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A HEMAGGLUTINATION TEST FOR MANSON'S SCHISTOSOMIASIS USING CHROMIUM CHLORIDE, FORMALIN TREATED HUMAN ERYTHROCYTES, SENSITIZED WITH WORM EXTRACTS

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SUMMARY

Antigens from *Schistosoma mansoni* were fixed to formalin-treated human erythrocytes through the action of chromium chloride. Hemagglutination tests with such sensitized cells were performed in plastic microplates, in 30 serums from patients showing eggs of schistosome in stools, and in 30 serums from individuals residing in non-endemic areas, who did not show clinical or parasitological evidences of schistosomiasis. Every patient in the first group gave positive results, and only negative tests were found in the second group. Titers in the positive cases were the same, or differed only by one dilution from those obtained in a similar hemagglutination test with tannic acid treated erythrocytes. Sensitized cells were stable at 4°C for at least 2 months.

INTRODUCTION

Serologic techniques may represent an important approach for the diagnosis of schistosomiasis, especially when applied to populational surveys. Sensitivity of the tests is certainly of utmost importance. A high specificity level is also necessary, but even when presenting relatively limited specificity, such tests could still be useful, at least as a screening technique. The application of parasitological methods could then be restricted to suspected serologically positive cases.

Hemagglutination techniques have been already employed for the serologic diagnosis of Manson's schistosomiasis [5, 6] with rewarding results [2]. However, the use of fresh erythrocytes in such tests is not practical for routine purposes, since new batches of cells have to be sensitized daily.

In our laboratory a hemagglutination technique with formalin-treated erythrocytes has been standardized [4]. Such cells after treatment with tannic acid, were sensitized with worm extracts. The test was found practical, since sensitized cells are stable for many months at 4°C, at —20°C or even at room temperature when freeze-dried.

In this paper we report initial results obtained in a similar test employing chromium chloride to bind *S. mansoni* antigens to formalin-treated erythrocytes.

MATERIAL AND METHODS

Antigenic extract of S. mansoni — Adult worms obtained through hepatic and portal perfusion of infected mice were washed successively in saline solution, cold ethyl alcohol and cold ether (—20°C) and dried. In a Potter-Evelyn tissue grinder, worms were ground for a few minutes in saline solution, about 1 ml for 10 worms. The resulting suspension was left at 4°C for 24 hours and

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then centrifuged in the cold. The supernatant was distributed in small vials, in 10 ml amounts, and kept at —20°C. Protein concentration in the extract was determined by the method of LOWRY et al. [7].

Serums — Were obtained from 30 patients showing eggs of *S. mansoni* in stools, and from 30 individuals residing in non-endemic areas and presenting no clinical and parasitological evidences of schistosomiasis. Such serums had been kept for varying periods at —20°C and were not inactivated before tests, nor absorbed with human erythrocytes. For use, serums were diluted at 1:40 and so on, in doubling dilutions, in saline solution containing rabbit normal serum at 2%, which had been previously absorbed with human erythrocytes.

Hemagglutination test — Formalin-treated, group 0 Rh negative human erythrocytes [1] were suspended at 2.5% in worm extract diluted in saline solution to contain 200 micrograms of protein per milliliter. Sensitization of erythrocytes was performed through the action of chromium chloride, as reported by GOLD & FUDENBERG, 1967 [3]. One tenth milliliter of a 1% solution of chromium chloride ($CrCl_3.6H_2O$) was added per milliliter of suspension and the mixture left for 5 minutes at room temperature. After this period, a large volume of saline solution was added to the mixture and this was centrifuged for a few minutes. Sedimented cells were twice washed and finally suspended at 2.5% in saline solution. Such sensitized cells were kept at 4°C.

The tests were performed in perspex plates * containing V-shaped wells of 0.15 ml capacity, or in disposable plastic trays **, with four drops (0.1 ml) of serum dilutions plus one drop (0.025 ml) of sensitized cell suspension. Blanks were prepared for serums (1:40 dilution plus non-sensitized cells) and for antigen (diluent plus sensitized cells). Plates were covered and left at room temperature and readings could be taken indifferently after 3 hours or overnight. Sedimentation patterns were evaluated as previously indicated for the hemagglutination test with tanned cells [4], negative results corresponding to a compact button of deposited cells. In strongly positive tests, an irregular carpet of sedimented cells formed, which was seen in weakly positive tests as a narrow ring around a smooth mat deposit of erythrocytes. Titers of serums were taken as the highest dilution giving any degree of hemagglutination.

RESULTS AND DISCUSSION

Positive tests were found with all the 30 serums from parasitized patients. For the other 30 individuals with no evidences of schistosomiasis, only negative results were obtained.

For the positive serums, titers were the same, or differed by only one dilution from those found in a similar test employing tannic acid treated erythrocytes, as previously described [4] (Table I). Both hemaggluti-

TABLE I

Comparative hemagglutination titers for *S. mansoni* antigens, with erythrocytes treated with chromium chloride or with tannic acid, in 30 serums

Titers with tannic acid	Titers with chromium chloride						
	640	1,280	2,560	5,120	10,240	20,480	40,960
640	6	3					
1,280		4	2				
2,560			3	2			
5,120			5				
10,240					1	2	
20,480							2
40,960							

* Microtitrator Takatsy, Labor, Budapest, Hungary
** Linbro Chemical Co., Conn., U.S.A., model IS-MVC-96

nation techniques were performed for each serum, using the same series of dilutions. In the serums from non-parasitized individuals, tannic acid hemagglutination tests were also negative.

Preliminary observations indicate that cells sensitized through the action of chromium chloride can be kept for at least 2 months (the largest period of observation) at 4°C, with no diminished activity.

Binding *S. mansoni* antigens to formalin-treated erythrocytes through the action of chromium chloride is an easier technique than with tannic acid, with comparable results regarding the sensitivity of the test and probably also the keeping qualities of the sensitized cells.

The *S. mansoni* hemagglutination test with preserved sensitized cells, seems practical for the serologic diagnosis of schistosomiasis, especially when large scale surveys are intended, because it is easy and economical. Antigenic suspensions are stored ready for use and, as the tests can be performed in microplates, antigen is spared and the minute blood samples collected from finger-tip on filter paper furnish eluates in sufficient amounts for the tests and can substitute venous blood serum.

RESUMO

Reação de hemaglutinação para a esquistossomose com hemácias formolizadas e tratadas pelo cloreto de cromo

O cloreto de cromo mostrou-se tão eficiente quanto o ácido tânico para a fixação de antígenos de *S. mansoni* a hemácias formolizadas. Idênticos resultados foram obtidos pelas duas técnicas em 60 soros, revelando que a sensibilidade de ambas é semelhante. A facilidade do processo de sensibilização das hemácias pelo antígeno e a possibilidade de conservá-las por longos períodos já prontas para uso, bem como a simplicidade da reação em microplacas fazem desta técnica um método prático para inquéritos sorológicos.

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