We hypothesized that placement of sterile catheter material into the peritoneal cavity results in a foreign-body response that varies with exposure duration.

Sterile medical Silastic catheter material was aseptically implanted into the abdomens of 42 anesthetized Sprague–Dawley rats. Controls (n = 18) underwent sham operations without catheter implantation. After 4, 8, or 20 weeks, the animals were anesthetized, the abdomen was opened, and the catheter material was recovered and processed to separate the cells adhering to the catheters. The cells, abdomen, and catheter material were all cultured to demonstrate sterility, and transport experiments were carried out. After euthanasia of the animals, abdominal wall tissue was examined for submesothelial thickness and vascular density, and immunohistochemistry (IHC) for cytokines was performed. Cells from the catheter material were processed for immunocytochemistry (ICC).

The catheter, adherent cells, and abdomen were free of bacteria. Inflammatory changes in peritoneal thickness and angiogenesis were highest at 4 weeks and declined thereafter to 20 weeks. Transport of mannitol was higher at 4 weeks in treated animals than in controls, and albumin transport was higher at 8 weeks in treated animals than in controls. The IHC for cytokines demonstrated changes paralleling the structural alterations (p < 10⁻⁵). The ICC of the catheter cell layer demonstrated mesothelial cells, macrophages, fibroblasts, and T cells.

Over 20 weeks, the foreign-body response to polymer catheters placed intraperitoneally in rats without injection of solution depends on exposure time, with an initial immune response evident at 4 weeks and decreasing thereafter.

Key words
Sterile inflammation, innate immunity, foreign-body response

Introduction
Most research concerned with preservation of the peritoneal barrier has focused on solution biocompatibility, but in previous work, we discovered that there are direct catheter effects that appear to be the result of a foreign-body response (1). Within 15 hours, white cells cover polymer discs implanted in the peritoneal cavity of mice, confirming the rapid reaction of the innate immune system to the foreign body (2–4).

Others examining foreign-body responses in rats have shown a resolution of these signs of inflammation after approximately 4 weeks (5). We showed that inflammation attributable to exposure to silicone materials progresses over 4 weeks, with greater changes in transport phenomena, angiogenesis, and thickening of the peritoneum occurring after 20 weeks of daily intraperitoneal (IP) injections of a solution low in glucose degradation product (GDPs), in rats with catheters than in animals injected without a catheter (4). We hypothesized that the observed inflammation in rats exposed to the dialysis fluid with a catheter was the result of a foreign-body response to the sterile polymer material within the cavity for the 20 weeks. To address the discrepancy, we implanted sterile rings of silicone material into rats for 4, 8, and 20 weeks, and we examined the response in the tissue and in the adherent cell layer on the catheter material.

Materials and methods
Most of our experimental techniques have been presented in previous papers (1–4). An abbreviated summary follows.

Medical Silastic catheters (7 French: Access Technologies, Skokie, IL, U.S.A.) were prepared with 96 hours of washing with ethyl alcohol, followed by sterile saline washes, and gas autoclave. The experiments used 60 Sprague–Dawley rats, including 18 controls. In each experimental animal, five rings (4 cm in length, approximately 1.5 cm in diameter) of catheter were implanted through a 1-cm midline incision in each animal under surgical anesthesia; control
animals underwent the surgical procedures without catheter placement. Using sterile technique, the rings were recovered at 4, 8, and 20 weeks. After ring recovery, ultrasonography was used to separate adherent cells from the catheter material, and the cells were either cultured with a segment of catheter or fixed with 2.5% glutaraldehyde. A sample of these separated cells, samples of the intact catheter, and a swab from the abdominal cavity were cultured for at least 48 hours.

After collection of the catheter rings and sampling of the fluid in the abdominal cavity, a transport chamber was affixed to the abdominal wall to determine the mass transfer coefficient for mannitol (MTCM), the flux of albumin (J Alb), and the osmotic filtration flux (J Osm) (3).

The catheter-adherent cell layer (ACL) was processed with immunocytochemistry (antibodies in parentheses) for presence of macrophages (ED1), mesothelial cells (cytokeratin), T cells and dendritic cells (CD3), fibroblastic cells (vimentin), and T cells or natural killer cells (CD8), in accordance with our previous paper (4). Portions of the abdominal wall from the side opposite the chamber were analyzed with immunohistochemistry for submesothelial thickening (trichrome), microvasculature (CD31), and cytokine activity (α smooth muscle actin, fibroblast growth factor 2, transforming growth factor β, and vascular endothelial growth factor). Submesothelial thickness is the average of determinations made by 3 different researchers in 5 different fields taken from the muscle layer of the abdominal wall up to the mesothelial layer. Angiogenesis (cells per millimeter of peritoneum) is the average of determinations made by 3 independent observers counting the number of CD31-positive cells below the mesothelial layer in 5 different fields. Cytokine staining was ranked 1 – 4 by 3 observers, with 1 being “no reaction” and 4 being “very heavy reaction.”

**Results**

Cultures of the cavity fluid and of intact and separated ACL were all negative at 120 hours. From this, we concluded that the ACL was free of bacteria.

Figure 1 shows the inflammatory effects of the catheter on submesothelial thickness and angiogenesis. At each interval of catheter exposure, a marked difference from control was observed. However, the reaction at 4 weeks was significantly greater than that at 8 or 20 weeks. Table I shows the semiquantitative scoring of the inflammatory cytokine staining in the abdominal wall peritoneum. The data indicate that inflammatory changes in the peritoneal abdominal wall were highest at 4 weeks and declined over subsequent periods.

Table II shows the analysis of transport data for the 3 time periods. The MTCM in catheter animals was significantly greater than that in controls for the 4-week exposure group only. The J Alb for catheter animals was significantly greater than that for controls at 8 weeks, but did not rise to significance at 20 weeks. Otherwise, there were essentially no changes in transport across the peritoneum of the abdominal wall.

Immunocytochemistry of the ACL demonstrated the presence of mesothelial cells, T cells, fibroblastic cells (fibroblasts and myofibroblasts), and macrophages at all intervals of exposure. Trends in staining were not quantifiable, but staining appeared to be qualitatively lighter for macrophages at 20 weeks.

**Statistics**

Statistical analyses were carried out as in earlier work (1). All results are presented as mean ± standard error of the mean. Data from rats with positive bacterial cultures were excluded from the analysis. Statistical analyses of effects in the various groups were performed using a one-way analysis of variance (ANOVA). The probability of a type 1 error was considered significant at \( p < 0.05 \). All tests were performed using the NCSS-97 software application (NCSS, Kayesville, UT, U.S.A.).
Discussion and conclusions

Our data demonstrate that inflammation of rat peritoneum from exposure to a foreign body is present at 4 weeks and then gradually declines. Peritoneal thickness and angiogenesis rise to high levels in the first 4 weeks, and then both decline in magnitude over 20 weeks. The observed inflammation alters transport of mannitol at 4 weeks, but only albumin has an increased rate of transport at 8 weeks. By 20 weeks, not only are the anatomic alterations in decline, but transport changes are not observed.

These results parallel, but do not duplicate, previous findings after implantation of a rubber foreign body (5), in which signs of inflammation were observed to resolve after 4 weeks. However, the analysis techniques in that study are not equivalent to our technique and may have missed subtle changes. Others have studied foreign-body response to polymers in mice (6), but no observations have been made of the effects of the foreign body on the surrounding peritoneal tissue. However, inflammatory cells in the ACL similar to those observed in mice are seen in rats (7). Studies in humans demonstrate a brisk reaction to the newly implanted catheter in the first week, with a decline in white cell counts in peritoneal effluent 1 week after catheter placement (8). Catheters removed for noninfectious reasons have an ACL composed of inflammatory cells without bacteria (9). Unfortunately, peritoneal biopsies were not obtained in either of the foregoing human studies.

Looking at previous data from solution injection with a catheter over 20 weeks (4), the structural changes apparently attributable to the catheter alone are similar, but the degree of inflammation in the submesothelium is far less than in the present experiment. We conclude that the initial immune response declines after 4 weeks of exposure and begins to approach control values after 20 weeks without injection of solution. It is interesting to hypothesize that the catheter reaction alone might resolve, but that the introduction of solution alters the resolving inflammatory process and results in chronic inflammation.

References

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