Human Intraperitoneal Response to a Left Ventricular Assist Device with a Ti-6Al-4V Alloy Surface

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The soft tissue reaction to long-term implantation of an intraperitoneal left ventricular assist device (LVAD) was investigated. The HeartMate 1000 (Thermo Cardiosystems, Inc., Woburn, MA) is a pneumatically driven blood pump with smooth titanium alloy (Ti-6Al-4V) outer surfaces that is placed intraperitoneally in the left upper quadrant and sutured to the anterior abdominal wall. It is being used currently as a bridge to cardiac transplant, which sometimes requires extended support times. We examined the tissue capsule that formed around the rigid circular pump housing of four LVADs (duration of implant: 61, 86, 128, and 153 days) for gross, histologic, immunohistochemical, and ultrastructural analysis. Immunostaining was performed using monoclonal and polyclonal antibodies against cytoskeletal tissue markers (vimentin, desmin, α-smooth muscle actin), T and B lymphocytes, carcinoembryonic antigen, factor VIII, and cytokeratins (CAM 5.2 AE1/AE3, 34βE12, and 35βH11). Direct fluorescent immunolabeling for fibrinogen was also performed to characterize cell and tissue type. Histologic analysis of the 3 to 4 mm thick capsule with white, glistening inner surfaces showed fibrovascular tissue with multipotential subserosal cells (MSCs), capillary endothelium, collagen, and a few mononuclear infiltrates. The immunohistochemical profile of the MSCs differed from myofibroblasts despite a morphologic similarity. Transmission electron microscopy revealed abundant rough endoplasmic reticulum and peripherally arranged myofilaments within the spindle shaped cells. It was hypothesized that capsule formation was initiated by fibrin deposition, followed by proliferation of MSCs and subsequent formation of fibrovascular tissue. Because no significant inflammatory response or nodular tumor-like masses were detected in the pump capsule, we concluded that long-term intraperitoneal implantation of a titanium alloy LVAD did not adversely affect the surrounding soft tissue. *ASAIO Journal* 1992; 38: M543–M549.

Two staged cardiac transplantation has become possible with the advent of left ventricular assist devices (LVADs) that provide temporary mechanical circulatory assistance to critically ill patients with end-stage heart disease. The abdominal placement of LVADs conveniently circumvents anatomic space limitations within the pericardial sac and significantly lowers the risk of perioperative infectious complications.[1] The potential risks, however, include neoplasmogenesis and progressive chronic inflammation secondary to sustained insult to the delicate peritoneal tissue. Thus, selection of materials and housing design are important factors affecting LVAD longevity.

Titanium and its alloys have been known for their remarkable resistance to corrosion and pitting and for their inertness, particularly in hard tissue replacement applications. Little is known, however, of the response elicited within the highly protective environment of the peritoneal cavity to an LVAD-sized titanium object. We attempted to answer this question by investigating the tissue capsule that forms around the pump housing of the HeartMate 1000 LVAD (Thermo Cardiosystems, Inc., Woburn, MA), by use of immunohistochemical and ultrastructural techniques.

Materials and Methods

Device Description

The HeartMate 1000 LVAD is a disc shaped pulsatile blood pump that weighs 570 g and measures 11 × 4 cm. The pump housing and other components are fabricated from

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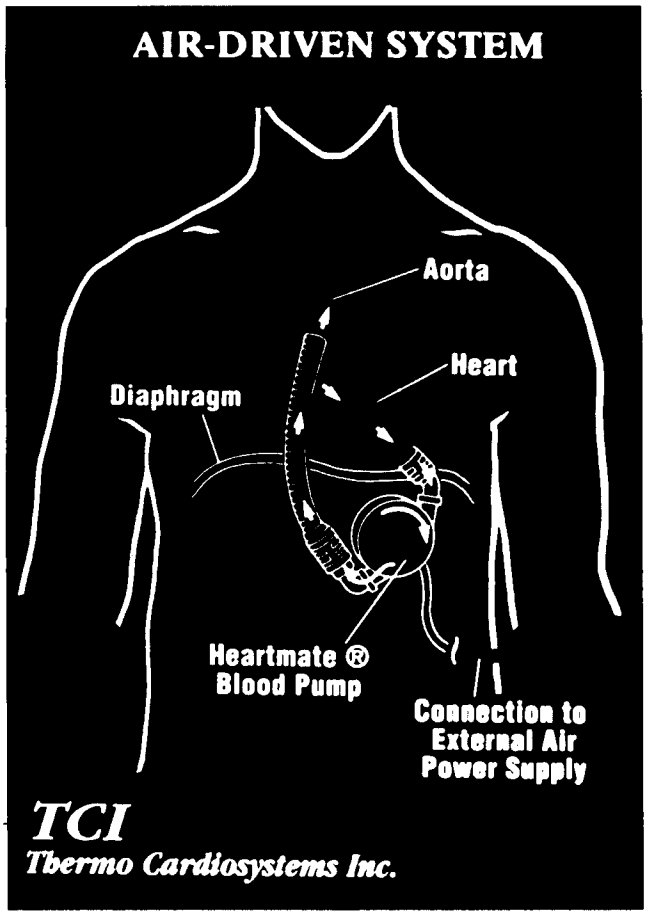


Figure 1. Anatomic positioning of the HeartMate 1000 LVAD.

titanium alloy (Ti-6Al-4V). The HeartMate is placed intraperitoneally in the upper left quadrant and sutured to the anterior abdominal wall (**Figure 1**). Pump inflow from the left ventricular apex is provided by a valved conduit that crosses the left anterior hemidiaphragm, whereas another valved conduit placed over the diaphragm directs pump outflow into the ascending aorta. The pump is connected to an external pneumatic drive console by way of a percutaneous tube with textured surfaces, which exits the skin through the left lower quadrant. A newer version of the LVAD can be operated by an internal electric motor. The maximum stroke volume of the LVAD is 83 ml, and it can provide a cardiac output of 11 L/min, in a fixed rate or automatic mode.

Study Protocol

We investigated the tissue capsule that formed around four HeartMate LVADs that had been implanted for extended periods (**Table 1**). At the time of cardiac transplant, circulatory support was discontinued, as previously described.[1] At LVAD explant, the tissue capsule surrounding the pump was identified and grossly inspected for color, consistency, thickness, attachment to the underlying pump housing surface, and presence of interfacial fluid. Several representative tissue samples were then taken from the ventral and caudal aspects of the capsule and submitted for histologic, immunohistochemical, or ultrastructural analysis.

Light Microscopy. Light microscopy was performed on tissue fixed in 10% buffered formaldehyde and processed into paraplast. Hematoxylin eosin and Masson's trichrome stained sections were evaluated.

Immunohistochemistry. Deparaffinized sections were labeled with commercially available antibodies to the following antigens (indicated in parentheses are clone, dilution, and manufacturer): vimentin (V9, 1:100, Dakopatts, Copenhagen, Denmark); desmin (D-33, 1:100, Dakopatts); α-smooth muscle actin (IA4, 1:50, Dakopatts); cytokeratin (CAM 5.2, 1:10, Becton Dickinson, Mountain View, CA); keratin (AE1/AE3, 1:1000, Boehringer, Indianapolis, IN); low molecular weight cytokeratin (35βH11, 1:100, Enzo, New York, NY); high molecular weight cytokeratin (34βE12, 1:100, Enzo); CD20 (L26, 1:100, Dakopatts); CD45RO (UCHL 1, 1:100, Dakopatts); carcinoembryonic antigen (CEA polyclonal, 1:600, Dakopatts); factor VIII related antigen (polyclonal, 1:800, Dakopatts). Pepsin digestion was used before labeling with all anti-cytokeratin and anti-factor VIII antibodies. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide with 0.1% sodium azide for 30 min. The sections were incubated with primary antibodies for 1 hr at room temperature. Biotinylated anti-mouse or anti-rabbit immunoglobulins were applied for 40 min, followed by streptavidin biotin peroxidase complex (Dakopatts) for 40 min. Diaminobenzidine was used as chromogen, and hematoxylin, for counterstaining. Appropriate positive and negative controls were used for each antibody. Direct immunofluorescence was performed on deparaffinized sections for fibrinogen (Diftest, undiluted, Shandon Lipshaw, Pittsburgh, PA).

Transmission Electron Microscopy. For ultrastructural examination, fresh tissue samples from two LVADs (Patients 2 and 3) were fixed in 3% buffered glutaraldehyde, postfixed in osmium tetroxide, then dehydrated and embedded in Epon (Polysciences, Inc., Warrington, PA). Ultrathin sections were examined in a Zeiss 10CA transmission electron microscope (Carl Zeiss, Oberkochen, Germany) at 60 kV.

Table 1. Patient Demographics

Patient No.	Age (yr)	BSA (m^2)	Duration of Support (days)	Status/Months After Transplantation
1	55	2.15	61	Alive and well/23
2	31	1.77	86	Alive and well/3.5
3	46	1.91	128	Alive and well/5
4	37	2.08	153	Alive and well/19
Mean (±SD)	42 (10)	1.98 (71)	107 (41)	—

BSA, body surface area.



Figure 2. Pump capsule (86 days after implantation). Tissue is 3–5 mm in uniform thickness without nodular masses. Note the white, glistening surfaces.

Results

Gross observation of the pump capsule at the time of explantation revealed a white, glistening tissue uniformly distributed around the pump, with no apparent nodular masses **(Figure 2)**. The capsule was loosely adherent to intestinal serosa but was not free to glide over the underlying metallic surface. Upon dissection, it was observed to be tightly apposed, but not adherent to the pump, with no serous effusion at the interface. The thickness of the capsule varied between 3 and 5 mm.

Light microscopy revealed that the capsular material was composed of fibrovascular tissue with an abundance of focally hyalinized collagen **(Figure 3)**. Focal fibrin deposits corresponding to homogenous oxyphilic material were revealed by means of immunofluorescent labeling **(Figure 3, inset)** and electron microscopy **(Figure 4)**. The focal perivascular inflammatory infiltrates consisted predominantly of UCHL1 positive monocytes and T lymphocytes, as well as L26 positive B lymphocytes. Occasionally, some plasma cells and a few polymorphonuclear and eosinophilic leukocytes were seen. Virtually no giant cells were observed, except in one instance around remnants of suture material (Patient 2). Numerous vascular channels formed by capillary endothelium expressing factor VIII related antigen were observed **(Figure 5)**. The predominant cellular type of the collagenous capsular matrix located further away from the capillaries was triangular or spindle shaped cells (SCs) with an oval or elongated nucleus **(Figure 6)**. These cells were characterized by simultaneous expression of vimentin, α-smooth muscle actin **(Figure 7)**, and low molecular weight cytokeratin **(Figure 8)**. Most of the SCs were stained with antibody AE1/AE3 and with the low molecular weight, cytokeratin specific monoclonal antibody, 35βH11, but almost none were labeled with high molecular weight cytokeratin antibody 34βE12. The percentage of cytokeratin reactive cells varied according to the type of monoclonal antibody used **(Table 2)**. A small number of the SCs expressed desmin, but none stained for carcinoembryonic antigen.

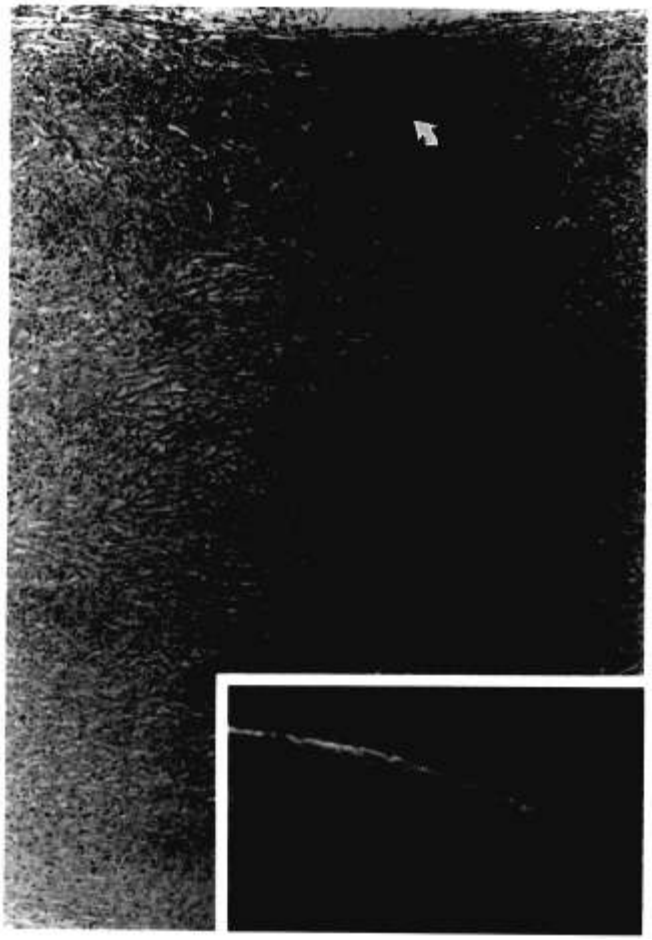


Figure 3. Photomicrograph of the capsule 153 days after implantation, with aggregates (arrow) of focal mononuclear cells (H&E, original magnification ×50). Deposits of organizing fibrin were positive for anti-FG antibody in direct immunofluorescence labeling (inset).

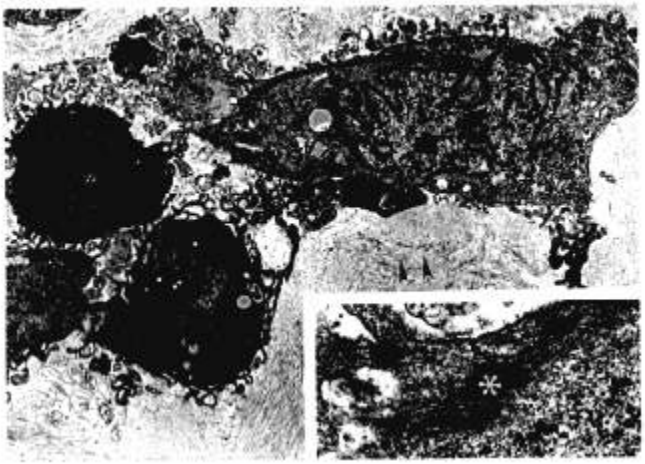


Figure 4. Transmission electron micrograph of capsule (86 days after implantation) showing spindle shaped cells with rough endoplasmic reticulum. Macrophages (M) and collagen (arrowheads) are in close proximity (original magnification ×2,500). Inset shows detail of cytoplasmic myofilaments (asterisk) (original magnification ×20,000).

Transmission electron microscopy revealed abundant rough endoplasmic reticulum and peripherally arranged myofilaments within the SCs (**Figure 4**). Golgi apparatus, some mitochondria, and randomly arranged 8 to 10 mm filaments were also present. No basal laminae were identified.

Discussion

Intra-abdominal placement of LVADs offers several advantages, such as surgical ease and freedom from repeat thoracotomy for implant removal.[2] In addition to being well tolerated in patients requiring long-term circulatory support,[3] patients with abdominal LVADs have fewer infectious complications associated with mortality or morbidity[4] than pa-



Figure 5. Capillary endothelium labeled with anti-factor VIII related antigen (original magnification ×640).



Figure 6. Capsule (61 days after implantation) showing triangular or spindle shaped cells embedded in collagen (H&E, original magnification ×640).

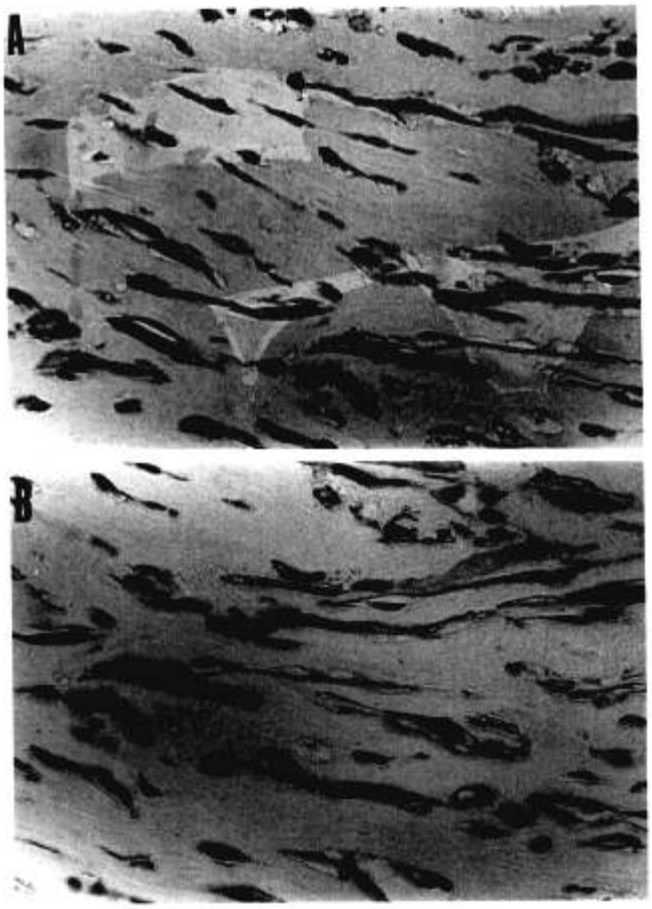


Figure 7. Capsule (61 days after implantation) showing spindle shaped cells and endothelial cells. (A) Anti-vimentin antibody (V9, original magnification ×640). (B) Anti-α-smooth muscle actin antibody (IE4, original magnification ×640).

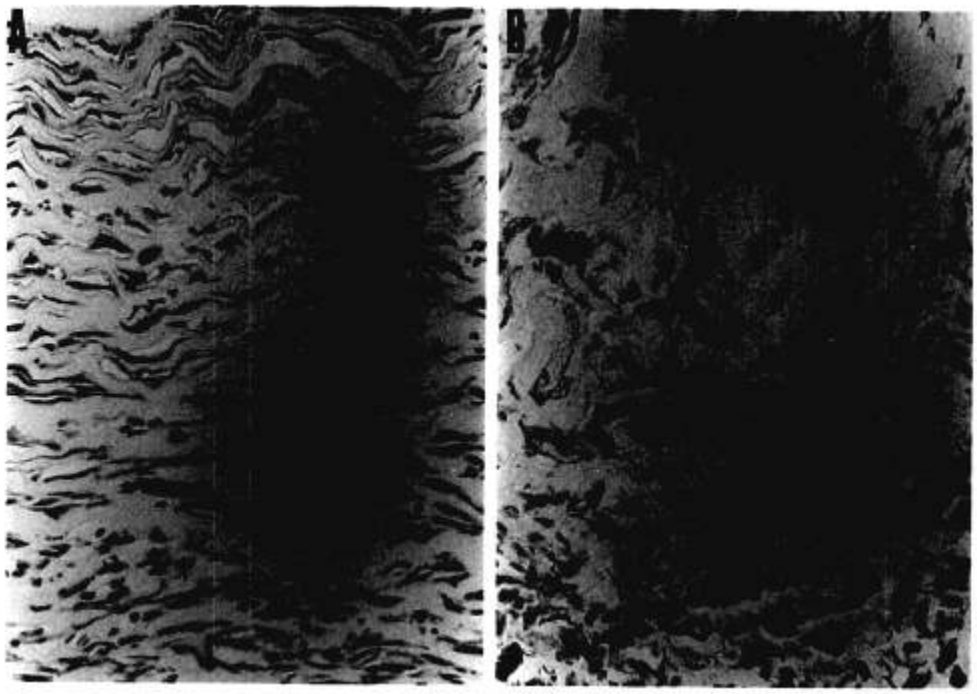


Figure 8. Capsule (128 days post implantation) showing cytokeratin expression in spindle shaped cells after labeling with anticytokeratin AE1/AE3 antibody. Both flat (A) and plump (B) cells present at the opposite superficial portions are positive (original magnification ×320).

tients with intrathoracic total artificial hearts[5,6] or subcutaneous, preperitoneal LVADs.[7,8] The omentum, richly supplied by vascular and lymphatic channels, possibly plays the major role in the containment of infections and rapid repair of potential insult secondary to trauma, obstruction, or foreign body presence, thereby contributing to LVAD longevity. Nevertheless, unfavorable material properties and poor implant design may lead to intensified reactivity of the peritoneal elements, which may lead in turn to a malignancy, such as foreign body sarcomas[9] and primary mesotheliomas.[10,11] In effect, the potential of some metals, such as cobalt, zinc, nickel, and cadmium, to induce proliferation or tumor promotion *in vivo* and, upon exposure, *in vitro*, to several soft tissue cell lines, is well characterized.[12]

In contrast to other metals, titanium appears to offer the advantage of soft tissue compatibility. Particulate titanium dioxide (TiO_2) has proved compatible with peritoneal cell populations *in vitro*[13] and *in vivo*.[14,15] Commercially pure titanium and Ti-6Al-4V alloy, both in particulate form, have been well tolerated by primary cultures of human synovial fibroblasts, mouse peritoneal macrophages, fetal rat cartilage, and synovial cells.[16] Pure[17] or ion implanted[18] titanium plugs have been tolerated well after transcutaneous and intraperitoneal implantation in a murine model. Clinically, the use of venous catheter tips for the treatment of intractable ascites in cirrhosis has been reported to result in prolonged long-term patency due to the thromboresistance of titanium.[19] Other areas where prostheses of titanium or its alloys have been used as replacement of, or adjacent to, human soft tissue include pacemaker cases, heart valves, chronic peritoneal dialysis catheter connections, and peritoneovenous shunts.

Table 2. Cytokeratin Expression in Spindle Shaped Cells in Relation to the Specificity of the Monoclonal Antibody Used

Monoclonal Antibody	Specificity	Cytokeratin Types	Labeled SCs (%)
AE1/AE3	Pankeratin	19, 16-14, 10, 8-1,	95–100
CAM 5.2	Lower molecular weights	19, 18, 8	90–95
35βH11	Low molecular weights	8	85–90
34βE12	High molecular weights	11, 10, 5, 1	0–1

SCs, spindle shaped cells.

The current report represents the first systematic description of the long-term interaction of a titanium alloy LVAD with human peritoneum. The close proximity of the capsule around the pump housing was the preliminary evidence, at the macroscopic level, of favorable acceptance of the titanium alloy housing by the peritoneal element. The tissue capsule itself, which represents the primary reaction of the latter to the former, was, upon microscopic observation, essentially reactive in nature. The identification of the cellular component responsible for the capsule formation was based on a combination of morphologic and immunohistochemical findings. The production of collagen, cell shape, and cytoplasmic oxyphilic material was initially suggestive of myofibroblastic origin. In addition, ultrastructural characteristics and complementary immunohistologic findings— such as

prominent rough endoplasmic reticulum, Golgi apparatus, mitochondria, clusters of peripherally arranged filaments that were 5 to 6 mm in diameter and that corresponded to α-smooth muscle actin, and 8 to 10 mm filaments randomly arranged in the cytoplasm corresponding to vimentin—were in agreement with this explanation. However, constant cytokeratin expression in the SCs was not compatible with the typical profile of a myofibroblast. The spectrum of cytokeratin expression was typical of low molecular weight cytokeratins (CAM 5.2 and 35βH11), which are also recognized by pankeratin antibody AE1/AE3. Almost no high molecular weight cytokeratins were detected with 34βE12 antibody. Keratins are a group of proteins contributing to the cytoskeletal network of epithelial cells. The term *cytokeratin* is usually used, instead, and 19 groups are recognized based on molecular weight and acidic or basic character.[20] Members of the acidic and basic subfamilies are found together in pairs. The low molecular weight 8/18 pair is characteristic of simple epithelia; the higher molecular weight cytokeratins are characteristic of epidermal differentiation.

Besides vimentin, ordinary (normal) mesothelium is known to express both low and high molecular weight cytokeratins. This directed us to explore the possibility that SCs are somewhat related to the mesothelium. Bolen et al[21] described immunologic and ultrastructural findings in nonneoplastic reactive serosal tissues. Most interestingly, normal peritoneum shows spindled MSCs, positive under normal circumstances for vimentin only. In cases of chronic inflammation, such as peritoneal scars or adhesions, however, these cells express vimentin together with low molecular weight cytokeratins.[10,21] Evidence for smooth muscle expression in MSCs was shown by Tsukada et al.[22] These findings are in complete agreement with our own observations. Therefore, we conclude that morphologic characteristics of spindle cells and simultaneous expression of vimentin, α-smooth muscle actin, and low molecular weight cytokeratin are consistent with the description of MSCs.

The analysis performed allowed us to suggest that the biologic response to an intraperitoneally placed LVAD includes initial deposition of fibrin, followed by proliferation of MSCs and vascular endothelium, with subsequent collagen production leading to the formation of a delicate, fibrous capsule. In regard to the possible carcinogenic properties of the titanium alloy implant, to this point there has been no gross or microscopic evidence of such an occurrence. Only a few cases of neoplastic mesothelioma have shown carcinoembryonic antigen-like immunopositivity,[23] which underscores the significance of carcinoembryonic antigen as a possible predictor of tumorigenesis. From this standpoint, the length of exposure to the alloy could be more important. The follow-up period is comparable to that of Heath,[12] who reported that after 4 to 5 months, tumors began to arise at the implantation site of metallic cobalt in the thigh muscle of rats.

The inflammatory infiltrate was mild, represented mostly by mononuclear cells. Granulomatous inflammation, explainable by the presence of foreign material, was identified in only one case. Sparse eosinophilic and polymorphonuclear leukocytes were of interest; however, at this point, their presence does not seem to represent any problem because of the extremely small number found. The biologic response to the LVAD, therefore, showed characteristics of a chronic proliferative reaction of serosal tissue, resembling a simple response of the peritoneum to mechanical injury.[24] Since no significant inflammatory response or nodular tumor-like masses were detected in the pump capsule, we concluded that long-term intraperitoneal implantation of a titanium alloy LVAD did not adversely affect the surrounding soft tissue.

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