitive. Human O cells are recommended.
MAMO, MATOSSIAN, DAKROUB (1975) Evaluated a slide technique against a tube method; noted little difference between the tests.
VARELA-DIAZ, LOPEZ-LEMES, PROZIOSO, COLTORTI, YARZABAL (1975b) Evaluated four technical variations of the IHAT. The methods employing tanic acid were superior to the techniques employing gluteraldehyde-, benzene- and formol-treated cells.
MATOSSIAN, ARAJ (1975) Employing IHAT, CF, and IF tests for the persistence of antibody in postoperative cases of hydatid infection, found that a combination of IHAT and CF led to the detection of recurrent illness.
MATOSSIAN, MAMO, DAKROUB (1976) Evaluated a slide IHAT with a tube IHAT, CFT, and IFT for the diagnosis of hydatidosis; the slide test proved to be a very acceptable procedure for diagnosis.
BOTROS, MOCH, BARSOUM, MAHMOUD, FAHMI, SABAA el LEIL (1975) Evaluated the IHAT in Egypt in 755 patients with chest problems. With IHAT and BFT, 6.1% were reactive. A combination of IHAT with BFT or LAT increased the specificity of the test.
VARELA-DIAZ, COLTORTI, RICARDES, PREZIOSO, SCHANTZ, GARCIA (1976) Used the IHAT and LAT in a survey of 622 individuals in an endemic area of Argentina. The LAT proved to be the screening test of choice.

Table 3

## Latex Test (LAT)

EJDEN (1970) Compared LAT with the IHAT and found that the LAT compared very favorably. Sensitized latex particles can be stored for 6 months at 4°C.
ZORIKHINA, BREGADZE (1970) In 282 proven cases, found the sensitivity of the LAT to be 94.3% and nonspecificity, was 3.3%.
ZORIKHINA (1970) Evaluated the tube and slide method for performing the LAT and found the two techniques to be comparable.
WILLIAMS, PREZIOSO (1970) Employed the Boerner slides in the LAT with good results; 53 of 65 (82%) sera were positive.

Table 3 (continued)

REISSENWEBER, LOZANO, SAMPIO, MUNIZ, ACEVEDO (1973) Using blood collected on filter paper and venipuncture, compared 76 paired samples in the LAT, IFT, and CEP test. The results obtained with the filter paper eluates were usable in all tests.
GENIS (1974) Studied the prevalence of hydatid disease in Kazakhstan; tested 1 689 persons by the LAT. The technique was found to be a useful method for seroepidemiologic study.
GUISANTES, VARELA-DIAZ (1975) Compared the LAT to the DDG and the IEP tests. These authors recommend the LAT as a diagnostic screening test because of its high specificity.
ZORIKHINA, BREGADZE (1975) Found that titer by LAT began to fall a few months after operation for removal of hydatid cyst and that it became negative in 1 to 3 years.
HOGHOOGHI, SABBAGHIAN, GHADIRIAN, KAGAN, SCHILLER (1976) Employing IDT and LAT, 51 patients were evaluated. The LAT was 82% sensitive and 5% nonspecific.

Table 4 Immunofluorescence Tests (IFT)

GORE, SADUN, HOFF (1970) Employed acetate impregnated with <u>E. granulosus</u> antigen fractions and developed a test that could be read by a fluorometer; it had a sensitivity of 82% and a nonspecificity of 4%.
AMBROISE-THOMAS, KIEN TRUONG (1970a, b) Evaluated 300 proven cases and reported a sensitivity of 96% for the IFT. Sera of patients with extirpated cysts became negative in 12 months.
RUITENBERG, van der SLEEN (1972) Reported a method of attaching whole scolices to glass slides.
KANE, MATOSSIAN, BATTY (1971) Utilizing monospecific IgG, IgM, and IgA in 9 cases of hydatid disease, found IFT positive for IgG in 8, for IgM in 4, and IgA in 6. The IHAT was positive in 8 of 9 cases, the CFT in 5 of 9 cases.
HULDT, LJUNGSTROM, AUST-KETTIS (1975) Found class-specific conjugates for hydatid infection to be useful in the diagnosis of infections in a population of Lapps in Scandinavia. In a group of 50 infected Laplanders, 60% showed IgM reactivity.
AMBROISE-THOMAS (1976) Reviewed immunofluorescence for the diagnosis of parasitic diseases.
FLOREZ, SANCHEZ, ALBALA (1976) Found IFT to be 90% sensitive in diagnosis.
WIKERHAUSER, DZAKULA, KUTICIC (1977) Using cryostat sections of protoscolices of <u>E. granulosus</u> , found 16 of 18 proven cases positive in the IFT.

Table 5 Immunoelectrophoresis (IEP)

CAPRON, YARZABAL, VERNED, FRUIT (1970a) Evaluated 400 sera by CFT, IHAT, LAT, IFT, and IEP test and found the IEP test to be the most sensitive (85%). Sera from patients with hydatid cysts due to <u>E. granulosus</u> had a 90% reactivity, and those with lung cysts, a 69% reactivity.
CAPRON, VERNES, FRUIT (1970b) In tests with 7 patients with <u>E. multilocularis</u> disease, identified a specific band for this parasite.
YARZABAL, CAPRON (1971) Reported very high specificity for Band 5 in human sera.
YARZABAL (1973) Compared DDG, IEP, and CEP and found IEP to be the method of choice for diagnosis.

Table 5 (continued)

<p>QUILICI, ASSADOURIAN, RANQUE (1971) Evaluated the CFT, LAT, IHAT, IFT, and IEP test on 68 proven cases in southern France. The nonspecificity was 8, 7, 6, 4, and 0%, respectively. Authors recommend either IEP and CF or IHAT and IF tests for diagnosis.</p>
<p>CASTAGNARI, SORICE (1971a) Compared specificity of IEP and countercurrent electrophoresis (CEP). Although CEP was more sensitive, the authors stressed the importance of using several tests in diagnoses.</p>
<p>CONTRERAS, KNIERIM (1974a) Evaluated IEP, DDG, and the IHAT in 67 patients with hydatid disease. The sensitivity of the tests were 56.7%, 48.2%, and 71.6%, respectively. Because of its clinical difficulties, the IEP test was not recommended for diagnosis.</p>
<p>VARELA-DIAZ, COLTORTI (1974a) Described techniques for performing the IEP, IHAT and LAT.</p>
<p>YARZABAL, LEITON, LOPEZ-LEMES (1974) Found the IEP more sensitive than the IHAT in the preoperative diagnosis of pulmonary hydatidosis in 54 patients.</p>
<p>COLTORTI, VARELA-DIAZ (1975) Modified the IEP by substituting normal serum for concentrated serum and filling the serum throughs several times.</p>
<p>GUISANTES, YARZABAL, VARELA-DIAZ, RICARDES, COLTORTI (1975) Proposed a standardized test for IEP, with 0.9% agarose over 1% agar used as a support medium. Rectangular wells were superior to circular wells. The optimal concentrate of crude hydatid fluid was 200 mg dry weight/ml and for purified hydatid cyst fluid antigen, 30 mg protein/ml.</p>
<p>LOPEZ-LEMES, VARELA-DIAZ (1975a) Detected Band 5 in 45% of 51 sera. Treating the slice with citrate brought out Band 5 more clearly, as did staining with Amino-Schwartz.</p>
<p>VARELA-DIAZ, GUISANTES, RICARDES, YARZABAL, COLTORTI (1975c) Found that although purified hydatid cyst antigen was more sensitive than crude cyst fluid antigen, the former was more nonspecific. Bands other than "Band 5" may be useful in the diagnosis of hydatidosis.</p>
<p>YARZABAL, RETAMAL, SEPULVEDA, GUACHALLA, KIGUEL (1975a) In 71 surgically confirmed patients with pulmonary hydatidosis in Chile, found the IEP positive in 55 (77.4%). The IEP is recommended for diagnosis, with IHAT and IFT as comparison tests.</p>
<p>YARZABAL, SCHANTZ, LOPEZ-LEMES (1975b) Compared the IDT with the IEP in 47 surgically confirmed cases. The IDT gave a few false positive reactions, but the IEP gave none. The high specificity of the IEP led these workers to recommend this technique over the skin test.</p>
<p>RAZZOKOV (1975) Evaluated DDG and IEP for species-specific components. Eight species-specific bands were found in human sera from patients with <i>E. granulosus</i>, of which 2 were diagnostically important. Species-specific bands for <i>E. multilocularis</i> were also found.</p>
<p>SCHANTZ (1976) Recommended that the IEP be included among those tests used for diagnosis of hydatid disease because of the presence of the specific "Band 5".</p>
<p>VARELA-DIAZ, COLTORTI, PREZIOSO, LOPEZ-LEMES (1975b) Evaluated the IEP test, LAT, and IHAT. The sensitivities of the LAT and IEP test were comparable; the IEP test gave a higher sensitivity than the IHAT. The LAT and IHAT, with tannic acid-treated cells, are recommended for screening, with the IEP as the test for use in confirming positive reactors.</p>
<p>RICKARD, ARUNDEL (1976) Reported that the program of Varela-Diaz et al. (1975) for the diagnosis of hydatid disease is being offered in Australia by the University of Melbourne, Veterinary Clinical Center.</p>

Table 6

## Countercurrent Electrophoresis (CEP)

SORICE, CASTAGNARI (1971) Reported the CEP to be slightly more sensitive (79%) than the IEP (63%)
CASTAGNARI, SORICE (1971b) Reported the CEP to be more sensitive than the IHAT, LAT, and IEP test.
LECUBARRI, CORROLLER, BELKAID (1971) Compared the CEP test with the LAT in a study of 160 patients with hydatid cysts in Algeria. The test was 97% specific and 80% sensitive.
CASTAGNARI, SORICE (1971b) Reported that the CEP test gave 94.7% sensitivity. With IEP, 8 specific bands were recognized, and 22 of 28 cases (78.5%) were diagnosed. In CEP, 4 specific bands were recognized, and 18 of 19 cases (94.7%) were diagnosed. In a comparison with the IEP test, the IHAT, and the LAT, the CEP test was most sensitive. The authors recommend IHAT and CEP for diagnosis.
GENTILINI, PINON (1972) Using cellulose acetate membranes in the CEP test, compared the technique to DDG and IEP. Of 40 confirmed hydatid cases, 38 were positive by IEP and CEP. More bands were visualized in CEP than IEP. "Band 5" was present in all positive sera.
TORRES, GUIANTES, ALVAREZ, YARZABAL (1973) Reported good sensitivity and specificity in the CEP test, with concentrated serum.
GEORGIEVA-BOJADZIEVA, DASKALOVA, STAROVA, BOJADZIEV, DEJANOV (1975) Compared CEP with CFT and found CEP to be more sensitive.
LOPEZ-LEMES, VARELA DIAZ (1975a) Found CEP less specific than IEP, since "Band 5" could not be readily detected.
SORICE, DELIA, CASTAGNARI (1975a) Found CEP, with concentrated hydatid fluid antigen, more sensitive than the IHAT.
APT (1975) Reviewed diagnostic methods.
KELKAR, KOTWAL (1975) Found 4 of 6 sera positive in CEP tests, 1 of 6 in DDG tests, and 2 of 5 in the IDT.
DRAPER (1976) Reviewed diagnosis of parasitic infections by CEP.
PINON (1976) Compared CEP to IEP and IHAT. CEP tests were positive in 81.4% of 400 cases of hydatid disease, and IEP tests in 76.6%. Seventy-five percent were positive in both. IHAT were positive in 91.8%. A characteristic "gloved finger" pattern in CEP indicates a cyst ready to burst.

Table 7

## Antigens

ORIOLO, WILLIAMS, PEREZ ESANDI, ORIOLO (1971) Isolated two lyoprotein antigens (A and B) from sheep hydatid fluid with minimum (1.6%) host serum proteins. One component (B) was obtained pure by gel diffusion.
WILLIAMS, PEREZ ESANDI, ORIOLO (1971) Found antigen A and B to be reactive in IHAT, the IEP test, and the IDT. The antigens, however, were not as sensitive as crude hydatid fluid.
MUNTIAN (1971) Examined the antigenic composition of hydatid fluid (HF), scolices (SC), and germinative membrane (GM). HF contained 10 antigens; SC, 17 antigens; and GM, 8 antigens.
FRONGILLO, DOTTORINI, PERFETTI (1971) Fractionated hydatid fluid with a Bio-gel P 300 column. IHAT activity was found in the first 15 tubes containing protein, but CF activity was found in almost all fractions.

Table 7 (continued)

- PAULUZZI, DOTTORINI, FRONGILLO (1972a) Found that fractionating hydatid fluid on a Bio-gel P 300 column did not produce fractions that protected Balb/C mice against experimental peritoneal infections.
- PAULUZZI, DOTTORINI (1972) By further analysis of the fractions from a Bio-gel P 300 column, showed two groups of substances, one of a high molecular weight, which was active in IF and IHA tests, and a second of low molecular weight.
- PAULUZZI, DOTTORINI, PIRAS (1972b) Using ultracentrifugation of the globulin fraction of hydatid fluid in a sucrose gradient, found that one of two fractions obtained inhibited the activity of the IHAT test.
- POZZUOLI, MUSIANI, ARRU, PIANTELLI, MAZZARELLA (1972) With Sephadex G-200, isolated a 400 000 and 150 000 molecular weight component from sheep hydatid fluid.
- BENEX (1972) By fractionating an extract of *Taenia saginata* on a Sephadex G-200 column, found that three fractions cross reacted with sera of patients with hydatid disease in the DDG, CFT, and LAT.
- VARELA-DIAZ, COLTORTI, RICARDES, GUI SANTES, YARZABAL (1974) Found antigen for Band 5 in 40 of 42 hydatid cyst pools.
- RUSSI, SIRACUSANO, VICARI (1974) Found that a glycoprotein isolated from hydatid cyst membrane with a high P<sub>1</sub> blood group activity was active in 11 of 21 sera from patients with pulmonary cysts.
- POZZUOLI, MUSIANI, ARRU, PATRONO, PIANTELLI (1974) With specific immunosorbents, absorbed sheep hydatid fluid for removal of host components on a Sepharose 4B column. Two major antigens, one of molecular weight greater than 400 000, was more immunoreactive than a smaller molecular weight component of 150 000.
- FEIZI, KABAT (1974) Isolated P<sub>1</sub> blood group substance plus I material from sheep hydatid fluid by immunoabsorption.
- AL CONTRERAS, KNIERIM (1974b) Determined protein and carbohydrate antigens in five lots of hydatid fluid. Protein varied from 14 to 65% and carbohydrate from 1.4 to 4.5%. Although two hydatid pools had similar protein and carbohydrate contents, only one was sensitive in the IEP test.
- BOUT, FRUIT, CAPRON (1974) With two dimensional immunoelectrophoresis, isolated the antigen responsible for the specific Band 5 in the IEP test. Monospecific antiserum to Band 5 was made. Use of this antiserum should facilitate the specific diagnosis of hydatid infections. The Band 5 antigen is a lipoprotein with a molecular weight of 60,000 and is similar to antigen A reported by Oriol et al. (1971).
- POZZUOLI, PIANTELLI, PERRUCCI, ARRU, MUSIANI (1975) With affinity chromatography on concanavalin A-Sepharose, isolated two specific antigens (Antigen 4 and 5). Antigen 4 was found in 58 of 75 (77%) hydatid sera by the IEP test, in 81% by CEP tests, and in 89% by IHAT.
- ROMBERT, FRAGO de AZEVEDO (1975) In a comparative study of hydatid fluid scolex and membrane antigens in serologic tests, found that membrane antigens were inactive, purified scolex antigens were active at the LAT and IHAT with low titers, and scolex antigen was active in the DDG test. Since scolex antigens detected antibodies that were not detected in the IHAT, the authors recommend the use of both antigens.
- YARZABAL, DUPAS, BOUT, CAPRON (1976) By using a monospecific anti-Band 5 antiserum, localized the antigen 5 by the IFT in the inner portion of the germinal membrane and in the parenchyma of the protoscolexes.

Table 8

## Immunoglobulins

PEREZ ESANDI (1970) Eluted antibodies from sera of patients with hydatid disease by using immunoabsorbents. The antibodies were found in the IgG, IgM, and IgA classes.
SEITANIDIS, ANGELOPOULOS (1971) Employing a radial immunodiffusion technique, noted increases in IgG and IgM in 28 adult patients with hydatid disease.
MATOSSIAN, KANE, CHANTLER, BATTY, SARHADIAN (1972) Reported that with nonspecific IF sera, IgG levels remain elevated. IgM and IgA levels diminished after the cyst was removed.
HULDT, GUNNAR, JOHANSSON, LANTTO (1973) Reported that in Fenno-Scandia, immunologic responses of Lapps infected with hydatid disease were marginal. A few individuals responded with IgE levels in a radioallergosorbent (RAST) test. IFT and IDT were also very nonspecific. Almost all cases were lung cysts.
SORICE, DELIA, CASTAGNARI (1975b) Measured, by radial immunodiffusion, IgE levels in 34 patients with active hepatic and extrahepatic hydatidosis and in 10 subjects with previous hydatidosis and 15 controls. IgE levels higher than 800 Mg/ml were detected in 33 of the 34 cases, 5 of the 10 with previous disease, and in 4 of 15 normal subjects.
DESSAINT, BOUT, WATTRE, CAPRON (1975) In a survey of 89 patients with hydatid disease, found high IgE levels by RAST. A purified hydatid antigen was found to be very reactive with IgE antibodies.
SORICE, DELIA, CASTAGNARI (1975b) Measured IgE levels in 34 patients with active hydatid infections by quantitative radial immunodiffusion. In 33 of 34, IgE levels were elevated.
VERVLOET, CHARPIN, DUMON, QUILICI (1975) Measured specific IgE antibody by RAST in the serum of patients with hydatid disease. In 20 of 24 cases, IgE was found in the hydatid fluid of the cyst. A significant quantitation correlation exists between the results of the RAST test and positive serology. In 6 of 24 patients with other diseases, false positive reactions were obtained.
MATOSSIAN, ALAMI, SALTI, ARAJ (1976) By using radial diffusion methods, measured IgG, IgM, IgA, IgD, and IgE levels in 83 patients with hepatic and pulmonary hydatidosis, 15 postoperative individuals, and 80 controls. IgG was elevated in all patients. IgM and IgA were elevated in pulmonary cases. IgD was normal, and IgE was elevated in 7 of 21 hydatid patients.
VERVLOET, DUMON, QUILICI, CHARPIN (1976) Used the RAST to evaluate 60 patients with hydatid disease for the presence of specific IgE antibody in the serum. Specific hydatid IgE antibody was found in 54 of 60 subjects.

Table 9

## The Intradermal Test (IDT)

ROY, BISWAS, CHATTERJEE, BASU MALLIK (1970) Evaluated the CFT, IDT, and IHAT in India with 25 proven cases of lung cysts. The IDT was positive in 80% in the immediate test and 3 of 5 patients had positive tests for 5 years. The CFT was positive in 60% and the IHAT in 52%. By using one of the three tests as a positive criterion, 88% were correctly serologically diagnosed.
WRAY (1970) Reported the IDT to be insensitive in the diagnosis of hydatid disease in Kenya in very chronic infections.
TODOROV, YONKOVA, JURUKOVA (1970) Standardized the IDT on the basis of protein content and found a protein content of 40 g/ml for dialized antigens to be best for epidemiologic investigations.

Table 9 (continued)

- TODOROV (1970) Found that although good sensitivity was obtained with the IDT (86.6%) in studies in Romania, the nonspecific response of 14.5% reduced the diagnostic value of the test.
- RAMIREZ, MACAYA, ROJAS, SCOZIA, SCHENONE, RODRIGUEZ, DIAZ, HESS (1971) Using and IDT antigen of 20 g N/ml and a reading of 1,6 cm<sup>2</sup> or greater at 30 minutes as a positive test, found the technique to be useful in a survey made in Chile.
- WILLIAMS, PEREZ ESANDI (1971) Using passive cutaneous anaphylaxis (PCA), examined the sera of 20 dogs infected with E. granulosus for homocytotropic antibody. Five of 20 sera were positive.
- JEZEK, RUSINKO, MINGIR, CERENSHIMID (1971) Using an antigen preferred in Germany, conducted a survey in Mongolia. The prevalence of positive reactions was 1.4% in urban areas and 2.2% in rural areas.
- WILLIAMS (1972) Evaluated diagnostic sensitivity and specificity for antigen of low N content derived from sheep hydatid cyst fluid. Boiling of the hydatid fluid produced an IDT antigen with one immunologically reactive component. An antigen containing 15 g/N/ml gave a sensitivity of 80%.
- KAHN, SPRUANCE, HARBOTTLE, CANNON, SCHULTZ (1972) Conducted a skin test survey of 399 individuals in Herriman, Utah, which revealed five positive reactors. These individuals had negative serologic tests (IHA and BF).
- SCHANTZ (1973a) Demonstrated homologous skin-sensitizing antibody in serum of 6 sheep by PRAUSNITZ-KÜSTNER (PK) and passive cutaneous anaphylaxis (PCA) reactions.
- SCHANTZ, WILLIAMS, POSSE (1973) Screened 1 669 persons in Rio Negro Province in Argentina by IDT; 8.3% were positive. Of the 49 positive, only 13 were positive in IHA, LA, or IEP tests.
- LASS, LAVER, LENGY (1973) Studied IDT in 28 patients before surgical intervention. The immediate reaction was 89% reactive and 15% nonspecific. The delayed response was 68% reactive and completely specific. Evaluating IHA, IF, LA, and CF tests with a group of patients after surgery, the only test that reverted to negative in 12 months was the CFT. The CFT was, however, quite insensitive.
- CHENITI, HALFON, GHARBI, JEGUERIM, BEN SALAH, BEN RACHID, MOYROUD (1973) Working in Tunis, studied pulmonary hydatid cysts by ID, IHA, and IEP tests. In 43 cases tested by IDT, 25 (58%) were positive. Twenty-nine (55%) of 52 lung cysts tested by IHAT were positive and 61 (50%) of 121 lung cysts tested by IEP were positive.
- KAGAN, KLOCK, SPRUANCE (1974) Carried out ID and serologic tests in a small endemic area in Alaska and Utah. In Alaska the IDT was more reactive than serologic tests; the reverse was true in Utah.
- VARELA-DIAZ, COLTORTI (1974b) Consider the IDT unsuitable for the immunodiagnosis of hydatid disease because of the high rate of nonspecific response.
- ROMBERT, FRAGA de AZEVEDO (1974) Reported good sensitivity in the diagnosis of hydatid infections with passive cutaneous anaphylaxis (PCA).
- GARCIA ALVAREZ, REVERTE CEJUDO, HERRUZO, PEREZ PENA (1974) Compared the IDT with the CFT in 60 proven cases. In 57 tested by IDT, 39 (68.4%) were positive, and in 24 tested by CFT, only 2 were positive (8%).
- LOPEZ-LEMES, GUI SANTES, TORRES, JOSEF (1975) Correlated the IDT with the IEP test. The sensitivity of the IEP was 74%; and that of the IDT, 54%. The authors do not recommend the IDT for diagnosis.
- SCHANTZ, ORTIZ-VALQUI, LUMBRERAS (1975) With a partially purified antigen prepared by boiling hydatid cyst fluid, found a high rate of nonspecific responses in a Peruvian population.

Table 9 (continued)

- YARZABAL, SCHANTZ, LOPEZ-LEMES (1975b) Reported that although the IDT is approximately as sensitive as the IEP test, it is much less specific.
- BARBOUR, FUKUSHIMA, NICHOLS (1975) Pointed out that the IDT is not sensitive enough for a hydatid cyst to be ruled out on the basis of a negative test.
- SCHANTZ (1975) Reaffirmed the statements by BARBOUR et al. and pointed out that, at present, no immunodiagnostic test can be used to "rule out" hydatid disease because many cyst carriers do not develop a detectable immune response.

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 Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health, Education, and Welfare.

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