Journal of Macromolecular Science, Part A
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597274

Nonthrombogenic Surfacing of Biological Membranes—A Review

Donald G. Stoffey[a]; Henry Lee[a]; William Stone Jr.[b]
[a] The Epoxylite Corporation, South El Monte, California [b] National Institute for Scientific Research, Los Angeles, California

To cite this Article Stoffey, Donald G. , Lee, Henry and Stone Jr., William(1969) 'Nonthrombogenic Surfacing of Biological Membranes—A Review', Journal of Macromolecular Science, Part A, 3: 1, 119 — 131
To link to this Article: DOI: 10.1080/10601326908053797
URL: http://dx.doi.org/10.1080/10601326908053797

J. MACROMOL. SCI.—CHEM., A3(1), pp. 119-131, Jan., 1969

Nonthrombogenic Surfacing of Biological Membranes—A Review*

DONALD G. STOFFEY and HENRY LEE

The Epoxylite Corporation
South El Monte, California

WILLIAM STONE, Jr.

National Institute for Scientific Research
Los Angeles, California

INTRODUCTION

One of the major problems present in devices used as blood vessel substitutes, cardiac assist devices, and heart-lung and hemodialysis systems is that of thrombosis which results from blood coagulation. This paper is a review of the pertinent experimental work which has been done to minimize this problem.

VASCULAR ARCHITECTURE

Arteries, veins, and the heart are not simple, solid, tube-like vessels conducting blood from one part of the body to another. Sawyer et al. [44] (see Fig. 1) have presented a hypothetical picture of the physical and chemical complexity of the arterial wall which indicates the many activities involving differential permeability, transport of ions, enzyme activity, etc., which are in constant use. The intimal surface is partly composed of mucopolysaccharides which possess sulfate and carboxylate groups. These anionic groups give the intima a net charge of −3 to −13 mV [50]. It is felt that this negative surface repels platelets, erythrocytes, and other blood components, which are also negatively charged.

---

*This paper was presented at a Symposium on Synthetic and Natural Polymer Membranes in Medicine at the Pacific Conference on Chemistry and Spectroscopy, October 1967, sponsored by the American Chemical Society and the Society of Applied Spectroscopy.

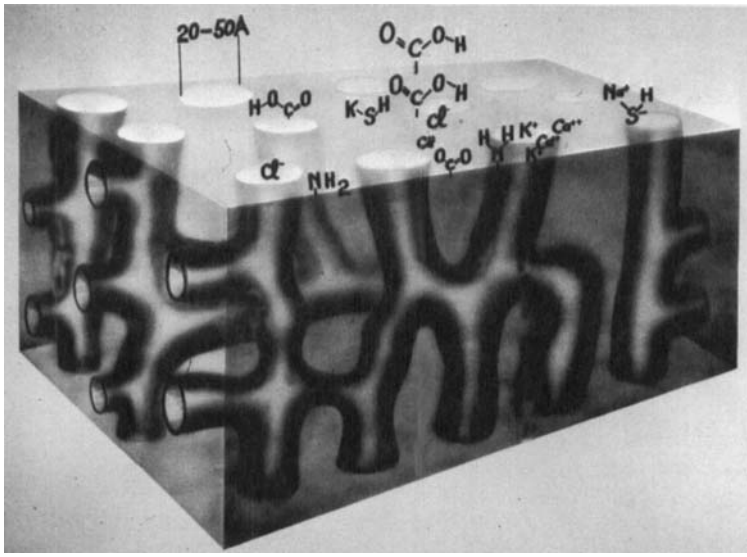


Fig. 1. Illustration of blood vessel wall. After Sawyer et al. [44].

Sawyer et al. [44] suggested that a good blood vessel wall interface must be porous, selectively permeable, negatively charged, semiconductive, and capable of reversibly absorbing and exchanging positively charged ions. However, at the present state of the art, it is not possible to reproduce all the above properties in one prosthetic material.

HISTORY

In 1885, Freund [13] reported that coating glass with vaseline extended the coagulation time of blood, Bordet and Gengou [2] found that paraffin had a similar effect. Early studies of other surfaces led to the belief that coagulation time was a function of the nonwettability of the surface. Materials which are more easily wetted than paraffin, however, such as poly(methyl methacrylate) and collodion, have longer coagulation times [5, 32]. It is no longer believed that coagulation time is a function of nonwettable surfaces, especially since the intima of natural vessels is highly wettable [40].

The relationship of the zeta potential, as calculated from the streaming potential, to clot formation has been studied by Leininger and co-workers [1, 18, 26, 28, 29]. The streaming potential is the voltage measured between the ends of a capillary while fluid is flowing through the capillary. The relationship between the streaming potential, S, and the zeta potential, Z, is shown in the following form of the Helmholtz equation:

$$Z = 4\pi\eta S/RDP$$

where R is the resistivity of fluid, esu; D, dielectric constant of fluid; P, pressure drop across capillary, dyne per square centimeters; η, viscosity of fluid, poise.

More recent results indicate that there is little correlation between zeta potentials and thrombi formation [23]. The zeta potential for most materials falls to zero when blood plasma or blood proteins are used in the test fluid [29]. These proteins are adsorbed on the surface, and the new surface formed by the proteins has very low zeta potential. However, zeta potentials are nonetheless useful for measuring the adsorption of protein on surfaces.

The effect of surface texture on clotting has involved many investigators. At first it was believed that a smooth surface would be clot free. In practice, smooth implants are more successful at some locations; at other locations rough implants are better [26]. In general, rough surfaces, having more area in contact with blood, cause local turbulence and stagnation. However, the new intimal tissue adheres better to the rough surface, thereby reducing the possibility of embolism caused by sloughed off pseudoendothelial cells.

WOVEN PROSTHESES

The ability to form a firm neointima has been used successfully to establish long-term prosthetic implants which are free from harmful thrombi. The prostheses are made of a mesh of Teflon, Dacron, or another substance. Platelets are rapidly deposited in and over the mesh. Fibrin is then formed and then replaced by connective tissue or pseudoendothelium. This neointima lines the prosthesis and inhibits further clot formation. Arterial replacements of woven Dacron are in routine use. Fibrin and fibroblastic tissue fill the pores of the fabric, thereby effectively stopping hemorrhaging. However, the neointima growths plug vessels under 5 mm in diameter [54]. Velour surfaces of Teflon, Dacron, and other materials also have been used to induce growth to strongly adhering neointima to the surfaces of prostheses. This technique has been quite successful; however, care has to be taken in the design of the prostheses since neointima has been known to grow a projection from the velour base of heart valve replacements through the valve seat, causing regurgitation [38]. Nevertheless, woven and velour-covered prostheses are very useful as long-term implants which remain free of thrombi.

THROMBOSIS FORMATION

The exact mechanism for blood clotting is not completely understood as yet. Davie and Ratnoff [4] offer a mechanism (see Fig. 2)

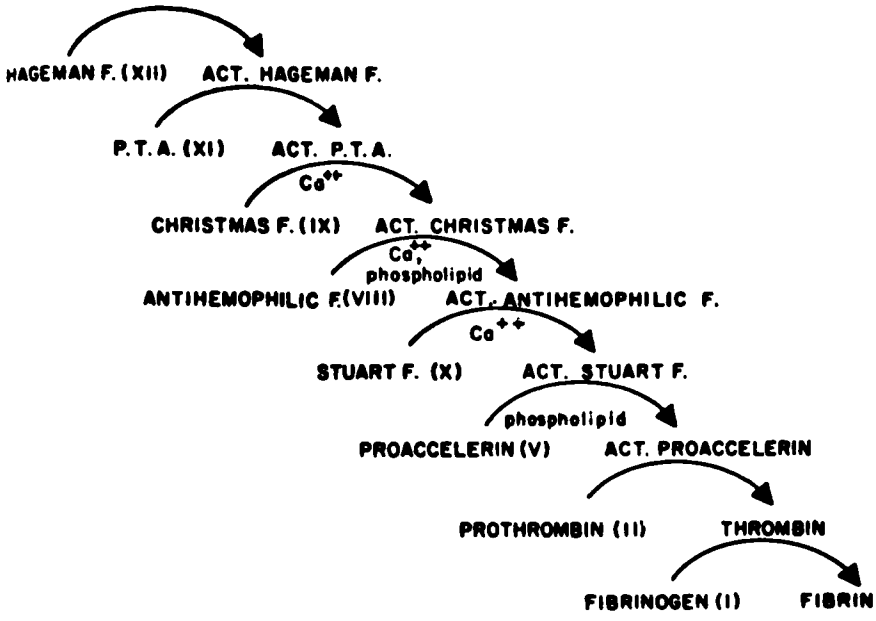


Fig. 2. Sequence for intrinsic blood clotting. After Davie and Ratnoff [4].

for intrinsic clotting which is consistent with most of the current available information. In this system, the Hageman factor, a plasma protein, is activated in some manner and the rest of this so-called "waterfall" sequence follows to ultimately produce fibrin. It is known that the Hageman factor is one of the plasma proteins adsorbed on foreign surfaces. The surface free energy could cause its activation. However, Falb et al. [9, 10] have shown that heparinized nonthrombogenic surfaces adsorb more Hagemen factor than unheparinized surfaces. Unless the surface free energy of heparinized surfaces is much lower than that of unheparinized materials, something more than adsorption and activation of Hageman factor is needed to start coagulation.

EFFECT OF SURFACE FREE ENERGY

The effect of the surface free energy of implants on clotting is being studied by Lyman et al. [34]. He feels that this energy is available to initiate coagulation, e.g., by furnishing the energy necessary

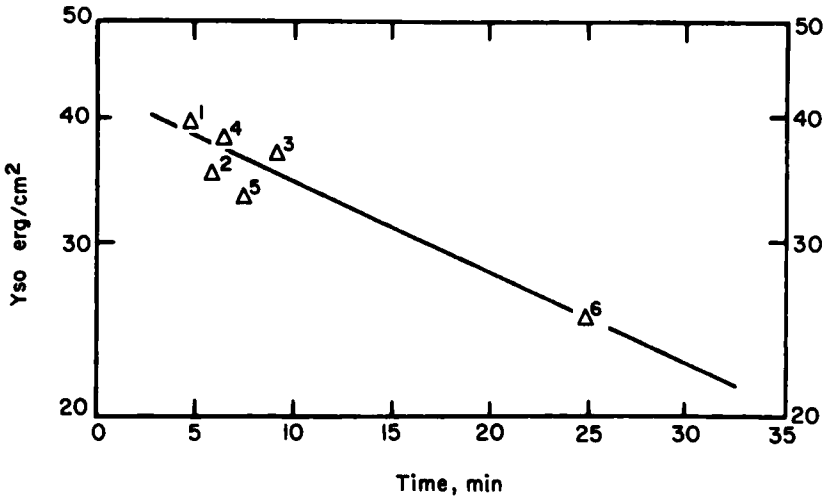


Fig. 3. (1) Poly(hexamethylene adipamide); (2) poly(vinyl chloride); (3) poly(methyl methacrylate); (4) poly(trifluorochlorethylene); (5) polyethylene; (6) paraffin.

to break hydrogen or other bonds in plasma proteins and thereby exposing active sites which could start the process of clot formation.

When the log of the surface free energy is plotted against the coagulation times for several surfaces, a linear relationship is shown [34] (see Fig. 3). This plot indicates that materials with a very low surface free energy should have a very long coagulation time. Great care would have to be taken with such materials in order that no flaw or sharp edge be present that would give anomalous locally high values to the free energy.

EFFECT OF ELECTRICAL CHARGE

It has been known for some time that the inner vascular wall is negative with respect to the outer surface. Also, applying an electrical charge to the vascular wall produces a clot at the positive electrode and inhibits clot formation at the negative electrode [52]. Further, it has been demonstrated that injury of the vessel wall reverses the sign of the charge, making it highly positive [52]. Active metals such as aluminum and magnesium, which supply electrons by oxidation, cause less clotting than do more inert metals such as platinum or copper [51]. However, active metals are not useful for implants since they dissolve in the blood, and they also develop harmful interfacial deposits.

From the foregoing observation, it was felt that inert plastic materials with a negative charge on their surface would have reduced clotting tendencies. Sulfonated styrene was tested in hopes that polyanionic materials would be antithrombic [36]; however, the coagulation time reported for this material is about the same as that of unsulfonated polystyrene and about half of that of siliconized glass.

Sharp postulated that implants of conductive plastics would become negatively charged by being in contact with intima [52]. He was able to prepare a conductive rubber by adding acetylene black to produce a material with a resistivity of "180 ohms per square." Not only did the elasticity of this material match that of normal arteries, implants of this material remained patent when implanted in the abdominal aorta for more than 50 days in eight out of 10 of the animals tested, but the average time of clotting was 24 min when these conductive rubbers were tested in the carotid artery of dogs. In another experiment, Sharp [53] implanted a latex graft with conductive rubber lining in the carotid artery. A $33\frac{1}{2}$ V battery was connected to the implant, and 1.0-1.3 and 3.0-3.5 mA of current were passed through the graft. With the higher current, all grafts remained patent for over 24 hr, three out of six for 6 days or more, and one remained patent for 12 days. However, medial necrosis was manifest in the artery next to the graft. Further study demonstrated that continuous direct current when applied to healthy tissue resulted in total destruction of the tissue [53].

However, conductivity in itself is not sufficient, since metals are conductive and, in general, thrombogenic. Also, the proteins which adsorb on all plastics, whether hydrophobic or hydrophilic, form a conductive surface [26], but nonetheless these surfaces are thrombogenic.

BIOELECTRIC POLYURETHANES

More successful implants were obtained by Sharp using a "bioelectric" polyurethane [53]. These polyester, polyether, diamine-cured polyurethanes have a naturally occurring negative static potential. By incorporating 10 parts of carbon black, the potential is increased to 200-300 mV. Implants of these materials in the carotid artery were patent for at least 3 days, three of the 15 grafts lasted 10 days, and one lasted 12 days. Occlusion of the grafts was attributed to detachment of the neointima.

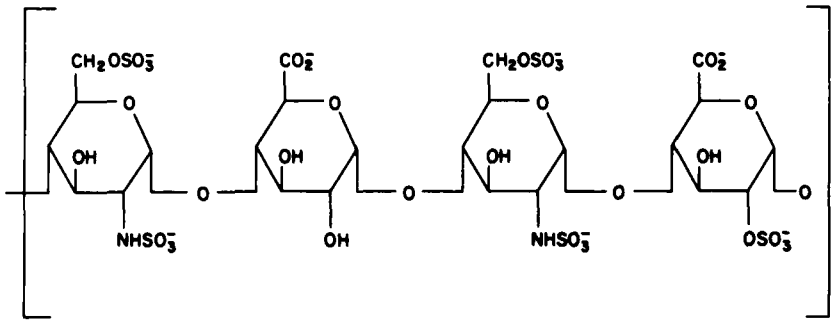
The "bioelectric" polyurethane can be prepared with various degrees of stiffness, from flexible rubbers to rigid materials. Short-term studies in rats showed no significant toxicity. The polyurethanes seem to be promising antithrombogenic materials.

INDUCED NEGATIVE CHARGE

A related type of surface was prepared by Sawyer et al. [48] and by Murphy et al. [37]. Negative charges were imposed on semimolten Teflon with an electrical field of approximately 30, 000 V/cm. While still in the field, the tubes were cooled. This technique produced permanent electrical fields on the inner surface. The tubes were implanted in the thoracic aorta and carotid artery of dogs. Only the first tube of the series thrombosed. The others remained patent. An electrically treated Dacron graft was also implanted, but this dog died 5 days postoperatively. No clots were found in the graft, but the graft never stopped oozing. About 1 liter of blood and clots were found in the dog's chest cavity.

GRAPHITE-BENZALKONIUM-HEPARIN-ADSORBED SURFACES

Gott and co-workers [17] first reported the coating of plastic materials with heparin in 1963. (See Fig. 4 for the structure of heparin.) A coating of colloidal graphite is applied to the surface. The graphite is saturated with a quaternary ammonium surface-active agent, benzalkonium chloride, and then with a heparin solution. The in vitro coagulation time of these surfaces, called GBH, is greater than 600 min. Under the same conditions, glass and siliconized surfaces coagulate blood in 7 and 17 min, respectively. Lexan polycarbonate rings prepared with GBH [60, 62] surfaces, when implanted in the pulmonary artery of dogs, remain patent for more than $2\frac{1}{2}$ years. It is interesting to note that the charge across the GBH surfaces is −10 to −16 mV [15]. This range is very close to the −3 to −13 mV reported for intima [50]. Whiffen and Beechler [56]



HEPARIN

Fig. 4.

used radioactive heparin to study the stability of GBH surfaces. He found that 65% of the heparin was removed by blood in 3 hr, 77% in 24 hr, but from 1 week to 3 months, 14-24% of the original heparin was left on the rings.

Sawyer and co-workers [22, 47] have demonstrated that natural blood vessel walls have $6\text{-}12 \times 10^{12}$ negative charges per square centimeter of surface. In the GBH surfaces, many of the negative charges of heparin are tied to the benzalkonium layer. Even taking this effect into account, Whiffen and Beechler [56] calculated that there were still 28.5×10^{14} free negative charges per square centimeter even after elution of the surface by blood. It was concluded that the GBH surfaces have charge densities comparable to normal vascular endothelium.

The above data are based on the retained radioactive heparin and should only be considered the minimum value since it is known that benzalkonium-treated graphite surfaces adsorb heparin from the blood system of dogs [61]. In fact, some early conclusions of the effectiveness of colloidal graphite in preventing clotting were misleading since the graphite coatings were sterilized with quaternary ammonium compounds [6]. When implanted, these devices complexed with natural heparin in the blood, thereby producing an antithrombic surface in vivo [61].

Heparin, a polyanion, is a very strong acid. In vivo, heparin is ionized. For every four sugar units in heparin, it is believed that there are three sulfate, two sulfamate, and two carboxylic groups. The molecular weight of heparin is 12,000-20,000. Considering the types of groups present, each ion pair in heparin should be dissociated and soluble, but the collective effect of the large number of ion pairs that would be formed with benzalkoniumized graphite would keep heparin strongly attached to the surface.

HEPARIN PAINTS

Fourt et al. [12] produced a water-insoluble complex of heparin with quaternary ammonium compounds which contained long alkyl chains. Antithrombogenic films of this complex can be applied to surfaces either pure or suspended in collodion. These workers also prepared poly(4-vinyl pyridine) paint films on which heparin was adsorbed. They studied both systems in comparison with the GBH surfaces and found all three systems effective in preventing clots in vitro.

Fourt et al. [11] also made some quantitative studies of several heparinized coatings and found that as little as 0.5 mg/cm^2 of heparin gave in vitro coagulation times greater than 100 min. On the other hand, they found that there was a marked nonuniformity in the concentration of the heparin layers studied. The ratio between

high and low concentrations of 4-cm^2 areas was 30:1. Greater uniformity was produced if electrolytes, such as sodium chloride, were used as leveling agents. The absolute amounts of heparin adsorbed were lower, but more effective surfaces were prepared.

CHEMICALLY BOUND QUATERNARY AMMONIUM SURFACES

Leininger and co-workers [7-10, 26-28] have reported the preparation of over 14 different plastic materials with heparinized surfaces. A quaternary ammonium iodide layer is chemically bonded to the base materials by one of several methods. Heparin is then complexed with the polycation layer. The best bonding of heparin is obtained when the quaternary ammonium groups are prepared from N, N-dimethylaniline. The heparin complex in this was less easily eluted than in the GBH surface. After 100 hr in 0.14 N sodium chloride solution, 69% of the heparin was removed from the GBH surface while less than 10% was removed from the surfaces prepared by Leininger's method [9]. Leininger's method allows the attachment of heparin to nonrigid materials. With the GBH-adsorbed surfaces, flexing or turbulence causes removal of graphite coating.

Salzman et al. [35, 42] have heparinized cellulose and cellulose acetate membranes by treating these materials with ethyleneimine and then heparin. The heparinized membranes dialyze urea as effectively as the unheparinized membranes do. These membranes also demonstrate antithrombogenic properties in both in vitro and in vivo testing.

CHEMICALLY BONDED HEPARIN

Recently, Halpern and Shibakawa [20] have reported forming a covalently bonded heparin layer on polystyrene. Isocyanate groups are placed on polystyrene by nitration, and then the nitro groups are reduced to amines. The amino groups are transformed into isocyanate groups by the action of phosgene, and these isocyanate groups react with the free hydroxyl groups of heparin. Heparin bonded in this way still maintains its antithrombic activity.

SUMMARY

At the present state of the art, two general methods of preparing antithrombogenic membranes seem possible. However, very little work on such membranes has been reported. Also, there is a disagreement about the action of heparin on these surfaces. Some feel that heparin is just a special case of a surface with negative charges,

but sulfonated polystyrene did not give a useful antithrombogenic material [36]. It would be expected that the sulfonate groups would have negative charges that are equally as effective as heparin in repelling the negatively charged blood components. Other investigators feel that heparin has a unique chemical function in preventing the blood from clotting.

There are also conflicts in the literature on the relative effectiveness of heparinized and charged surfaces in preventing clots in vivo. It would be highly useful to prepare prostheses with the same shapes, using the different methods, and implanting them in the same locations in dogs to give a truly accurate comparison.

REFERENCES

[1] R. E. Beck, V. Mirkovitch, P. G. Andrus, and R. I. Leininger, *J. Appl. Physiol.*, **18**, 1263 (1963).
[2] J. Bordet and O. Gengou, *Ann. Inst. Pasteur*, **17**, 822 (1903).
[3] N. S. Braunwald, J. C. A. Fuchs, and L. I. Bonchek, *Trans. Am. Soc. Artificial Internal Organs*, **13**, 111 (1967).
[4] E. W. Davie and D. Ratnoff, *Science*, **145**, 1310 (1964).
[5] T. J. Donovan and B. Zimmermann, *Blood*, **4**, 1310 (1949).
[6] Edmark, discussion after paper, *Trans. Am. Soc. Artificial Internal Organs*, **10**, 225 (1964).
[7] R. D. Falb, G. A. Grode, M. T. Takahashi, and R. I. Leininger, "Characteristics of Heparinized Surfaces," *Org. Coatings Plastics Preprints, ACS Meeting, Sept. 1967*, p. 123.
[8] R. D. Falb, G. A. Grode, and R. I. Leininger, *Rubber Chem. Technol.*, **39**, 1288 (1966).
[9] R. D. Falb, M. T. Takahashi, G. A. Grode, and R. I. Leininger, *J. Biomed. Mater. Res.*, **1**, 239 (1967).
[10] R. D. Falb, G. A. Grode, M. T. Takahashi, and R. I. Leininger, *Development of Blood-Compatible Polymeric Materials*, U.S. Govt. Rep. PB 175668, March 1967.
[11] L. Fourt, A. M. Schwartz, R. H. Becker, and E. O. Kairinen, *Org. Coating Plastic Preprints, ACS Meeting, Sept. 1967*, p. 127.
[12] L. Fourt, A. M. Schwartz, A. Quasius, and R. L. Bowman, *Trans. Am. Soc. Artificial Internal Organs*, **12**, 155 (1966).
[13] E. Freund, *Med. Jahrb. Wien*, **3**, 259 (1885).
[14] I. Gore and B. J. Larkey, *J. Lab. Clin. Med.*, **56**, 839 (1960).
[15] V. L. Gott, *J. Surg. Res.*, **6**, 274 (1966).
[16] V. L. Gott, D. E. Koepke, R. L. Daggett, W. Zarnstorff, and W. P. Young, *Surgery*, **50**, 382 (1961).
[17] V. L. Gott, J. D. Whiffen, and R. C. Dutton, *Science*, **142**, 1297 (1963).
[18] V. L. Gott, J. D. Whiffen, R. C. Dutton, R. I. Leininger, and W. P. Young, "Biophysical Studies on Various GBH Surfaces," in

Biophysical Mechanisms in Vascular Homeostasis and Intravascular Thrombosis (P. N. Sawyer, ed.), Appleton-Century-Crofts, New York, 1965, p. 297.

[19] V. L. Gott, J. D. Whiffen, D. E. Koepke, R. L. Daggett, W. C. Boake, and W. P. Young, *Trans. Am. Soc. Artificial Internal Organs*, **10**, 213 (1964).

[20] B. D. Halpern and R. Shibakawa, *Org. Coatings Plastics Preprints, ACS Meeting, Sept. 1967*, p. 132.

[21] D. Harshaw, H. Hiskind, S. Wesolowski, and P. N. Sawyer, *Trans. Am. Soc. Artificial Internal Organs*, **9**, 317 (1963).

[22] D. H. Harshaw and P. N. Sawyer, "Electro-osmotic Characteristics of Mammalian Aorta and Vena Cava," in *Biophysical Mechanisms in Vascular Homeostasis and Intravascular Thrombosis* (P. N. Sawyer, ed.), Appleton-Century-Crofts, 1965, p. 61.

[23] F. E. Horan, F. G. Hirsch, L. A. Wood, and I. S. Wright, *J. Clin. Invest.*, 29, 202 (1950).

[24] D. Hubbard and G. L. Lucas, *J. Appl. Physiol.*, **15**, 265 (1959).

[25] R. A. Iadeglia, F. D. Dorman, A. R. Castaneda, R. L. Varco, and E. F. Bernstein, *Trans. Am. Soc. Artificial Internal Organs*, 12, 166 (1966).

[26] R. I. Leininger, "Surface Effects in Blood-Plastic Compatibility," in *Biophysical Mechanisms in Vascular Homeostasis and Intravascular Thrombosis* (P. N. Sawyer, ed.), Appleton-Century-Crofts, New York, 1965, p. 288.

[27] R. I. Leininger, C. W. Cooper, R. D. Falb, and G. A. Grode, *Science*, **152**, 1625 (1966).

[28] R. I. Leininger, M. M. Epstein, R. D. Falb, and G. A. Grode, *Trans. Am. Soc. Artificial Internal Organs*, **12**, 151 (1966).

[29] R. I. Leininger, V. Mirkovitch, R. E. Beck, P. G. Andrus, and W. J. Kolff, *Trans. Am. Soc. Artificial Internal Organs*, **10**, 237 (1964).

[30] R. I. Leininger, V. Mirkovitch, A. Peters, and W. A. Hawks, *Trans. Am. Soc. Artificial Internal Organs*, **10**, 320 (1964).

[31] J. E. Lovelock and J. S. Porterfield, *Nature*, **4326**, 39 (1951).

[32] E. L. Lozner and F. H. L. Taylor, *J. Clin. Invest.*, **21**, 241 (1942).

[33] D. J. Lyman, *Rev. Macromol. Chem.*, **1**, 355 (1966).

[34] D. J. Lyman, W. M. Muir, and I. J. Lee, *Trans. Am. Soc. Artificial Internal Organs*, 11, 301 (1965).

[35] E. W. Merrill, E. W. Salzman, B. J. Lipps, E. R. Gilliland, W. G. Austin, and J. Joison, "Antithrombogenic Cellulose Membranes for Blood Dialysis," *Trans. Am. Soc. Artificial Internal Organs*, 12, 139 (1966).

[36] V. Mirkovitch, R. E. Beck, P. G. Andrus, and R. I. Leininger, *J. Surg. Res.*, 4, 395 (1964).

[37] P. Murphy, F. Holly, L. van Someren, M. Bankole, S. Singh, and W. F. Bernhard, *Trans. Am. Soc. Artificial Internal Organs*, 13, 131, (1967).

[38] Nelson, discussion after paper, *Trans. Am. Soc. Artificial Internal Organs*, **13**, 115 (1967).
[39] J. C. Rose and H. P. Broida, *Proc. Soc. Exptl. Biol. Med.*, **86**, 384 (1954).
[40] J. Ross, Jr., L. J. Greenfield, R. L. Bowman, and A. G. Morrow, "The Chemical Structure and Surface Properties of Certain Synthetic Polymers and Their Relationship to Thrombosis," *Prosthetic Valves for Cardiac Surgery* (K. A. Meredino, ed.), Charles C. Thomas, Springfield, Ill., (1961), pp. 212-23.
[41] J. R. Sadd, D. E. Koepke, R. L. Daggett, W. C. Zarnstorff, W. P. Young, and V. L. Gott, *Surg. Forum*, **12**, 252 (1961).
[42] E. W. Salzman, W. G. Asisten, B. J. Lipps, E. W. Merrill, E. R. Gilliland, and J. Joison, *Surgery*, **61**, (1967).
[43] P. N. Sawyer, W. H. Brattain, and P. J. Baddy, *Proc. Natl. Acad. Sci. U.S.*, **51**, 428 (1964).
[44] P. N. Sawyer, C. Burrowes, J. Ogoniak, A. O. Smith, and S. A. Wesolowski, *Trans. Am. Soc. Artificial Internal Organs*, **10**, 316 (1964).
[45] P. N. Sawyer and B. Deutch, *Am. J. Physiol.*, **187**, 473 (1956).
[46] P. N. Sawyer and D. H. Harshaw, *Surgery*, **56**, 846 (1964).
[47] P. N. Sawyer and E. Himmelfarb, "Studies of Streaming Potential in Large Mammalian Blood Vessels In Vivo," in *Biophysical Mechanisms in Vascular Homeostasis and Intravascular Thrombosis* (P. N. Sawyer, ed.), Appleton-Century-Crofts, New York, 1965, p. 69.
[48] P. N. Sawyer, M. Kaplitt, A. Afshar, M. Miller, and R. H. Postal, *Trans. Am. Soc. Artificial Internal Organs*, **12**, 183 (1966).
[49] P. N. Sawyer and J. W. Pate, *Am. J. Physiol.*, **175**, 103 (1953).
[50] P. N. Sawyer, J. W. Pate, and C. S. Weldon, *Am. J. Physiol.*, **175**, 108 (1953).
[51] P. N. Sawyer, K. T. Wu, S. A. Wesalowski, W. H. Brattain, and P. J. Boddy, *Arch. Surg.*, **91**, 735 (1965).
[52] W. V. Sharp, *Trans. Am. Soc. Artificial Internal Organs*, **10**, 223 (1964).
[53] W. V. Sharp, *Surgery*, **61**, 763 (1967).
[54] W. V. Sharp, D. L. Gardner, and G. J. Anderson, *Trans. Am. Soc. Artificial Internal Organs*, **12**, 179 (1966).
[55] C. W. Walter, W. P. Murphy, A. G. Jessiman, and R. M. Ahara, *Surg. Forum*, **2**, 289 (1951).
[56] J. D. Whiffen and D. C. Beechler, *J. Thoracic Cardiovascular Surg.*, **52**, 121 (1966).
[57] J. D. Whiffen, R. Dutton, W. P. Young, and V. L. Gott, *Surgery*, **56**, 404 (1964).
[58] J. D. Whiffen and V. L. Gott, *J. Thoracic Cardiovascular Surg.*, **50**, 31 (1965).
[59] J. D. Whiffen and V. L. Gott, *J. Surg. Res.*, **5**, 51 (1965).
[60] J. D. Whiffen and V. L. Gott, *Proc. Soc. Exptl. Biol. Med.*, **116**, 314 (1964).

[61] J. D. Whiffen and V. L. Gott, *Surg., Gynecol. Obstet.*, **121**, 287 (1965).
[62] J. D. Whiffen, W. D. Young, and V. L. Gott, *J. Thoracic Cardiovascular Surg.*, **48**, 317 (1964).

Accepted by editor October 30, 1967
Received for publication August 29, 1968