

Table 1 The  $a$  values and symmetrized force constants ( $10^2 \text{ Nm}^{-1}$ )

Molecule	$a$ values							
	$a(A_1)$	$a(E)$	$F_{11}(A_1)$	$F_{22}(A_1)$	$F_{12}(A_1)$	$F_{33}(E)$	$F_{44}(E)$	$F_{34}(E)$
NH <sub>3</sub>	-0.0700	0.0245	7.1147	0.2318	0.2092	6.9779	0.3328	-0.1146
ND <sub>3</sub>	-0.1300	-0.0094	7.0709	0.2660	0.2175	7.0559	0.3316	-0.1241
NT <sub>3</sub>	-0.0345	0.0007	7.2039	0.2407	0.1968	6.8154	0.3300	-0.1380

Table 2 Centrifugal distortion constants (MHz)

Molecule	$D_J$	$-D_{JK}$	$D_K$	Ref.
NH <sub>3</sub>	24.0210	43.9950	22.4321	PW
	24.2700	43.6500	—	9
	24.3100	45.2700	—	16
ND <sub>3</sub>	5.8322	10.8921	4.8212	PW
	5.8500	10.9800	—	9
	5.9100	10.4900	—	17
NF <sub>3</sub>	2.5802	4.4092	2.0481	PW
	2.5981	4.4720	—	18

to a very high degree of approximation", it is significant to note that the numerical values of the symmetrized force constants for the three isotopically substituted ammonia molecules are close to each other. Using the set of potential constants, derived here, the centrifugal distortion constants for these cases have been evaluated. In table 2, the evaluated values are compared with the observed values<sup>9,16-18</sup>. The close agreement between the calculated and observed values of centrifugal distortion constants brings out the validity of the method of parametric representation described in this note.

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1. Wilson, E. B., Decius, J. C. and Cross, P. C., *Molecular vibrations*, McGraw Hill, New York, 1955.
2. Cyvin, S. J., *Molecular vibrations and mean amplitudes*, Elsevier, Amsterdam, 1968.
3. Meal, J. H. and Polo, S. R., *J. Chem. Phys.*, 1956, **24**, 1119, 1126.
4. Jahn, H. A., *Phys. Rev.*, 1939, **56**, 680.
5. Bhoopathy, T. J., *Theoretical and experimental spectroscopic studies of some polyatomic molecules*, Ph.D. Thesis, Anna University, 1985.
6. Mohan, S., Bhoopathy, T. J. and Asha Selvaraj, *Proc. Indian Nat. Sci. Acad.*, 1986 (in press).

7. Mohan, S., Ravikumar, K. G., Bhoopathy, T. J. and Gunasekaran, S., *Acta Cien. Indica*, 1986 (in press).
8. Krupnov, A. F., Gershtein, L. I. and Shustrov, V. G., *Opt. Spectrosc. (U.S.A.)*, 1971, **30**, 427.
9. Benedict, W. S. and Plyler, E. K., *Can. J. Phys.*, 1957, **35**, 1235.
10. De Alti, G., Costa, G. and Galasso, V., *Spectrochim Acta*, 1964, **20**, 965.
11. Kozulin, A. T. and Chebykin, N. A., *Sov. Phys.*, 1972, **8**, 142.
12. Jache, A. W., Blevins, G. S. and Gordy, W., *Phys. Rev.*, 1955, **97**, 680.
13. Ponamarev, Yu. I. and Khovrin, G. V., *Opt. Spectrosc.*, 1971, **30**, 122.
14. Thirugnanasambandam, P. and Guanasekaran, S., *Bull. Des. Soc. Chim. Belges (Belgium)*, 1977, **86**, 11.
15. Herzberg, G., *Infrared and Raman spectra of polyatomic molecules*, Van Nostrand, New York, 1956, p.227.
16. Mould, H. M., Price, W. C. and Wilkinson, G. R., *Spectrochim. Acta*, 1959, **15**, 313.
17. Helminger, P., De Lucia, F. C. and Gordy, W., *J. Mol. Spectrosc.*, 1971, **39**, 94.
18. Helminger, P., De Lucia, F. C., Gordy, W., Morgan, H. W. and Staats, P. A., *Phys. Rev.*, 1974, **A9**, 12.

### LECTIN-IMMOBILIZED ALBUMINATED POLYETHER URETHANE UREA: TISSUE COMPATIBILITY

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It was known<sup>1</sup> that the cell growth is normal on albuminated substrates and was that lectins are

present<sup>2</sup> and associated with the membranes of various cells. An effort is therefore made in this study to understand the growth of the tissue (*in vivo*) onto lectin-immobilized albuminated polyether urethane urea (PEUU). Since the porosity also plays an important role<sup>3</sup> in tissue ingrowth and the stability of the implant, the results have been compared with bare PEUU having pore size  $\sim 70 \mu$ . It is expected that such efforts may throw some light on implant surface modification, requiring tissue compatibility and also in the development of artificial skin.

PEUU was synthesized in this laboratory<sup>3</sup>. Albumin (human, essentially fatty acid, fraction V, 96–99% pure, Sigma Co.) and lectin (from *Phaseolus vulgaris* (kidney bean) type V (phytohemagglutinin, Sigma Co.) were used to coat the PEUU material.

The samples were fabricated from 20% PEUU solution in dimethyl formamide on clean and dry glass rods. They were washed with distilled water and dried in a vacuum oven at 60°C. The clean grafts were divided into the following groups (size  $2 \times 2 \text{ cm}^2$ ).

- I. Cleaned bare samples.
- II. Cleaned samples immersed and shaken for about an hour in 0.1 M phosphate buffer (pH 7.4) and then albumin solution (pH 7.4) was added to make a final concentration of 100 mg%. After 3 hr the samples were removed and rinsed with distilled water, exposed to 2.5% glutaraldehyde for 1 hr, rinsed and then again exposed to 100 mg% albumin solution for 3 hr and rinsed with distilled water.
- III. After glutaraldehyde crosslinking (instead of exposing to albumin) they were immersed in 5 mg% lectin solution for 3 hr and then rinsed with distilled water.

Octane contact angle was taken using a goniometer under water before and after implantation. In brief, the samples are fixed on a microscopic slide and can be immersed inverted in a container containing double-distilled water. After the surface is fully hydrated, a hypodermic syringe containing pure *n*-octane can be lowered under the surface. A drop can be formed at the tip of the needle and allowed to touch the polymer/water interface. The angle can then be measured. Several readings on different parts of the surface are taken and averaged. Higher octane contact angle in the above system reflects the hydrophilicity of the surface.

**Tissue compatibility test:** Six young male Albino rats weighing between 80 and 100 g were selected. The

fur over their dorsal side was clipped closely and the skins were exposed for further surgical procedure. Rats were anaesthetized by intraperitoneal injection of Nembutal (sodium pentobarbital) 30 mg/kg.

For implantation of test materials (duplicated samples of three types of sterile clean polyether-urethane urea polymers of  $1 \times 1 \text{ cm}$  size strips – 6 Nos) into the subcutaneous space of these six rats, a 3 cm skin incision was made in each rat on the clipped dorsal area, parallel to the line over vertebral column (figure 1).

Subcutaneous pockets were made by blunt dissection to accommodate the test material. One strip of the test material was inserted securely into the subcutaneous space in each rat. After the insertion of test materials the skins were closed with interrupted surgical suture. Preparation of test samples and implantation procedures were performed under aseptic conditions. The animals were killed at the end of 23 days after implantation using overdose of Nembutal.

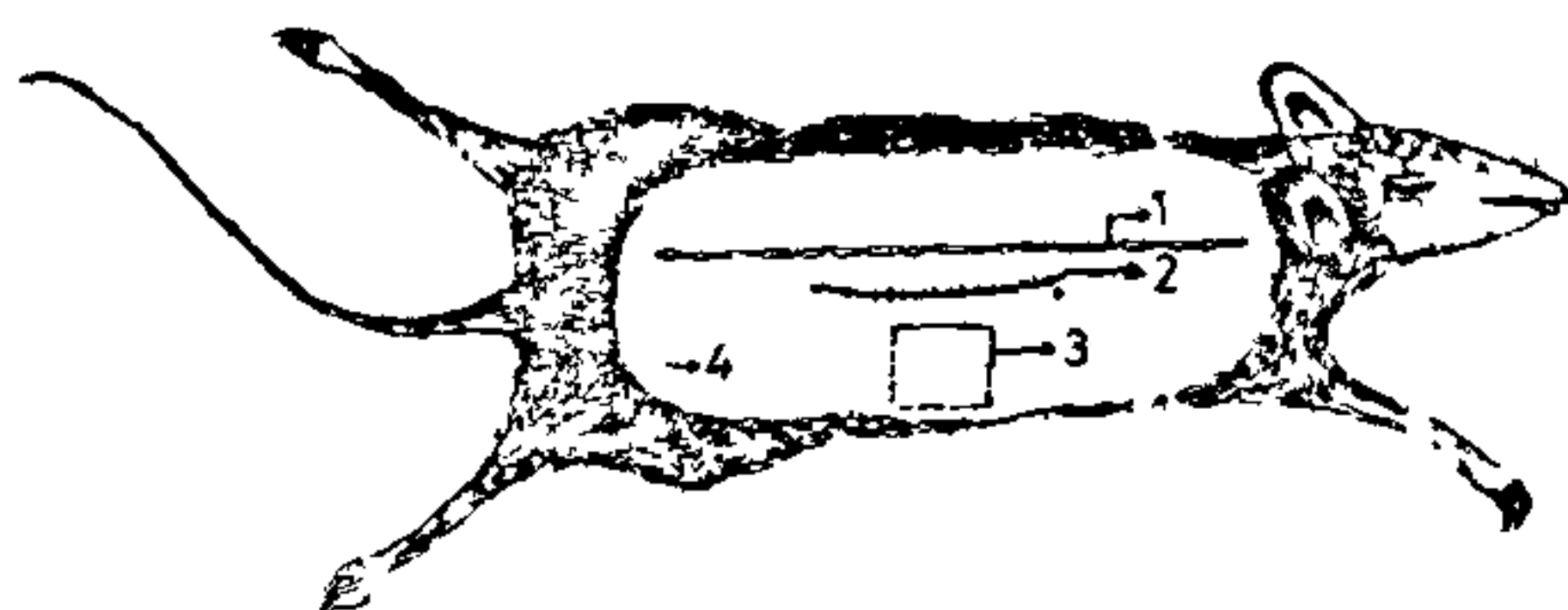
All the portions of the skin carrying subcutaneous implants were excised carefully and removed for biocompatibility assessment.

The parameters looked for the tissue material compatibility under subcutaneous 'milieu interieur' of the animals are:

(i) Formation of new layer (neointima) of tissue over the implanted material and establishment of vascular circulation (neovascularization) over and into the implanted material.

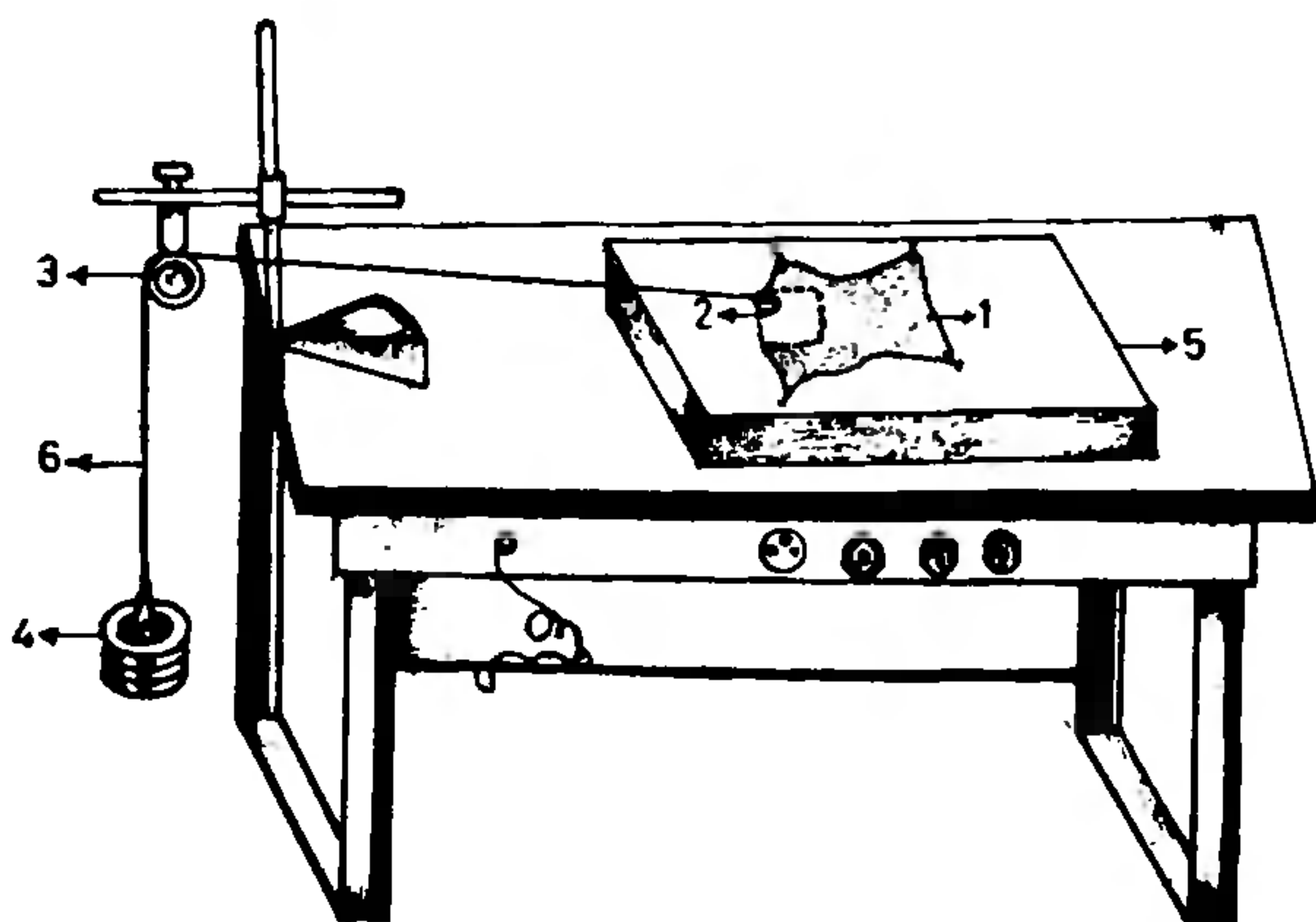
(ii) Tissue ingrowth (evaluation of pull strength) and bonding strength (pull strength) established between the material implanted and the tissue was tested by the experimental set-up (figure 2).

Tissue compatibility study revealed the presence of highly grown-up tissue (Neodermal tissue layer)



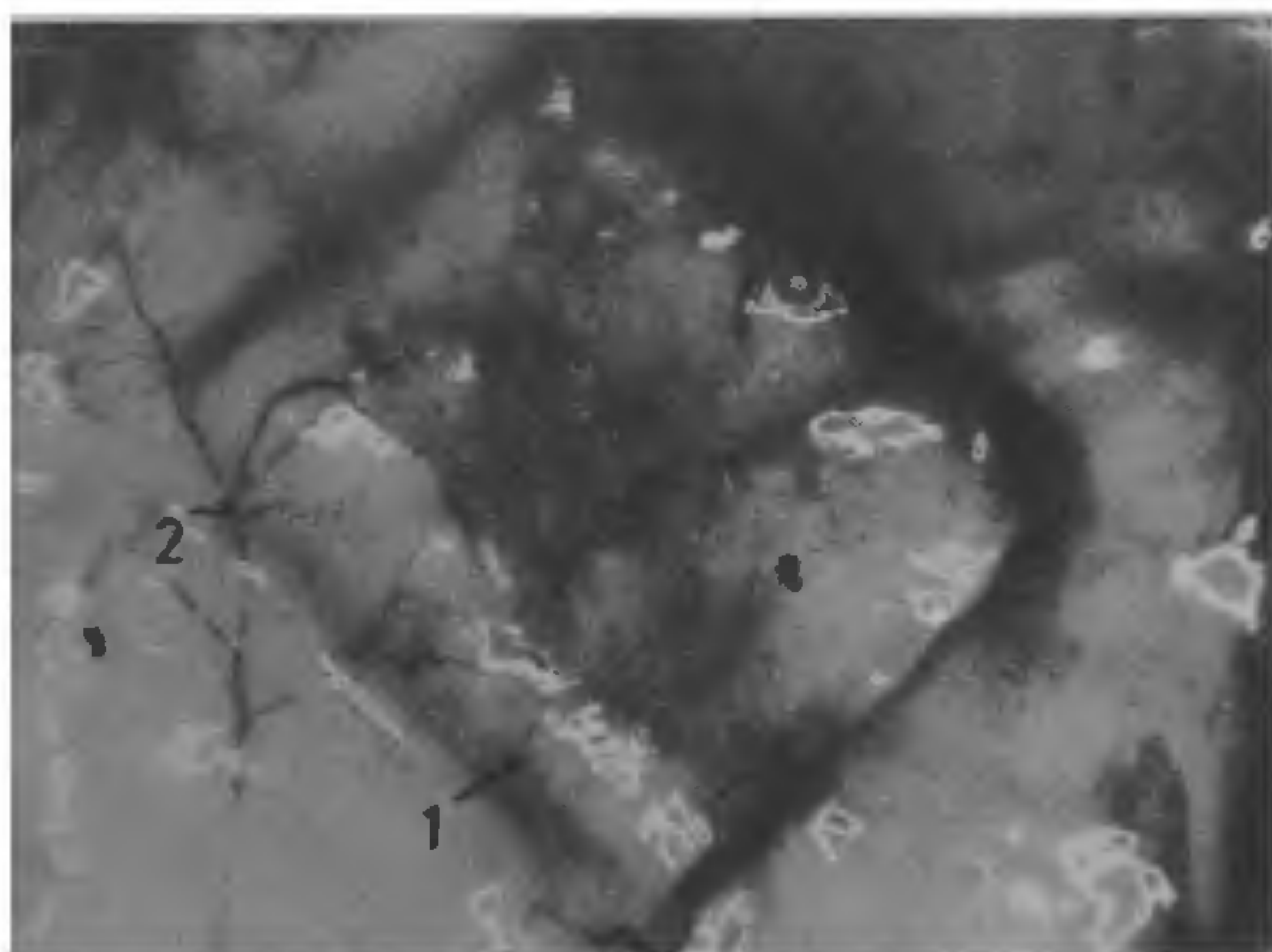
**Figure 1.** Anaesthetized and subcutaneously implanted rat: 1. Vertical column; 2. Incised and sutured skin; 3. Subcutaneously implanted material; 4. Clipped area.





**Figure 2.** Experimental set-up to test pull strength.

1. Excised skin with subcutaneous implant;  
2. Hook; 3. Pulley; 4. Aluminium pan;  
5. Wooden block; 6. String.



**Figure 3.** The growth of new layer of dermal intimal tissue (1); and formation of the new blood vessels (2).

over the implanted material and well-developed new blood vessel supply (Neo vascularization) over and into the material (figure 3). There was no evidence of infection, inflammation and necrosis in the tissues adjacent to implanted materials. Pull strength differences was seen between bare PEUU and albumination lectin-immobilized PEUU.

Lectin-immobilized surfaces seem to demonstrate advantage from the tissue linkage point of view, since octane contact angle is high indicating enhanced tissue adhering to the surface as compared to albuminated substrate. Octane contact angle (table 1) is high indicating hydrophilicity in the case of bare

**Table 1** Octane contact angle on substrates

Surface	Before implantation in degrees $\pm$ S.D	After implantation in degrees $\pm$ S.D
I Bare	111.60 $\pm$ 1.07	148.50 $\pm$ 1.29
II Albuminated	140.17 $\pm$ 2.08	137.50 $\pm$ 1.93
III Lectinated	131.00 $\pm$ 1.31	142.58 $\pm$ 4.10

**Table 2** Pull strength for subcutaneously implanted materials

Type of material	Pull strength* (g)
Bare PEUU	35.8
Albumin coated	24.2
Lectin plus albumin coated	24.0

\* The amount of weight (g) required to pull the implanted material out of subcutaneous tissue.

substrates also. Obviously, it is due to the ingrowth of tissue into the pores. Our observations demonstrate the importance of pores as far as the stability of the implant is concerned, e.g. pull strength (table 2) in the case of the bare is quite high compared to albuminated and lectinated surfaces; however as far as the biological interface with normal tissue bonding is concerned, lectin-immobilized albuminated surfaces may be preferable. We believe that such surfaces with optimum unfilled pores may be good for further studies now in progress.

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1. Copper, S. L., (ed.) *Biomaterials: Interfacial phenomena and application*, ACS Publications, Washington, 1982.
2. Monsigny, M., Kieda, C. and Roche, A. C., *Membrane Lectins Biol. Cellulaire*, 1979, **35**, 289.
3. Boretos, J. W., *Cell. Polymers*, 1984, **3**, 345.
4. Sharma, C. P., *Curr. Sci.*, 1983, **52**, 1057.