ELECTROOSMOTIC CHARACTERISTICS OF CANINE AORTA AND VENA CAVA WALL

PHILIP N. SAWYER *and* DAVID H. HARSHAW

From the Vascular Surgical Services, Departments of Surgery and Surgical Research, State University of New York, Downstate Medical Center, Brooklyn

ABSTRACT Experiments in which canine aorta and vena cava walls are subjected to electroosmosis in an open system at constant pressure are described. Electroosmosis reveals that the blood vessel walls studied have a negative zeta potential. The calculated zeta potentials are different for aorta and vena cava, -9.0 ± 5.0 mv compared with -4.7 ± 1.2 mv, respectively, and again of different magnitude with different bathing solutions. The calculated membrane pore charge per centimeter of effective pore surface in statcoulombs is approximately 6.2×10^8 for aorta compared with 3.5×10^8 for vena cava. The implications of the negative electroosmotic zeta potential in terms of the surrounding electric double layer, ion transport, and thrombosis are briefly discussed.

INTRODUCTION

Electroosmosis of blood vessel wall was carried out to determine whether or not blood vessel wall, cells, and fibers have a measurable and calculable surface charge. The importance of these phenomena was first suggested by Abramson in 1927 when he measured the electrophoretic characteristics of erythrocytes, leukocytes, and platelets, and effectively demonstrated their negative charge. He stressed the significance of electrical factors in the migration of leukocytes to an area of inflammation and tissue necrosis and suggested the importance of cell charge in vascular homeostasis (1, 2).

Additional experiments carried out by members of our laboratory from 1951 to 1956 indicated the role of oriented electrical currents in the production and prevention of intravascular thrombosis (3, 4, 5). That currents of the correct sign and order of magnitude of physiologic injury currents would also produce vascular thrombosis (6, 7) was subsequently demonstrated. It was suggested, therefore, that an electric double layer which yields streaming potentials during blood flow could account for the charge of blood vessel wall measured in the intact system in vivo. A properly oriented interfacial potential difference at the blood-intimal surface would serve to repel negatively charged blood cells from the vessel wall.

Four experiments resulted. In two of these, ion metabolism and turnover rates

by blood vessel wall (8) and ion flux across the wall (9, 10) were measured to determine types of ions available to the vessel interface. In the third experiment, attempts were made to measure streaming potentials produced by blood flow in the normal blood vessel in vivo (11). The last experiment investigated wall pore charge by an in vitro electroosmosis experiment described in this communication.

MATERIALS AND METHODS

The Electroosmosis Cell. A lucite double cell with six screw ports was used to determine the electroosmotic characteristics of blood vessel wall (Fig. 1). A 1 cm^2

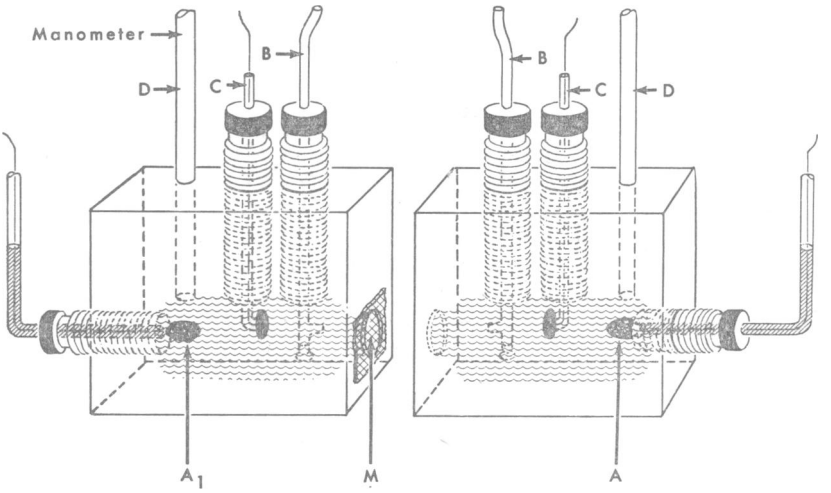

FIGURE 1 Drawing of electroosmosis cell. Labelled components are referred to in text.

opening between the two half-cells could be varied in area by inserting discs with smaller orifice area. The vascular membrane to be tested was placed across the opening. Silver-silver chloride electrodes (*A*) with a surface area of approximately 3 cm^2, screwed into the back of each half-cell, were used to apply voltage across the membrane.

A pair of calomel cells was attached on either side of the membrane (*M*) by using threaded inserts containing modified Krebs agar bridges (*B*). The voltage generated across the membrane and solution at the interfaces of the tissues by the current flow was measured via the calomel cells by using a Millivac MV17B potentiometer.

A pair of platinum disc electrodes was inserted behind the potentiometric electrode bridges to measure the specific conductance of the cell, bathing solution, and contained membrane at the beginning and end of each experiment (*C*). These conductance electrodes acted as the unknown arm of a Wheatstone bridge, which was balanced by using an audiometric circuit at 2 kc. The capacitive component of this determination was shown to be small by balancing the circuit at several frequencies from 200 to 2000 cycles.

In early experiments reported here, vertical calibrated pipettes (0.082 ml/cm) (*D*) were inserted into the most posterior aspect of each cell perpendicular to the long axis

of the cell. Later experiments were carried out by using horizontal pipettes calibrated to 0.01 ml/div. These recorded the increase or decrease of bathing fluid volume in each half-cell due to movement of fluid across the membrane during the electroosmosis experiment. The direction and rate of fluid flow across the membrane permitted determination of both the sign of the membrane interface potential, as zeta potential (in millivolts), and calculation of the approximate charge density per unit area of the wall pores (in electrostatic units) (12, 13).

Tissues. The aortae and vena cavae were taken from unanesthetized normal mongrel dogs sacrificed by either air embolism or exsanguination. The vessels were immediately placed in either modified Krebs saline solution (14), or 0.154 *N* saline solution at a pH of 7.4 and a temperature of 37°C. The vessels were prepared for use by splitting them longitudinally into a flat membrane. This was cut into segments large enough to be placed over the opening between the two half-cells.

Solutions. Either modified Krebs saline solution consisting of cations: Na^+, 145 meq; K^+, 4 meq; Ca^{++}, 5 meq; Mg^{++}, 2 meq; and anions: Cl^-, 118 meq; HCO_3^-, 35 meq; $SO_4^=$, 2 meq; and $H_2PO_4^-$, 1 meq; or unbuffered 0.154 N NaCl were used to bathe the membranes during the experiment. Glucose or other sugar was not added to the solution in these experiments because of its effects on viscosity and, hence, on conductance.

Technique. After placing the vessel wall between the two lucite half-cells, the entire cell was placed in a constant temperature Faraday cage, and the two sides of the cell were filled with physiologic solution at 37 to 38°C until the measuring pipettes were equally filled.

The cell was tested for leaks and for satisfactory function by using collodion membranes. Several determinations without impressed voltage were then made with the vessel wall in the cell for 12 to 48 hr periods to determine whether or not the vessel might display spontaneous bulk water transport, which would be measurable in the manometers used in the cells. For periods up to 48 hr, no net fluid movement across the membrane was measurable with the pipettes used.

Air bubbles were carefully excluded from the cell. The cell was then allowed to equilibrate at 37 to 38°C ambient temperature within the Faraday cage for approximately 1 hr, and frequent readings of the pipette fluid levels were taken. After the fluid levels were found to be stationary for a 30 min period, implying temperature stability between the plastic cell and its contained fluid, the conductance was measured. Next, voltage from a constant current source was applied to the silver-silver chloride electrodes at either end of the cell to produce a constant current, usually 10 ma, through the fluid and the membrane. Voltage drop across the membrane and the liquid-tissue interface between the two attached measuring bridges varied between 1.5 and 2.5 v. Simultaneous reading of current flow produced by the applied voltage, the potential difference across the membrane, the fluid levels in each pipette, and cage temperatures were recorded every 10 min thereafter for the duration of the experiment. In selected experiments the polarity of the Ag-AgCl electrode was reversed at the end of the first hour. The experiment was then run for another hour or part, with current polarity reversed in order to measure possible differential water transport characteristics (rectification) of the vessel wall.

The temperature change within the cell was measured during trial experiments. Temperature was found to change less than 1°C during a 60 min electroosmosis experiment carried out at 10 ma of current, the change averaging 0.8°C, equivalent to 10^{-6} ml change in manometer reading due to the thermal expansion.

The net volume of fluid transferred through the membrane during current flow was measured by the changes in fluid level in the pipettes. Zeta potential of blood vessel wall was calculated by using the Helmholtz-Smoluchowski electroosmosis equation (15). Thus,

$$\zeta = \frac{4\pi K\eta F}{Di} \tag{1}$$

where: ξ = zeta potential in volts; K = the specific conductance of the bulk solution in e.s.u., in the cell, approximately 1.62×10^{10} esmhos/cm for 0.154 N saline and 1.71×10^{10} esmhos/cm for modified Krebs saline serum substitute; η = viscosity in poises, 0.00695 poise for water and approximately the same for saline and Krebs solutions; F = rate of fluid flow across the membrane in milliliters per second; D = the dielectric constant, 74.4 for saline and physiologic solutions; and i = the current applied in electrostatic units.

In order to determine whether the membranes were physically intact at the end of the experiment, conductivity measurements were taken before and after each experiment. These did not show a change greater than 5 to 10%.

A possible source of error during early experiments was bellowing of the wall membrane, invariably toward the positive electrode in the experimental cell, which produced a spurious and variable apparent increase in flow of bathing solution across the membrane. In later experiments bellowing was prevented by encasing the membrane between rigid multiperforated discs or by decreasing the orifice size between the cells so that less membrane distention toward the positive pole was possible. These experiments yielded a more consistent, calculated zeta potential. As shown in Fig. 2, change in orifice size did not change the values of zeta potential calculated from the data.

Visible deflection of the vessel wall preparation toward the positive pole early in the pilot experiments suggested that the vessel wall at a pH of 7.4 exhibited a negative charge. Since neither the total number of pores nor the pore diameter within the membrane is known absolutely, it is not possible to calculate the surface charge per square centimeter of vessel wall. However, it is possible from the zeta potential calculations to make a rough approximation of the electrostatic charge *per square centimeter of pore surface* of exposed blood vessel wall. The equation for this calculation was derived by Debye and Hückel (12).

$$\sigma = \sqrt{\frac{DRT}{2000\pi}}\ \sqrt{C_c(\epsilon^{-Z_c\epsilon\zeta/KT} - 1) - C_a(\epsilon^{-Z_a\epsilon\zeta/KT} - 1)} \tag{2}$$

where: σ = electrostatic charge per pore of surface area, D = dielectric constant, R = molar gas constant, T = absolute temperature, C_c = moles, cations per liter, C_a = moles, anions per liter, Z_c = valence, cations, Z_a = valence, anions, ϵ = elementary charge, ξ = measured zeta potential, and K = Boltzman constant.

For *univalent solutions* the corrected equation becomes

$$\sigma = +\frac{(2KT\eta' D)^{1/2}}{(\pi)} \operatorname{Sin} h\zeta/2KT \quad \text{at} \quad 25°\text{C} \tag{3}$$

where η' is the net charge per milliliter of solution or tissue.

If one uses a univalent solution such as 0.154 N NaCl to carry out electroosmosis determinations, it is possible to use the simple form of this equation (3) to determine the approximate net charge per unit pore. While the use of the equation to calculate

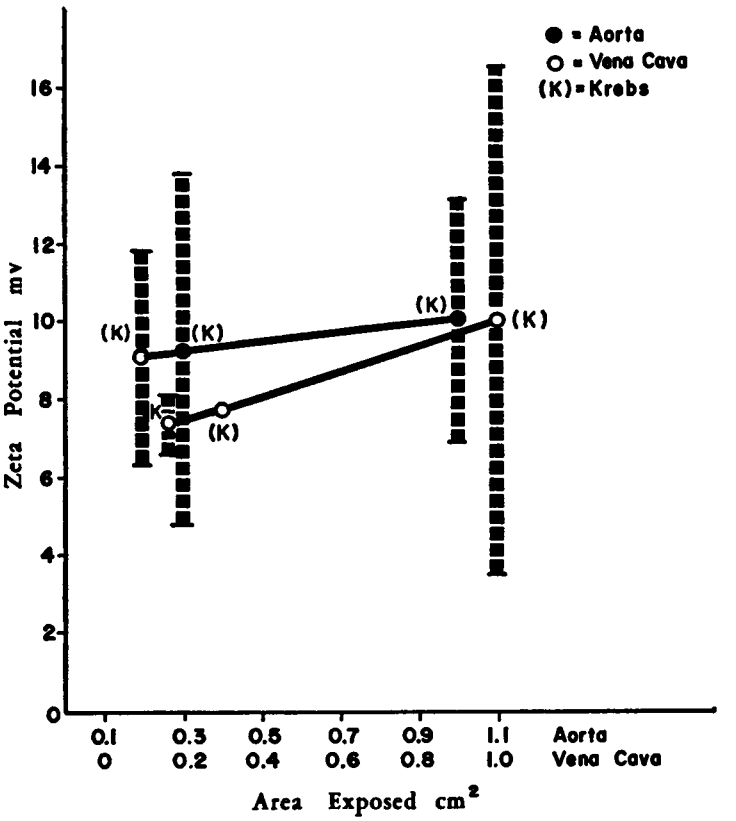

FIGURE 2. Varying the area of aorta or vena cava, exposed to a constant electric current, from 1 to 0.1 cm² produced no change in electroosmosis water transport or the resultant calculated zeta potential. This is characteristic of classical electroosmosis.

pore charge per square centimeter of pore surface in the experiment is not entirely unambiguous, it gives the only available approximation for this quantity. A more serious restriction is found in the fact that the total number of pores per square centimeter of tissue also can only be approximated. Thus the charge per pore cannot be calculated with precision because the total number of effective pores is not known absolutely. In order to minimize the problem of a very complex double layer, physiological saline, 0.154 N NaCl, was used in many of the electroosmosis experiments. The charge density calculated from this equation can only be considered part of the interface surface charge, ψ_o. The exact fraction will depend on the position of the plane of shear in the electrical double layer produced by electroosmosis.

RESULTS

The initial electroosmosis experiments indicate that the canine aorta and vena cava walls are negatively charged. This is in general agreement with the studies of Mudd (16–18) on other mammalian membranes.

Effects of Physical and Chemical Factors on the Electroosmotic Determinations and Calculated Zeta Potentials

Effect of Boiling (Table I). The effect of boiling the tissue for five minutes in saline was tested in a separate group of experiments to determine whether this would destroy the surface charge characteristics which produced the zeta potential. A statistically significant decrease in zeta potential resulted when compared to

TABLE I

EFFECT OF BOILING ON TISSUE ZETA POTENTIALS

Condition of tissue	No. of exps.	Bathing solution	Perfusing gas of O_2	Mean zeta potential	Charge/cm² of pore surface statcoulombs × 10^3	Net negative ions × 10^{12}	*P* values Normal vs. boiled
			%	*mv at 10 ma*			
Aorta							
Normal	16	0.154 N NaCl	100	−5.0 ± 1.4	3.4	6.8	$P = < 0.01$
Boiled	26	0.154 N NaCl	100	−2.1 ± 0.4	1.4	2.6	$T = 3\ .01$
Vena Cava							
Normal	10	0.154 N NaCl	100	−3.8 ± 2.8	2.6	5.1	$P = <0.01$
Boiled	16	0.154 N NaCl	100	−0.7 ± 0.1	0.5	0.94	$T = 3.69$

unboiled vessel which did not, in the time heat was applied, completely destroy tissue surface charge.

Effect of Varying Membrane Areas Exposed to the Electroosmosis Current. The effect of decreasing the exposed membrane area in steps progressively from 1 to 0.19 cm^2 at a constant current did not result in a significant change in the measured mean aortic and vena caval transmural zeta potential (19).

Effect of Varying Current on the Fluid Flow Across the Experimental Membrane. Currents of 1, 5, and 10 ma produced a proportional linear increase in fluid transport across the membrane and thus a constant calculated zeta potential (19).

Effect of Changing pH on the Zeta Potential Determinations. The pH was changed by increasing CO_2 in the perfusing gases from 5% at a pH of 8.1 to 50% at a pH of 6.5. Decreasing the pH produced a decrease in the calculated zeta potential for canine aorta of approximately 4.1 mv/pH unit change and for vena cava of approximately 3.0 mv/pH unit change at constant current (19). All experimental groups were run at 10 ma. The use of physiologic buffer limited the range to pH's between 6.5 and 9.

Quantitative Results of Canine Aorta and Vena Cava Electroosmosis Experiments

Statistical analysis: Analysis of variance of all data herein was carried out by multiple comparison techniques described by Tukey (20).

Canine Aorta

Thirty-four electroosmosis experiments using modified Krebs solution to bathe the canine aorta wall, carried out in vitro, gave a mean zeta potential and standard deviation of -9.0 ± 5.0 mv and an approximate equivalent charge of 6.2×10^3 statcoulombs/cm^2 of pore surface (Table II). The calculated net negative ions per

TABLE II

CUMULATIVE ELECTROOSMOSIS EXPERIMENTS: +CANINE AORTA

Perfusing gas	Bathing solution	No. of determinations	Direction of electro-osmosis (+) → (−)	Mean zeta potential	Charge/cm² of pore surface (statcoulombs) × 10^3	Net negative ions × 10^{12}
				mv		
85% + 15% O_2 CO_2	Krebs	18	$I \rightarrow A$	− 8.1 ± 4.2	5.6	11.0
85% + 15% O_2 CO_2	Krebs	16	$A \rightarrow I$	−10.0 ± 5.7	6.9	14.2
Mean and SD				− 9.0 ± 5.0	6.2	12.3
100% O_2	0.154 N NaCl	7	$I \rightarrow A$	− 4.2 ±0.71	2.8	3.7
100% O_2	0.154 N NaCl	9	$A \rightarrow I$	− 5.7 ± 1.4	3.6	7.1
Mean and SD				− 5.0 ± 1.4	3.4	6.8

pH = 7.2 to 7.4

square centimeter of pore surface was 12.3×10^{12} at a pH of 7.4 based on equation (3).

Effect of Using Different Solutions

When the electroosmosis was carried out on canine aorta wall bathed with 0.154 N NaCl, the mean zeta potential decreased to -5.0 ± 1.4 mv (Table II). Net negative ion charge per square centimeter of pore surface correspondingly decreased to 6.8×10^{12} net negative ions/cm^2 of pore surface. The zeta potential difference when using Krebs bathing solution as opposed to saline was found to be statistically significant ($P < 0.0001$, Table IV).

Canine Vena Cava

Table III gives the results for canine vena cava walls bathed in both modified Krebs and 0.154 N NaCl. The mean zeta potential for vena cava wall when bathed with

modified Krebs was -5.2 ± 1.6 mv with an approximate equivalent charge of 3.5×10^3 statcoulombs or 7.0×10^{12} net negative ions/cm^2 of pore surface.

Electroosmosis of the canine vena cava (Table III) carried out by using either 0.154 N NaCl or Krebs solution as the bathing solution (in comparison with those carried out by using aorta wall) again gave zeta potentials of smaller magnitude by

TABLE III

CUMULATIVE ELECTROOSMOSIS EXPERIMENTS: CANINE VENA CAVA

Bathing solution	Perfusing gas	No. of determinations	Direction of electro-osmosis (+) → (−)	Mean zeta potential	Charge/cm^2 of pore surface (statcoulombs) × 10^3	Net negative ions/cm^2 of pore surface × 10^{12}
				mv at 10 ma		
Krebs	85% + 15% O_2 CO_2	7	$I \rightarrow A$	−5.5 ±1.2	3.8	7.5
Krebs	85% + 15% O_2 CO_2	1	$A \rightarrow I$	−2.4	1.7	3.3
Mean and SD				−5.2 ±1.6	3.5	7.0
0.154 N NaCl	100% O_2	4	$I \rightarrow A$	−3.3 ±0.85	2.3	4.5
0.154 N NaCl	100% O_2	6	$A \rightarrow I$	−4.0 ±3.6	2.7	5.5
Mean and SD				−3.8 ±2.8	2.6	5.1

TABLE IV

EFFECT OF VARIOUS BATHING SOLUTIONS ON ELECTROOSMOSIS DETERMINATIONS

Bathing solution	Perfusing gas	No. of exps.	Mean zeta potential	Charge/cm^2 of pore surface statcoulombs × 10^3	Net negative ions × 10^{12}	P value Krebs vs. saline
			mv at 10 ma			
Aorta						
Krebs	85% + 15% O_2 CO_2	34	−9.0 ± 5.0	−6.2	12.3	$T = 4.54$
0.154 N NaCl	100% O_2	16	−5.0 ± 1.4	−3.4	6.8	$P < 0.0001$
Vena Cava						
Krebs	85% + 15% O_2 CO_2	8	−5.2 ± 1.6	−3.5	7.0	$T = 1.47$
0.154 N NaCl	100% O_2	10	−3.8 ± 2.8	−2.6	5.1	$P \approx 0.07$

using saline solution. However, the resultant differences are not quite significant ($p \approx 0.07$, Table IV). The mean calculated zeta potential and standard deviation with 0.154 N NaCl was -3.8 ± 2.8 mv. Equivalent net charge was 2.6×10^3 statcoulombs or 5.1×10^{12} net negative ions/cm^2 of pore surface.

For ion concentrations in the range of 0.1 M the calculated thickness of the electrical double layer is of the order 5 to 10 A (12, 21).

DISCUSSION

Electroosmosis appears to be an effective technique for determining the basic structural charge of blood vessel wall pores even though it has obvious limitations in terms of differentiating the location within the membrane of the net charges measured and though the relationship of electroosmotic characteristics of vessel wall to tissue metabolism is unknown.

Evidence that the blood vessel wall displays classical electroosmotic activity of significant magnitude is demonstrated by the findings reported herein and previously:

1. Fluid transport across canine aorta and vena cava is proportional to current flow (22).
2. Fluid transport reverses when the electric current is reversed (Tables II and III).
3. Fluid transport (and calculated zeta potential) across the membrane for a constant current is independent of membrane area exposed within the limits of the experimental technique (23).

The zeta potential as determined by electroosmosis represents only a segment of the vascular interfacial potential (ψ_o) in vivo at the solid liquid interface and is not necessarily the only in vivo factor leading to vascular interfacial potential. Teorell (24, 25) has analyzed and summarized the various phenomena responsible for total interfacial charge (ψ_o), which he carefully distinguishes from the zeta potential. At least four related phenomena occur simultaneously in porous biological membranes, the end result of which produces the interfacial charge. These phenomena are: (*a*) ionization of various chemical groups because of their structural characteristics; (*b*) Donnan equilibrium phenomena as hypothesized in the Meyer-Teorell theory (25, 26); (*c*) active ion transport (This has been demonstrated in blood vessels by measurement of active ion transport of Na^{24}, Cl^{36}, K^{42}, and Ca^{45} (9, 10, 27) across normal canine aorta and vena cava walls.); and (*d*) electroosmosis.

In addition, pressure produces electroosmotic effects through charged pores (pressure and stretch effects). The blood vessel wall can be expected to display transvascular ion sieving under in vivo conditions. The size of the calculated zeta potential will depend on the level of the shear of blood with reference to the various characteristic potential profiles produced by each of the aforementioned phenomena.

In this discussion it has been assumed that active ion transport, ion absorption, and electroosmosis all take place through or on the surface of pores. Since fluid in the extracellular space (29–31) has higher conductance than have the vessel wall cell membranes and tissues, it would seem most probable that both ion and water flow take place largely through these extracellular spaces (pores), which are the source of least resistance to water and ion movement.

Both active transport of various ions and selective ion sorption onto blood vessel wall pores and surfaces suggest an explanation for the different electroosmotic activities observed when using modified Krebs as opposed to physiologic saline as the bathing solution.

The finding that modified Krebs solution produces greater net water transport per unit current under the conditions of the experiment (Tables II, III, and IV) is in agreement with previous findings by Sawyer and coinvestigators that Na^+ (diameter of hydration sphere approximately 2.5 A) (32) and CL^- ions traverse the blood vessel wall far more freely than either Ca^{++} or K^+ (8–10, 28). Because of the larger hydration sphere around Ca^{++} (hydration sphere diameter approximately 7 A) and Mg^{++} ions contained in Krebs solution, it is possible that more water molecules will be transported per coulomb of current flow when large numbers of divalent ions are present in solution. In addition, the greater number of ion species in modified Krebs solution will provide a more complex electric double layer at the pore interface, as indicated in ion sorption studies which show that Ca^{++}, Mg^{++}, and K^+ are selectively sorbed and bound more tightly by the blood vessel wall, tissue, and pore surfaces (8). The additionally sorbed ions produce more charge per unit area of pore, the end result being greater water transport per coulomb of current passed in the experiment.

In view of the imposing evidence that has accrued concerning the role of electric charges in the production and prevention of intravascular thrombi, one would seem compelled to consider that the surface charge of pores and fibers of blood vessel wall has a causal relationship to the antithrombotic characteristics of the wall. The blood cells and proteins, which are negatively charged at the pH of blood (1, 33), carry a similar charge and one clearly of sign, like that on the surface of blood vessel wall as shown by measurements of streaming potential (11). Under these conditions, like charges would produce mutual repulsion of the flowing blood elements and the blood vessel walls over short ranges.

Evaluation of data obtained in this experiment and the experiments indicated above, as well as others (8–11, 28, 33) such as measurement of streaming potentials produced by blood flow through blood vessels, should shortly permit a more detailed and integrated description of the physical chemical structure of the blood-intimal interface and the electric double layer at the surface.

This work was supported in part by the National Heart Institute (Grants HE-3879-C4 and C5 and HE-07371-01, -02, and -03.

Dr. Sawyer is the Markle Scholar in Medical Science. Dr. Harshaw's work was carried out in part while he was a Postgraduate Research Fellow of the National Heart Institute.

Received for publication 8 March 1966.

BIBLIOGRAPHY

1. ABRAMSON, H. A., and MOYER, L. S., *J. Gen. Physiol.*, 1936, **19**, 601.
2. ABRAMSON, H. A., *J. Exp. Med.*, 1927, **66**, 1003.
3. SAWYER, P. N., and PATE, J. W., *Am. J. Physiol.*, 1953, **175**, 103.
4. SAWYER, P. N., and WESOLOWSKI, S. A., *Surgery*, 1961, **49**, 486.
5. SAWYER, P. N., and DEUTCH, B., *Am. J. Physiol.*, 1956, **187**, 473.
6. SAWYER, P. N., SUCKLING, E. E., and WESOLOWSKI, S. A., *Am. J. Physiol.*, 1960, **198**, 1006.
7. SAWYER, P. N., and WESOLOWSKI, S. A., *Ann. Surg.*, 1961, **153**, 34.
8. SAWYER, P. N., ZISKIND, H. S., and HARSHAW, D. H., Fundamentals of Vascular Grafting, New York, McGraw-Hill Book Company, Inc., 1963, 3.
9. SAWYER, P. N., and VALMONT, I., *Nature*, 1961, **189**, 470.
10. SAWYER, P. N., LEVINE, J., MAZLEN, R., and VALMONT, I., *J. Gen. Physiol.*, 1961, **45**, 181.
11. SAWYER, P. N., and HIMMELFARB, E., Biophysical Mechanisms in vascular Homeostasis and Intravascular Thrombosis, (P. N. Sawyer, editor), New York, Appleton-Century and Appleton-Century Crofts, 1965, 69.
12. DEBYE, P., and HÜCKEL, E., *Z. Physik*, 1923, **24**, 185.
13. CREETH, J. M., Electrical Phenomena at Interfaces, (J. A. V. Butler, editor), New York, The MacMillan Company, 1951, 75.
14. KREBS, H. A., *Biochem. et Biophysica Acta*, 1950, **4**, 429.
15. SMOLUCHOWSKI, M., Handbuch der Elektrizität und des Magnetismus II, (L. Graetz, editor), Leipzig, J. A. Barth, 1912, 366.
16. MUDD, S., *J. Gen. Physiol.*, 1925, **7**, 389.
17. MUDD, S., *J. Gen. Physiol.*, 1926, **9**, 73.
18. MUDD, S., *J. Gen. Physiol.*, 1926, **9**, 361.
19. HARSHAW, D. H., and SAWYER, P. N., Biophysical Mechanisms in Vascular Homeostasis and Intravascular Thrombosis, (P. N. Sawyer, editor), New York, Appleton-Century-Crofts, 1965, 61.
20. TUKEY, J. W., *Biometrics*, 1949, **5**, 99.
21. BULL, H. B., Physical Biochemistry, New York, John Wiley & Sons, Inc., 1951.
22. REUSS, F., *Mem. Soc. Impériàle Naturalistes Moskou*, 1809, **2**, 327.
23. WIEDEMANN, G., *Ann. Physik u. Chem.*, 1852, **11**, 321.
24. TEORELL, T., Biophysical Mechanisms in Vascular Homeostasis and Intravascular Thrombosis, (P. N. Sawyer, editor), New York, Appleton-Century and Appleton-Century-Crofts, 1965, 19.
25. TEORELL, T., *J. Gen. Physiol.*, 1959, **42**, 831.
26. MEYER, K. H., and STRAUS, W., *Helv. Chim. Acta*, 1940, **23**, 795.
27. SAWYER, P. N., LYONS, H. A., and WESOLOWSKI, S. A., *Bull. Internat. Surg. Soc. and Internat. Cardiovascular Soc.*, 1962, **21**, 352.
28. SAWYER, P. N., BURROWES, C. B., OGONIAK, J. C., SMITH, A. O., and WESOLOWSKI, S. A., *Trans. Am. Soc. Artificial Internal Organs*, 1964, **10**, 316.
29. COLE, K. S., and CURTIS, H. J., Medical Physics, (O. Glasser, editor), Chicago, Year Book Publisher, 1950, **2**, 82.
30. GRAM, H. C., *J. Biol. Chem.*, 1924, **59**, 33.
31. STEWART, G. N., *Am. J. Physiol.*, 1929, **90**, 194.
32. PAULING, L., Nature of the Chemical Bond, 2nd edition, Ithaca, New York, Cornell University Press, 1940.
33. SAWYER, P. N., editor, Biophysical Mechanisms in Vascular Homeostasis and Intravascular Thrombosis, New York, Appleton-Century and Appleton-Century Crofts, 1965.