Notes

A Preserved Antigen for the Hydatid Fluorescent-Antibody and Other Tests Utilizing Scolices*

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Good results have been obtained with the fluorescent-antibody (FA) test in the diagnosis of human hydatid disease. Fresh suspensions of scolices gave best results, but put a limitation on the performance of the test, while preservation by formalin reduced sensitivity (Sorice et al., 1966; Moroni & Sorice, 1966). In this laboratory the test has been performed with scolex suspensions stored frozen. This suspension is suitable for a limited period only, and the sensitivity was lower than that of the whole-scolex complement-fixation test (SCCF) (Fischman, 1968).

In this study a modified procedure has been developed using scolices preserved by freeze-drying, retaining high sensitivity in the FA test. The preserved scolices have been used in the SCCF also.

Materials and methods

Preparation of preserved scolices. Fresh hydatid cysts were obtained from the abbatoir, the fluid was withdrawn and refrigerated for 24 hours. The supernatant fluid was separated from the scolices and the scolices were then washed 3 times in ice-cold physiological saline by gentle centrifugation and suspension. To 1 volume of final deposit were added 3 volumes of 4% sucrose solution and the suspension was freeze-dried [3] in 0.25-ml volumes. Ampoules were sealed under vacuum and stored at 2°C–4°C.

Antigen for the fluorescent-antibody test. Preserved scolices were reconstituted with distilled water and fixed in 10 volumes of 2% formol–saline for 30 minutes. They were then washed twice in phosphate-buffered saline, pH 7.2. Scolices which did not sediment after standing for 10 minutes in the second wash were discarded. The remaining scolices were suspended in phosphate-buffered saline at an approximate concentration of 5000 per ml (100–200 per drop of suspension).

Procedure. For each test 2 drops of scolices suspension were pipetted to the bottom of a 10-ml tapered centrifuge tube and 2 drops of serum diluted 1 : 10 with phosphate-buffered saline were added and carefully mixed. Tubes were then incubated at 37°C for 30 minutes. The scolices were washed 3 times in physiological saline. To wash the scolices they were suspended in saline, allowed to settle for 2 minutes, then centrifuged for 10 seconds at slow speed (approximately 5 *g*). After the last wash as much saline as possible was removed from the tubes. Then 0.2 ml of optimally diluted fluorescent anti-human globulin, prepared by conjugating rabbit anti-human globulin with fluorescein-isothiocyanate and absorbing the conjugate with hog-liver powder and bovine bone marrow, was added. Tubes were incubated at 37°C for 20 minutes and the scolices again washed as before. A drop of glycerol, buffered to pH 9.0 with carbonate buffer, was added to each tube. The scolices were transferred on to slides for examination under the microscope. A Leitz Laborlux microscope was used with HBO 200W ultraviolet lamp, primary fluorescence filters 4 mm BG 38 and 2 mm UGI, ocular filter K430, bright ground condenser na 1.4, ×10 objective na 0.25, binocular phototube and ×6 eyepieces. Cover-slips were not used on the preparations. Siliconized test-tubes were used in all procedures to prevent excessive loss and deterioration of scolices. Positive sera produced bright fluorescence of the scolices, mostly at the periphery of the organism. Normal sera either produced no fluorescence or a weak cytoplasmic fluorescence. They showed no peripheral staining.

Whole-scolex complement-fixation test. This test was performed as described previously (Fischman, 1968), except that the stock antigen was made by reconstituting with distilled water an ampoule of freeze-dried scolices. (No formalin was added as in the FA test). A 1 : 10 dilution of the stock antigen suspension constituted the working antigen in the SCCF.

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[3] Speedivac centrifugal freeze-drier, model L5 series, Edwards High Vacuum Ltd, Sussex, England.

Results and discussion

The preserved scolices have been in use for several months, giving excellent, reproducible results in the FA test, with an antigen available whenever required.

It should be noted here that hydatid cysts, as well as containing the typical live scolices as described in standard text-books, also contain a varying proportion of what are presumably dead or degenerating scolices. These scolices are more triangular in shape and are about half the diameter of the normal scolices. Other workers (Pauluzzi, 1965; Coudert et al., 1967) have used the terms " metascolices " and " orthoscolices " for these dead and living scolices respectively, and these terms are used herein to differentiate the two forms.

The tube FA technique has been criticized on the grounds that only the metascolices stain, coexistence of meta- and orthoscolices making reading difficult. In the slide test there is loss of scolices, mainly orthoscolices, during processing, causing difficulties in reading, unless a tissue-embedding method is used (Pauluzzi, 1965; Coudert et al., 1967). The modified tube test described above appears to overcome some problems previously encountered, giving high sensitivity and clear-cut easy reading.

In initial experiments using unfixed scolices, stored both frozen at −15°C and freeze-dried at 2°C–4°C, it was observed that the orthoscolices were more sensitive in the reaction than the metascolices. However, the cell wall, or the antigen on the cell wall, of the orthoscolices appeared to be very easily dislodged or disintegrated in some way, so that at times only the metascolices appeared to react very well. Fast or prolonged centrifuging during washing procedures was partly responsible for this disintegration but most of it was found to be caused by the use of cover-slips on the final preparation.

The disintegration was not very pronounced when strongly positive sera were used, but with weaker sera scolices which showed fluorescence quickly became negative when a cover-slip was applied. Formalin fixation for 30 minutes was found to increase the sensitivity of the orthoscolices, possibly by helping to retain the antigen at the periphery of the organism. However, even formalin-fixed scolices lost their peripheral fluorescence fairly readily if cover-slips were applied. Using the present technique, the orthoscolices are more sensitive than the metascolices. As the metascolices are smaller and settle less readily, most of them are removed during the washing procedures and the final preparation consists primarily of orthoscolices.

Retesting of available sera (27) from surgically proven cases used in the previous study (Fischman, 1968) has shown increased sensitivity in the FA test: 88% positives, approaching that of the whole-scolex complement-fixation, which again gave 92% positives. Three sera only showed disagreement, 2 having a negative FA and positive SCCF, while 1 gave a positive FA and negative SCCF.

To maintain highest sensitivity, the two main technical points are: refraining from using cover-slips in the final reading, and applying the short formalin procedure to the preserved scolices. This enhances sensitivity, in contrast to previous methods where a high concentration of formalin (10%–20%) has been used as a preservative, with loss of sensitivity. Freeze-dried scolices can be used after reconstitution without formalin treatment, but a slight loss of sensitivity results.

The availability of preserved scolices is of value also if one wants to perform other tests utilizing scolex antigens. The whole-scolex complement-fixation test using freeze-dried scolices gave identical results with frozen antigen. While it is useful to have freeze-dried scolices for the SCCF also, it is not as important as for the FA test. In the SCCF it is not essential that scolices remain intact. Actually, the frozen antigen described previously (Fischman, 1968) does contain antigen released from scolices in its suspending fluid but this has no effect on sensitivity and titres.

Postscript

Current experiments have shown that the inclusion of 3% dextran in the suspending medium may further increase the stability of the freeze-dried scolices.

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REFERENCES

Coudert, J. et al. (1967) *Bull. Soc. Path. exot.*, **60**, 555
Fischman, A. (1968) *Bull. Wld Hlth Org.*, **39**, 39
Moroni, M. & Sorice, F. (1966) *G. Mal. infett.*, **18**, 471
Pauluzzi, S. (1965) *Ann. Fac. Med. Perugia.*, **56**, 469
Sorice, F., Castagnari, L. & Tolu, A. (1966) *G. Mal. infett.*, **18**, 192