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LEWIS RESEARCH CENTER
National Aeronautics and Space Administration

GRANT NSG-3126



Annual Report
December, 1977

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# First Annual Report

## Effect of Surface Texture by ion beam sputtering on implant biocompatibility and soft tissue attachment.

The need to determine the effectiveness of surface texture as a means to improve the stability of a number of clinically used devices and prostheses in soft tissue was documented in the original proposal. The objective of the surface texture is to either increase the positional stability of the device, as in the case of the power and control package of pacemaker electrodes and electrodes for functional stimulation of muscles or control of the epithelial cell attachment and sealing on percutaneous connectors and tooth implants at the gingiva.

## Introduction

The objectives which were proposed for the research presented in this report were to use the ion beam sputtering technique to produce surface textures on polymers, metals and ceramics. The morphology of the texture (Fig. 1) was altered by varying both the width (w) and depth (d) of the square pits which were formed by ion beam errosion. The width of the ribs (R) separating the pits is defined by the mask used to produce the texture (1). The area of the surface containing pits varies as w is changed; Table I summarizes the pertinent texture parameters.

The biological parameters which were used to evaluate the biological response to the texture are,

a) fibrous capsule and inflamatory response in subcutaneous soft tissue using histological techniques (Biocompatibility),

b) strength of the mechanical attachment to the textured surface by the soft tissue (Mechanical), and

c) morphology of the epidermal layer interfacing the textured surface of percutaneous connectors.

Because the sputter yield on teflon$^{R}$ is approximately an order of magnitude larger than any other material (polymer, metal or ceramic) the majority of the measurements presented in the report, on the effect of the ratio w/d, were obtained with teflon.

## Results

### a) Biocompatibility

Table I summarizes the materials and texture parameters which were used in the evaluation of the biocompatibility. All of the observations and measurements reported were made after six (6) weeks of implantation. The implants were introduced into a pocket, produced by blunt dissection, between the skeletal muscle and the panniculus muscle layer on the dorsal surface of Sprague-Dawley rats. The implants were approximately 8mm x 8mm, cut from sheet of the same thickness used for the mechanical attachment measurements, namely 0.020" to 0.040" thick. The actual thickness depending upon the depth of pit to be evaluated.

Polymers:

Because of the low sputter rate in all other materials tested, teflon is the only one on which the evaluation of the effect of w/d ratio has been achieved during the first year of this program. The low sputter rate of other materials made extensive evaluation of small w/d ratios prohibitive in terms of time commitment of the sputtering chamber. The histological sections were prepared by both normal paraffin embedding as well as by direct frozen sections from unfixed tissue.

Polyurethane was only evaluated with the w/d ratios in the range 2.0 to 4.0 for w of 21μ and 50μ respectively. The thickness of the fibrous capsule against a textured surface exhibited a slightly smaller thickness (27-32μ) as compared with the control surface (50-65μ). Because of the artifacts produced by the presence of the polymer sample during sectioning, many more samples would be required to demonstrate whether a statistically significant difference was present. The fibrous capsule was well defined and surrounded by a moderate capilliary net work, Fig. 2. Use of a trichrome stain for collagen showed that collagen has been laid down up to the surface of the polyurethane. The fibrous capsule and surrounding tissue showed a normal mature fibrocyte population with only an occasional lymphocyte or histeocyte. There appeared to be a monolayer of cells at the surface of both the textured and control materials. It was not possible to make a definitive identification of these cells which could be either fibroblasts or monocytes. The capsule extended into the pits, however the cells in these regions appeared to be more active fibroblasts, Fig. 3.

Teflon was evaluated with a range of values for w/d at widths (w) of 21μ and 50μ. The thickness of the fibrous capsule appeared to be independent of the texture parameters and similar to the control surface (55-65μ). The cellular response however was sensitive to the presence of texture; the capsule against the textured surface showed active fibroblast morphology (Fig. 4) as compared with the quiescent fibrocyte morphology against the control, nontextured, surface (Fig. 5). Use of a trichrome stain for collagen demonstrated no collagen deposition against the non textured control. The fibrous capsule in all cases was surrounded by a moderate capillary network. The cellular response otherwise was unremarkable, with occasional lymphotcytes and histocytes and occasional to sparse eosinophils.

Cells had migrated to the bottom of all pits irrespective of pit width or ratio of w/d. The cell type could not be determined unambiguously and special thin sectioning techniques will have to be used to document the cell types and behaviour in the pits. With the data available at present it would appear doubtful that collagen was produced within the pits. The cellular behaviour in the pits may well be influenced by the presence of teflon filaments, of the order of 1μ diameter, which are produced by the sputtering process Fig. 6. The influence of a pit on the local cell morphology is clearly visible on some sections from frozen tissue samples. (Fig. 7).

Metals:

Because of the technique used for making tissue sections it was necessary to remove the sample prior to preparing sections for histology. It was not possible therefore to obtain any data on tissue infiltration into the pits nor was it possible to make unambiguous statements regarding the tissue layer immediately adjacent to the surface.

The thickness of the fibrous capsule around a naturally textured surface (i.e. no mask, Table I) of Haynes 25 was approximately 81μ. This capsule thickness is greater than that normally reported for Haynes 25, however, a rough surface is known to enhance the cytoplasm enzyme activity of fibroblasts (2) and also leads to increased fibrous capsule thickness due to mechanical trauma (3). The textured surface also produced a similar effect to that which was elicited by the textured teflon, namely an active fibroblast response after 6 weeks of implantation. Trichrome stain for collagen also demonstrated a lack of collagen deposition near the textured surface. The cellular response in the capsule and surrounding tissue was otherwise unremarkable with only occasional lymphocytes and histocytes. Occasionally however, small particles of metal either from the mask or the specimen were observed in the capsule and these particles produced a local monocyte macrophase infiltration togetner with foreign body giant cells.

The fibrous capsule response to the large samples used for the mechanical attachment experiments was quite marked. As a result of the extreme mechanical trauma effect in the soft tissue (3), the fibrous capsule thickness, for all the metal alloys around the large specimens varied from 175μ to 250μ. The overall cellular response,as well as the capsule cell response adjacent to the implants, were however identical to those around the smaller samples used for the evaluation of the tissue response.

Ceramics:

Ceramic specimens as for the metals had to be removed prior to sectioning for histology. Alumina, the only ceramic used in this investigation, does not exhibit any natural (no mask) texture and the samples evaluated for tissue response were taken from the mechanical attachment experiments and had a texture pit width w of 50μ and a w/d of ∿2.0. The overall cellular response was similar to the metals. The thickness of the fibrous capsule exhibited the mechanical trauma response and averaged 150μ adjacent to the textured surface and 260μ on the control surface.

b) Mechanical attachment experiments

All specimens used for the measurement of the mechanical attachment strength were made from sheet specimens 0.75 mm thick. The samples were 0.5 cm wide and 3.0 cm long. The textured region extended 2.0 cm along one surface, the remaining 1 cm being untextured and used for griping the specimen in the tensile machine. Two specimens were implanted subcutaneously on the dorsal surface of Sprague-Dawley rats, one on either side of the midline. After six weeks the animals were sacrificed and the pelt immediately removed from the dorsal surface. Strips of the pelt approximately 1.0cm wide containing the specimen were cut with a "prep" blade. The non textured end of the specimen was dissected free and inserted in one grip of the instron tester and the pelt was inserted into the other grip. The load necessary to extract the specimen from its capsule was measured with a rate of 2"/min crosshead speed.

Initial tests with polyurethane (w = 50μ , w/d ∿2.0) and using the peel test (ASTM D903-49) showed no significant increase in the mechanical attachment over that of the control. However these tests demonstrated that the peel test was impractical. The mechanical properties of the subcutaneous tissue immediately after sacrifice are such that it is not possible to dissect out and prepare specimens for a peel test without severely compromising the integrity of the fibrous capsule at the textured surface. Accordingly the method for evaluating mechanical attachment was changed to a direct "pull out" test. Since most of the

specimens had been prepared for the peel test prior to the change in protocol the pull out measurements were made on specimens textured on only one surface. These preliminary data also demonstrated the need for increased texture pit depth, i.e. smaller values of w/d. As indicated in the introduction, the remainder of the polymer tests were made on Teflon which exhibits a much greater sputter errosion rate.

Figure 8 shows a typical load-displacement curve for the Teflon samples. Table II summarizes the values of maximum load required to extract the specimen from its fibrous capsule on all the materials used in these initial tests. These data demonstrate a wide variation in values among the polymer specimens and therefore it is not appropriate to make any predictions regarding the effect of w/d ratio. The metal alloy specimens consistently exhibit higher values for the maximum pull out load.

A factor which undoubtedly contributed to the wide variation in the mechanical attachment data was noted while observing the specimen and tissue during a test; collagen fibers from the capsule were occasionally snagged in small rough areas on the edge of the specimen or in the case of metal specimens where the mask had been tacked in position for the texturing. More attention must be paid, therefore, to the preparation of specimens so as to ensure that the edges of all specimens are completely smooth and remnants of the mask material completely removed.

c) Percutaneous connectors

Dumbell type percutaneous connectors fabricated from teflon and 316 stainless steel have been textured without use of a mask, along the shaft. Teflon strips textured on both sides were also prepared. The specimens have been implanted on the dorsal surface of cats. The specimens have as yet not been recovered sincethey have only been in place for three weeks.

## Discussion

The data obtained from the research so far have clearly defined the methods and techniques necessary to answer specific questions which have emerged and which are necessary to the understanding of the effect of surface texture on soft tissue response.

The effect of implant size and mechanical stiffness which produces localized mechanical trauma and results in the increased fibrous capsule thickness are well appreciated in the literature. This effect was maximized by extreme tissue mobility at the specific implantation site used in this study. Similar capsule effects, as compared with smaller test specimens, also exist around pacemaker power and control packages which are implanted clinically in regions of relatively mobile tissue.

None of the textured materials elicited anything more than an acceptable level of chromic inflamatory type cellular response and therefore at this level surface texturing has not altered the soft tissue response of the materials. The surface textures produced by ion beam sputtering have however been shown to alter the behavior and morphology of the cells adjacent to the textured surface. This manifests itself as a region of cells, either active fibroblasts or monocytes, which do not exhibit the usual elongated, spindle, morphology of quiescent fibrocytes. Smooth implants usually demonstrate a monolayer of cells of unidentified type at their surface and the effect of the

texture may have been to extend this layer. The second and probably correlated observation is the absence of collagen fibers, as defined by trichrome stain, in this region. This suggests that the surface region of cells, if fibroblasts, are not secreting collagen, or at least not "normal" collagen fibers.

The cells which infiltrate and line the surface of the textured pits also appear not to produce collagen fibers. However, since we have been able to make observations in the pits only on teflon this could be influenced by the fine filaments of teflon within the pits. These filaments are characteristic of the natural texture produced by ion beam sputtering on teflon.

During the coming year we intend to clarify some of these observations by the use of other techniques including histochemical (4) (to identify changes in the cytoplasmic enzymes), scanning electron microscope (to make observations on the cell population as well as the presence of collagen fibers in pits) and transmission electron microscopic (to identify changes in the cells at the ultra structural level). An understanding of the tissue responses to texture will also be attacked by increasing the width of the texture morphology to include widths of 100μ and 150μ, as well as investigate the influence of the natural surface texture.

The mechanical attachment strength experiments will be improved by the use of the pull out test on specimens textured on both sides. It is anticipated that this improved protocol together with expanding the range of w to include specimens with w of 100μ and 150μ will define the effect of w and w/d on mechanical attachment strength. Since collagen at the implant surface and within the pits is necessary for strong mechanical attachment these data will also provide information on the nature of the cellular behavior within the textured pits.

The lower values for the mechanical attachment on teflon are also influenced by the very low surface energy of teflon. The low surface energy is responsible for the poor attachment of cells to its surface which has been observed with woven teflon vascular grafts(5,6) and why they are usually considered inferior to those fabricated from dacron(7,8). The high sputter yield of teflon however made it an attractive material with which to explore the effect of pit depth, i.e. smaller w/d ratios. Recent experiments have established that polyoxymethylene, Delrin®, also exhibits a high sputter yield. Use of Delrin specimens will therefore permit us to evaluate the role of polymer surface energy on mechanical attachment strength.

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TABLE I

| Material | Texture | | | | |
|---|---|---|---|---|---|
| | With Mask | | | Without Mask* | |
| | w | w/d | R | w | w/d |
| Polyurethane, Biomer® | 25μ | ∿2.0 | 80μ | | |
| | 50μ | ∿4.0 | 85μ | | |
| Polytetrafluoroethylene Teflon® | 25μ | 1.0-0.1 | 80μ | 8μ | 0.3 |
| | 50μ | 1.0-0.1 | 85μ | | |
| Alumina | 50μ | ∿2.0 | 85μ | | |
| Titanium 6Al-4V | 48μ | ∿1.0 | 80μ | 0.5μ | ∿0.5 |
| | 70μ | ∿1.0 | | | |
| Haynes 25 (Co-Cr-W) | 70μ | ∿1.0 | | 3μ | ∿1.0 |
| 316 S.S. | 70μ | ∿1.0 | | 2μ | ∿0.7 |

* The definition of w and d is somewhat modified in this category since sputter errosion, without a mask, produces a texture consisting of spikes or pillars of width, w, and of height, d, standing up on the surface.

Table II

Mechanical Attachment Strengths

| Material | w | w/d | Max. load on Pull out Test (gm.) |
|---|---|---|---|
| Teflon$^{R}$ | 25μ | 0.5 | 40 ; 65 |
| | | 0.25 | 27 ; 36 |
| | | 0.16 | 19 ; 30 |
| | | 0.1 | 33 ; 22 |
| Teflon$^{R}$ | 50μ | 1.0 | animal died before test completed |
| | | 0.3 | 26 ; 13 |
| | | 0.16 | 140 ; 55 |
| | | 0.1 | single sample showed no attachment |
| Alumina | 50μ | 2.0 | 97 ; |
| Haynes 25 (Co-Cr-W) | 70μ | ∿3.0 | 110 ; 140 ; 156 ; 152 |
| Titanium 6Al-rV | 70μ | ∿3.0 | 98 ; |
| | 48μ | ∿2.0 | 175 ; 176 |
| 316 Stainless Steel | 70μ | ∿3.0 | 194 ; 255 ; 285 |

## Figure Legends

Figure 1 — Diagram of texture geometry with symbols used to characterize the variables.

Figure 2 — Fibrous capsule and adjacent capillaries surrounding polyurethane (Biomer$^{R}$). (x260)

Figure 3 — Fibrous capsule adjacent to textured polyurethane ($^{W}/d \approx 2.0$). Note fibroblast activity in pit. (x260)

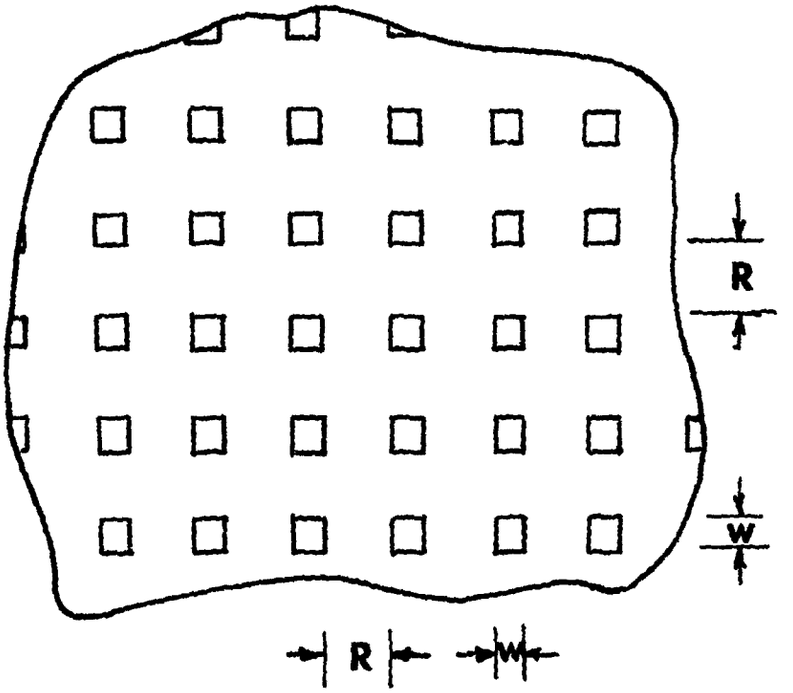
Figure 4 — Fibrous capsule adjacent to textured surface of teflon$^{R}$ ($^{W}/d \approx 0.15$), illustrating fibroblast activity. (x1000)

Figure 5 — Fibrous capsule adjacent to smooth surface of teflon$^{R}$ illustrating quiescent fibrocytes. (x1000)

Figure 6 — SEM image of section through pits ($^{W}/d \approx 0.25$) in teflon$^{R}$, illustrating 1μ dia. filaments projecting from base of pit. (x300)

Figure 7 — Photomicrograph from frozen section showing influence of pits on cell morphology. (x260)

Figure 8 — Load-displacement curve for pull out test on textured teflon$^{R}$ specimen (w = 21μ; $^{W}/d$ 0.1).



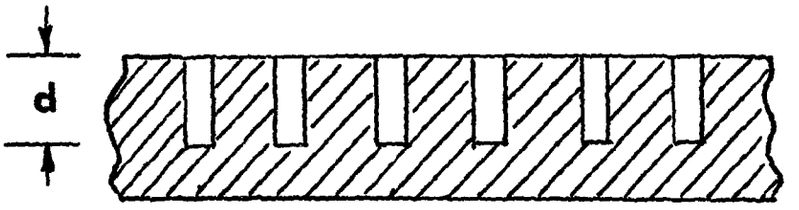




FIGURE 1

Fig. 2

Fig. 3

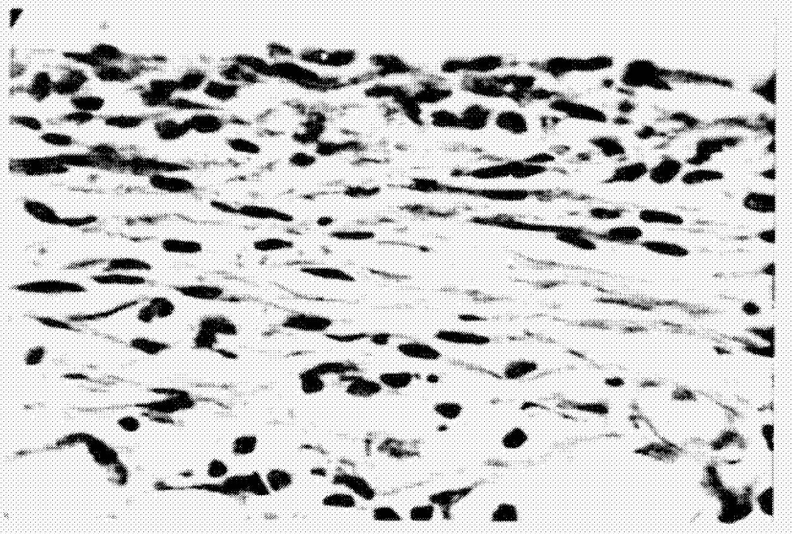


Fig. 4

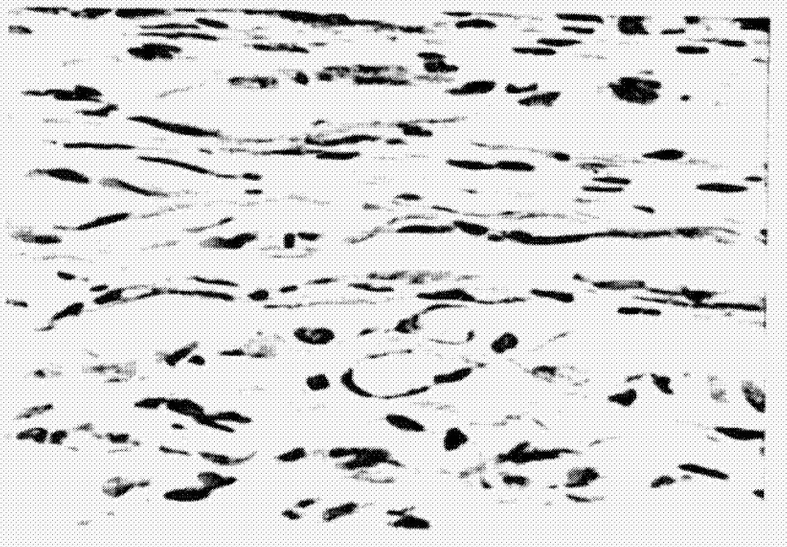


Fig. 5

Fig. 6

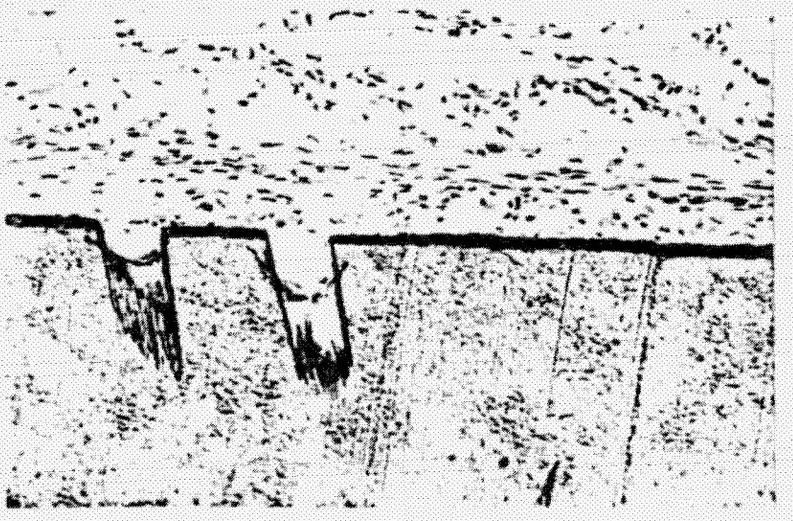


Fig. 7

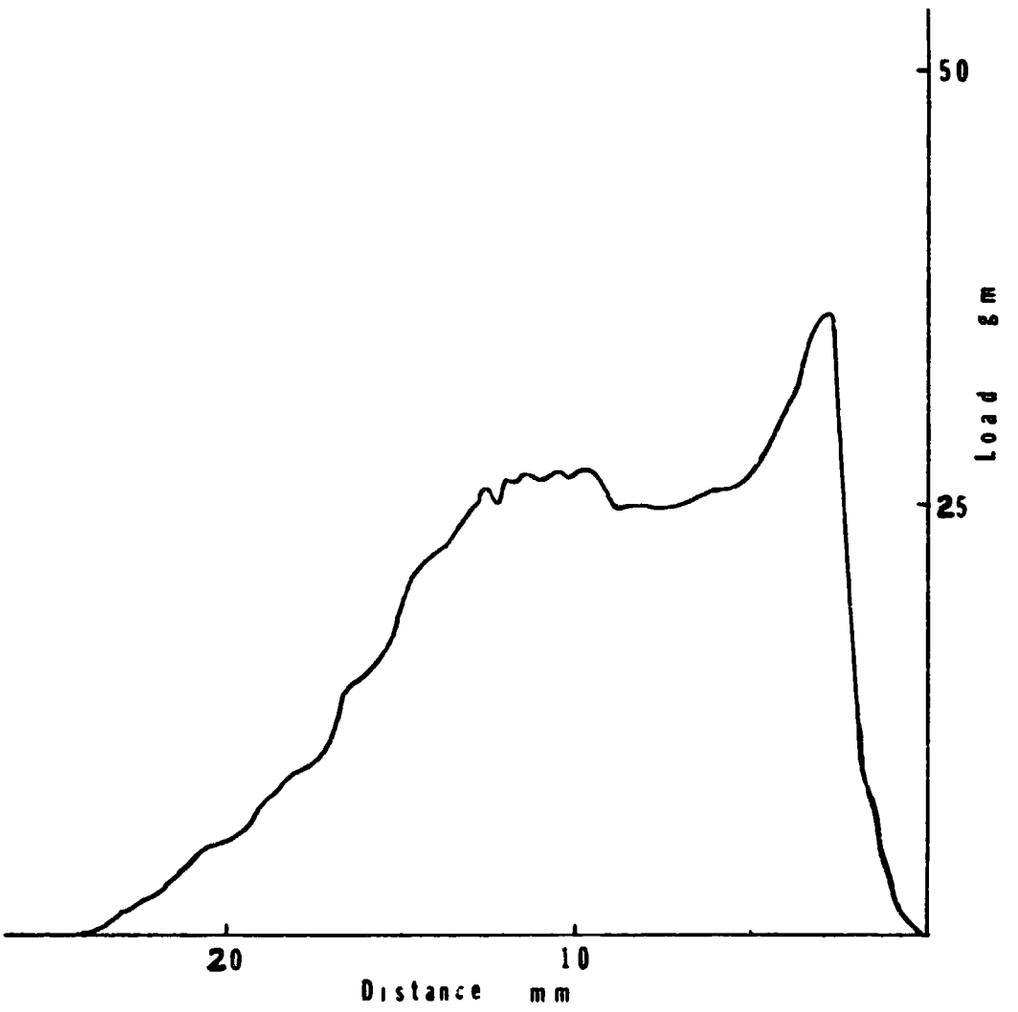



FIGURE 8