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## Suspension Cultures of Mononuclear Phagocytes in the Teflon Culture Bag<sup>1</sup>

JOS W. M. VAN DER MEER, JOKE S. VAN DE GEVEL, INE ELZENGA-CLAASSEN,  
AND RALPH VAN FURTH

*Department of Infectious Diseases, University Hospital, Leiden, The Netherlands*

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The Teflon culture bag (TCB) provides a cheap and simple method for culturing mononuclear phagocytes in suspension. The cells can easily be recovered intact and used in further experiments. The Teflon membrane is permeable to O<sub>2</sub>, CO<sub>2</sub>, and water vapor. Therefore, gas exchange is guaranteed when the bags are sealed after being filled with medium and cells. The risk of infection is minimized since the cultures are incubated in closed bags.

### INTRODUCTION

Mononuclear phagocytes cultured *in vitro* on a glass or plastic surface adhere and attach to the surface. When cultured on a Teflon<sup>®</sup> surface, these cells adhere very poorly and can easily be recovered intact for experimental purposes (6).

In the present paper a Teflon<sup>®</sup> culture bag (TCB) is described that provides a cheap and easy method for culturing mononuclear phagocytes in suspension and offers two additional advantages: it minimizes the risk of infection of the cultures and facilitates transportation of the cultured cells.

### MATERIALS AND METHODS

*The Teflon culture bag (TCB).* Disposable non-toxic hydrophobic Teflon FEP films (fluorinated ethylene propylene resin, Dupont de Nemours and Co., Geneva, Switzerland; gauge 25  $\mu$ m, supplied by Janssens' M & L, St. Niklaas, Belgium) are sealed with a diathermic sealing apparatus (Super Sealboy 235; 210 watt, Audion Electro, Amsterdam, The Netherlands) such that a triangular bag (approximately 4  $\times$  15  $\times$  15 cm) with one open angle is formed (Fig. 1). A glass bead (diam. 4 mm) is sealed in this angle to allow easy introduction of the needle used to fill the bag with a cell suspension. The bags are sterilized by autoclaving for 20 min at 120°C and three atmosphere. After the bag has been filled with medium and cells, the opening is sealed diathermically. During incubation substances can

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be added or samples taken by perforating the Teflon near this seal with the needle of the syringe and then resealing the hole.

*Preparation of cell suspensions.* The techniques for the harvesting and culturing of proliferating murine bone marrow cells have been described elsewhere (3). Conditioned medium from cultures of embryonic mouse fibroblasts (2) or L-cell conditioned medium (1) are used for these bone marrow cultures. Two macrophage cell lines (WEHI-3, PU 5-1.8) (5) are cultured in RPMI 1640 (Flow Laboratories, Irvine, Scotland) containing 10% fetal bovine serum (Flow Laboratories, Irvine, Scotland), 1% glutamine (Microbiological Associates, Bethesda, Maryland), and 0.5% glucose 1000 U/ml penicillin G (Mycofarm, Delft, The Netherlands) and 50  $\mu\text{g}/\text{ml}$  streptomycin (Mycofarm). The cell lines are maintained by incubating about  $5 \times 10^5$  cells, which are replated every 5 or 7 days.

*Incubation.* The TCB containing the cell suspension is placed in an incubator with 10%  $\text{CO}_2$  in air in a humidified atmosphere at  $37^\circ\text{C}$ .

*Recovery of the cells.* After gentle kneading of the TCB, a needle (19 gauge) attached to a syringe is introduced and the culture fluid is aspirated; this fluid is gently reinjected and aspirated several times to remove all loosely adherent cells. The recovered cells are then counted in a hemocytometer and viability is determined by trypan-blue (0.1%) exclusion.

*Permeability of the TCB.* To estimate carbon dioxide transport through the Teflon membrane, TCBs containing cells and culture medium were placed in the incubator together with petri dishes filled with the same suspension, which served as controls. The  $\text{O}_2$  and  $\text{CO}_2$  tension and the pH of the culture fluid were measured in a blood gas analyzer (IL 213; Instrumentation Laboratories, Lexington, Mass.). Permeability for water or water vapor was determined from the weight increase of silicagel in a dry sealed TCB during incubation in a container with water or in the humidified  $\text{CO}_2$ -incubator at  $37^\circ\text{C}$ , respectively. Permeability for sodium and potassium ions and glucose was evaluated by measuring these compounds before and after submersion in distilled water of a TCB filled with culture medium at  $37^\circ\text{C}$ . Determination of sodium and potassium ions was performed in a flame

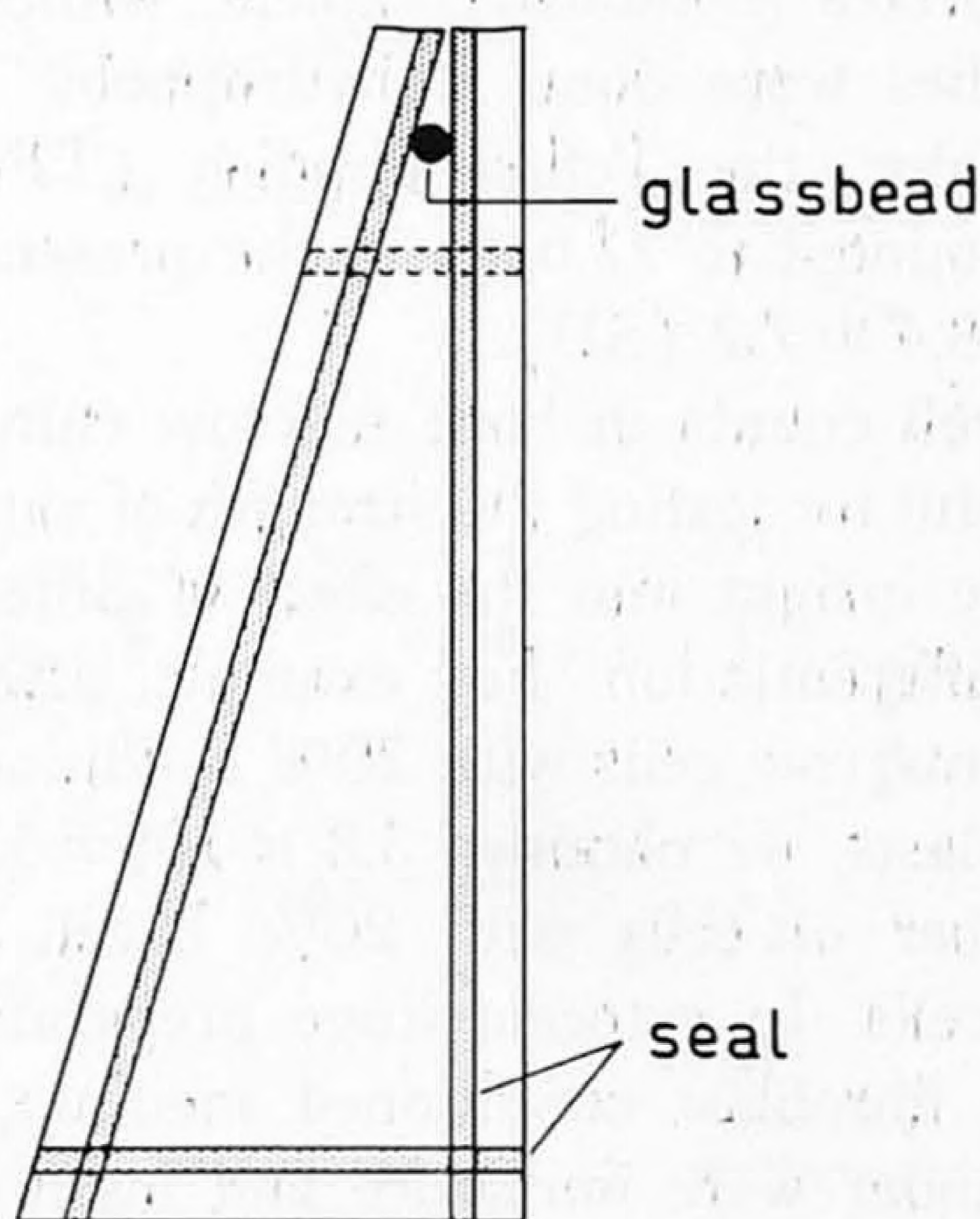


FIG. 1. The Teflon culture bag (TCB), a triangled bag made by diathermic sealing of two layers of Teflon FEP film. A glass bead allows easy introduction of a needle to fill the bag.

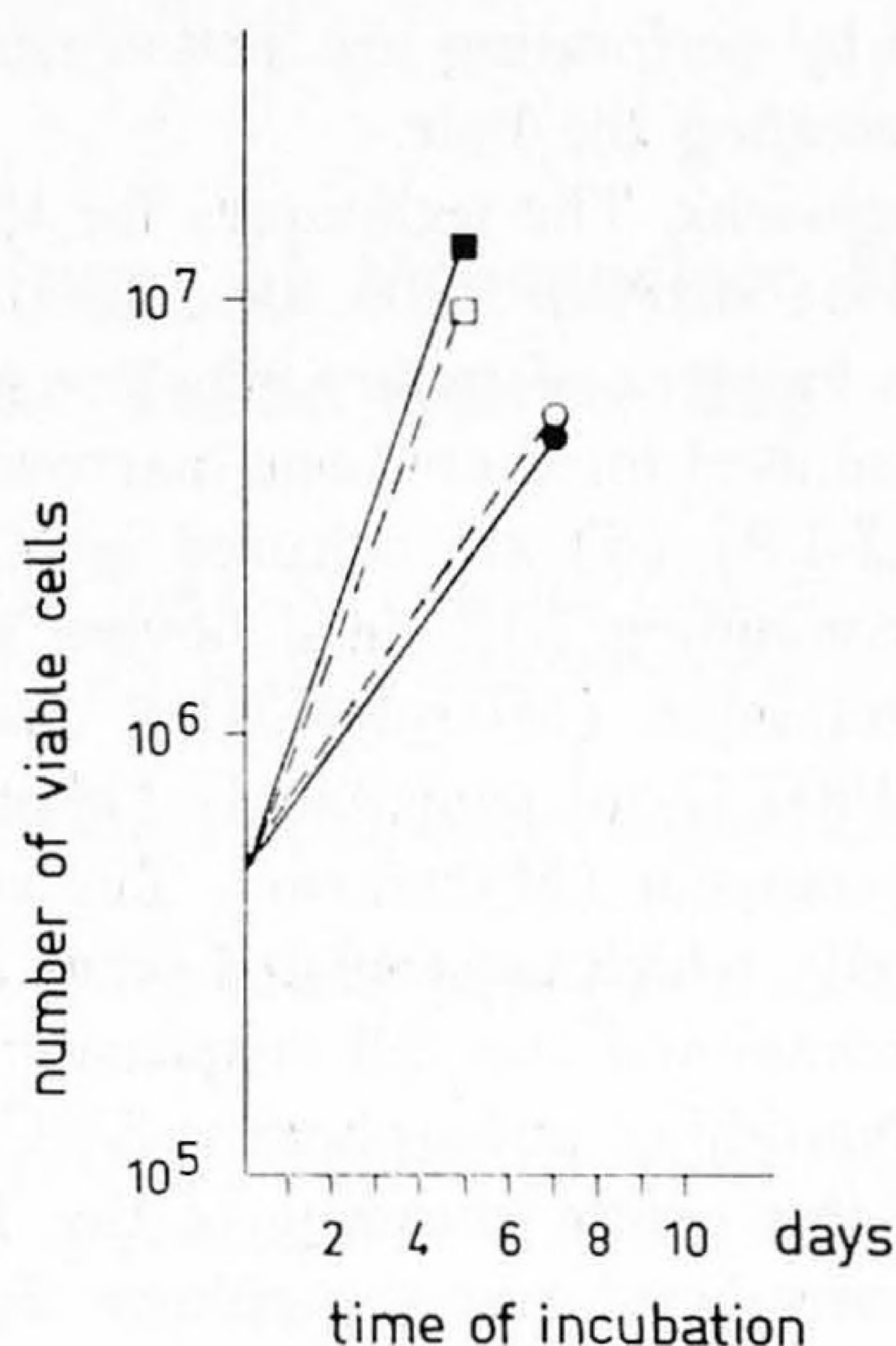


FIG. 2. Proliferation of macrophage cell lines in TCB (—) and plastic petri dishes (---). ○, cell line WEHI-3 and □, cell line PU 5-1.8.

photometer (IL 543, Instrumentation Laboratories, Lexington, Mass.). For glucose measurement, a Technicon Auto Analyzer AAII (Technicon instruments corporation, Tarrytown, N.Y.) was used.

*Resistance to infection.* Sealed TCBs containing culture medium were incubated in a flask with broth containing  $10^9$  *Staphylococcus aureus* per milliliter. After 24 hr of incubation the TCBs were removed, the outer surface of the bags was washed thoroughly, and the culture medium was aspirated and inoculated in broth.

## RESULTS AND DISCUSSION

In a previous paper (6) it was shown that recovery of mononuclear phagocytes cultured on a Teflon surface is almost complete, without cellular damage or loss of function. These studies were done in hydrophobic Teflon-film mounted in a reusable aluminium holder, the Teflon film dish (TFD). In these studies the recovery percentage amounted to 77.6%; in the present study with the TCB the recovery percentage is  $78.4 \pm 7.2$  (SD).

The combination of cell counts in bone marrow cultures with colony counts in semi-solid media are useful for testing the strength of various batches of conditioned media and provide more insight into the effect of different conditioned media on cell proliferation and differentiation. For example, after 7 days of incubation of  $5 \times 10^4$  nucleated bone marrow cells with 20% conditioned medium prepared with embryonic mouse fibroblasts, we obtained  $3.8 \times 10^4 \pm 1.1$  (SD) cells, and incubation of the same number of cells with 20% L-cell conditioned medium gave  $4.1 \times 10^4 \pm 0.2$  (SD) cells. In cytocentrifuge preparations of the cells recovered from the cultures with fibroblast conditioned medium, 14.2% of the cells were granulocytes; the remainder were immature and mature mononuclear phagocytes, as described elsewhere (3). The culture with L-cell conditioned medium had 8.2% granulocytes, the other cells being mononuclear phagocytes.

Figure 2 shows the findings for various macrophage cell lines. Proliferation of

TABLE 1

Electrolyte and glucose concentrations in culture medium in TCB<sup>a</sup>

	Na <sup>+</sup> (mmol/l)	K <sup>+</sup> (mmol/l)	Glucose (mmol/l)
Before incubation	161	5.1	5.2
After incubation for 24 hr	160	5.3	5.2
After incubation for 48 hr	160	5.1	5.3

<sup>a</sup> Incubation in distilled water at 37°C.

these cells was of the same order of magnitude as on plastic, but counting of the cells and replating are much easier after culture on Teflon.

TCBs filled with culture medium and incubated in broth containing bacteria did not become infected.

The Teflon film is permeable for CO<sub>2</sub> and O<sub>2</sub> (Fig. 3), but not for water and electrolytes (Table 1). The increased weight of TCBs filled with silicagel (19.5 mg in 48 hr per gram silicagel in a TCB) indicated permeability for water vapor, but permeability for water in the liquid phase could not be demonstrated. These results are in accordance with data provided by the manufacturer and published in the literature (4). The permeability of the Teflon membrane for gasses is dependent on the thickness of the membrane. The 25 μm-thick membrane seems to be optimal in this respect.

Munder *et al.* (4) used hydrophilic Teflon film for cell support but cells attach to such chemically etched films and the advantage of complete recovery is lost. The properties of the film we use are not the same as those of the cuprophane sheet used by van Ginkel *et al.* (7) to prevent adherence of monocytes. Cuprophane,

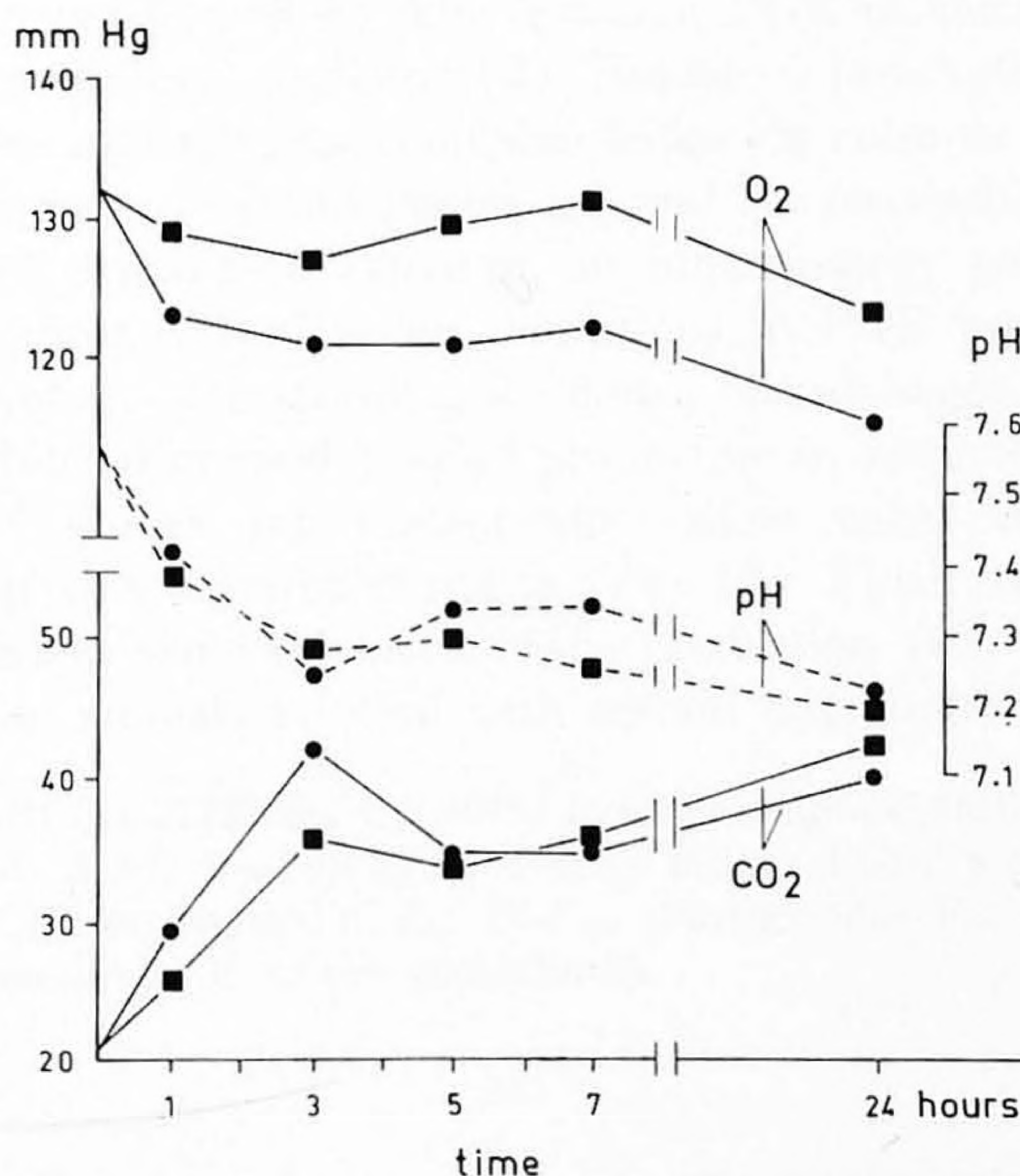


FIG. 3. CO<sub>2</sub> and O<sub>2</sub> tension and pH in culture medium in TCB (■) and in petri dishes (●) during incubation with 10% CO<sub>2</sub> in air in humidified atmosphere at 37°C.

which is used as a membrane in renal dialysis, has the disadvantage of permeability for water and electrolytes. The Teflon-film dish (TFD) (6) also has some disadvantages; the aluminium holder is expensive, cleaning and assembly of the device is laborious, and the cultures can easily become infected in the incubator. The TCB, on the contrary, is cheap and disposable, can be made quickly in the laboratory, and the size can be varied; moreover, the risk of infection is reduced and gas exchange is guaranteed.

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