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Brief Definitive Report

CULTURE OF MONONUCLEAR PHAGOCYTES ON A TEFLON SURFACE TO PREVENT ADHERENCE*

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Mononuclear phagocytes are usually cultured on a glass or plastic surface to which these cells readily become attached and spread. Both glass and plastic have, however, the disadvantage that it is very difficult to recover mononuclear phagocytes without injuring the cells. The present paper describes a method for culturing on a Teflon membrane to which cells do not attach and from which they can easily be recovered intact for experimental purposes.

Materials and Methods

The Teflon Film Dish (TFD). The TFD is a tissue-culture chamber consisting of a reusable aluminium holder with a Teflon ring (inner diameter 40 mm) in which a disposable nontoxic Teflon-FEP film (fluorinated ethylene propylene resin, Du Pont de Nemours and Co., Geneva, Switzerland; gauge 25 μm, supplied by Janssens' M & L, St. Niklaas, Belgium) is mounted (Fig. 1). The cover consists of a metal ring in which a transparent glass disk is fixed. This tissue-culture chamber has been described in detail elsewhere (1, 2), and can be obtained from Tecnomara AG, Zürich, Switzerland. The assembled chamber can be sterilized by autoclaving. Control experiments were done in glass and plastic Petri dishes (Falcon, Becton, Dickinson and Co., Oxnard, Calif.) and in Leighton tubes with a flying cover slip.

Preparation of Mononuclear Phagocytes. The techniques used for the harvesting and incubation of murine peritoneal cells have been described elsewhere (3).

Bone marrow cells were harvested from the mouse femur and cultured with an embryonic mouse fibroblast conditioned medium as already described in detail (4, 5).

Three macrophage cell lines (6, 7) ($P388D_1$, J774-1, and N6H1-3) were cultured in Dulbecco's modified Eagle's medium (Grand Island Biological Co., Grand Island, N. Y.) with 10% fetal bovine serum (Flow Laboratories, Ayrshire, Scotland) and 1% L-glutamine (Microbiological Associates, Bethesda, Md.), to which 1,000 U/ml penicillin G (Mycofarm, Delft, The Netherlands) and 50 μg/ml streptomycin (Mycofarm) had been added. The cell lines are maintained by incubating about 10^6 cells which are replated every 9–10 days.

Fibroblasts. Fibroblasts from mouse embryos were prepared and cultured as described earlier (5).

Recovery of the Cells. The cells were recovered from the TFD by aspirating the culture fluid before gently rinsing the Teflon surface with culture medium to remove all loosely adhering cells, after which the cells in the suspension were counted in a hemocytometer. The viability was tested by trypan blue (0.1%) exclusion.

Peritoneal cells cultured in glass Petri dishes were recovered by scraping with a rubber policeman at 4°C. Peritoneal cells cultured in plastic Petri dishes were treated with 10.7 mM lidocaine (Astra Chemicals & Pharmaceuticals, Södertalje, Sweden) and 3 mM EDTA in culture medium for 15 min at room temperature (M. Rabinovitch, personal communication), after which

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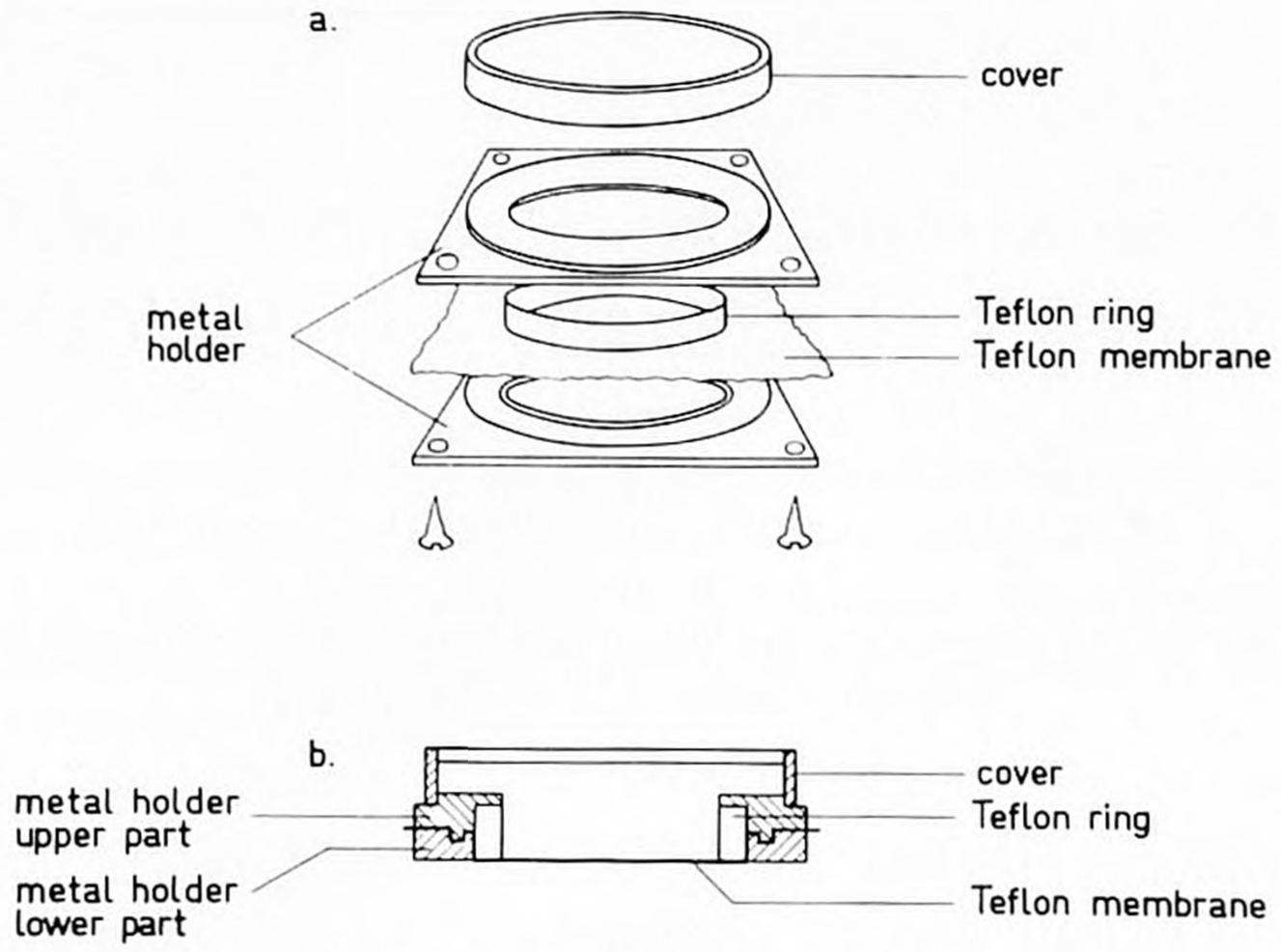

FIG. 1. Schematic drawing of the Teflon film dish. a. dish in unassembled state, b. dish in cross-section.

the surface was rinsed with 10 jets of medium from a Pasteur pipette. The fluid was aspirated, the number of cells counted, and the viability tested.

Light and Phase-Contrast Microscopy. During incubation, the cultures were studied in an unfixed state with an inverted phase-contrast microscope. Light-microscopical studies were done in cytocentrifuge preparations (8) made with cells recovered from the TFD or in 24-h cultures of these cells on a flying glass cover-slip in a Leighton tube. The cells on the glass surface were rapidly air dried and, unless stated otherwise, fixed in methanol and stained with Giemsa stain.

Cytochemistry. Esterase activity was investigated with α-naphthyl butyrate (Sigma Chemical Co., St. Louis, Mo.) as substrate at pH 6.1 (9); the incubation time was 25 min at room temperature. Peroxidase staining was performed according to Kaplow (10).

Receptors at the Cell Surface. In cells recovered from TFD and cells attached to glass, the presence of IgG receptors and complement receptors was determined as described elsewhere (4).

Phagocytosis and Intracellular Killing. The methods used to measure phagocytosis and intracellular killing separately are described in detail elsewhere (8). To test phagocytosis, about 10^7 viable cells were incubated with an equal number of preopsonized *Staphylococcus albus* (8) and 10% newborn calf serum under continuous rotation (4 revolutions/min) in a siliconized glass tube. At several time points the number of viable bacteria in the supernate was determined by a plating method.

Intracellular killing was assessed after 15 min of phagocytosis of preopsonized *S. albus*. The washed cells were then reincubated at 37°C, and at various time points the number of viable intracellular bacteria was determined (8).

Pinocytosis. Pinocytosis was studied by incubating the cells in the TFD with 10% dextran sulphate (mol wt 500,000; Pharmacia, Uppsala, Sweden) for 24 h, after which the cells were recovered from the dish for cytocentrifuge preparation, fixed in absolute methanol, and stained with Giemsa 2.5% vol/vol. Control experiments were done with cells cultured in Leighton tubes with a flying cover slip.

Results

Characteristics of Peritoneal Cells Incubated in TFD. Macrophages attach very loosely or not at all to a Teflon surface. Consequently, most of the cells do not stretch during incubation but remain round, as can be observed with phase-contrast microscopy. When the macrophages from TFD cultures are replated on a cover slip and incubated for 24 h, they can still attach to the glass surface and show the typical stretched shape of such macrophages.

Esterase and peroxidase staining and the percentage of macrophages with Fc

TABLE I

Characteristics of Mononuclear Phagocytes Cultured on a Teflon Film and on a Glass Surface

	Peritoneal macrophages*		Bone marrow mononuclear phagocytes‡		
			Cultured on Teflon		
	Cultured on Teflon	Cultured on glass	Cyto-centrifuge preparation	Recultured for 24 h on glass	Cultured on glass
	%	%	%	%	%
Peroxidase	0	1	1	7	1
Esterase	100	100	100	100	100
Fc Receptors	100	100	99	ND	100
C Receptors	98	100	100	ND	100
Pinocytosis	100	100	95	100	100

* Cultured for 24 h.

‡ Cultured for 7 days in the presence of conditioned medium.

and complement receptors, are the same in TFD- and glass-cultured macrophages (Table I).

Phagocytosis and intracellular killing of bacteria by TFD-cultured macrophages and noncultured cells obtained directly from the peritoneal cavity are comparable (Fig. 2).

Recovery and Viability of Peritoneal Cells in TFD Cultures. A recovery percentage of 77.6 (range 67.0–96.6%) is found after 24 h of incubation in the TFD. After harvesting, very few cells are left on the Teflon surface.

The viability of the recovered cells amounts to 93.3 ± 4.9% (SD) after 24 h of incubation in the TFD. In cultures on a glass or plastic surface, 100% viability is found for attached cells and 84% for the cells present in the supernate, more than 90% of which are lymphocytes. The recovery of cells cultured on glass and scraped off with a rubber policeman is about 25% with a viability of less than 55%.

The recovery of cells cultured on plastic after treatment with lidocaine and EDTA is 59.5% (range 46.2–79.3%) with a viability of 94.2 ± 4.3% (SD).

Characteristics of Bone Marrow Mononuclear Phagocytes Grown in TFD. The rate of proliferation of bone marrow mononuclear phagocytes on Teflon in the presence of conditioned medium is similar to that of cultures on a glass or plastic surface (Fig. 3). In TFD the mononuclear phagocytes do not grow in typical colonies (4), and because the cells do not attach and stretch, the various cell types (monoblast, promonocyte, and macrophage) cannot be recognized easily. The characteristics of bone marrow mononuclear phagocytes cultured for 7 days on Teflon and studied in cytocentrifuge preparations or after 24 h reculturing on glass are similar to those of cells cultured for 7 days on glass (Table I).

Macrophage Cell Lines Grown on TFD. The rate of proliferation of the three macrophage cell lines in TFD, from which the cells can easily be recovered in an undamaged state, proved to be of the same order of magnitude as on plastic. The cell lines P388D_1 and J774-1 showed a 25-fold increase and cell line N6H1-3 a 6-fold increase over a 10 day period of incubation. The viability after culture in TFD is more than 95% for these cell lines.

Fibroblasts Grown in TFD. Fibroblasts grown in TFD do not attach and

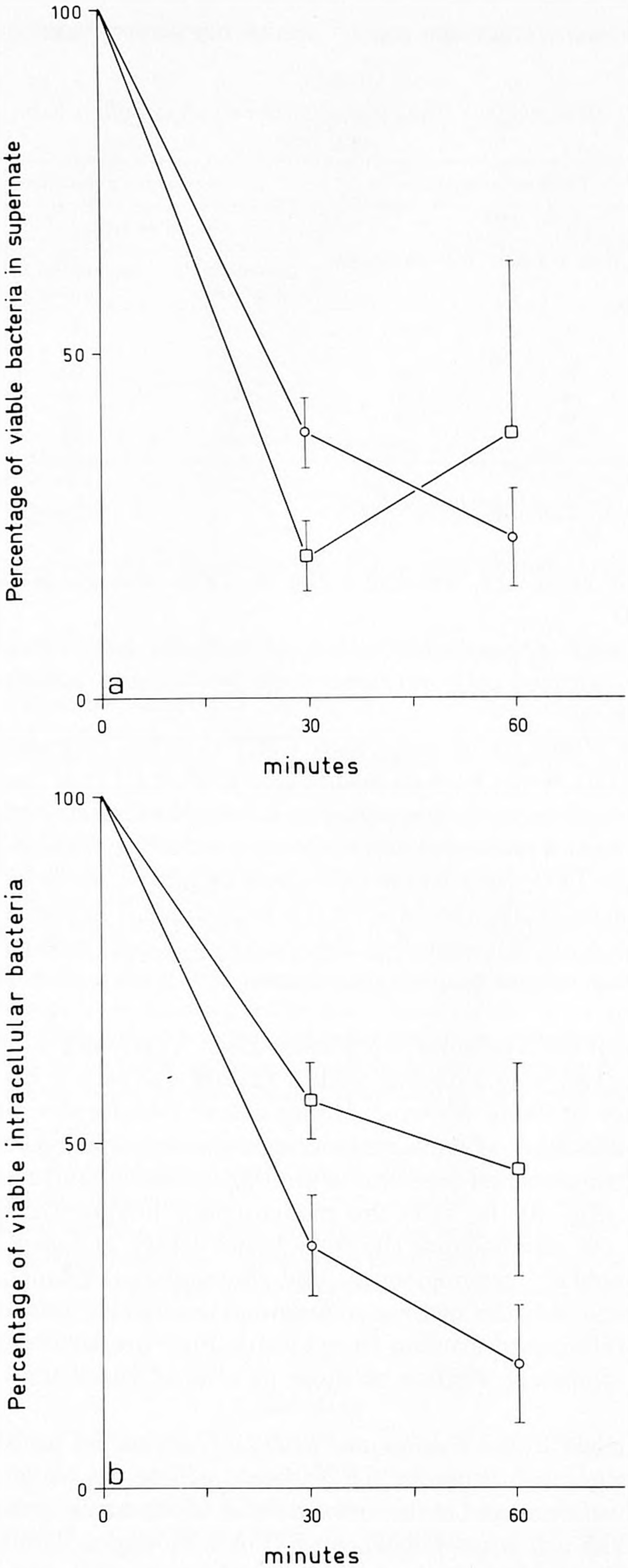

FIG. 2. Kinetics of phagocytosis (a) and intracellular killing (b) of *S. albus* by murine peritoneal macrophages. □ Cells recovered from TFD after 24 h of culture, ○ Cells directly harvested from the peritoneal cavity (data from reference 8). Bars indicate standard deviation.

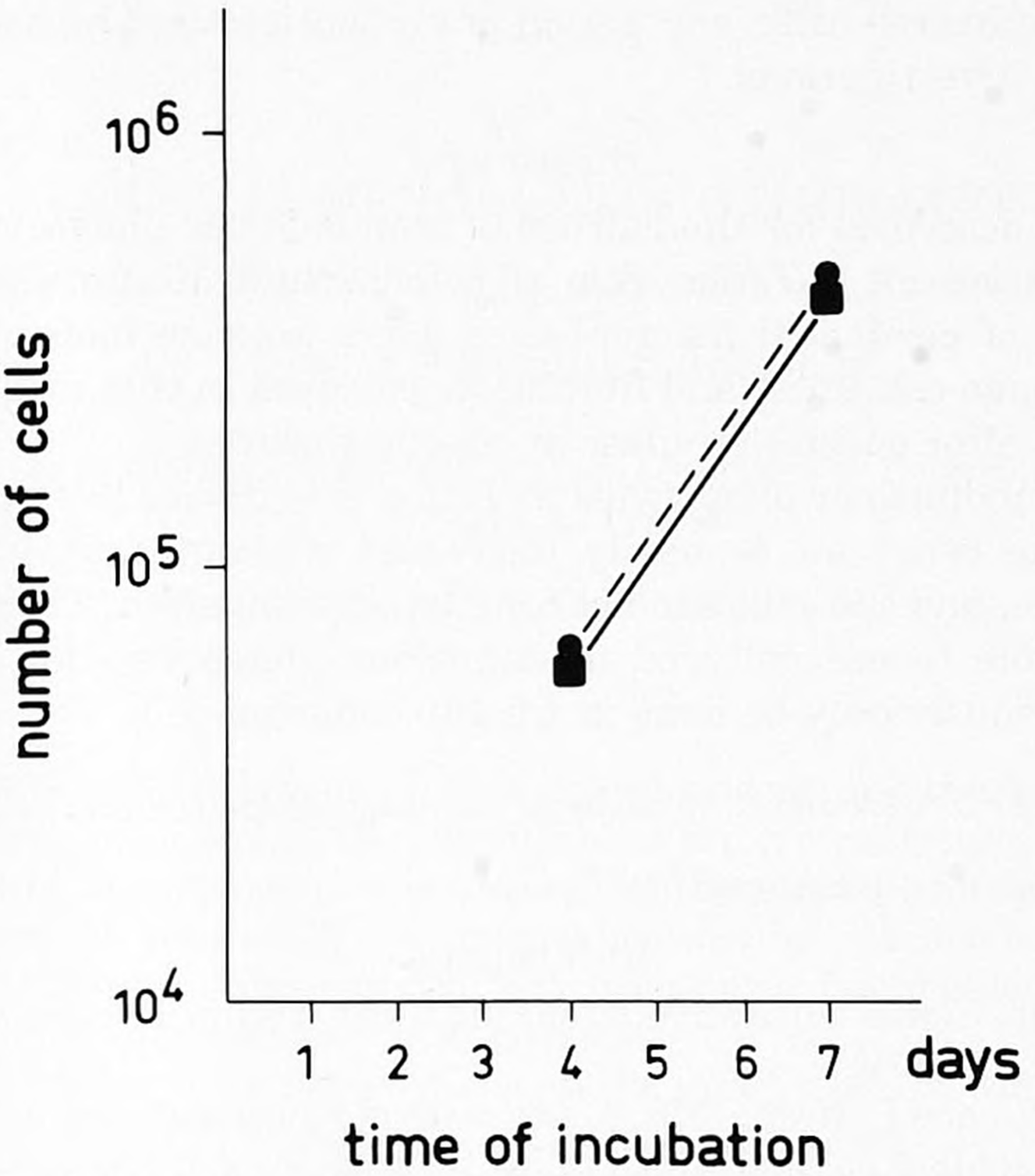

FIG. 3. On day 0, 2.5×10^5 nucleated bone marrow cells were incubated in Dulbecco's modified Eagle's medium with 20% horse serum and 20% conditioned medium (● cultures on plastic surface, ■ cultures on Teflon surface).

stretch on the Teflon surface either, but clump together during growth and retain a rounded shape.

Discussion

The disadvantage of culturing mononuclear phagocytes on a glass or plastic surface lies in the difficulty encountered in recovering the cells from the surface into suspension. Several methods are used to recover adherent cells, such as scraping with a rubber policeman at 4°C and treatment with trypsin, chelating agents (11, 12), or cationic anesthetics (13, 14). Under these conditions, however, recovery is far from complete or the cells are damaged by the procedure.

To avoid attachment of cells to the surface, a number of different techniques have been tried. Cultures of cells in tumbler tubes or in siliconized glassware (12) are only partially successful in avoiding attachment of these cells to the surface. The culture of mononuclear phagocytes on a surface coated with collagen or in a plasma clot (11, 12) and recovery of the cells by enzymatic digestion of the coating has several disadvantages, such as activation of the cells by the material on the surface, ingestion of the coating substance, and difficulties in the direct microscopical observation of the cells during incubation. Furthermore, study of the morphology of fixed, cultured cells is hampered by the coating material. Even cells which normally do not easily attach to glass or plastic (e.g. lymphocytes) may attach to the coated surface.

The culture chamber with the Teflon membrane (TFD) makes it possible to culture mononuclear phagocytes mainly in suspension, which means that they

can be easily recovered after any period of incubation, and be used for further immunological investigations.

Summary

A method is described for the culture of mononuclear phagocytes in suspension by incubation on a Teflon film to which the cells do not adhere. The characteristics of peritoneal macrophages, bone marrow mononuclear phagocytes, macrophage cell lines, and fibroblasts cultured in this way are similar to those observed after culture on glass or plastic surfaces.

Culture of mononuclear phagocytes in Teflon film dishes has three important advantages: the cells can be easily harvested without damage, recovery is almost complete, and the cells are not functionally impaired. Thus, this method makes it possible to use cultured mononuclear phagocytes for many studies that could previously only be done in freshly collected cells.

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