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Purification of *Giardia muris* Cysts by Velocity Sedimentation

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***Giardia muris* cysts were separated from fecal contaminants in primary isolates by unit gravity velocity sedimentation. Crude isolates obtained by centrifugation over 1.0 M sucrose were overlaid onto a Percoll density gradient, 1.01 to 1.03 g/ml. *G. muris* cysts were well separated from faster-sedimenting fecal debris and slower-sedimenting *Spironucleus muris* and bacteria in 1.5 h.**

Investigations with *Giardia muris* as a model of *Giardia lamblia* are often hampered by the difficulty in obtaining purified cyst preparations from primary fecal isolates. These isolates are often contaminated with the common rodent parasite *Spironucleus muris* (synonym, *Hexamita muris*) as well as other fecal debris. *G. muris* and *S. muris* have similar buoyant densities, but they do differ in size. *G. muris* cysts measure ca. 9 by 6 μm (7), and *S. muris* cysts measure ca. 6 by 3 μm (1). Primary isolates, obtained by centrifugation of fecal material over a 1.0 M sucrose step gradient, can be further purified by a simple unit gravity velocity sedimentation procedure capable of separating *G. muris* from *S. muris* and other dissimilar-size fecal debris.

Percoll (colloidal silica coated with polyvinylpyrrolidone; Pharmacia Fine Chemicals, Uppsala, Sweden) was chosen as the gradient medium because of its minimal contribution to the osmolarity of the solution, its low toxicity to cells, and its low viscosity (3, 4). The buoyant densities of both *G. muris* and *S. muris* were found to be between 1.049 and 1.096 g/ml when determined on isotonic Percoll-sucrose step gradients at 20°C with density marker beads (Pharmacia Fine Chemicals) to calibrate the gradient. The unit gravity gradient media densities were therefore set as low as practical (1.01 to 1.03 g/ml) to minimize any separation based on buoyant density differences, yield the fastest separation, and still stabilize against convection and mixing during the separation.

The solutions of Percoll were made in the following way. Low-density Percoll solution (1.01 g/ml) was made by adding 77.3 ml of undiluted Percoll (1.13 g/ml) and 10 ml of phosphate-buffered saline concentrate (80 g of NaCl, 2 g of KH_2PO_4, 29 g of $Na_2HPO_4 \cdot 12H_2O$, 2 g of KCl, distilled water to make 1,000 ml [pH 7.4]) to 900 ml of distilled water containing 0.01% polyoxyethylene sorbitan monolaurate (Tween 20; Sigma Chemical Company, St. Louis, Mo.). High-density Percoll solution (1.03 g/ml) was made by adding 230.8 ml of undiluted Percoll and 10 ml of phosphate-buffered saline concentrate to 700 ml of distilled water containing 0.01% Tween 20. Both solutions were adjusted to pH 7.0 with 1 N HCl before adjusting the final volumes to 1,000 ml with Tween-distilled water.

The velocity sedimentation apparatus is illustrated in Fig. 1. The sedimentation chamber size and sample size were chosen so that the sample layer when initially applied was less than 2 mm thick. In practice, a Nalgene 250-ml polymethylpentene cylindrical sample container (no. 2117; 6.4 cm [diameter] by 10.8 cm [height]) was used at a 10° tilt for the sedimentation chamber. A 100-μl glass pipette was taped to the inside, 0.5 mm from the lowest point. Fine flexible tubing was used to interconnect the gradient mixer with the separation chamber. The flow rate indicator was a drop-counting device similar to that used to control fluids administered intravenously.

G. muris cysts were initially harvested by centrifugation on a 1.0 M sucrose solution (5). The crude isolate was then washed several times with distilled water, and finally, cyst numbers were adjusted to no more than 10^7 cysts per ml. In velocity sedimentation procedures there is a maximum concentration limit for particulates in the starting sample. Beyond this limit a phenomenon known as streaming occurs (2), which mixes the sample with the gradient. In practice, the maximum concentration limit for this system was ca. 10^7 cysts per ml in most crude isolates and was somewhat less in highly contaminated isolates.

An 80-ml gradient (1.01 to 1.03 g/ml) was formed in the sedimentation chamber, with a final depth of 3.5 cm. The flow rate was adjusted with the drip counter to form the gradient without turbulence. Up to 7 ml of the sample was carefully layered on the gradient, forming a sample band ca. 2 mm thick and well defined on the bottom edge.

The sample band was then allowed to sediment at room temperature for 1.5 h. The *Giardia* cyst band fell at a rate of ca. 1.0 cm/h. When the *Spironucleus* concentration was heavy, it could be seen as a band ca. 0.9 cm above the *Giardia* band.

After closing the clamp (G) and breaking the connection at F (Fig. 1), fractions were rapidly collected by syphon flow from the bottom of the chamber. Fraction 1 (ca. 15 ml) preceding the *Giardia* band contained large-sized fecal debris and therefore was discarded. The rest of the fractions (5 ml each) were examined for *G. muris*, *S. muris*, bacteria, and debris by phase or bright-field microscopy. The fractions that were free from contamination, as determined by microscopic examination, were pooled and washed three times with distilled water by centrifugation for 2 min at 650 × *g* to remove Percoll. *Giardia* cyst viability, as determined by excystation and infectivity for CF-1 mice, was not affected by exposure to sedimentation in Percoll (F. W. Schaefer, personal communication).

Table 1 shows the reduction in *Spironucleus* contamination and *Giardia* recovery data for four different sample separations. The range of values reflects not only variability in this technique, but also qualitative differences in the original samples (i.e., amount of fecal debris, bacteria, and *S. muris*). A typical separation profile of *Giardia* and *Spironucleus* cysts is illustrated in Fig. 2. In this separation, the original sample contained a ratio of approximately one *Spironucleus* cyst to four *Giardia* cysts. Of the total number of *Giardia* cysts layered onto the gradient, 73% were recovered in fractions 2 to 6. *Spironucleus* contamination in

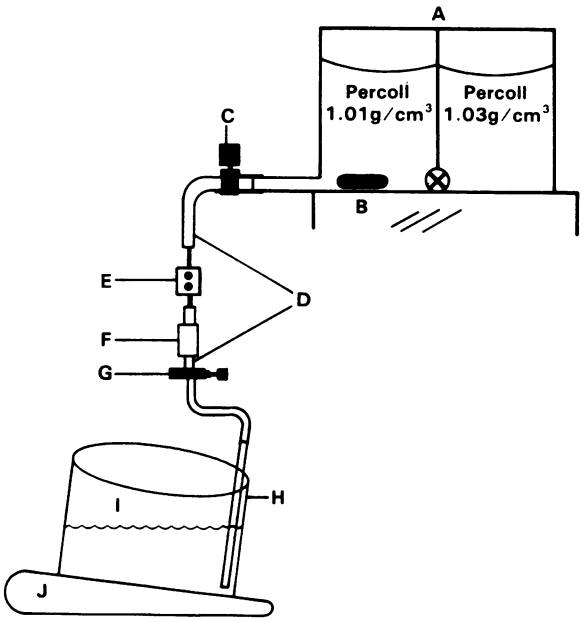

FIG. 1. Cyst separation apparatus. The letters represent the following: A, gradient maker; B, magnetic stirrer; C, screw clamp for flow rate adjustment; D, flexible tubing; E, drip counter; F, connector or three-way valve used for fraction collection; G, screw clamp for shutoff; H, 100-μl glass micropipette; I, sedimentation chamber; J, wedge-shaped support with ca. 10° tilt.

these pooled fractions was reduced to 1 for every 1.6×10^3 *Giardia* cysts. Bacteria and fine-sized debris remained at the top of the gradient, and as determined by microscopic examination, the pooled *Giardia* cyst fractions were free from fecal contaminants.

The unrecovered *Giardia* cysts were probably lost either by mixing with the fast-sedimenting debris or by adherence to the walls of the sedimentation chamber. Tween 20 and plastic containers were used to reduce adherence. The increase in cysts in the last fraction may have been caused by the detachment of adhering cysts as the air-liquid interface passed over the chamber wall and floor.

This technique can be performed on a larger scale with a large sedimentation chamber (Nalgene no. 2117; 500 ml; 11 cm [diameter] by 7.5 cm [height]) filled to a depth of 5 cm with gradient media. Here, the sample size can be increased to 40 ml (maximum of 4×10^8 cysts in a 0.4-cm-thick band) with a sedimentation time of 2.5 h.

TABLE 1. Reduction of *S. muris* contamination in recovered *G. muris* cyst suspensions after unit gravity sedimentation

Trial no.	% of applied *G. muris* recovered in cleanest pooled fractions[a]	Ratio of *G. muris* to *S. muris* in:	
		Original sample[b]	Cleanest pooled fractions[a]
1	68	4:1	1,500:1
2	82	4:1	>900:1[c]
3	60	3:1	>4,200:1[c]
4	81	5:1	>3,900:1[c]

[a] Cleanest pooled fractions result from the unit gravity sedimentation of original samples isolated on 1.0 M sucrose.
[b] Original sample is the product of centrifugation over 1.0 M sucrose.
[c] No *S. muris* cysts were observed in five hemacytometer fields.

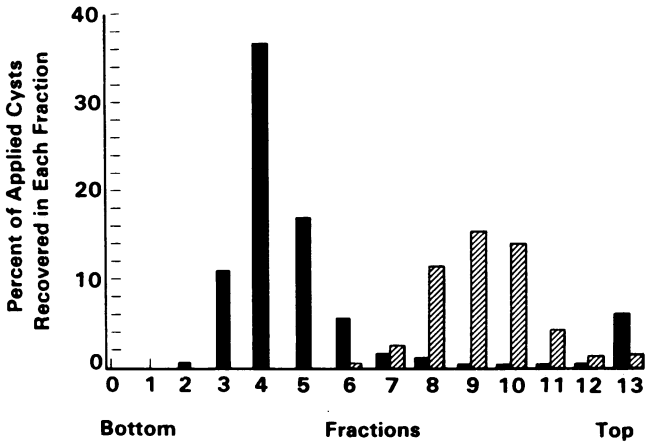

FIG. 2. Separation profile of *G. muris* (■) and *S. muris* (▨). Five-milliliter fractions (no. 2 to 13) were collected after the first 15 ml (fraction 1) was discarded.

The most important factors affecting the success of the separation are the concentration of particulates in the starting band and the starting band thickness.

This separation procedure with Percoll is simple and yields good recoveries of viable *G. muris* cysts that, as determined by microscopic examination, are virtually free from fecal debris and intestinal flora. Provided that the crude isolates, produced by centrifugation over sucrose, are not heavily contaminated, this procedure also may be used for *G. lamblia*. Given the success of this technique, it is likely that centrifugal elutriation techniques (6) could be successfully applied to the purification of both *G. muris* and *G. lamblia*.

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