EVALUATION OF STABILISED CELLS IN THE INDIRECT HAEMAGGLUTINATION TEST FOR ECHINOCOCCOSIS

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SUMMARY. We have evaluated the use of sheep red-blood cells stabilised in various ways in indirect haemagglutination tests on the sera of 21 surgically confirmed cases of echinococcosis, and on control sera. Tests with Double-Aldehyde-Stabilised cells—treated sequentially with pyruvic aldehyde, tannic acid and glutaraldehyde—were more sensitive than tests with the cells treated only with formaldehyde, glutaraldehyde or pyruvic aldehyde, and subsequently tanned.

INTRODUCTION

The indirect haemagglutination test (IHA), by virtue of its simplicity, specificity and sensitivity, is being increasingly used by many workers for the serodiagnosis of echinococcosis (Iacona, Pini and Vicari, 1980; Chemtai, Bowry and Ahmad, 1981). Red blood cells stabilised either by formaldehyde, glutaraldehyde or pyruvic aldehyde are routinely used in the test in different laboratories. Formalised cells have been used by Parodi (1960), Mandras and Addis (1960), Allain and Kagan (1961) and Chordi *et al.* (1962) (all cited from Kagan, 1968), whereas glutaraldehyde-treated cells have been used by Ben-Ismaïl *et al.* (1980).

Earlier, Ling (1961) showed a superiority for pyruvic aldehyde and glutaraldehyde over other reagents in stabilisation of the cells. Later, Farshy and Kagan (1972) observed that cells stabilised by a combination of these two aldehydes separated by a tanning procedure were better than cells stabilised by treatment with a single aldehyde for use in IHA for malaria. The present study was undertaken to evaluate the use of Double Aldehyde Stabilised (DAS) cells and of cells stabilised by treatment with formaldehyde, pyruvic aldehyde or glutaraldehyde in an IHA test for echinococcosis.

MATERIALS AND METHODS

Antigen. Hydatid-cyst fluid was obtained from patients at surgical operation and was processed by the method of Arabatzis and Papapanagiotou (1963).

Sera. Blood samples were collected from 21 surgically confirmed cases of echinococcosis in our hospital during the period 1981–83 and the sera separated. Negative control sera were obtained from 25 normal healthy persons. All the sera were stored at -20°C until required.

Sheep red blood cells (SRBC). Blood was collected into Alsever's solution, the cells thrice

washed in phosphate-buffered saline 0.15 M, pH 7.2 (PBS) and treated with the stabilising agent within 48 h.

Stabilisation with formaldehyde. The method of Fulthroe *et al.* (1961, cited by Herbert, 1978) was used. To a suspension of SRBC (10 ml of packed cells in 500 ml of PBS), 50 ml of formalin (aqueous formaldehyde, 40% w/v) was added slowly from a burette, over a period of 30 min. The mixture was left overnight at 4°C, and a further 50 ml of formalin was then added in one lot. Twenty-four hours later the preserved cells were washed thrice in PBS and stored at 4°C as a 50% v/v suspension in a 1 in 200 dilution of formalin in PBS.

Stabilisation with glutaraldehyde. The suspension was prepared as described by Avrameas, Taudou and Chuilon (1969). Briefly, 10 ml of 5% v/v aqueous glutaraldehyde was added to 1 ml of a 10% suspension of SRBC in PBS, and the mixture was stirred by a magnetic stirrer for 4 h at room temperature. The cells were washed thrice in PBS and stored at 4°C as a 30% v/v suspension in PBS.

Stabilisation with pyruvic aldehyde. The method was modified from that of Farshy and Kagan (1972). A reaction mixture of the following substances, added in the order given, was prepared and subsequently kept in the cold: 1.7% NaCl, 12 ml; 40% v/v aqueous pyruvic aldehyde, 4 ml; 1.0% Na₂CO₃, 35 ml; Sorensen's phosphate buffer (pH 7.2) 7 ml. To this, 10 ml of a 50% suspension of SRBC was added. The mixture was stirred for 30 min and stored for 24 h at 4°C. The cells were then washed thrice in PBS and stored in this as a 2.5% v/v suspension at 4°C.

Double-aldehyde stabilisation (DAS) was performed as described by Farshy and Kagan (1972). The cells were first stabilised with pyruvic aldehyde (as above). To a 2.5% suspension of these an equal volume of cold tannic acid 1 in 25000 in PBS was added and the mixture kept at 4°C for 30 min. The cells were then washed thrice in PBS and made up to a 4% suspension in the same diluent. To this was added an equal volume of 2% v/v aqueous glutaraldehyde in PBS over a magnetic stirrer which was run for 2 h. The cells were then washed thrice with PBS and stored as a 50% v/v suspension in this diluent.

The IHA test. Cells treated with formaldehyde, glutaraldehyde or pyruvic aldehyde were treated with tannic acid before being sensitised with antigen (Herbert, 1978). With DAS cells this step was omitted because the cells had been tanned during stabilisation.

The optimum sensitising dose of antigen was determined for each type of sensitised cells by chequer-board titration with known positive and negative sera. The values obtained were: 1 in 16 for formaldehyde-, 1 in 8 for glutaraldehyde-, 1 in 8 for pyruvic-aldehyde-treated cells, and 1 in 16 for DAS cells. The IHA test was performed as described by Herbert (1978).

RESULTS

The table shows the IHA titres obtained with the four types of stabilised SRBC. With 19 of the 21 sera from patients with echinococcosis, the DAS cells gave titres 2-4 times higher than those obtained with any other type of cell. We concluded from the results obtained with sera from persons with no evidence of echinococcosis that a titre of 128 or more could be taken as evidence of this disease. Such a titre was given by 19 sera with DAS cells but by only 16 with formaldehyde-treated and 15 with pyruvic aldehyde- and glutaraldehyde-treated cells.

DISCUSSION

IHA tests are inexpensive and easy to perform, and can be read without errors of subjectivity. This makes them potentially useful for investigations in areas endemic for echinococcosis (Lewis, Koss and Kerstein, 1975) such as the one in which we work (Parija *et al.*, 1983).

When used for the diagnosis of malaria (Kagan, 1972), the sensitivity of the test depended upon the method of stabilising the cells and the antigen employed (Farshy

TABLE

Results of IHA tests on the sera of confirmed cases of echinococcosis and normal persons

Serum no.	Titre of haemagglutination of sheep RBC stabilised with			
	Formal-dehyde	Pyruvic aldehyde	Glutar-aldehyde	Double aldehyde
<i>Cases of echinococcosis</i>				
1	128	128	128	256
2	32	8	16	128
3	256	256	256	1024
4	128	128	128	512
5	32	16	8	8
6	128	128	128	256
7	128	128	128	256
8	256	128	128	512
9	128	128	128	256
10	128	128	128	256
11	512	256	256	1024
12	128	128	128	256
13	8	8	16	32
14	64	32	16	128
15	128	128	128	256
16	32	16	32	128
17	128	32	64	128
18	256	128	128	512
19	256	256	128	512
20	256	256	256	1024
21	128	128	128	256
<i>Normal persons*</i>				
1	16	16	32	32
2	32	16	16	16
3	32	32	32	32
4	16	32	32	32
5	32	32	16	16
6	16	16	16	32
7	16	16	16	16
8	16	16	32	32
9	64	32	64	64
10	16	32	32	64

*Sera from another 15 normal persons showed a titre of 8 or less with all the stabilised cells.

and Kagan, 1972). Coupling agents such as aldehydes not only stabilise the cells but react with free amino groups, sulphydryl groups and, to some extent, imidazole groups, rendering the surface of the RBC more anionic and so facilitating the attachment of protein antigens to them (Farshy and Kagan, 1972). The experience of these authors with malaria, and of Jagannath and Sengupta (1981) with leprosy, showed that the use of DAS cells greatly increased the sensitivity of the IHA tests. Our experience with echinococcosis is in conformity with this; in tests with DAS cells we obtained a diagnostic titre in 90.4% of surgically confirmed cases.

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