Is Percoll innocuous to cells?

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Peritoneal macrophages from mice, isolated rat liver Kupffer cells and rat testis Leydig cells ingested large numbers of Percoll particles, a gradient medium widely used for separation of cells and subcellular organelles by density-gradient centrifugation. A decrease in the percentage of macrophages adhering to plastic also occurred after exposure of the cells to Percoll, even at 4°C, a temperature at which Percoll was not ingested. The effect of Percoll on macrophage adherence may involve a loose association between the density medium and the cell surface. Other cell-surface-related phenomena may also be affected by prior exposure of cells to Percoll.

Percoll was introduced in 1978 as a gradient medium for density-gradient centrifugation of cells and subcellular organelles (Pertoft *et al.*, 1978). It is now widely used; more than 100 references to its properties and use were collected by the manufacturer (Pharmacia Fine Chemicals, Uppsala, Sweden) by 1980. Percoll consists of polyvinylpyrrolidone-coated colloidal silica-gel particles, which form a polydisperse population of nearly spherical particles with a mean diameter of 28–30 nm when hydrated with 0.15 M-NaCl (Laurent *et al.*, 1980). When introduced, Percoll was reported to be non-toxic to cells, and little evidence to the contrary has appeared, to our knowledge. We have studied the effect of Percoll on the adherence to plastic of cells obtained from peritoneal exudates induced in mice. We report here that prior incubation with Percoll substantially decreases the percentage of adherent cells when compared with controls. Furthermore, at 20–37°C peritoneal macrophages, as well as rat Kupffer cells and rat Leydig cells, ingest large numbers of Percoll particles.

Materials and methods

Macrophage-rich peritoneal exudates were collected from female CBA mice injected 6 weeks previously with 20 *Mesocostoides corti* larvae. The cells were pelleted by centrifugation (at 400 g for

5 min at 22°C) and resuspended at 10⁷ cells/ml in 0.14 M-NaCl/20 mM-sodium phosphate buffer, pH 7.2, with or without 45% (v/v) iso-osmotic Percoll. After incubation for 30 min, the cells were diluted with 2.5 vol. of Dulbecco's modified Eagle's medium (Grand Island Biological Co., Grand Island, NY, U.S.A.) washed twice and either prepared for electron microscopy or assayed for their ability to adhere to plastic. Recovery of cells after exposure to Percoll was quantitative. Kupffer cells were isolated by collagenase perfusion of rat liver followed by mechanical separation of the cells (Seglen, 1972), and were then incubated with 80% (v/v) iso-osmotic Percoll for 30 min at 20°C, washed once in 25 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid; Sigma Chemical Co., St. Louis, MO, U.S.A.]/NaOH buffer, pH 7.4, and prepared for electron microscopy. Leydig cells were isolated from rat testes by a procedure that involved collagenase treatment to prepare a crude interstitial-cell preparation (Purvis *et al.*, 1978), which was then subjected to density-gradient centrifugation in Percoll for 20 min at 20°C (Gale *et al.*, 1982). The isolated Leydig cells were washed twice in Dulbecco's modified Eagle's medium and then prepared for electron microscopy.

Cells were fixed for electron microscopy with one-half-strength Karnovsky's fixative (Karnovsky, 1965) followed by OsO₄ (1%, w/v). Percoll was embedded in agar. All samples were sectioned on an LKB Ultratome III, stained with uranyl acetate and lead citrate, and examined in a Siemens 102 electron microscope.

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Results and discussion

Peritoneal exudates, collected from mice infected with *Mesocestoides corti* larvae, contained monocytes/macrophages (55%), polymorphonuclear leucocytes (3%), eosinophils (38%) and lymphocytes (4%). Most (96%) of the cells excluded Trypan Blue. Prior exposure of the cells to Percoll at 4°C and at 37°C resulted in a marked decrease in the percentage of cells that adhered to the plastic (Table 1, Expt. 1). Small molecules in the Percoll suspension obtained from the manufacturer were not responsible for the inhibition of cell adherence that was observed. The diffusate from a Percoll suspension was without significant effect, whereas the dialysed Percoll still exhibited a substantial (though smaller) inhibitory effect (Table 1, Expt. 2).

Electron micrographs showed that incubation with Percoll at 37°C, but not at 4°C, resulted in the ingestion by macrophages of large numbers of Percoll particles, which were tightly packed within large vacuoles (Plate 1). The other cell types in the peritoneal exudate did not take up Percoll.

Leydig cells from rat testis and Kupffer cells from rat liver were also found to ingest Percoll. Under the

electron microscope, the isolated Leydig cells were seen to contain particle-filled vacuoles, which were not observed in Leydig cells in the interstitial-cell suspension before contact with Percoll (Plate 2a). The size and appearance of the particles within the vacuoles are similar to those of Percoll (Pertoft *et al.*, 1978; and Plate 1a inset). Ingestion of Percoll by isolated Kupffer cells from rat liver in incubations at 20°C, but not 4°C, was also observed (Plate 2b). Percoll was not ingested by isolated rat hepatocytes or rat testicular tubule cells incubated at 20°C for 30 min, or by human polymorphonuclear leucocytes incubated at 37°C for 30 min.

The two major components of Percoll, silica and polyvinylpyrrolidone, are known to be taken up by cells, presumably by pinocytosis (Williams *et al.*, 1975; Allison *et al.*, 1966). Uptake of Percoll by cells has been infrequently reported, however. Rat peritoneal mast cells, isolated by centrifugation (at 40g for 5 min at 4°C) in a density gradient of Percoll, displayed occasional endocytic vesicles containing Percoll in the peripheral parts of the cells (Enerbäck & Svensson, 1980). Others have reported that incubation of isolated rat liver lysosomes with Percoll results in uptake that increases with time and temperature (Marzella *et al.*, 1980). The paucity of reports of Percoll uptake by cells may be related to the fact that many density-gradient centrifugations are performed at 4°C, a temperature at which endocytosis is inhibited, and to the failure of investigators to obtain electron micrographs of the isolated cells. Furthermore, we have shown that certain cell types do not ingest Percoll under conditions favourable for its uptake by other cells.

The uptake of Percoll by rat Leydig cells was surprising, since, in contrast with macrophages and Kupffer cells, Leydig cells are not considered to be actively phagocytic *in situ*. We suspected that the collagenase treatment used to prepare the crude interstitial-cell preparation stimulated phagocytosis by the Leydig cells. Further work has shown, however, that rat Leydig cells prepared without collagenase also ingest large quantities of Percoll at 20°C (J. St.J. Wakefield, unpublished work). Browning *et al.* (1981) centrifuged a collagenase-derived interstitial-cell preparation from rat testis in a Percoll gradient at 4°C and did not observe particles of the gradient medium within the isolated Leydig cells. Phagocytosis of Percoll by the isolated rat Leydig cell thus appears to be primarily a temperature-dependent phenomenon. Mouse Leydig cells, on the other hand, apparently do not ingest Percoll, even at 20°C (Schumacher *et al.*, 1978).

A number of investigators have studied the function of monocytes and macrophages after isolation in Percoll gradients under a variety of conditions. Nathanson *et al.* (1977) noted a significant depression of chemotactic and random

Table 1. *Effect of prior exposure to Percoll on the adherence of mouse peritoneal macrophages to plastic*
Washed macrophages were prepared as described in the text, and 20000 cells were suspended in Dulbecco's modified Eagle's medium (without serum) and were plated in flat-bottomed plastic microtitre wells (Nuncclon, Roskilde, Denmark). After incubation for 1 h or 3 h in an atmosphere of 5% CO₂, non-adherent cells were resuspended by tapping or with a Pasteur pipette and removed by aspiration. The adherent cells were counted in four to ten microscopic fields (magnification ×400). Dialysis of Percoll was performed overnight at 4°C against an equal volume of 0.14M-NaCl/0.22M-sodium phosphate buffer, pH 7.2. Different batches of Percoll were used in Expts. 1 and 2. The values are the means ± S.E.M.

Cell treatment	Adherent cells/microscopic field (% of cells added)	
	1 h	3 h
Expt. 1		
Buffer, 4°C	77 ± 9	55 ± 5
Buffer, 37°C	68 ± 7	64 ± 4
Percoll, 4°C	27 ± 4	33 ± 3
Percoll, 37°C	15 ± 2	29 ± 4
Expt. 2		
Buffer, 4°C	74 ± 6	
Percoll, 4°C	28 ± 1	
Percoll, dialysed, 4°C	41 ± 3	
Percoll, diffusate, 4°C	72 ± 5

EXPLANATION OF PLATE 1

Electron micrographs of parts of mouse peritoneal macrophages after incubations with Percoll

Conditions of incubation with Percoll and method of preparation of cells for electron microscopy are described in the text. (a) After incubation at 4°C (magnification $\times 22\,400$); (b) after incubation at 37°C (magnification $\times 21\,600$). Inset to (a) is an electron micrograph of Percoll (magnification $\times 38\,400$).

EXPLANATION OF PLATE 2

Electron micrographs of cells after exposure to Percoll

Conditions of exposure to Percoll and method of preparation of cells for electron microscopy are described in the text. (a) Rat Leydig cell (magnification $\times 10\,400$); (b) part of a rat Kupffer cell (magnification $\times 16\,800$). Inset to (a) is part of a rat Leydig cell (magnification $\times 40\,000$).

motility of monocytes separated on a Percoll gradient, as compared with cells separated on a Ficoll/Hypaque gradient, and Serio *et al.* (1979) observed a 6% inhibition of phagocytic activity after incubation of mouse peritoneal macrophages in an 8% (v/v) suspension of Percoll. Others have reported that monocyte/macrophage activities are unimpaired after isolation in Percoll gradients, including Trypan Blue exclusion and ability of monocytes to transform into macrophages (Fluks, 1981), phagocytosis (Gmelig-Meyling & Waldmann, 1980), cytotoxic ability (Farram & Nelson, 1980) and Fc receptor activity (Pertoft *et al.*, 1980). Fc receptors, phagocytic activity and non-specific esterase activity were found to be present in cultures of Kupffer cells previously isolated by centrifugation in a density gradient of Percoll (Pulford & Souhami, 1980).

In a study with mouse spleen cells, Dettman & Wilbur (1979) reported that few cells (1–6%) adhered to the walls of centrifuge tubes when colloidal-silica/aluminium-modified polyvinylpyrrolidone was used as a medium for density-gradient centrifugation, whereas 52–94% adhered when Ficoll or bovine serum albumin was used. In the present study, it is noteworthy that the peritoneal-exudate cells exposed to Percoll at 4°C displayed decreased adherence but did not take up Percoll. Therefore the inhibitory effect was not dependent on the cellular ingestion of Percoll. In no case were we able to demonstrate Percoll particles attached to the surface of the cells. Thus the results presented here suggest that a loose association between Percoll particles and the cell surface is sufficient to mediate an inhibitory effect on cell adherence. The mechanism of this effect remains to be determined, but it is likely that other surface-related phenomena are also affected by exposure of cells to Percoll. Also, the long-term effect of Percoll ingestion on cell viability and growth characteristics has yet to be assessed quantitatively.

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