

## Technical method

### Purification of *Toxoplasma gondii* from host cells

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*Toxoplasma gondii* is a protozoan parasite that can cause stillbirth or abortion in humans and domestic animals if first contracted during pregnancy. Because it is an obligate intracellular parasite and multiplies only in living cells, host cell contamination is a problem when research is performed on *Toxoplasma*. Therefore, a method of separating the tachyzoite from host cells that results in as little damage as possible to the tachyzoite is necessary to facilitate analysis of the parasite.

Although techniques involving preferential physical and/or chemical disruption of host cells do not appear to affect the viability of such purified tachyzoites,<sup>1-3</sup> the possibility exists that ultrasonic vibration and/or enzyme or antiserum effects may alter cell membrane antigens without affecting the viability of the tachyzoites. Consequently, different types of differential centrifugation and filtration have been developed for purification of the parasite.

Zonal centrifugation and density-gradient centrifugation have given recoveries of *Toxoplasma* up to 70% with less than 1% host white blood cell (WBC) contamination,<sup>4,5</sup> but these techniques are expensive, time-consuming and technically complex.<sup>6</sup> Of the filtration techniques available, the original method of Fulton and Spooner<sup>7</sup> using sintered glass filters of 15–40  $\mu\text{m}$  porosity has gained wide acceptance, as these filters are readily obtainable and allow filtration of large volumes of material in a short time. Although Fulton and Spooner obtained 100% recovery of *Toxoplasma* in their filtrate, other workers<sup>1,6,8</sup> have reported only 40% to 58% recovery of parasites and there is doubt as to whether all filters give similar results.<sup>1,9</sup>

Recently, Wilson *et al*<sup>10</sup> and Handman and Remington<sup>11</sup> have reported good success (less than one leucocyte/10<sup>4</sup> tachyzoites) using filtration

through a polycarbonate membrane of 3  $\mu\text{m}$  porosity, although percentage recovery of the parasites was not stated. We have been unable to find detailed results for this new technique, and report here a comparison between sintered glass filtration and polycarbonate membrane filtration for the purification of *Toxoplasma* tachyzoites.

#### Material and methods

##### MICE

Specific-pathogen-free female outbred LACA mice (6–8 weeks old) were used for growth of the parasite. They were kept under conventional conditions for at least one week prior to use.

##### PARASITE

The RH strain of *T gondii* was grown in LACA mice and harvested when parasite numbers were maximal.<sup>12</sup> Viability of the tachyzoites before and after filtration was measured by 0.1% trypan blue exclusion.

##### FILTRATION

Sintered glass filter: the pooled exudate washings of infected mice were passed through a sintered glass filter of 15–40  $\mu\text{m}$  porosity, diameter 90 mm, total volume 600 ml (Buchner Funnel, size 3, catalogue No SF3B/33 Corning Ltd, Laboratory Division, Staffordshire, England). The viable cells present before and after filtration were counted using a Neubauer haemocytometer.

Nuclepore polycarbonate filter: the pooled exudate washings of infected mice were passed through a Nuclepore polycarbonate membrane of 3  $\mu\text{m}$  porosity, diameter 47 mm (stock No 111112, lot No 62C1A1, Nuclepore Corp, Pleasanton, California) under light vacuum. The viable cells present before and after filtration were counted as above.

#### Results

Table 1 shows the mean values obtained for five filtrations through a sintered glass filter. The recovery of tachyzoites and red blood cells (RBC) were about the same (60% to 70%), but greater than 99.5% of the WBC were retained by the filter. *Toxoplasma* tachyzoites constituted greater than 93% of the total cells in the filtrate whilst WBC were reduced to less than 0.05% of the total cells present.

Table 2 shows the mean values obtained for five filtrations through 3  $\mu\text{m}$  polycarbonate membranes.

Table 1 Effect of filtration through a sintered glass filter on the numbers of cells present in the pooled peritoneal exudate washings of mice infected with the RH strain of *Toxoplasma gondii* (values = mean ± SD for five experiments)

Volume filtered (ml)	Cell type	Concentration of cell		% recovery	% of filtered cells
		Before filtration	After filtration		
113.00 ± 65.24	<i>Toxoplasma</i>	6.61 ± 1.37 × 10 <sup>7</sup> /ml	4.10 ± 1.46 × 10 <sup>7</sup> /ml	62.48 ± 22.91%	93.60 ± 1.01%
	White blood cells	4.08 ± 0.82 × 10 <sup>6</sup> /ml	1.52 ± 0.89 × 10 <sup>6</sup> /ml	0.40 ± 0.20%	0.04 ± 0.02%
	Red blood cells	3.83 ± 1.24 × 10 <sup>6</sup> /ml	2.69 ± 0.95 × 10 <sup>6</sup> /ml	71.00 ± 17.04%	6.36 ± 1.01%

Table 2 Effect of filtration through a Nuclepore polycarbonate membrane filter on the numbers of cells present in the pooled peritoneal exudate washings of mice infected with the RH strain of *Toxoplasma gondii* (values = mean ± SD for five experiments)

Volume filtered (ml)	Cell type	Concentration of cell		% recovery	% of filtered cells
		Before filtration	After filtration		
40.0 ± 21.95 ml	<i>Toxoplasma</i>	3.54 ± 1.58 × 10 <sup>7</sup> /ml	2.18 ± 1.03 × 10 <sup>7</sup> /ml	61.43 ± 11.37%	99.03 ± 0.47%
	White blood cells	3.30 ± 1.54 × 10 <sup>6</sup> /ml	7.40 ± 4.22 × 10 <sup>6</sup> /ml	2.16 ± 1.18%	0.32 ± 0.14%
	Red blood cells	2.64 ± 1.62 × 10 <sup>6</sup> /ml	1.40 ± 0.73 × 10 <sup>6</sup> /ml	6.56 ± 5.63%	0.64 ± 0.35%

The recovery of tachyzoites was similar to that obtained with sintered glass filtration, but the removal of RBC was markedly better using polycarbonate filtration. The retention of WBC by the polycarbonate membranes was less than for the sintered glass filter. The most important feature of the polycarbonate membrane filtration was that the *Toxoplasma* tachyzoites constituted greater than 99% of the cells in the filtrate, whilst WBC and RBC were reduced to less than 0.5% and 1% respectively of the total cells present.

The viability of the tachyzoites before and after filtration was greater than 95% for both methods.

**Discussion**

The results obtained suggest that filtration through a 3 µm polycarbonate membrane produces a filtrate with a similar yield of tachyzoites to sintered glass filtration, but with less contamination with host WBC and RBC. The disadvantages of the system are the cost of the membranes, and the fact that they tend to become blocked after about 10 ml of peritoneal exudate has been passed through.

Sintered glass filtration allows the filtration of larger volumes of peritoneal exudate, but the filter must be thoroughly cleaned with boiling 1% (wt/vol) sodium hydroxide prior to use, and the filtrate contains more host cell contamination than that obtained by polycarbonate membrane filtration. Therefore, sintered glass filtration appears suitable for removing host cells from large volumes of peritoneal exudate in situations such as the preparation of tachyzoites for use in the indirect

immunofluorescence test, where a small amount of host cell contamination may be acceptable. The new technique of polycarbonate membrane filtration appears ideal for the filtration of small volumes of peritoneal exudate to be used in immunological or biochemical analyses of the parasite, where host cell contamination should be reduced to a minimum.

**References**

- 1 Tsunematsu Y. Purification of *Toxoplasma* by means of sonic vibration and tryptic digestion. *Am J Trop Med Hyg* 1960;9:556.
- 2 Takeuchi T. Purification of *Toxoplasma* trophozoites from the peritoneal exudate of infected mice. *Jpn J Parasitol* 1971;20:205.
- 3 Tryon JC, Weidner E, Larson AD, Hart LT. A rapid method for isolating purified *Toxoplasma* tachyzoites from peritoneal exudate and cell culture. *J Parasitol* 1978;64:1127.
- 4 Masihi KN, Mach O, Valkoun A, Jira J. *Toxoplasma gondii*: large-scale purification by zonal density centrifugation. *Exp Parasitol* 1976;39:84.
- 5 Masihi KN, Jira J. Density gradient centrifugation for separation of different stages of *Toxoplasma*. *Folia Parasitol (Praha)* 1979;26:9.
- 6 Gracheva LI, Masihi KN, Valkoun A, Jira J. Separation of *Toxoplasma gondii* trophozoites from mouse exudate cells: comparison of methods. *Folia Parasitol (Praha)* 1973;20:203.
- 7 Fulton JD, Spooner DF. Preliminary observations on the metabolism of *Toxoplasma gondii*. *Trans R Soc Trop Med Hyg* 1957;51:123.
- 8 Kumar PS, Kumar R, Mohapatra LN. Purification of *Toxoplasma* haemagglutination antigen. *Indian J Med Res* 1978;68:44.
- 9 Masihi KN. A simple method for separating *Toxoplasma gondii* from infected mouse peritoneal exudate cells. *Zbl Bakt Hyg I Abt Orig A* 1975;233:556.
- 10 Wilson CB, Tsai V, Remington JS. Failure to trigger the oxidative metabolic burst by normal macrophages. *J Exp Med* 1980;151:328.

<sup>11</sup> Handman E, Remington JS. Serological and immunochemical characterization of monoclonal antibodies to *Toxoplasma gondii*. *Immunology* 1980;40:579.

<sup>12</sup> Johnson AM, McDonald PJ, Neoh SH. Kinetics of the growth of *Toxoplasma gondii* (RH strain) in mice. *Int J Parasitol* 1979;9:55.

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## Letters to the Editor

### Internal birefringence and the recognition of *Leishmania* parasites

The parasites of visceral leishmaniasis may be found in stained smears of bone marrow or splenic aspirates, predominantly in macrophages or monocytes but frequently scattered loosely about the intercellular spaces. When they are numerous there may be little difficulty in their detection and recognition; they appear with Romanowsky staining as oval bodies, about 2–5  $\mu\text{m}$  by 1–3  $\mu\text{m}$ , and the leishmanial or amastigote forms seen in direct smears have a dark staining round nucleus and a smaller, more rod shaped, kinetoplast (Fig. 1). When parasites are infrequent, as is usually the case in bone marrow smears, they may not

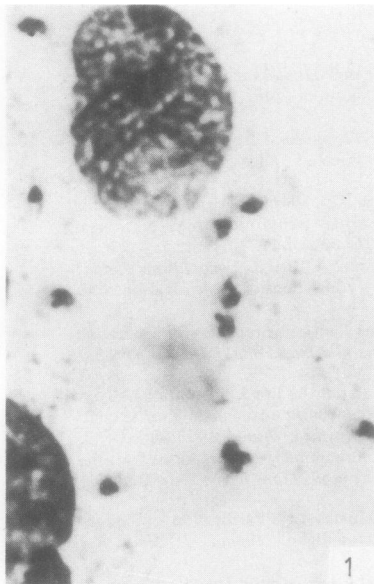


Fig. 1 *Leishman* stain of bone marrow smear showing *L donovani* bodies in the disrupted cytoplasm of a pair of macrophages.

be easy to recognise; in size and shape they closely resemble platelets and their internal structure is not always discernible or clearly distinguishable from the granular content of platelets.

We have recently noted, in the bone marrow of a patient admitted to Addenbrooke's Hospital under the care of Dr D Rubenstein and found to have kala azar, that the kinetoplast of the amastigote form of *Leishmania donovani* is sharply birefringent under polarised light (Fig. 2). Study of several specimens of different *Leishmania* species in Romanowsky-stained smears (one of *L donovani* (man) kindly provided by Professor W Peters of the London School of Hygiene and Tropical Medicine and others of hamster tissues infected respectively with *L tropica* major (USSR, rodent), *L braziliensis braziliensis* (Brazil, man), *L donovani* infantum (Ethiopia, man) and *L mexicana amazonensis* (Brazil, rodent) kindly provided by Dr PJ Gardener of the Molteno Institute, Cambridge) has confirmed this observation as valid for all parasites studied. We report it here in the hope that others may find this a helpful feature, as we have, in confirming the suspected presence of the parasites. Platelets do not show birefringence. No doubt the same feature would be visible in the parasites seen in skin biopsies from cutaneous leishmaniasis, but such material has not been available for us to study.

As far as we are aware the birefringence of the kinetoplast in *Leishmania* has not previously been reported. Gardener<sup>1</sup> drew attention to the presence of refractile granules in one isolate of *L braziliensis braziliensis* (LV63) though they were not present in other isolates from the same species (LV20 and LV64). Scorza *et al*<sup>2</sup> described similar refractile inclusions, separate from the kinetoplast, in a new species of *Leishmania* (*L garnhami*) found in cases of cutaneous leishmaniasis in the Venezuelan Andes region. These bodies,

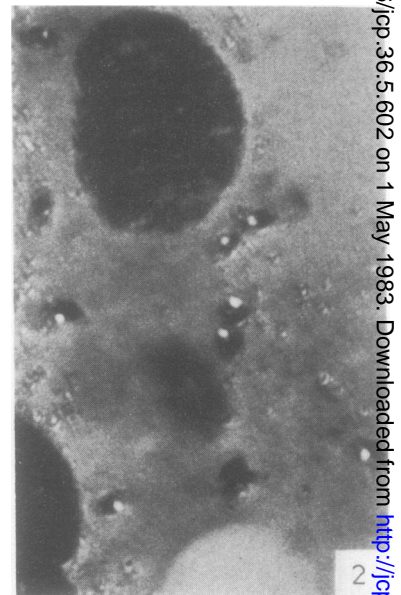


Fig. 2 The same area under polarised light showing sharp refringence in the parasites at a point corresponding to the kinetoplast. With rotation typical birefringence from blue to orange was seen.

which appear so far to be restricted to the above isolates, may perhaps correspond to liposomes visible by electron microscopy, at least in *L garnhami*.<sup>2</sup> In the LV63 isolate of *L braziliensis braziliensis* the position, staining and refractile characteristics of these granules can be clearly distinguished from the birefringent kinetoplasts, as shown in Figs. 3 and 4.

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