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Isopycnic isolation of African trypanosomes on Percoll gradients formed in situ

Short communication

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Several methods can be used to isolate trypanosomes from infected blood; namely hemagglutination and differential centrifugation (Simmons et al., 1964), sucrose gradient centrifugation (Williamson and Cover, 1966) and anion exchange chromatography (Lanham, 1968; Lanham and Godfrey, 1970). The principle of separation by the latter method which is the simplest and most widely used rests on the fact that the ionic charges on the cell surface of salivarian trypanosomes differ significantly from the surface charges of the cellular components in mammalian blood (Lanham, 1968; Lanham and Godfrey, 1970). Although relatively simple, this method is not optimal because the yields and viability of the parasites vary, especially with *Trypanosoma vivax* organisms, and the method can be time consuming.

Colloidal silica gradients have recently been employed for isopycnic isolation of cells and cellular organelles with high viability from a variety of sources (Pertoft and Laurent, 1977). Silica solutions are polydisperse and when they are centrifuged at high speeds in an angle head rotor the colloidal particles will sediment at different rates, generating a density gradient (Pertoft and Laurent, 1977). When silica solutions are mixed with an inert polymer, the gradients approach linearity. Variation of both the silica and polymer concentration as well as the g-force and centrifugation time, allows the generation of various gradient profiles (Pertoft and Laurent, 1977). Recently, a new technique was introduced for the isolation of African trypanosomes on Percoll[1] (polyvinylpyrrolidone coated silica) gradients (Bwayo and Hirumi, in press). We now present in greater detail a general method which exploits the self-generating gradient property of Percoll for the isolation of *T. brucei*, *T. congolense* and especially

[1] Percoll, Pharmacia Fine Chemicals AB, Uppsala, Sweden

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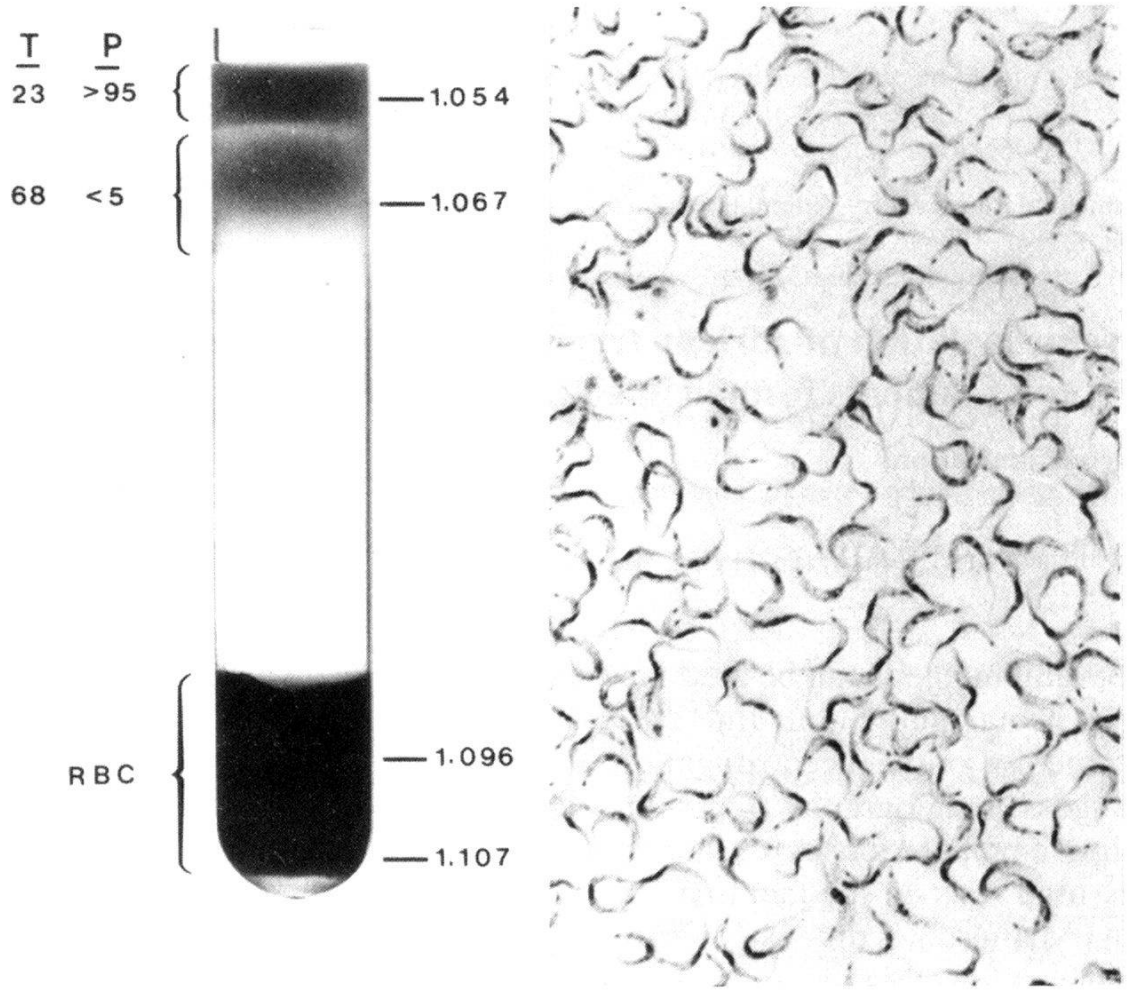

A B

Fig. 1. – A. *T. vivax* was prepared from 7.5 ml infected blood (3.3×10^8 parasites/ml) as described in the text. A sham gradient containing serum (in place of whole blood) and Percoll density marker beads (having buoyant densities ranging from 1.054 to 1.107 g/ml) was also run. The trypanosome containing bands were fixed in 0.37% formaldehyde in Dulbecco's phosphate buffered saline and counted. The % recovery of trypanosomes and of platelets are shown as well as the buoyant densities throughout the gradient. T = trypanosomes; P = platelets; RBC = red blood cells. – B. Low power light micrograph of Giemsa stained *T. vivax* isolated in the lower trypanosome containing band shown in Fig. 1 A.

T. vivax from infected rat and mouse blood in high yields (approximately 90% or better).

Trypanosome infected whole blood (in citrate or heparin) is mixed with an equal volume of Percoll made up as follows: 8.55 g sucrose, 2 g glucose, and 100 ml 100% Percoll (density 1.130 /ml). The pH is then adjusted to 7.4 by the addition of solid HEPES. After mixing, the blood-Percoll suspension is centrifuged for 15–20 min at 17,500 × *g* (4° C) in an angle head rotor (34 or 40°; e.g. Beckman JA-20 or JA-21, or Sorvall SS-34 rotors). Under these conditions, the concentrated trypanosomes usually float as one band near the top of the gradient, well separated from the red blood cells which sediment to the bottom. With blood highly infected with trypanosomes, contamination by platelets is

negligible; however, platelet contamination can be significant if the parasitaemia is low. The latter problem can be alleviated by passing the Percoll isolated organisms through a small DEAE-cellulose column (Lanham, 1968; Lanham and Godfrey, 1970; Lumsden et al., 1977).

Although we routinely use the proportions of Percoll-blood and the buffer system outlined above, other proportions and buffer systems can be utilized. The important observation is that the final Percoll concentration should be approximately 50% in isotonic sucrose buffers (60% if isotonic salt based buffers are used; e.g. culture media). It is equally important that the centrifuge tubes be at least 80% full and the best results are obtained when the blood is immediately mixed with the Percoll after removal from the animal. Alternatively, modifications of the above isolation conditions have been tried. For example, the infected blood can be layered either on top of or below (in the latter case the blood is in a denser solution of Percoll) a 50% isotonic Percoll solution followed by high speed centrifugation. However, mixing the blood into the Percoll is simpler and greater amounts of infected blood can be processed.

Trypanosomes do not appear to be affected by the high *g*-forces involved in the in situ formed gradient separation. This has also been found with other cell systems (Pertoft and Laurent; 1977). This technique has proved particularly useful in the isolation of rodent adapted *T. vivax* which is recovered in low yield when subjected to the DEAE-cellulose isolation method, while more than 90% of viable parasites are recoverable with the Percoll method (Fig. 1). The viability and integrity of Percoll isolated rodent infective *T. vivax* has been assessed by their capacity to incorporate ^{35}S-methionine into protein in a linear fashion similar to that found for *T. brucei* and *T. congolense*, by binding of surface specific antibody in immunofluorescence assay indicating that the coat is intact, and by infectivity titrations (Suman Mahan, personal communication).

The simplicity and speed of isolation, as well as the higher yields obtainable by the new method for separation of African trypanosomes from infected blood should prove useful in many biochemical and parasitological studies.

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Bwayo J. J., Hirumi H.: An improved method for the separation of African trypanosomes from infected rat blood. Proceedings of the 2nd Annual Medical Conference of Kenya Medical Research Institute and Kenya Trypanosomiasis Research Institute (in press).

Lanham M. S.: Separation of trypanosomes from the blood of infected rats and mice by anion-exchanges. Nature (Lond.) *218*, 1273–1274 (1968).

Lanham M. S., Godfrey D. G.: Isolation of salivarian trypanosomes from man and other mammals using DEAE-cellulose. Exp. Parasit. *28*, 521–531 (1970).

Lumsden W. H. R., Kimber C. D., Strange M.: *Trypanosoma brucei:* a miniature anion exchange centrifugation technique for detection of low parasitaemias in mice. Trans. roy. Soc. trop. Med. Hyg. *71*, 421–424 (1977).

Pertoft H., Laurent T. C.: Isopycnic separation of cell and cell organelles by centrifugation in modified colloidal silica gradients. In: Methods of cell separation, ed. by N. Catsimpoolas, Vol. I, p. 25–65. Plenum Press, New York 1977.

Simmons V., Knight R. H., Humphreys K. C.: Separation of trypanosomes from rat blood components. 10th Meeting, International Scientific Committee for Trypanosomiasis Research, Kampala, p. 81–82. C.C.T.A., London 1964.

Williamson J., Cover B.: Separation of blood-cell-free trypanosomes and malaria parasites on a sucrose gradient. Trans. roy. Soc. trop. Med. Hyg. *60*, 425–427 (1966).