
In vivo testing of crosslinked polyethers. I. Tissue reactions and biodegradation

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The *in vivo* biocompatibility and biodegradation of cross-linked (co)polyethers with and without tertiary hydrogen atoms in the main chain and differing in hydrophilicity were studied by means of subcutaneous implantation in rats. After 4 days, 1 month, and 3 months postimplantation, the tissue reactions and interactions were evaluated by light microscopy (LM) and transmission electron microscopy (TEM). Poly(tetrahydrofuran) (poly(THF)), poly(propylene oxide) (poly(POx)), and poly(tetrahydrofuran-co-oxetane) (poly(THF-co-OX)) were tested as relatively hydrophobic polyethers, and poly(ethylene oxide) (PEO) and a poly(THF)/PEO blend were used as more hydrophilic materials. In general, all polyethers showed good biocompatibility with respect to tissue reactions and interactions, with low neutrophil

and macrophage infiltration, a quiet giant cell reaction, and formation of a thin fibrous capsule. For the relatively hydrophobic polyethers studied, the biostability increased in the order poly(POx) < poly(THF-co-OX) < poly(THF), probably indicating that the absence of tertiary hydrogen atoms has a positive effect on the biostability. Concerning the more hydrophilic materials, crosslinked PEO showed the highest rate of degradation, probably due to the mechanical weakness of the hydrogel in combination with the highest presence of giant cells as a result of the high porosity. A frayed surface morphology was observed after implantation of the cross-linked poly(THF)/PEO blend, which might be due to preferential degradation of PEO domains. © 1996 John Wiley & Sons, Inc.

INTRODUCTION

Recently several studies have been published concerning the *in vitro* and/or *in vivo* degradation of poly(ether urethane)s used in biomedical applications.¹⁻¹⁴ Proposed mechanisms for biodegradation are (enzymatic) hydrolysis of the urethane and urea bonds^{2,3} and oxidation of the polyether soft segment.^{2-4,6-9,13}

Bots et al. found that small-diameter vascular grafts, made from crosslinked blends of poly(propylene oxide) (poly(POx)) and poly(ethylene oxide) (PEO), had lost their dimensional stability after implantation in rats.¹⁵ It was suggested that this phenomenon might be due to oxidative degradation caused by the sensitive tertiary hydrogen atoms in the poly(POx) main chain. Our hypothesis was that the biostability might be increased by using polyethers without tertiary hydrogen atoms, and as an example poly(tetrahydrofuran) (poly(THF)) was chosen (Table I). Because poly(THF) is semicrystalline at 20°C, it was crosslinked

at 80°C in order to obtain elastomeric networks at body temperature. In addition, a noncrystalline copolyether, poly(tetrahydrofuran-co-oxetane) (poly(THF-co-OX)), was synthesized and tested. Next to these hydrophobic (co)polyethers, crosslinked (hydrophilic) PEO was studied to investigate the effect of hydrophilicity. As a potential material for vascular grafts, a crosslinked blend of poly(THF) and PEO also was studied.

To evaluate the suitability of the crosslinked polyethers as materials for the manufacture of (small-diameter) vascular grafts, the *in vivo* biocompatibility and stability of the above-mentioned polyether systems (Table I) was investigated by means of subcutaneous implantation in rats. We were in particular interested in the influence of the chemical structure and the hydrophilicity of the crosslinked (co)polyether systems. This article deals with the results of our light microscopy (LM) and transmission electron microscopy (TEM), study of tissue reactions, cytotoxicity, and biodegradation. In Part II, the weight loss, the changes in the chemical structure of the surface, and the swelling behavior of the crosslinked polyethers after implantation will be discussed.¹⁶

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TABLE I
Structures of (Co)Polyethers

Poly(POx):	$-(\text{CH}_2\text{CH}-\text{O})_n-$ CH_3
Poly(THF):	$-(\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-\text{O})_n-$
Poly(THF-co-OX):	$-(\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-\text{O})_n-(\text{CH}_2\text{CH}_2\text{CH}_2-\text{O})_m-$
PEO:	$-(\text{CH}_2\text{CH}_2-\text{O})_n-$

MATERIALS AND METHODS

Materials

Poly(propylene oxide) (Poly(POx), $M_w = 541,000$ g/mole, $M_w/M_n = 2.21$ (GPC)) was synthesized using a procedure, reported by Bots et al., in which the catalyst was suggested to have the following structure: $\text{R}(\text{C}_6\text{H}_5)_2 - \text{Sn}(\text{SZn})_2\text{R}$ with $\text{R} = (\text{CH}_2)_3\text{N}(\text{CH}_3)_2$.¹⁷

Poly(tetrahydrofuran) (poly(THF), $M_w = 358,000$ g/mole, $M_w/M_n = 2.51$) was synthesized by bulk polymerization with SbCl_5 as a catalyst.¹⁸

Poly(tetrahydrofuran-co-oxetane) (Poly(THF-co-OX), $M_w = 389,000$ g/mole, $M_w/M_n = 2.63$, 17 mole% OX units) was prepared by bulk copolymerization of a THF/OX mixture, with $\text{Al}(i\text{-Bu})_3-0.5\text{H}_2\text{O}$ complex as a catalyst.¹⁸

The PEO sample consisted of a 3:1 (w/w) mixture of PEO's with a molecular weight of 600,000 g/mole and a molecular weight of 8,000,000 g/mole, respectively. Both PEO's were purchased from Aldrich, Brussels, Belgium. The composition of the poly(THF)/PEO blend was: poly(THF)/PEO(600,000)/PEO(8,000,000), 80/15/5 (w/w).

Methods

Film preparation

Polymers or mixtures of polymers and dicumyl peroxide (DCP, the crosslinking agent) were dissolved in dichloromethane, and films were cast from viscous 2.5–5% (w/v) solutions, followed by evaporation of the solvent under a hood overnight. Except for the PEO sample, for which 6 phr (parts per hundred rubber) DCP was used, all other samples were prepared with 2 phr DCP. Films were crosslinked under nitrogen by UV irradiation with a set of two parallel-placed Sterisol® 503P lamps (15W each, wavelength, 254 nm). Films of poly(POx) and poly(THF-co-OX) were irradiated 2 h on each side at 20°C; films of poly(THF) and of the poly(THF)/PEO blend were irradiated 2 h on each side at 80°C; and the film of PEO was irradiated 4 h on each side at 20°C.

In order to remove all nonbound material (non-bound polymer, unreacted DCP, and decomposition

products of DCP), crosslinked samples were extracted in dichloromethane for 3 days at room temperature, with refreshment of solvent every day, and subsequently dried *in vacuo* at 35°C.

Small disks with a diameter of 2–3 mm were punched from the dried films (2 mm for PEO and 3 mm for the others), allowing fixation and embedding of the whole disk).

The disks were sterilized with ethylene oxide by a standard procedure (5 h at 55°C in an ethylene oxide atmosphere at a relative humidity of 70%), followed by aeration of the samples with an air flow (of $\pm 50^\circ\text{C}$) at atmospheric pressure for 48 h to remove residual ethylene oxide. Subsequently the disks were equilibrated in phosphate-buffered saline (PBS, NPBI, Emmer-Compascuum, The Netherlands) at 37°C for 3 days.

The films were dried by extraction with dichloromethane, and then the water content was determined after equilibrating parts of the films in water at 37°C for 2 days. The water content was calculated as the weight percentage of water in the swollen sample. The values for poly(POx), poly(THF), and poly(THF-co-OX) amounted to 1–3 wt %, whereas PEO and the poly(THF)/PEO blend contained 63 and 30 wt % water, respectively.

Implantations

NIH guidelines for the care and use of laboratory animals have been observed. Male PVG-E rats of approximately 3 months of age were used. The rats were ether anesthetized and subcutaneous pockets were made to the right and left of two midline incisions. The poly(THF)-containing specimens were heated to 50°C for 5 min to ascertain complete absence of crystallinity for all samples. The disks were implanted in the pockets at a distance of about 1 cm from the incisions. The rats were sacrificed after 4 days, 4 weeks, or 12 weeks, and the implants with surrounding tissue were harvested.

Microscopy

Explants were fixed in 2% (v/v) glutaraldehyde in 0.1M PBS. After at least 24 h of fixation at 4°C, specimens were postfixed in 1% OsO_4 , 1.5% $\text{K}_4\text{Fe}(\text{CN})_6$ in PBS, dehydrated in graded alcohols, and embedded in Epon 812. For light microscopy, semithin sections ($\pm 2 \mu\text{m}$) were cut and stained with toluidine blue. For TEM, ultrathin sections (70 nm) were cut and stained with uranyl acetate and lead citrate and examined with a Philips EM 201 transmission electron microscope operated at 40 kV.

RESULTS

Because of pronounced differences in hydrophilicity, in terms of water content, which might be of great

influence on the surface texture, a separation was made between the (relatively) hydrophobic materials—poly(POx), poly(THF), and poly(THF-co-OX)—and the more hydrophilic samples, PEO and the poly(THF)/PEO blend.

After 4 days implantation

Hydrophobic materials

Poly(POx) had been folded with three bends. Since the tissue interface was not folded, it was not clear if the lining tissue actually had been adhering to the surface or whether it was an artifact. Part of the lining tissue was quiet; one cell layer of macrophages had adhered to the surface with fibrin, with loosely present collagen bundles and fibroblasts representing the following layers. However, other sites showed a more reactive, i.e. indented, cellular lining consisting of more layers of macrophages and giant cells and migration of increased numbers of mononuclear cells. These were macrophages and fibroblasts, but not (neutrophilic) granulocytes.

The tissue at the edge of poly(THF) showed a quiet reaction. In general, macrophages had adhered to the surface [Fig. 1(a)]. Similar to the quiet regions described for poly(POx), this usually consisted of one cell layer with fibrin, with loosely present collagen bundles and fibroblasts representing the following layers. However, locally some more layers of macrophages also were observed. Sometimes a giant cell was present that contained lipid droplets. Few granulocytes were observed.

The reaction to poly(THF-co-OX) also generally consisted of both macrophages and collagen bundles. Especially the macrophages showed high migrative activity. Fibroblasts also were present. The tissue seemed to be more tightly packed in cellular/collagen layers, i.e. the loose connective layers were farther away compared to those for poly(THF). At some sites a fibrin network directly lined the surface [Fig. 1(b)]. Granulocytes were hardly observable.

Hydrophilic materials

PEO at 4 days had induced an intense tissue reaction. The material had been broken into three larger and several smaller parts [Fig. 2(a)]. This and the not-very-sharp material interface are indications of biodegradation. Many small blood vessels with high migrative activity of mononuclear cells were observed near the edges. A complete lining of giant cells trying to invade the material was present [Fig. 2(b)]. Some segments of the implant already were separated from the bulk. Macrophages, also those farther away from the material, and giant cells contained lipid droplets. At some

sites giant cells from one side had contacted giant cells at the other side of the material [Fig. 2(a)]. At one site increased fibrin and loose erythrocytes could be observed as signs of hemorrhages. Granulocytes were present, but not in high numbers.

At one side of the poly(THF)/PEO blend a clear foreign body reaction with a compact layer of macrophages and giant cells could be observed at the interface [Fig. 3(a)]. Many cells contained vacuoles, at LM level called foams cells. This edge was not sharp, but had a somewhat frayed morphology. Fibroblasts and some eosinophilic granulocytes also were present [Fig. 3(b)]. The vacuoles could be an indication of biodegradation. A second layer contained blood vessels in loose connective tissue. At the other side the reaction was more quiet, with less cellular activity. Neutrophilic granulocytes were hardly observable.

After 1 month implantation

Hydrophobic materials

The poly(POx) sample obviously had flipped double, possibly at implantation, since two parts of film were found. Areas that showed a quiet reaction, with one small layer of giant cells almost immediately followed by loose connective tissue, and areas with a larger giant cell reaction were observed [Fig. 4(a)]. At one site some mast cells were present; however, this seemed coincidental and not an indication of some allergic reaction.

Poly(THF) was encapsulated with, at first, one or two layers of giant cells, then by 5 or 6 layers with active blasts, and then by layers with elongated fibroblasts, collagen bundles, and blood vessels. Cellular invasion into the material was not observed. The active blasts were macrophages and fibroblasts. Some cells contained lipid droplets. Collagen bundles were present between the blasts. Active cellular migration from the layers with blood vessels occurred.

The tissue reaction to poly(THF-co-OX) seemed at first glance similar to the one to poly(THF). This consisted of the giant cell layer and the blast layer. The blast layer seemed smaller than that of poly(THF). However, on closer observation indications were found of a degradation process that had proceeded further. There were several locations with giant cellular invasions into the material [Fig. 4(b)] while somewhat farther away, giant cells with many inclusions were found.

Hydrophilic materials

At 1 month the PEO sample had broken into six larger and ten smaller fragments (as observed at low magnification). All over the specimen were found signs

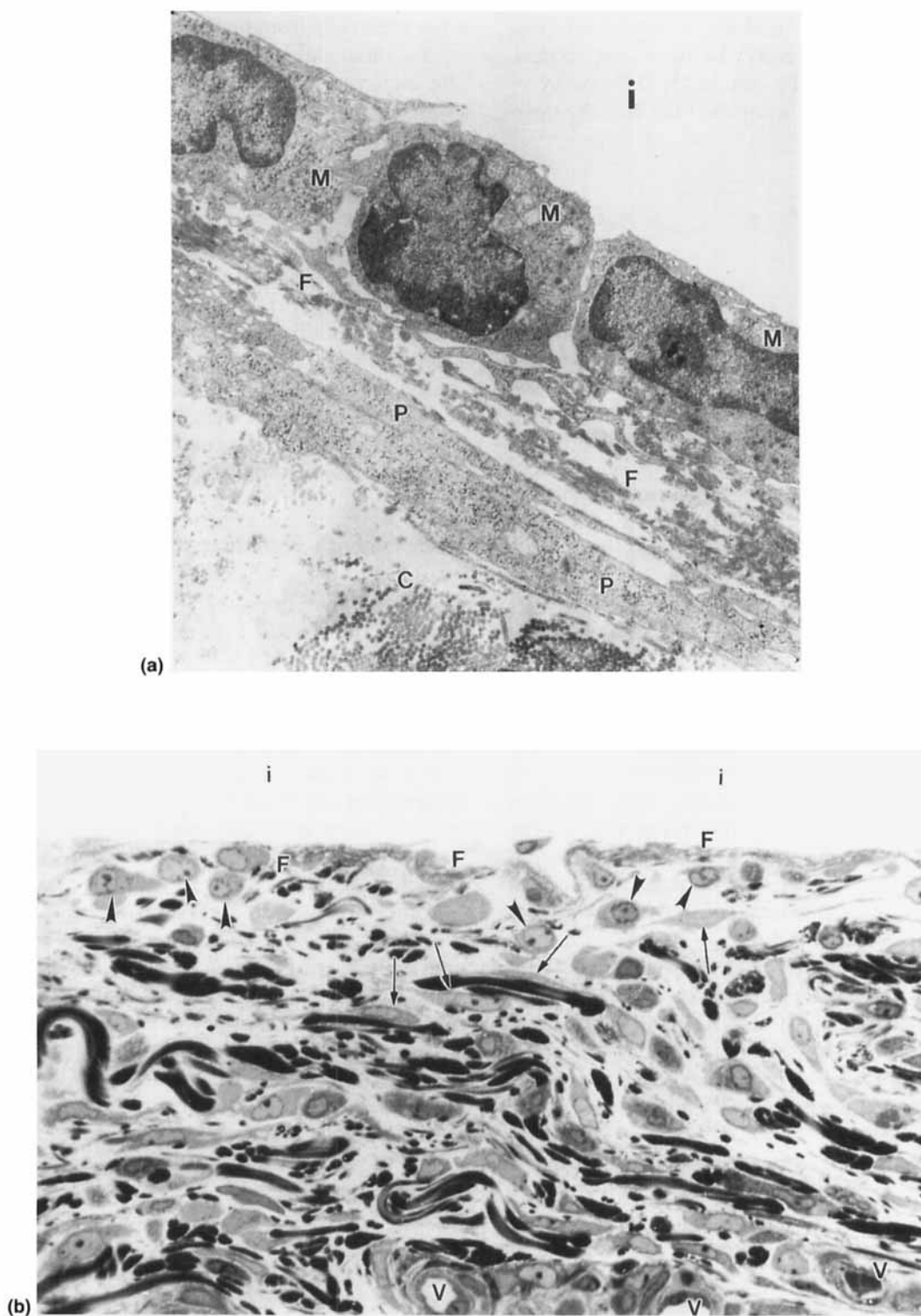


Figure 1. (a) TEM micrograph (original magnification $\times 7000$) of the interface (i) of poly(THF) at day 4. A quiet tissue reaction was observed in this case, with three adhering macrophages (M), followed by some fibrin (F), pseudopods (P) of fibroblasts, and collagen (C) fibrils in cross-section. (b) LM micrograph (original magnification $\times 400$) of the interface (i) of poly(THF-co-OX) at day 4. A quiet tissue reaction with adhering fibrin (F) and some migration of macrophages (arrow heads) and fibroblasts (arrows) was observed. Collagen bundles are dark-stained and nearby the interface, usually somewhat smaller when farther away at the level containing some blood vessels (V).

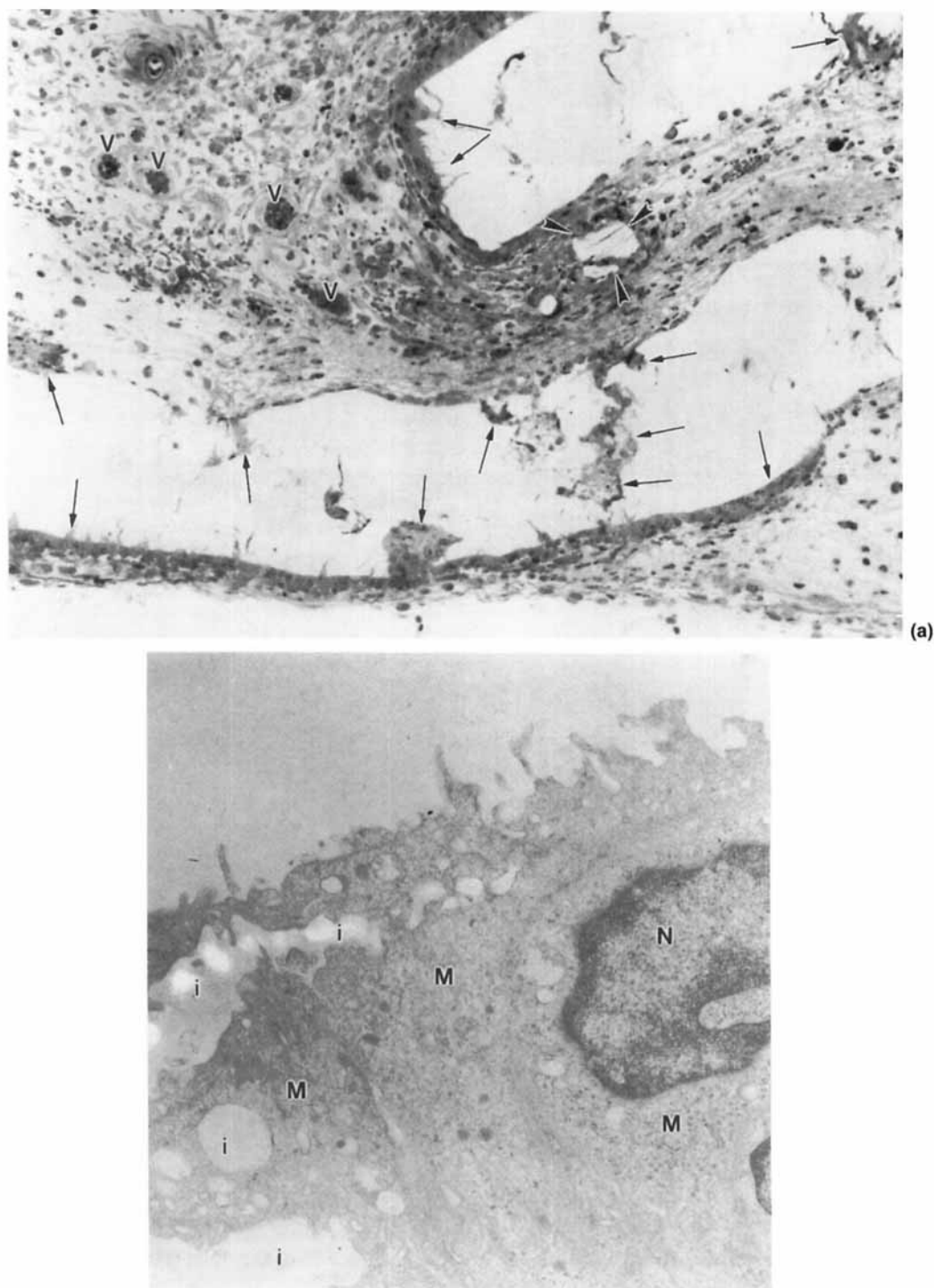


Figure 2. (a) LM micrograph (original magnification $\times 100$) of PEO at day 4. The material was present in several larger and smaller (arrow heads) segments. Mononuclear cells had migrated from blood vessels (V) to the interface, where giant cell formation occurred (arrows). At some points giant cells even contact the other side. (b) TEM micrograph (original magnification $\times 10000$) of PEO at day 4, showing parts of several macrophages/giant cells (M) that had invaded the material. Some segments of the implant (i) already had been separated. N, nucleus.

of an ongoing biodegradation by way of cellular invasions (which very often contacted the other side) and by way of the many small-sized fragments. The material/tissue interface always consisted of giant cells with invading pseudopods but not very many vacuoles or

lipid droplets, indicating that these mostly were rather young, maybe newly arrived or newly formed giant cells [Fig. 5(a)]. In between fragments, an area with mononuclear cells, much newly formed collagen, and arterioles, recognized by the elastic lamina, was found;

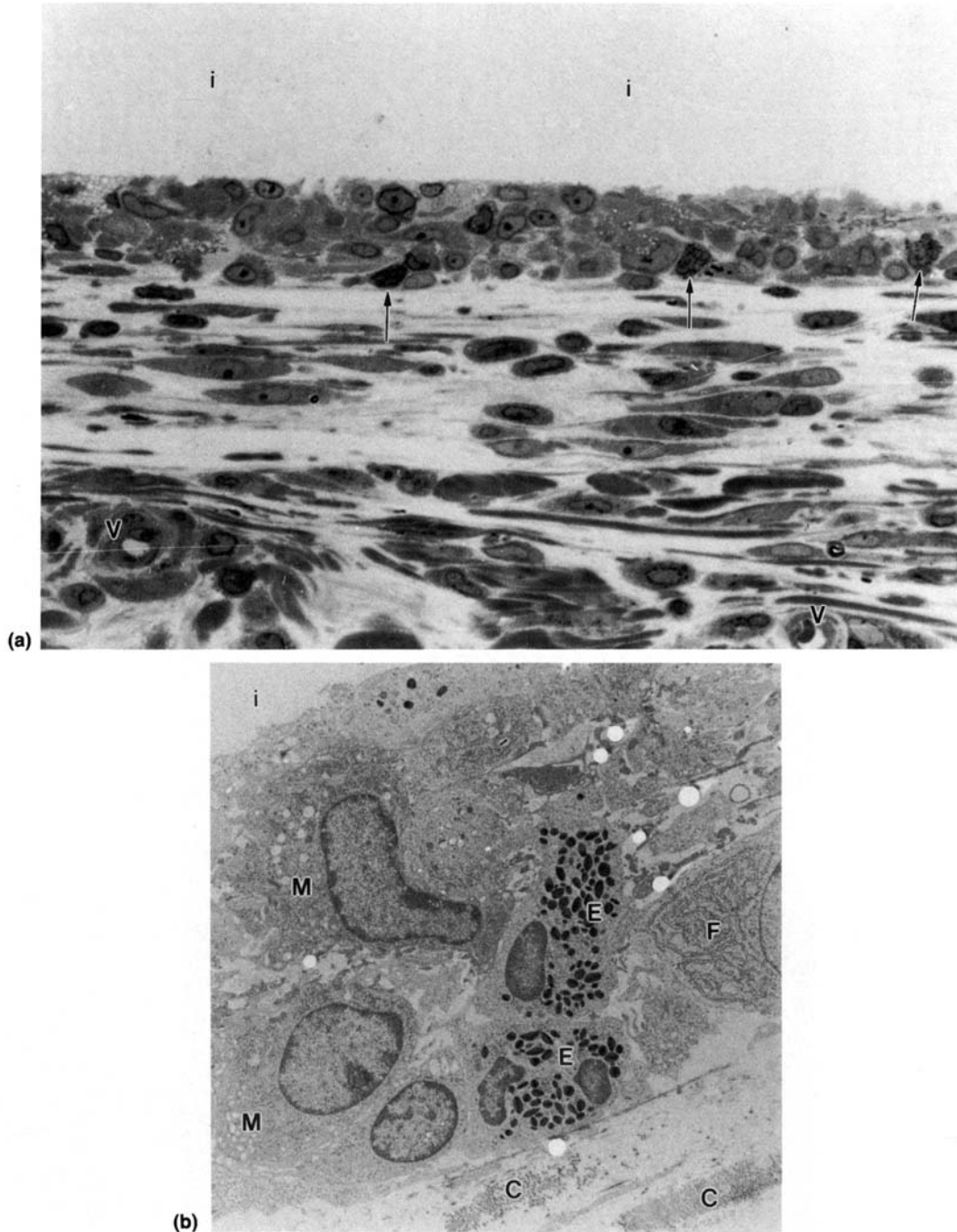


Figure 3. (a) LM micrograph (original magnification $\times 400$) of the interface (i) of the poly(THF)/PEO blend at day 4. First a compact layer consisting mainly of macrophages/giant cells was observed. Some fibroblasts and eosinophilic granulocytes (arrows) also were present. The next layer consisted mainly of parallel-oriented fibroblasts and collagen bundles, followed by a layer with blood vessels (V) and less orientation. (b) TEM micrograph (original magnification $\times 3000$) of the interface (I) of the poly(THF)/PEO blend at day 4 showing the compact layer with some macrophages (M), two eosinophilic granulocytes (E) with their specific granules and a fibroblast (F), recognized from the rough endoplasmic reticulum, followed by collagen bundles (C).

in addition, an area with numerous cells containing vacuoles [Fig. 5(a)] was found. The cells in Figure 5(a) mostly were mononuclear, and it is unclear whether the vacuoles represent true vacuoles, lipid droplets from the lipid that had been removed during the embedding procedure, or remnants of phagocytosed particles. Figure 5(b), at TEM level, shows that all vacuoles in macrophages/giant cells were lysosomes with

phagocytosed particles. Farther away from the interface, lysosome fusion resulted in enlargement.

At first glance the interface of the poly(THF)/PEO blend looked very straight. It consisted of one large giant cell layer and one layer of blasts, which immediately was followed by loose connective tissue with fibroblasts and collagen [Fig. 6(a)]. In all layers vacuoles could be observed inside cells. These seemed to

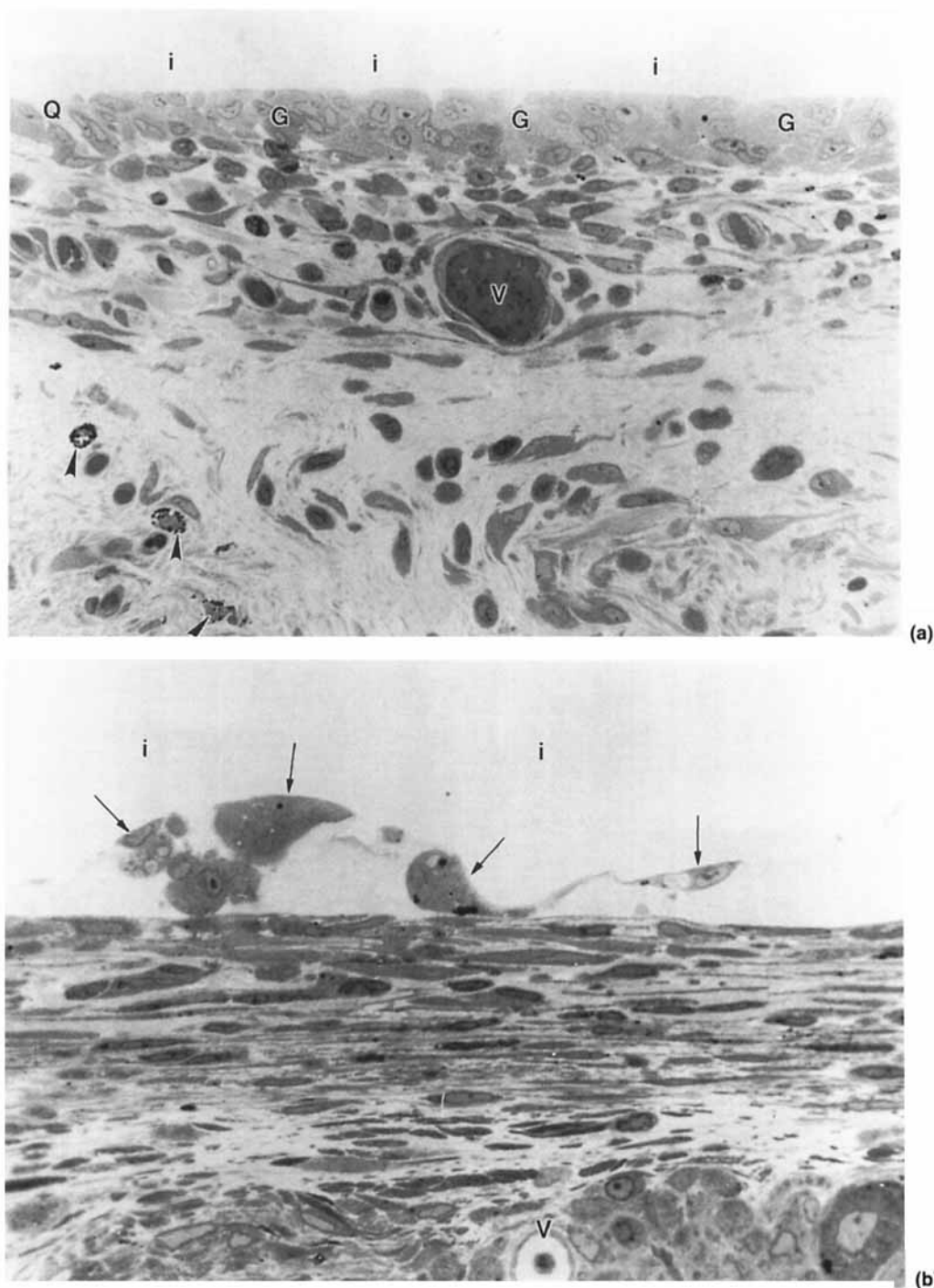


Figure 4. (a) LM micrograph (original magnification $\times 250$) of the interface (i) of poly(POx) at 1 month. Areas with a quiet (Q) tissue reaction as well as with a clear giant cell reaction (G, arrows) were observed. Migrative activity occurred from the blood vessel (V), beneath which a more or less loose connective tissue was present. A few mast cells (arrow heads), as indicated from the specific granules, were present, which seemed, however, coincidental. (b) LM micrograph (original magnification $\times 400$) of the interface (i) of poly(THF-co-OX) at 1 month. Although the interface in general was straight, at some sites clear signs of degradation, as observed from invading macrophages/giant cells (arrows), could be observed. This process was confirmed by the activity of cells with phagocytosed particles observed at the level of the blood vessels (V).

originate from phagocytosis, a process that clearly occurred at the interface, as indicated by the frayed morphology, resulting from small pseudopods and possibly some (extracellular matrix) protein deposition into the material. Also, a few small material fragments surrounded by giant cells were observed [Fig. 6(b)].

After 3 months implantation

Hydrophobic materials

Poly(POx) had broken into three larger and six smaller fragments [Fig. 7(a)]. The tissue reaction

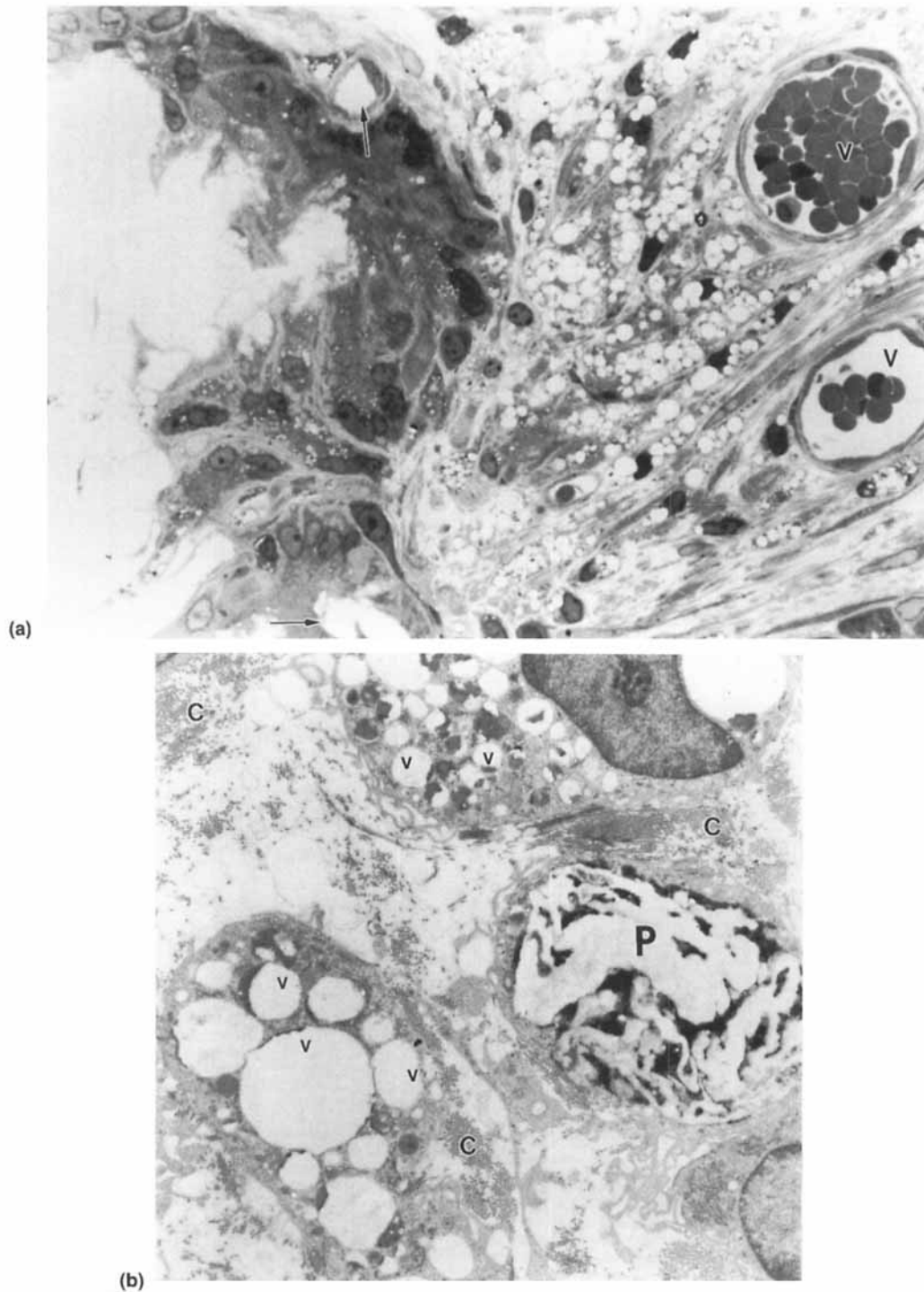


Figure 5. (a) LM micrograph (original magnification $\times 400$) of PEO at 1 month. Giant cells were actively invading and phagocytosing the material, of which at least two smaller fragments (arrows) were separated from the "bulk." The following layers of tissue showed mostly "vacuoles" of phagocytosing/degenerating cells moving towards blood vessels (V), from which new cells also had migrated to the interface to assist degradation. (b) TEM micrograph (original magnification $\times 4500$) of PEO at 1 month showing the "vacuoles" (V), i.e., lysosomes of phagocytosing/degenerating cells, which clearly contained remnants of the material. One large particle (P) seemed only surrounded and not yet internalized. Collagen fibrils (C) were present in between the cells.

looked like a mild version of the 1-month reaction to PEO. At the interface the reaction was intense, but a bit farther away already loose connective tissue could be recognized [Fig. 7(b)]. The interface consisted of

giant cells trying to invade and to degrade the material [Fig. 7(b)]. Smaller-sized fragments also were surrounded by giant cells while small particles had been phagocytosed. Many cells shown in Figure 7(b) con-

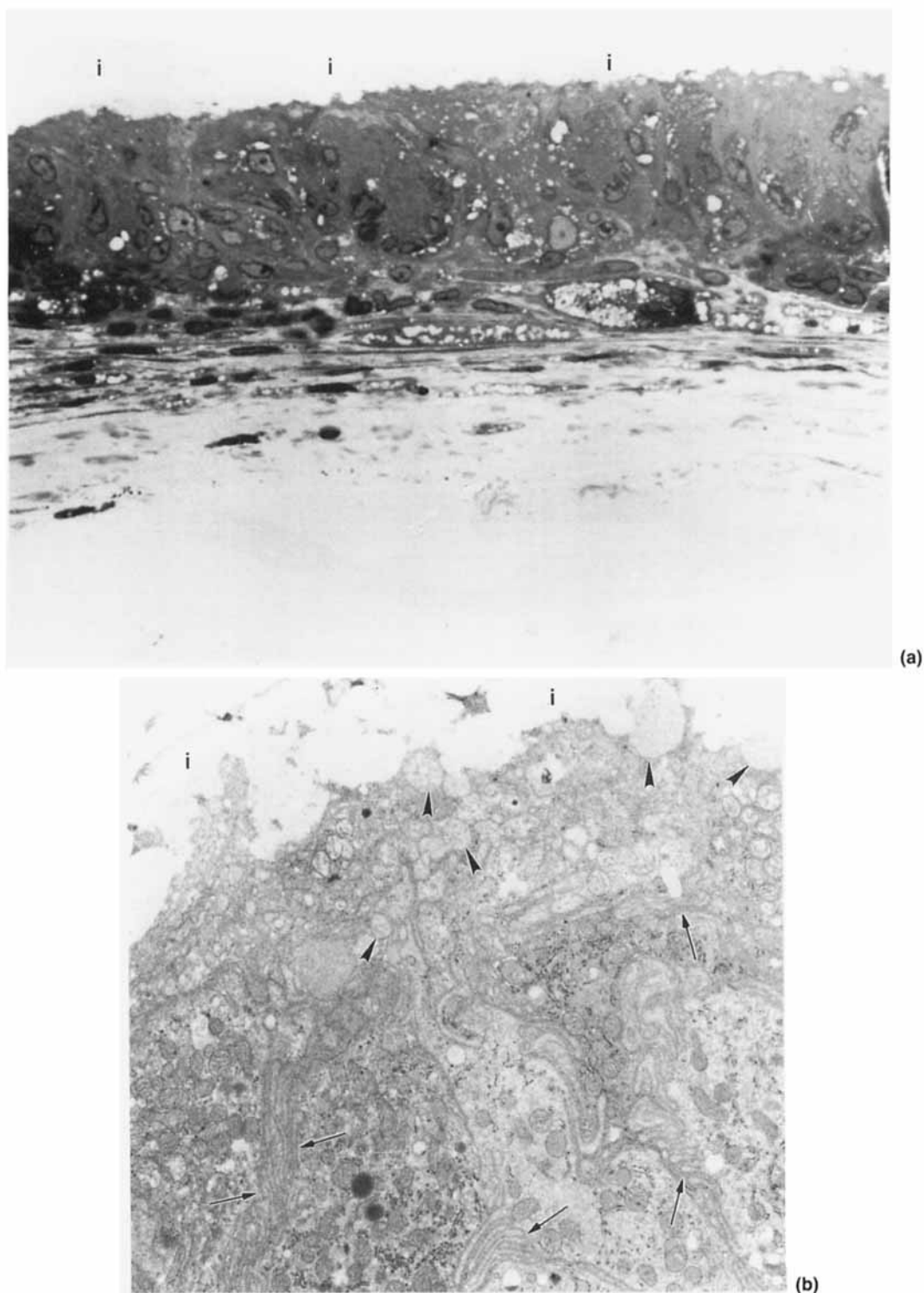


Figure 6. (a) LM micrograph (original magnification $\times 400$) of the poly(THF)/PEO blend at 1 month showing the ongoing giant cell formation in the compact layer. "Vacuoles" indicated the process of phagocytosis and degeneration of the material, but cells were unable to create large passage ways in the material, as indicated from the frayed morphology at the interface (i). (b) TEM micrograph (original magnification $\times 7000$) of the poly(THF)/PEO blend at 1 month showing that the frayed morphology at the interface (i) resulted from many pseudopods, protein deposition, and phagolysosomes with phagocytosed material (arrow heads). Furthermore the tight interaction between giant cells, as represented by many lamellopodia, was observed (arrows).

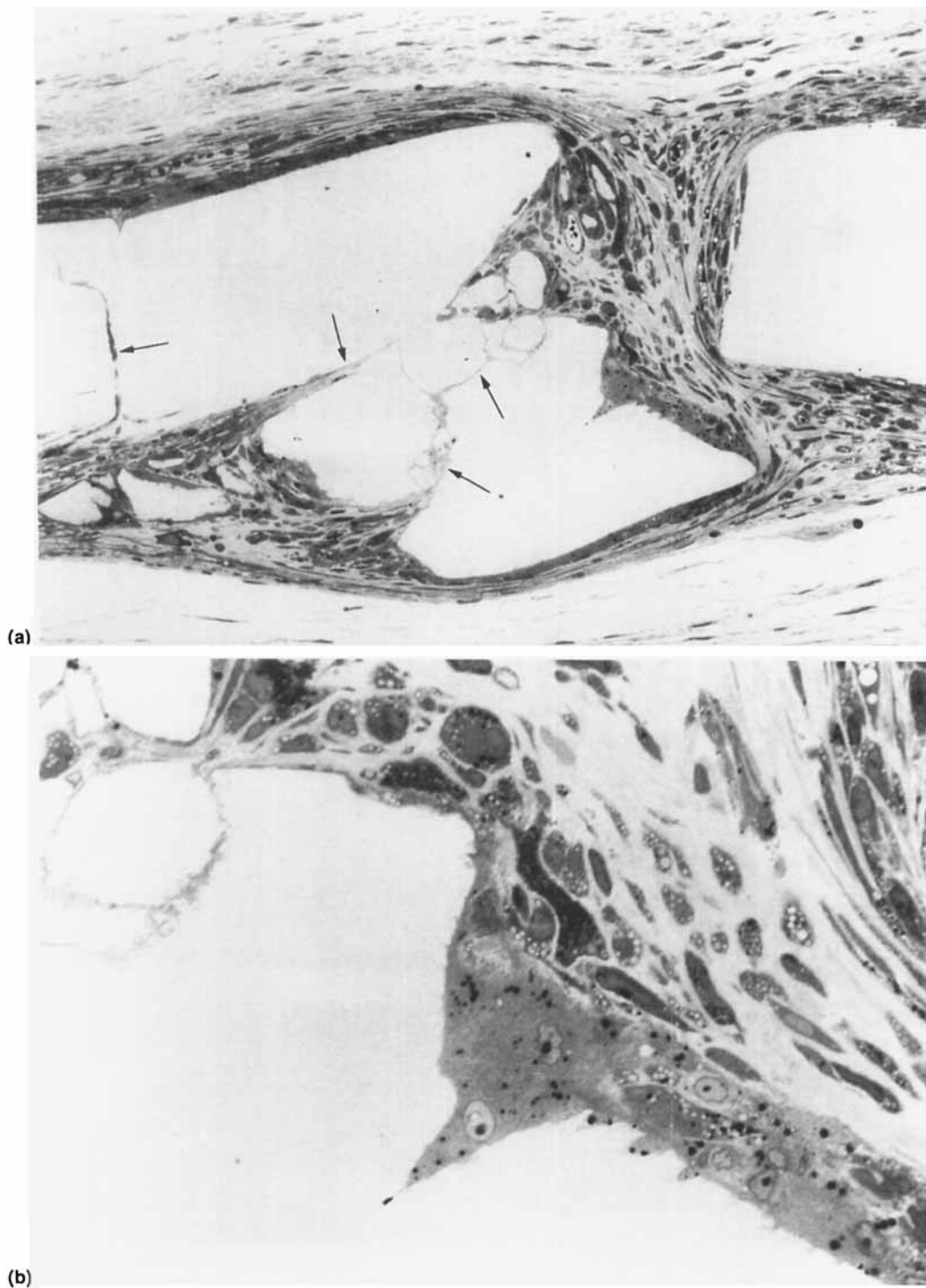


Figure 7. (a) LM micrograph (original magnification $\times 100$) of poly(POx) at 3 months. The material had broken into three larger and six smaller fragments, which soon was followed by quiet loose connective tissue. At some sites contact from one side to the other was observed (arrows). (b) LM micrograph (original magnification $\times 400$) of poly(POx) at 3 months showing the foreign body reaction with a first layer of giant cells in which dark spots represent lipid droplets. The following layer contained collagen and dispersed phagocytosing cells, as observed from vacuoles.

tained lipid droplets and vacuoles representing phagolysosomes.

Poly(THF) had been encapsulated by one or two layers of giant cells, followed by one to five layers of

blasts and a layer with elongated fibroblasts, collagen, and blood vessels [Fig. 8(a)]. Hardly any signs of biodegradation could be found. The exceptions were a few irregularities at the interface, which at these sites

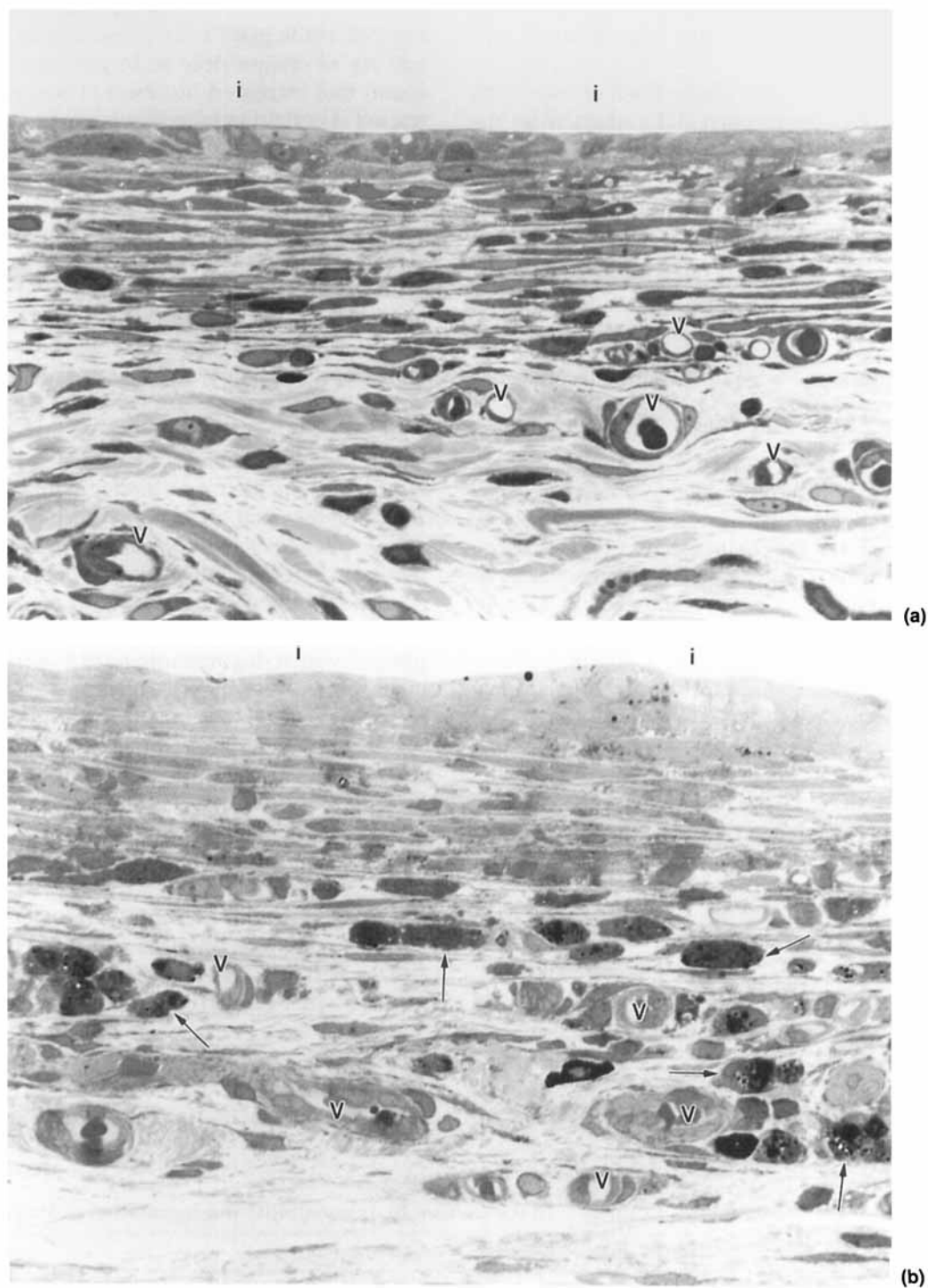


Figure 8. (a) LM micrograph (original magnification $\times 250$) of poly(THF) at 3 months. A capsule consisting of one or two layers of giant cells was observed, followed by (parallel to the interface (i)) oriented fibroblasts and collagen bundles and a less oriented layer with a.o. blood vessels (V). (b) LM micrograph (original magnification $\times 400$) of poly(THF-co-OX) at 3 months. Signs of degradation were observed, as indicated from the slightly "waving" interface (i) with giant cells and, more important, from higher cellular activity at the level of the blood vessels (V). Here many macrophages (arrows) with phagocytosed particles were observed.

was not straight but showed an invading giant cell (as a whole, not by pseudopods). Vacuolization was hardly observable.

For poly(THF-co-OX) the tissue reaction was very similar to the reaction to poly(THF), apart from the additional presence within the fibrotic capsule/blood vessel layer of macrophages/giant cells, which actively had been phagocytosing particles [Fig. 8(b)]. These clear signs of biodegradation were not observed for poly(THF).

Hydrophilic materials

After 3 months some large PEO fragments still were found. These were surrounded by giant cells, often followed by smaller giant cells that obviously had phagocytosed a lot, and then by loose connective tissue. In between collagen bundles, remnants of many cells clearly containing phagocytosed particles were observed. In general, the tissue reaction for this specimen gave the impression of not being very intense anymore.

The tissue reaction to the poly(THF)/PEO blend had proceeded. A thick giant cell layer consisting of one to six smaller layers could be observed. Immediately at the interface the same frayed morphology, as observed at 1 month and also somewhat present at day 4, could be recognized. Vacuolization was widespread, and often vacuoles clearly related to phagocytosed particles could be recognized (Table II).

DISCUSSION

Usually during the first days after implantation of biomaterials, neutrophilic granulocytes and macrophages are attracted. This occurs as a result of the wounding of tissues, e.g., the skin and the subcutis, and as a result of the insertion of a foreign material.

Somewhat later fibroblasts will start to encapsulate the implant while giant cells, formed from macrophages, will try to phagocytose it. In previous studies it was found that increased numbers of neutrophils are attracted if certain cytotoxic substances are released by the biomaterial.¹⁹⁻²¹ Other reasons for increased neutrophil infiltration may be complement activation,²² the presence of bacteria,²³ or late-phase biodegradation resulting in a secondary inflammation reaction, as observed with certain poly(L-lactide) bone plates.²⁴

The most striking result in the present study was that none of the (co)polyethers attracted many neutrophilic granulocytes. Apart from the PEO at day 4, neutrophils were observed hardly at all or not at all, not at 4 days nor at 3 months when, for example, poly(POx) clearly showed biodegradation. For PEO at 4 days the presence of neutrophilic granulocytes probably is caused by the high incidence of biodegradation at that time. This suggests that all (co)polyethers show practically no complement activation²² and/or that from these materials no cytotoxic substances²⁰ are released. This was confirmed by the absence of (massive) cell degeneration.²⁰ Especially in contrast to poly(L-lactide) bone plates,²⁴ which degrade into hard mechanically irritating fragments, another reason might be that the (co)polyethers are smooth and soft elastomeric structures.

Considering granulocytes in general, the only type of granulocyte clearly present, although not in very high numbers, was the eosinophilic granulocyte observed at day 4 with the poly(THF)/PEO blend. Eosinophils are usually related to the presence of bacteria or to allergic-type reactions.^{25,26} However, in this case the reason for their presence is not clear.

Concerning macrophages and fibroblasts, the tissue reactions to the different (co)polyethers represent normal and quiet foreign body reactions. Fibroblasts tend to encapsulate, and macrophages and giant cells formed from them tend to phagocytose foreign material.²⁶⁻²⁹ Obviously differences in surfaces did not provoke differences in the presence of giant cells; all

TABLE II
Survey of the Microscopical Evaluation of the *In Vivo* Biocompatibility/Biodegradation of Polyethers

Implantation:	4 Days	1 Month	3 Months
Poly(POx):	Some sites: indented interface and giant cell formation	Some sites: strong giant cell reaction	Broken, intense interface reaction; giant cell invasion and phagocytosis
Poly(THF):	General: quiet reaction, some sites: multilayer of macrophages	One to two layers of giant cells	One to two layers of giant cells, some of them invading
Poly(THF-co-OX):	Macrophages with high migrative activity	Some giant cellular invasions; blood vessels; phagocytosis	Some giant cellular invasions; blood vessels; phagocytosis
PEO:	Broken, giant cell invasion	Broken, giant cell invasion; high extent of phagocytosis	Broken giant cell invasion; high extent of phagocytosis
Poly(THF)/PEO: blend	Onset of frayed morphology at interface	Frayed morphology; thick giant cell layer; phagocytosis; vacuoles	Frayed morphology; thick giant cell layer; phagocytosis; widespread vacuolization

(co)polyethers seemed attractive to macrophages, inducing them to form giant cells, which may indicate that all materials had adsorbed protein layers that enabled cell adhesion. Thus varying wettability seems not to be a determinant in this respect. Thus far our group has found only one material that does not provoke giant cell reaction, and that is a noncrosslinked collagen²⁰ that obviously turned into a gelatinous mass, thereby combining high wettability with not being 'foreign' anymore. The more intense reactive sites are related to the breaking of materials into different fragments, resulting in the presence of more surface area and thus provoking more normal foreign body reactions. Depending on the material, phagocytosis and transport are observed earlier, or not at all in the case of poly(THF). Around the biomaterial (-fragments), however, a more or less quiet situation with loose connective tissue and blood vessels soon was observed. This was in contrast to, for example, in the case of an abscess, the formation of numerous layers with fibroblasts and collagen.²³

Very interesting phenomena were found with regard to the rate and way of degradation and the concomitant tissue reaction. Of the hydrophobic materials, poly(THF) was the least susceptible to degradation, showing at 3 months only some denoting irregularities at the surface, while poly(POx) was the most susceptible, resulting in several fragments after 3 months and a pronounced invasion of giant cells into the material. Poly(THF-co-OX) allowed invasion of complete cells at 1 month, but was not fragmented even after 3 months. As will be discussed in Part II, the presence of tertiary hydrogen atoms or a high fraction of α -methylene groups in a polyether increases the biodegradation.¹⁶

Biofragmentation and degradation of poly(POx) also was reported by Bots et al. upon implantation in the middle ear of rats.¹⁵ In their study, fragmentation was observed after only 1 month. In the first few weeks after implantation they observed only few (polymorphonuclear) granulocytes. However, for periods longer than 1 month, increased numbers of granulocytes were found together with an ongoing fragmentation and phagocytosing activity. High *in vivo* degradation rates for poly(POx) in rats also were reported by Bakker et al., who found a decrease of more than 50% of the cross-sectional area of implanted poly(POx) samples 13 weeks postimplantation and a high extent of phagocytosis.^{30,31} Next to the presence of tertiary hydrogen atoms, catalyst residues may be a possible cause for the poor stability of poly(POx).¹⁵ In contrast to the results obtained by Bots et al. and by Bakker et al., we found a lower degradation rate for poly(POx). This is probably the result of a lower residual catalyst content and might be related to the absence of (considerable amounts of) granulocytes even after 3 months of implantation.

Among the hydrophilic materials, the PEO network already showed severe degradation 4 days after implantation. The fragmentation at this early time interval might have been the result of the mechanical weakness of the hydrogel, and fragmentation results in an increased surface area exposed to degradative attack by macrophages and/or giant cells. Furthermore, due to the high swelling ratio in aqueous media, the PEO samples were very porous. Therefore they were much more accessible to invasion by cellular material compared to the hydrophobic polyethers. A high degree of phagocytosis was observed after 1 and 3 months. The poly(THF)/PEO blend from day 4 on appeared to degrade via a surface erosion process, as indicated by the frayed morphology of the cell layers lining the surface. This frayed morphology might be the result of preferential degradation of the PEO domains.¹⁶ The blend showed an intermediate degradation behavior between that of poly(THF) and PEO. The phase-separated crosslinked blend had an (initial) water content of 30 wt%, considerably less than that of the neat PEO network (63 wt%). Therefore the blend will be much less sensitive to invading cells.

CONCLUSIONS

The *in vivo* biocompatibility and biostability of several crosslinked polyethers was studied by means of subcutaneous implantation in rats. In general, all polyethers showed good biocompatibility with respect to tissue reactions and interactions, i.e. no indications of release of cytotoxic compounds and/or complement activation were found.

For the relatively hydrophobic polyethers studied, the biostability increased in the order poly(POx) < poly(THF-co-OX) < poly(THF). This confirmed our hypothesis that the absence of tertiary hydrogen atoms would improve the biostability.

Concerning the more hydrophilic materials, crosslinked PEO showed the highest rate of degradation, probably due to the mechanical weakness of the hydrogel in combination with the invasion of phagocytic cells as a result of the high porosity. The frayed morphology observed after implantation of a crosslinked blend of poly(THF) and PEO might be due to preferential degradation of PEO domains.

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