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A MODEL FOR A MODEL: PRESSURE JACKETS ON NUDE MICE WITH IMPLANTS OF HYPERTROPHIC SCARS

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Abstract

The hypertrophic scar, a consequence of deep surface injury, is peculiar to humankind. Subcutaneous implants of these tissues in nude mice provide a quasi-animal model for experimental use. An efficacious treatment to promote acceleration of maturation of the hypertrophic scar is controlled topical pressure produced by flexible dacron fabric wraps. Implants in the nude mouse provide a model for study of the effects of pressure. Preliminary results indicate that those implants in nude mice, responding favorably to pressure jackets, show changes consistent with those previously obtained in human studies. The collagen matrix shows reduced density and parallel layering of collagen fascicles. Fibroblast cells, endothelial cells and pericytes show degenerative changes attributed to the effects of pressure treatment. Thus, pressure jackets properly applied to nude mice over hypertrophic scar implants constitute a model for the study of the effects of pressure therapy on the model of implants in the nude mice for the study of the dynamics of the hypertrophic scar.

**Key Words:** nude mice, hypertrophic scar, implantation, histology, scanning electron microscopy, transmission electron microscopy, collagen, pressure (mechanical)

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Introduction

The hypertrophic scar is the inevitable sequel to deep injury of the skin. It is a fibrotic lesion restricted to humans, in which the collagen is organized into discrete anatomical units called nodules. Nodular development appears dependent on what happens to the microvessels in the granulation tissue established immediately after injury (Kischer et al., 1991).

The fate of the hypertrophic scar is, ultimately, one of self limitation (Hunt, 1979), but the time for this maturation to occur is not necessarily predictable from a clinical point of view. Surgeons are not inclined to remove hypertrophic scars if still in the stage of hypertrophy because clinical experience has shown under such a condition they tend to recur (Peacock and van Winkle, 1976).

Various clinical treatments have been tried, including injections of steroids, x-rays and surgical revisions or excisions, but with mixed success (Rockwell et al., 1989). Larson et al. (1974) reported the use of elastic wraps on selected sites, usually the extremities, which exert topical pressure of approximately 30-50 mm Hg to accelerate maturation of the hypertrophic scar. Leung and co-workers (Leung and Ng, 1979; Clark et al., 1987) have reported the successful application of pressure on patients with hypertrophic scars for the past many years.

The site for pressure treatment must be such that subsequent movement would not compromise the constancy of the pressure which, according to experience, must persist for 10 months to 2 years (Leung and Ng, 1979; Clark et al., 1987). The magnitude of effective pressure has been established as 10 to 35 mm Hg (Cheng et al., 1983), depending on the body site.

It is generally agreed that no *bona fide* animal model for the hypertrophic scar or keloid is known, although there have been a few reports to the contrary (Marcenac, 1951; Silverstein et al., 1976). Those reports have not been subsequently supported in the literature.

However, in 1985, Shetlar et al. reported the use of a possible quasi-animal model for the study of the hypertrophic scar, and its related lesion, the keloid, by implanting pieces of

each, subcutaneously, into the nude mouse. The nude mouse is also athymic. Hence it would not be likely that implanted scar tissue from the human would be rejected (Krueger and Briggaman, 1982). More extensive investigations into this possible model were completed by Kischer and co-workers (1989a; 1989b). They confirmed the usefulness of this model and extended it to the study of another related fibrosis, Dupuytren's Contracture (Kischer et al. 1989c).

A variation of the model has been reported by Estrem et al. (1987) in which cultured fibroblasts and very thin slices from keloids were implanted into nude mice and grown successfully.

These models lend themselves to an attack on the dynamics of hypertrophic scars through experimental applications of steroids, lathyrogens, other appropriate chemicals, or antibodies to effect resolution of the lesions.

As it became clear that use of the nude mouse model achieved credibility, we decided to attempt to use the implanted nude mouse as a model for the study of pressure treatment. To this end, nude mice, implanted with pieces of hypertrophic scars were fitted with specially-made pressure jackets.

This pilot study reports the effects of constant topical pressure, over time, on the implants in the nude mouse, and evaluates the results against previously known data from human trials of pressure-treated hypertrophic scars.

#### Methods

The Jobst Institute of Toledo, Ohio, prepared strips of flexible tridimensional dacron fabric, of the same design and material which they manufacture for human clinical burn scar control (Malik and Carr, 1980). The strips were 12 mm wide and approximately 72 mm in circumference with velcro tabs on the ends for closure.

The Jobst Institute determined that the closed strip on their model would produce approximately 35 mm Hg. This pressure was compatible with that previously reported for human trials (Cheng et al., 1983).

Ten male nude (athymic) mice, classified as juveniles and weighing approximately 20-25 grams each were obtained from Charles River Co. (Massachusetts) and selected for the pressure trials. They were kept in a special room, in sterilized clear plastic pans, one mouse to a pan, fitted with filter bonnets. The pans were kept on a rack equipped with laminar air flow. The mice were supplied with sterilized water and chow and observed daily.

Five of the mice were fitted with Jobst pressure jackets while the other five served as controls. All 10 mice were implanted with pieces of the same hypertrophic scar, obtained as excess tissue from a surgical procedure. The scar had originated from a thermal injury and was of 3 years duration. Each tissue piece was obtained in sterile fashion and was dissected from the nodular areas of the scar specimen. Each mouse received two implants, one placed subcutaneously, over each suprascapular area,

right and left. Each implant was of a standard size, 5x8x5 mm. The surgical implant and harvesting procedures have been previously described (Kischer et al., 1989a).

The implants were allowed to vascularize and stabilize for 44 days, at which time five mice were selected for wearing the pressure jackets. Other pieces of nodular tissue implanted in companion mice of the same age and weight were harvested at this time for additional controls.

The pressure jackets were placed over the implant areas and closed on the abdominal side of the mouse (Fig. 1). They were worn continuously and the mice were observed daily for signs of slippage of the jackets or any other compromising condition to constant pressure.

Two implants from one mouse were harvested after 37 days of pressure treatment. Four implants from two mice were harvested after 44 days of pressure. Four implants from two other mice were harvested after 55 days of pressure. Control mice had their implants removed on the corresponding days, as above.

Each implant was sized upon harvesting. Each harvested implant was dissected so that portions were obtained for morphological analyses by light microscopy (LM) and by scanning (SEM) and transmission electron microscopy (TEM).

All tissues reserved for study by microscopy were fixed at room temperature in Karnovsky's fixative, and remained in the fixative up to 4 days. Tissues to be studied by light microscopy were embedded in paraffin, sectioned, and stained by hematoxylin and eosin, and by Masson's trichrome stain. Those subsequently studied by SEM were dehydrated by transfer through graded alcohols, 50% to absolute alcohol, then placed in the chamber of a Tousimis Samdri-790 Critical Point Dryer. The intermediate fluid was alcohol and the transitional fluid was liquid CO<sub>2</sub>. Tissues were mounted on platforms, coated with approximately 300 Angstroms of gold using a Polaron Sputter Coater, Model #5100, with argon gas. The tissues were examined in an ETEC Autoscan using 20 kV.

Those specimens to be studied by TEM were post-fixed in 2% OsO<sub>4</sub> buffered with sodium cacodylate, for one hour, then washed in cacodylate buffer, dehydrated through graded alcohols, passed through propylene oxide, and embedded in Epox 812 (Ladd Research Co.). Thick and thin sections were cut on a diamond knife and thin sections were stained with lead citrate and uranyl acetate and examined in a Philips 300 electron microscope. At least two grids with multiple sections per tissue were examined by TEM from each tissue specimen. Thick sections were cut at 1 µm, and stained with 1% Toluidine Blue O.

#### Results

The jackets were tolerated well, although from time to time some had to be readjusted, or replaced when found wet. Representative pieces of the hypertrophic scar used for the implants,

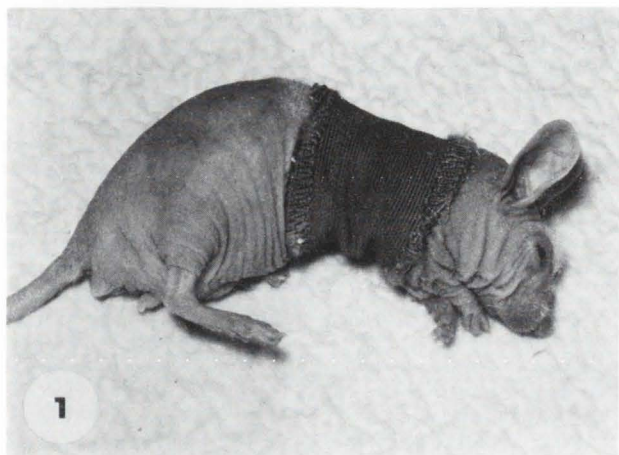


Fig. 1. The fabric jacket is shown on a nude mouse.

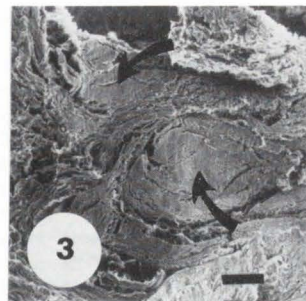
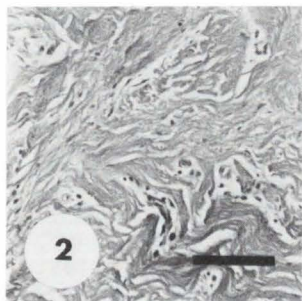


Fig. 2. Histological section through the original hypertrophic scar tissue used for the implants. The lesion was 3 years post-injury. Dermis shows typical pattern for hypertrophic scarring. H&E stain. Bar = 100  $\mu$ m.

Fig. 3. SEM of piece of the original hypertrophic scar tissue used for implants. Nodules (  $\blacktriangleright$  ). Bar = 100  $\mu$ m.

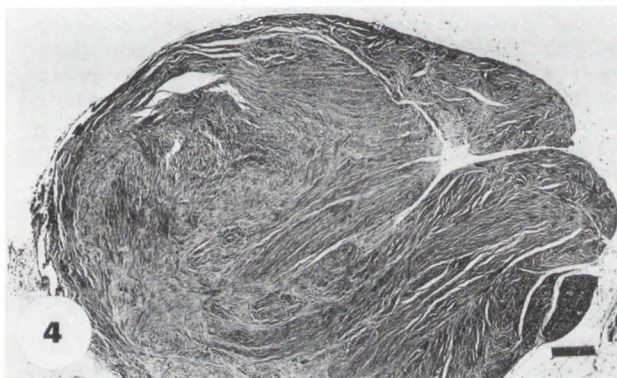
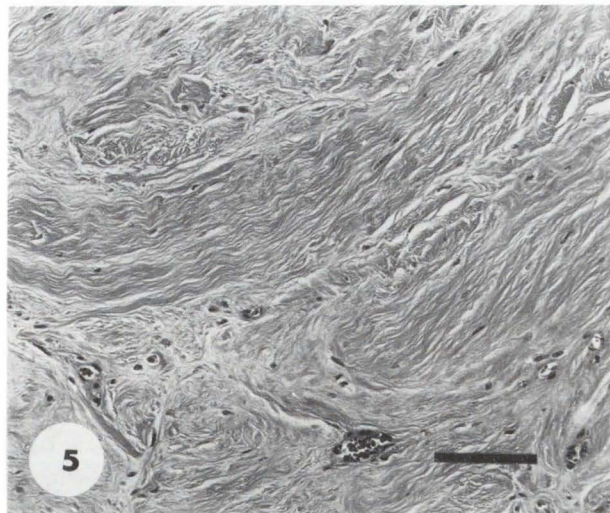


Fig. 4. Histological section of control implant of 88 days. The morphology does not essentially differ from the zero time control. H&E stain. Bar = 100  $\mu$ m.

Fig. 5. Section of a control implant after 88 days at a higher magnification than Fig. 4. Note the dense collagen in parallel alignment. H&E stain. Bar = 100  $\mu$ m.



examined prior to implantation demonstrated typical nodular structure by light microscopy (Fig. 2) and by SEM (Fig. 3).

Implant controls examined after each of the trial periods demonstrated retention of the hypertrophic scar characteristics, which did not significantly differ from the preimplant morphology (Figs. 4 & 5).

Four implants, from two mice, each with two implants (right and left), treated with pressure, demonstrated morphological changes which were different from the controls. One was treated for 44 days, the other treated for 55 days. There was increased parallel layering of the collagen about and within the implant by light microscopic section study as exemplified by figure 6. The extent of the parallel rearrangement of the collagen is considerably greater than is observed in the control (no pressure) implants (compare Figs. 4 & 6). These areas viewed at a greater magnification show a considerable increase in interstitial space and smaller dimensions of collagen fascicles (Fig. 7). By SEM, the effect of pressure reflecting the parallel layers of collagen fascicles separated one from another is clearly seen (Fig. 8). This pattern contrasts markedly

with the appearance of the collagenous matrix in the control (no pressure) implants, in which virtually no separation is observed (Fig. 9).

A study by TEM of sections of the implants adjacent to those studied by LM and SEM demonstrated typical changes in morphology attributed to the effects of pressure. This was true for each of the four implants. The incidence of degenerating forms of fibroblasts, pericytes, and endothelial cells appeared increased in the four implants responding to pressure treatment (Fig. 10) over those observed in the controls (Fig. 11). These degenerating characteristics were manifest mostly in the form of cytoplasmic vesiculation or evacuation.

### Discussion

Our previous studies of hypertrophic scars under pressure included analyses by light and electron microscopy and by glycosaminoglycans assays (Kischer et al., 1975; 1978; Kischer and Shetlar, 1979). The data obtained from those studies indicated that endothelial cells and pericytes of the microvessels, and many fibroblasts demonstrated degenerative characteristics with a magnitude above that observed in the nonpressure-treated scars. Changes in the levels of certain GAGs were detected after only 30 days of pressure.

The observations made by TEM on the four implants responding to the pressure treatment demonstrate increased interstitial space, small fascicles of collagen fibrils and parallel arrangement of the fibrils, especially near the surface of the implant. These observations are compatible with observations made in previous studies of mature scar and studies of human scars under pressure treatment (Kischer et al., 1975, 1978; Kischer and Shetlar, 1979; Berry et al., 1985).

Our experience is that daily observations are mandatory. This is due to the mechanics of the jacket and the activity of the mouse which, at times, promote a loosening of the jacket. Adjustments had to be made periodically. Sometimes a jacket would become soaked from contact with the tip of the water bottle and have to be changed. The jacket might be modified so as to place a pad on the underside, of sufficient size, to cover the width of the implant and to better ensure a constant pressure. While it is true that only four implants demonstrated any effect of the pressure treatment, we believe that the use of pressure jackets on implanted nude mice presents a usable, workable model for studies on the effects of topical pressure for therapeutic resolution of the hypertrophic scar.

### Acknowledgments

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Fig. 6. Section of implant treated with pressure for 55 days. Note increased parallel layering of collagen extending deep into the implant (➡). Bar = 100 μm.

Fig. 7. Section of implant treated with pressure for 55 days. Parallel layering of collagen bundles is obvious. Note increased interstitial space and small profiles of collagen fascicles indicating reduced stress alignment. Bar = 100 μm.

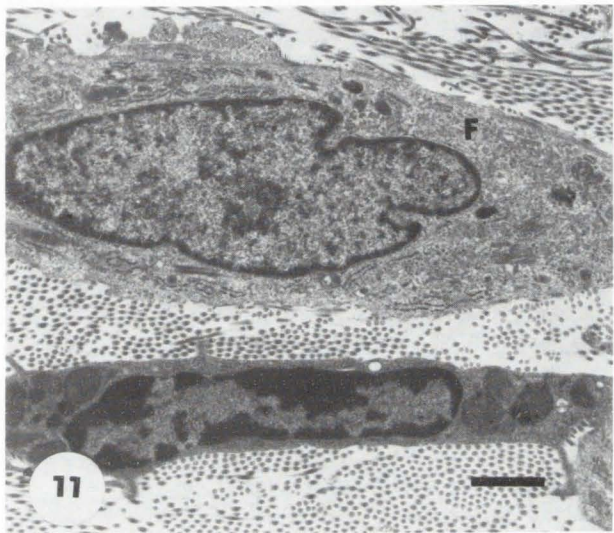
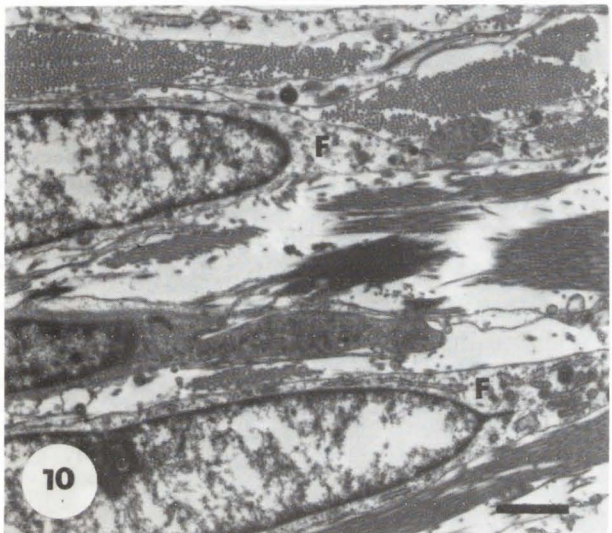
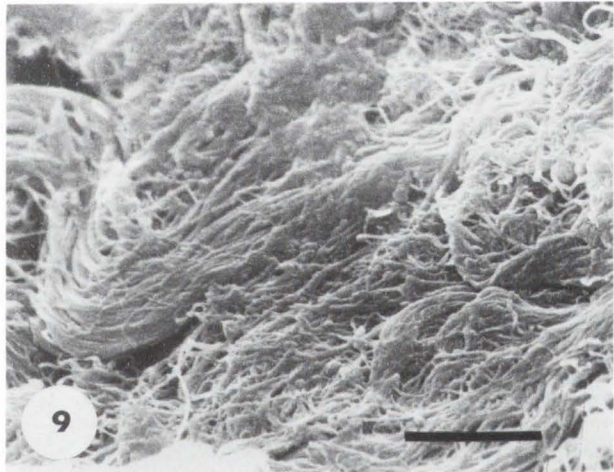
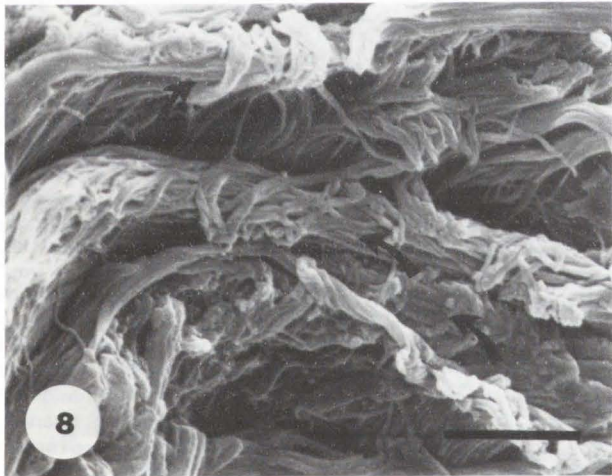
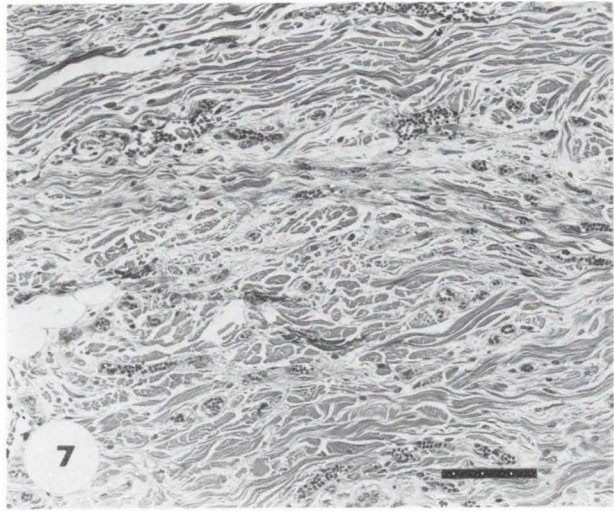
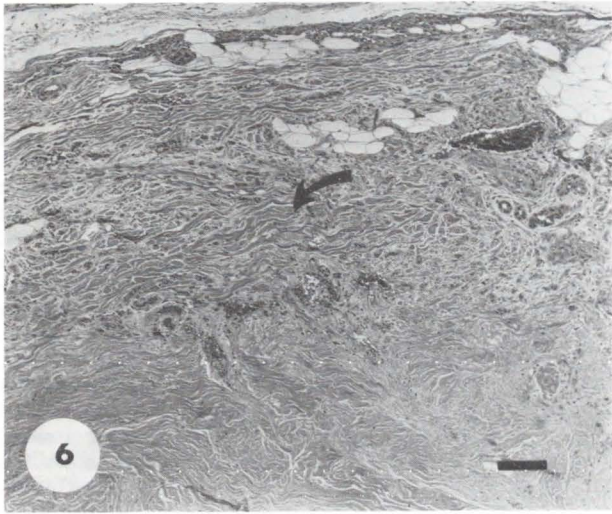
Fig. 8. SEM of implant treated for 55 days with pressure. Collagen bundles are in parallel layers clearly separated one from another (➡). Bar = 5 μm.

Fig. 9. SEM of control implant harvested after 99 days post implantation. The collagenous matrix appears homogeneous and typical of that of a hypertrophic scar. Bar = 5 μm.

Fig. 10. TEM of implant treated with pressure for 55 days. Profiles of fibroblast-type cells (F) show loss of cytoplasmic structures as a degenerative characteristic. Matrix of collagen fibrils includes increased interstitial space. Bar = 1 μm.

Fig. 11. TEM of control implant harvested after 99 days. Fibroblast-type cell appears normal (F). Collagenous matrix is more dense than that in Fig. 10. Bar = 1 μm.

Pressure Model for Hypertrophic Scars



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#### Discussion With Reviewers

A.J. Wasserman: During wound healing it has been shown that there is a dramatic transition in proteoglycan morphology as the developing matrix matures. How do these events compare to the changes taking place in the pressure treated animals you describe? How do you think the proteoglycans differ between your controls and experimental groups? How would you expect this atypical connective tissue to compare to normal skin with regards to biomechanical properties?

Authors: In this study we have not observed any clear-cut differences in the interfibrillar or perifibrillar areas which could be attributed to suspected changes in the proteoglycans. Furthermore, samples from two of the four implants which demonstrated morphological changes consistent with effects of pressure therapy were analyzed for their distribution of chondroitin sulfates, and compared with the distribution of same from the controls. There were no significant differences noted. However, the sample size should be larger and should be repeated, because we would expect there to be differences which should be observable and which may, indeed, render some differences in such properties as tensile strength.

C.J. Doillon: In comparing figure 4 with figure 6, it seems that in the control group the scar implant is surrounded by a loose connective tissue (newly synthesized maybe?) while under pressure, a fibrotic capsule with a dense connective tissue is observed with fatty cells. In figure 6, for example, it seems that

hypertrophic tissue is below the arrows. Could you comment on this morphological observation and also could you describe the tissue reaction around the implant?

Authors: Each implant undergoes a surface remodeling of about 30 micrometers, so that the cells parallel the surface of the implant. The loose connective tissue outside this surface belongs to the mouse. No real capsule, as such, forms around the implant. However, at certain locations vascular plexi, belonging to the mouse, appear, some of the tributaries of which will anastomose with peripheral implant microvessels. But, this anastomosis may extend up to 300 micrometers from the implant surface. The fat cells seen at the periphery of the pressure-treated implants may or may not be consistent with pressure. A larger sample number would have to be examined because we have occasionally seen these in non-treated implants.

R.J. Goss: Why do you think only four out of ten hypertrophic scar implants exhibited effects of the pressure treatment? Were such results "all of nothing", or did some of the other six implants show partial effects?

Authors: As explained in the Discussion, there were some problems encountered in maintaining constant pressure on some of the mice. This probably explains why only four responded to the treatment. In some sense the response would equate to an "all or nothing" because the response is to a constancy of pressure. Intermittent relief of this constancy, through neck stretching (which some do much more than others), wetting of the wrap, etc., would compromise the effect of the pressure. We believe that in some sense what we see as positive responses to the pressure are, in fact, partial effects. As Doillon pointed out, the effect is peripheral to begin with and the implant remains hypertrophic below this change. In time, we would expect the entire implant to mature.

R.J. Goss: Since both implants in each mouse reacted the same, could this imply operation of a systemic factor?

Authors: This is not likely. The simple explanation is an effect which is directly derived from the mechanical pressure of the wrap, which should be equivalent on both sides. From our studies on human patients we can speculate the same kind of phenomenon occurring in this model, that there is sufficient microvascular occlusion to drive the hypoxic state of the implant towards anoxia. This would promote degeneration of the fibroblast-type cells, releasing, precociously, lysosomal enzymes, which may, in turn, digest interfibrillar material (perhaps proteoglycans), producing more interstitial space. This is speculation at the moment without hard evidence. But, the important aspect of this study appears to be the establishment of a model by which such speculation could be tested.

## Pressure Model for Hypertrophic Scars

B. Forslind: How is the pressure of the jacket determined in mice and man? Have you any indications that there may be a pressure gradient from the center of the jacket towards its rim? Will all jackets provide constant pressure during the entire application or is there a creep in the jacket material causing a continuous decrease in pressure?

Authors: The pressure effected by the jacket is derived from two sources: 1) deformation measurements of the fabric material itself involving LaPlace's law, and 2) pressure measurements made on a LegForm model using a strain guage. The Jobst Institute routinely conducts these measurements. We do not suspect, nor have any evidence, that there is a pressure gradient under the wrap, given that it is the proper size. Indeed, there is a creep of some jackets produced by the movements of the mouse, some more than others. That is why we had to make daily observations so that we could predict which mice would be more likely to produce consistent results. As indicated in the text, from time to time we did have to make certain adjustments in some mice.

B. Forslind: It is not clear if the jacket may have occlusive properties in addition to exerting pressure. If so, to what extent would occlusion influence the results you obtain?

Authors: The jacket is woven dacron fabric and should not be occlusive. We do not believe that occlusion is involved in the results.