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SEPARATION OF TOXOPLASMA GONDII TROPHOZOITES FROM MOUSE EXUDATE CELLS: COMPARISON OF METHODS

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Abstract. The efficiency of methods used in separating *Toxoplasma gondii* from mouse peritoneal exudate cells was compared. The following techniques were employed: centrifugation in saccharose polymere gradients, ultrasonic vibration with tryptic digestion and filtration through glass filters. Recovery of *Toxoplasma* cells was traced during the comparison. The contamination with leucocytes was checked by counting on the one hand and by immunodiffusion tests on the other. The gradient centrifugation proved to be the most efficient, but technically very complicated method.

Trophozoites of *Toxoplasma gondii* are mostly obtained from the infected mouse peritoneal exudate. Besides the parasites, the latter also contains a considerable amount of host cells. For various immunological studies removal of the cells is desirable and for this reason several methods of separation of toxoplasmas from exudate cells were employed. The techniques mostly used at present are filtration (Fulton and Spooner 1957), ultrasonic vibration (Tsunematsu 1960), differential centrifugation (Lycke and Lund 1964) and filtration through glass wool (Maekelt 1970). In this study, a comparison of *Toxoplasma* suspensions purified by filtration, ultrasonic vibration and zonal centrifugation was made.

MATERIAL AND METHODS

The P strain of *Toxoplasma gondii* was used in the Prague laboratory. It is a virulent strain isolated from a child who died of congenital toxoplasmosis. In the Moscow laboratory the RH strain was used. *Toxoplasma* strains were maintained by serial passage in mice.

Zonal centrifugation was performed in the MSE Mistral 6 L centrifuge using zonal rotor A (MSE, London). Centrifugation was carried out using 5—25 % dextran (National Enterprise Léčiva, Prague) or Ficoll (Pharmacia, Uppsala) density gradients for 20 min. at 1,700 rpm. During centrifugation *Toxoplasma* cells and leucocytes were separated. Pure suspension of *Toxoplasma* trophozoites was obtained from the peak fraction concentrated by centrifugation. (For details see Masihi and Jíra, in press).

Ultrasonic vibration was performed according to Tsunematsu (1960). 100 Watt Ultrasonic Desintegrator (MSE, London) was operated for 20 sec. at 18—24 KH, followed by trypsin (2 %) digestion for 1 hr at 37 °C. The suspension obtained was centrifuged and washed in physiological saline.

Filtration was performed after Fulton and Spooner (1957). Jena G 3 sintered glass filter was used (diameter 90 mm, porosity 15—40 (μ).

Toxoplasmas and leucocytes were counted in a Bürker counting chamber using phase contrast microscope at 360× magnification. Antigen was prepared by extracting desintegrated toxoplasmas in tris buffer pH 7.4. For desintegration a glass homogenizer (model Eppendorf 50 ml, Braun Co. Melsungen) or ultrasonic desintegrator were used. After centrifugation the supernatant fluid was used as antigen.

Immunodiffusion tests were performed as a slide microtechnique (Wadsworth 1957, Hübner and Uhlíková 1969). To detect host components, 1 mg/ml of protein in tris buffer was placed in the first well, followed by serial dilutions in other remaining wells. The central well was filled with mouse antiserum and each of the four peripheral wells were filled with antigen of varying dilution.

The antiserum against mouse leucocytes was prepared from two rabbits which had been immunized with mouse spleen homogenates in an adjuvant (60 % Bayol F, 30 % Arlacel, 10 % Tween 80 mixed with antigen at the 1 : 1 ratio). Before the experiment the rabbits were tested on the presence of *Toxoplasma* antibodies by indirect fluorescent antibody test with a negative result. The adjuvant was injected subcutaneously in the flank at 14 days intervals, totally in 5 doses. Each dose contained 25—30 mg of protein. The animals were bled 10 days after the last immunization. In order to get gamma globulin the sera obtained were fractionated by ammonium sulphate precipitation. The gamma globulin was lyophilized. The dry weight of 140 mg of gamma globulin per ml was used for immunodiffusion tests.

The protein estimation was done employing modifications by Lowry et al. (1951).

RESULTS

The particular methods of separation of *Toxoplasma* trophozoites from mouse peritoneal exudate cells are compared in Table 1. Recovery represents the percentage of toxoplasmas obtained after the procedure as compared with the initial amount before the procedure. The same applies to leucocytes. The immunodiffusion titre represents the reciprocal value of the last dilution, where the lines between central well with the mouse antiserum and the peripheral well with the diluted *Toxoplasma* antigen were still discernible.

Table 1. Comparison of methods used for separation of *Toxoplasma* trophozoites from leucocytes in mouse peritoneal exudate

Method	% Recovery		Immunodiffusion titres with antimouse serum
	Toxoplasma cells	Leucocytes	
Centrifugation in gradient (peak fraction)	38—42	0	0—2
Ultrasonic vibration with tryptic digestion	25—40	0	2—4
Filtration	40	0—1.5	4—8
Unpurified exudate sediment	100	100	16—32

Centrifugation in gradients: if dextran is used, the peak of *Toxoplasma* fraction is in gradient of 1.02 density, while the peak fraction of mouse leucocytes is in gradient of 1.06 density. The *Toxoplasma* peak is contaminated with mouse erythrocytes. No leucocytes were found during the count in Bürker counting chamber, but some of them were observed sporadically during the sediment control following the concentration. Toxoplasmas are not damaged by gradient contrifugation as demonstrated by tests on mice.

Ultrasonic vibration: host leucocytes but no erythrocytes may be sometimes found in the resulting suspension. The infectivity of toxoplasmas is decreased. The mice infected with the treated toxoplasmas die 2-3 days later than the control animals.

Filtration: Jena glass filter used in our experiments was chosen as the best among 16 filters (with 15-40 μ porosity) of English, German and Czech production. The filtrate usually contains mouse leucocytes as well as erythrocytes.

DISCUSSION

In order to separate toxoplasmas from the host cells Lycke and Lund (1964) employed gradient centrifugation. They used cellulose, saccharose, various salts as gradient but without any success. In our laboratory dextran and Ficoll were used as gradient. Ficoll gives somewhat better results than dextran. The peak of toxoplasmas and that of leucocytes are well separated. Recovery of peak fraction is usually about 40 %. Removal of mouse débris is considerable, but not 100 %, as demonstrated by immunodiffusion. The method of gradient centrifugation is technically quite complicated.

While using the ultra-sound method described by Tsunematsu (1960), recovery of toxoplasmas was 30-60 % and removal of host cells almost 100 % in all cases. In our experiments the recovery did not exceed 40 %. The removal of host cells was not 100 %, as apparent from the result of immunodiffusion. The decrease of *Toxoplasma* infectivity was in agreement with the results of above mentioned authors.

Filtration tests were described by several authors, such as Fulton and Spooner (1957), Neimark and Blaker (1967), Remington et al. (1970), Bloomfield and Remington (1970). In order to check the purity of filtrate Bloomfield and Remington used immunodiffusion in agar. The immunodiffusion demonstrated no host cell impurities, although the authors described the presence of erythrocytes in the filtrate. This may have been caused by the fact that the authors used antiserum against mouse serum and not antiserum against mouse leucocytes and erythrocytes. In the paper of Fulton and Spooner (1957) the recovery is given to be as high as 100 %, while in our experiments it did not exceed 40 %.

The result of immunodiffusion shows that the absence of host cells ascertained by microscope does not imply that no host impurities are present. In the recent paper of Takeuchi (1971) dealing with centrifugation of toxoplasmas in saccharose containing mouse antiserum the recovery given ranges from 25 to 40 % (exceptionally also 50 %).

All these methods may be of limited importance for the studies on antigen structure and biochemical studies as well. To obtain pure toxoplasmas a greater attention should be paid to *Toxoplasma* cultivation in tissue cultures (Akinchina and Gracheva 1964).

ОТДЕЛЕНИЕ ТРОФОЗОИТОВ *TOXOPLASMA GONDII* ОТ КЛЕТОК МЫШИНОГО ЭКСУДАТА: СРАВНЕНИЕ МЕТОДОВ

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Резюме. Сравнивалась эффективность методов для отделения трофозоитов *Toxoplasma gondii* от клеток мышиного брюшинного эксудата. Были использованы следующие методы: центрифугация в градиентах полимеров сахарозы, обработка ультразвуком и трипсином, и фильтрация через стеклянные фильтры. При сравнении наблюдали выход токсоплазм. Наличие лейкоцитов проверялось с одной стороны учетом, а с другой путем иммунодиффузной пробы. Самой эффективной, но зато технически весьма сложной оказалась центрифугация в градиентах.

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