MEASUREMENT OF STREAMING POTENTIALS OF MAMMALIAN BLOOD VESSELS, AORTA AND VENA CAVA, IN VIVO

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ABSTRACT Attempts to measure streaming potentials in large rabbit blood vessels in vivo have been carried out. Streaming potentials, V_s, were measured by the introduction of microelectrodes through the wall of the blood vessel at separations greater than 1 cm. The outputs from these electrodes fed through calomel cells were amplified and recorded directly by using an Electronics for Medicine photorecorder (White Plains, N. Y.). "Effective streaming currents" were determined by running the output through a low impedence galvanometer while simultaneously measuring the resistance of the circuit V_s were, therefore, calculated from two measurements and compared. Flow through vessels studied was measured using two different electromagnetic flowmeters. The results indicate that V_s present in both aorta and vena cava are of the order of 5 to 10 mv. By using the Helmholtz-Smoluchowski equation into which flow was reintegrated, the numbers yield zeta potentials approximating 0.1 to 0.4 v in both aorta and vena cava. This number approaches the apparent upper limit for zeta (actually "interfacial potentials") potentials in biological systems. The measured "i.f." potential is considered as the interreaction of several physical and metabolic factors operating at the blood intimal interface. The polarity of the potential suggests that the interface is negative with respect to the blood flowing through the vessel. Interfacial potential and related V_s are discussed in terms of their possible importance as a mechanism for maintaining vascular homeostasis in the living animal.

INTRODUCTION

More than ten years ago the effects of oriented direct electric currents to produce and prevent vascular thrombosis (1, 2) in vivo were demonstrated and have since been described in detail (3). More recently, because of discrepancies in potential measurements measured across blood vessel wall in vivo and in vitro (4–6), attempts have been made to determine whether or not streaming potentials caused by blood flow in vivo might account for the measured discrepancy in the potentials

measured. In the initial communication concerning measurement of streaming potentials in vivo (7) it was reported that streaming potentials had finally been measured successfully in biological systems but that a number of problems were presented in evaluating the streaming potentials and fitting the data to the classical Smoluchowski (8) equation. One problem was the inability to measure the pressure difference, P, between the two measuring electrodes in blood flow systems in vivo. This problem is partially solved in this report by substituting Poiseuille's Law into the Helmholtz-Smoluchowski equation. This substitutes vessel radius and length and blood flow rate for the factor P. In combination with streaming potential (V_s) measured in the high impedance photorecorder, zeta can then be calculated. Using the new equation, measurement of the diameter of the vessel is critical, because radius in the equation is taken to the fourth power. The second problem is related to determination of absolute magnitude of streaming potentials in the measuring system. This problem is again partially resolved by carrying out a simple Kirchoff's (Ohm's) Law experiment in which the resistance of the system and the current produced through the measuring micropipettes by the streaming potential are determined. The streaming potential can then be calculated from the two basic determinations.

In the series of experiments presented in this communication, calculated streaming potentials obtained by using both techniques, current-resistance determinations and direct measurement of streaming potential in a high impedance system, are compared.

MATERIALS AND METHODS

Experimental Animals. Streaming potentials were measured in both rat and rabbit aortas and vena cavae. Rabbits have become the standard animal for this experiment because their vessels are of significantly increased diameter and more easily manipulated. The aortas, vena cavae, and their branches in the posterior retroperitoneal space of rabbits weighing 2.2 to 4 kg were exposed by a standard transabdominal, transperitoneal approach (7).

Occlusion Technique. Vena cava was commonly occluded by using a compression technique caudad to the applied streaming potential measurements, micropipettes, and flowmeter. Aortic occlusion was commonly carried out by using a circumferential sling sometimes proximal to the flowmeter transducer head and micropipettes, sometimes distal, and sometimes both. Micropipettes drawn from a 1 mm diameter Kimble capillary glass had a tip diameter of 5 μ or less. They were filled with 3M KCl and inserted into holders attached to micromanipulators. The electrodes were inserted into the aorta or vena cava separated by distances ten times or more the radius of the blood vessel.

Techniques for Measurement of Streaming Potentials and Streaming Currents. In each rabbit two types of measurement were carried out. In most instances V_s were measured directly following insertion of micropipettes into the blood vessel to be used, with either a Suckling high impedence (10^{13} ohms input impedance) preamplifier from which the output fed directly into a Tektronix 502 oscilloscope or a multichannel Electronics for Medicine oscilloscope photorecorder (7).

Effective streaming currents (I_s) were measured by using the second technique. In

this instance the current produced by the blood flow streaming potential was passed through a galvanometer so that the actual current produced by V_s was measured (7). Total circuit resistance (R_T), including glass microelectrodes in the blood stream, was measured by a Keithley No. 610 electrometer resistance circuit. Inserting the current and resistance measurements into Ohm's Law permitted calculation of the effective streaming potential. The measured currents are not *true* streaming currents since the output was fed into a system which has a high intrinsic resistance, at least 10^{5-6} ohms. The term "effective streaming current" is, therefore, the term adopted for convenience by our group to represent the current produced by V_s through the resistance of the system used, R_T.

Blood flow was measured directly using a Carolina Medical Electronics square wave flowmeter, Model 2010 (Carolina Medical Electronics, Winston-Salem, N. C.) In each instance, probes supplied by the manufacturers and calibrated by the investigators were used. Probes, 2 and 3 mm in diameter, were used to measure aorta and vena cava flows, respectively. Diameter of vessels was measured immediately after exposure and serially during the course of the experiments. The electrodes, flowmeter probe, and electronic arrangement were similar to that previously described (7).

Experiments. The following experiments were performed.

1. In rat aorta and vena cava, the recording of V_s in vivo by using a Tektronix 502A oscilloscope, blood flow rate, and also vessel length and diameter.
2. Simultaneous measurement of relative changes of V_s and blood flow in vivo during occlusion-opening experiments in rabbit aorta and vena cava, by using calibrated oscilloscope photorecorder and flowmeter.
3. Measurement of effective streaming currents and associated R_T.
4. Evaluation of the effect of varying distance between the microelectrodes upon the streaming potential.

RESULTS

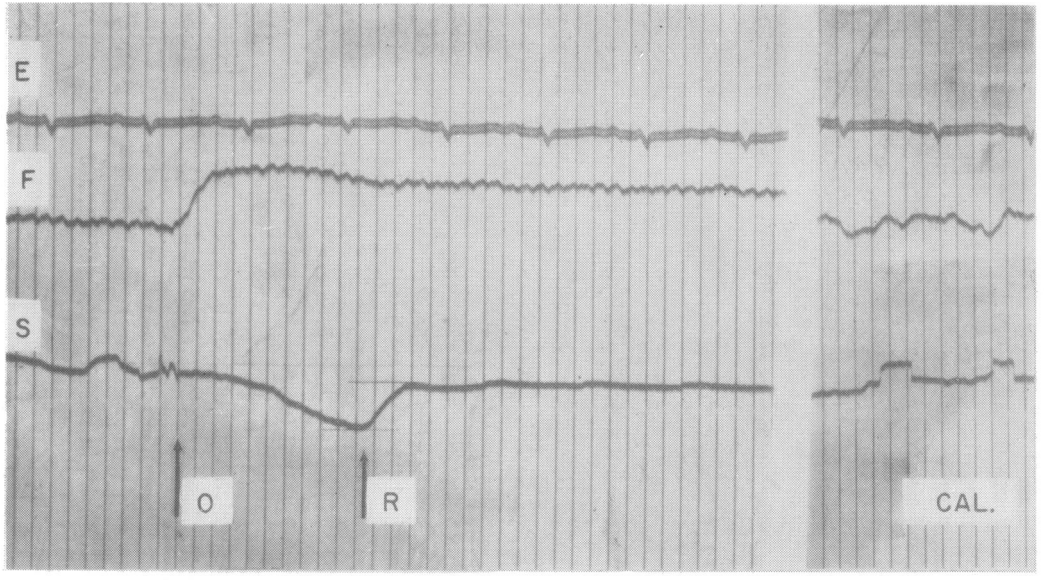
The sign before each number refers to interface polarity in the following results:

1. Streaming potential determined from recent recordings and traces in rat vena cava and aorta averaged -2.3 ± 0.5 mv in 10 rat vena cavae and -5.6 ± 1.0 mv in 5 rat aortas. Occlusion of either aorta or vena cava resulted in a fall of blood flow.

2. Streaming potentials, measured by using an Electronics for Medicine oscilloscope photorecorder, of rabbit vena cava averaged -4.0 ± 0.5 mv in 19 vessels and of aorta averaged -3.7 ± 0.2 mv in 14 vessels. Radius, length of vessel, and blood flow were also determined. One of the experiments is illustrated in dual traces in streaming potential and flow (Fig. 1). Streaming potential and blood flow change simultaneously, which indicates the strong correlation between the two determinations.

3. Data from experiments in which cessation of blood flow in both flowmeter and V_s traces in dying rabbits has been previously reported (7). The relationship between potential and flow is again linear, consistent with a system exhibiting streaming potentials.

4. Using Ohm's Law to calculate V_s in rabbit aorta and vena cava from values



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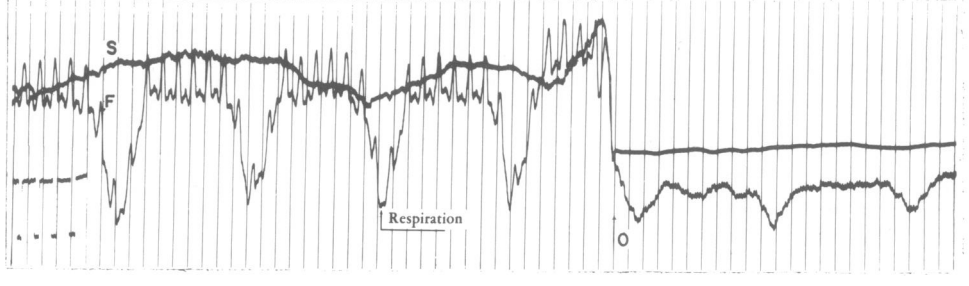

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FIGURE 1 *a* Photorecording from the vena cava of a rabbit showing streaming potential, *S;* electrocardiogram, *E;* and blood flow, *F*. *O* indicates occlusion. *R* indicates release. Calibration (*CAL.*) = 3 mv; linear division = 0.1 sec; change in flow = 10 ± 3 cc/min; and streaming potential change approximately = 8.5 mv.

(*b*) Simultaneous change in recorded flow, *F*, and streaming potential, *S*, from rabbit aorta. Large dips in flow trace are caused by diaphragmatic movement during respiration. Both streaming potential zero and flowmeter zero are down. Single mechanical occlusion, *O*, does not produce complete cessation of blood flow due to the collateral system inflow. Decrease in blood flow = 9 cc/min. Calibration = 3 mv; and division = 0.4 sec.

of I_s and R_T, for vena cava, $V_s = -8.3 \pm 1.5$ mv; for aorta, $V_s = -12.1 \pm 1.9$ mv, as seen in Table I. Streaming potentials calculated by this technique are of the same magnitude as streaming potential measurements made by using the cathode follower-oscilloscope recorder high impedance technique. Table II summarizes mean changes of V_s calculated from I_s and R_T during occlusion opening experiments. The changes resulting from occlusion are variable since the same level of partial or complete occlusion cannot be obtained in each experiment. The changes paralleled those found during direct measurement of V_s.

5. Finally, a representative result of the effect of varying the distance between the microelectrode tips within the aorta or vena cava is illustrated in Table III.

DISCUSSION

The streaming potential may be defined as the potential difference occurring along the axis of a tube when a hydrostatic pressure differential exists between the ends of the tube causing flow of liquid through it (9). A liquid phase is in motion with respect to a stationary solid phase. This condition, of course, is duplicated in the blood vessels of all living animals.

Of importance in the quantitative study of electrokinetic effects is the concept of the electrokinetic or zeta potential (ζ) (10–12). This quantity is defined as the potential at the surface of shear due to the combined effects of the charges on the surface and the equal and opposite charges of the ionic atmosphere. The term is rather ambiguous with respect to biological surfaces (13). The situation existing at the blood-intimal interface is complex and can only be approximately fitted to the equations now available since the interfacial potential (ψ_o) results from summation of all the interreacting phenomena at the vascular interface. According to Teorell these include (13): (*a*) net ion fluxes, (*b*) selective pressure filtration of ions through vessel wall pores, (*c*) net structural charge of vessel wall pores and tissues, (*d*) selective absorption and desorption of various ions from vessel wall tissues,

TABLE I

CALCULATED STREAMING POTENTIALS IN RABBIT AORTA AND VENA CAVA,* CALCULATED BY USING OHM'S LAW

Determinations	Effective streaming current (I_s)	Total resistance (R_T)	Streaming potentials (V_s)
	amp $\times 10^{-9}$	*ohms* $\times 10^{6}$	*mv*
Aorta			
8	5.7 ± 3.5‡	2.6 ± 1.3	−12.1 ± 1.9
Vena Cava			
8	3.2 ± 2.1	3.4 ± 2.0	−8.3 ± 1.5

*Measured by using the Keithley electrometer and the Leeds and Northrup galvanometer.
‡Mean and SD
The sign before the reported streaming potentials in data refers to the polarity of the vascular interface (negative) with respect to the flowing stream.

TABLE II

CHANGES IN STREAMING CURRENT AND CALCULATED STREAMING POTENTIAL IN RABBIT VENA CAVA AND AORTA WITH CHANGES IN FLOW (PARTIAL OCCLUSIONS CARRIED OUT BY MECHANICAL TECHNIQUES)*

Determination	Effective streaming current (I_s)	Resistance (R_T)	Streaming potential (V_s)	ΔV_s, *mv*
	amp $\times 10^{-9}$	*ohms* $\times 10^{6}$	*mv*	
Vena Cava				
I 1) Initial streaming current (I_s)	1) 1.8	5.0	−8.8	
2) I_s upon occlusion	2) 0.75		−3.8	−5.0
3) I_s upon release	3) 1.5		−7.5	−3.8
4) I_s 3 min after release (equilibration)	4) 1.8		−8.8	−1.3
II				
	1) 1.2	7.5	−9.4	
	2) 0.2		−1.8	−7.7
	3) 1.1		−8.8	−7.0
	4) 1.2		−9.4	−0.7
III	1) 4.5	1.8	−8.1	
	2) 3.5		−6.3	−1.8
	3) 4.5		−8.1	−1.8
	4) 4.5		−8.1	0
IV				
	1) 3.0	3.0	−9.0	
	2) 0.75		−2.8	−6.8
	3) 2.8		−8.3	−6.0
	4) 3.0		−9.0	−0.8
Aorta				
I 1) Initial streaming current (I_s)	1) 3.8	4.0	−15.0	
2) I_s upon occlusion	2) 2.5		−10.0	−5.0
3) I_s upon release	3) 4.0		−16.0	−6.0
4) I_s 3 min after release (equilibration)	4) 3.8		−15.0	−1.0
II	1) 5.3	3.0	−15.8	
	2) 3.8		−11.3	−4.5
	3) 5.5		−16.5	−5.3
	4) 5.3		−15.8	−0.8

The sign before the reported streaming potentials in the data refers to the polarity of the vascular interface (negative) with respect to the flowing stream.
*As measured with the Keithley electrometer and the Leeds and Northrup galvanometer.

and (*e*) pH and other physical-chemical effects. All the phenomena undoubtedly occur across the complex blood-intimal interface. Moreover, the equations used to calculate zeta potential were derived from experiments on rigid, usually narrow, infinitely resistant walled capillary tubes, through which flowed Newtonian fluids at a constant rate. Salt concentration was kept low so that the plane of shear intercepted a relatively large fraction of the interfacial potential (ψ_0).

TABLE III

THE EFFECT OF SEPARATION BETWEEN THE MICROELECTRODE TIPS AND MEASURED STREAMING POTENTIAL IN RABBIT VESSELS CALCULATED FROM HIGH IMPEDANCE MEASUREMENTS

	Determination	Distance between probes	Streaming potential
		mm	*mv*
Vena Cava			
	1	3	−6.0
	2	3	−7.0
	3	3	−6.5
	4	3	−8.4
	5	3	−3.0
	6	5	−6.0
	7	5	−4.0
	8	10	−8.0
	9	10	−5.5
	10	10	−4.5
	11	15	−3.4
	12	20	−1.8
Aorta			
	1	3	−6.0
	2	3	−6.0
	3	3	−7.0
	4	3	−8.0
	5	5	−6.5
	6	10	−6.0
	7	10	−5.0
	8	10	−6.0
	9	10	−7.0
	10	20	−3.3
	11	20	−1.9
	12	20	−2.3

The sign before the reported streaming potentials in the data refers to the polarity of the vascular interface (negative) with respect to the flowing stream.

Potentials measured in this experiment were determined in an elastic, porous, low resistance blood vessel, through which flowed (in a pulsatile manner on the aortic side) a non-Newtonian protein-containing fluid having the properties of a colloid and a relatively concentrated (approximately 0.154 N) ionic solution. Thus zeta potential may be only approximately calculated from the streaming potential by the following equation:

$$\zeta = \frac{(4\pi\eta K)}{D}\frac{(V_s)}{P} \tag{1}$$

where ζ is zeta potential in volts; η is viscosity in poises (0.027 for blood); K is specific conductance in statmhos per centimeter approximately 2×10^9 for rabbit blood; D is the dielectric constant (120 for blood at 25°C, 74 for blood at 37°C);

P is the pressure differential between measuring electrodes in dynes per square centimeter; and V_s is the streaming potential in volts.

By substituting the various constants into equation (1)

$$\zeta = (3.02 \times 10^7) \frac{(V_s)}{P}. \tag{1a}$$

By rearrangement of equation (1)

$$\frac{\eta}{P} = \frac{\zeta D}{4\pi K V_s}. \tag{2}$$

By rearrangement of Poiseuille's Law,

$$\begin{aligned} \mathfrak{F}_T &= \frac{P\pi r^4}{8\eta l} \\ \frac{\eta}{P} &= \frac{\pi r^4}{8l\mathfrak{F}_T} \end{aligned} \tag{3}$$

Where $\mathfrak{F}_T$ is flow in milliliters per second; r is radius in centimeters (0.075 ± 0.025 cm for aorta, 0.2 ± 0.1 for vena cava); and l is length of tube (capillary) in centimeters, 1.0 to 2.0 cm in these experiments. By substitution of equation (3) into equation (2)

$$\zeta \approx \frac{\pi^2 r^4 K V_s}{2Dl\mathfrak{F}_T}. \tag{4}$$

or

$$\zeta \approx \frac{\pi^2 r^4 K}{2Dl} \frac{V_s}{\mathfrak{F}_T}.$$

From equation (1a), it can be seen that V_s/P should be constant for a given system. Since P, the difference in pressure between the two electrodes, is extremely difficult to measure satisfactorily, the equation cannot be used effectively in biological experiments. The factors in equation (4) can be quantitatively determined. Therefore, equation (4) represents a more satisfactory form of the Helmholtz-Smoluchowski equation in the system when vessel length, radius, blood flow rate, and V_s are measurable.

By substituting into equation (4)

$$\zeta \approx 8.2 \times 10^7 r^4 \frac{V_s}{\mathfrak{F}_T}$$

the ratio of $V_s : \mathfrak{F}_T$ becomes a constant for the system studied.

Solution of equation (4) with the data at hand yields a zeta potential of the magnitude of 100 to 400 mv. This number approximates the expected order of magnitude. It could probably be improved in situations where the radius of the blood vessel could be more rigidly controlled.

Change in vessel radius, r, could be observed in these experiments due to micropipette puncture, stimulation to the wall, blood loss, or drop in systemic pressure during the procedure or during cessation of cardiac output. These variables could be kept to a minimum by gentle handling during dissection and during micropipette puncture.

Since flow and streaming potential are directly related, the considerations described above made it essential to demonstrate that changes in V_s during experiments relate closely to changes in flow. This relationship was repeatedly shown to exist in the studies demonstrating concomitant variation in streaming potential and blood flow (Fig. 2).

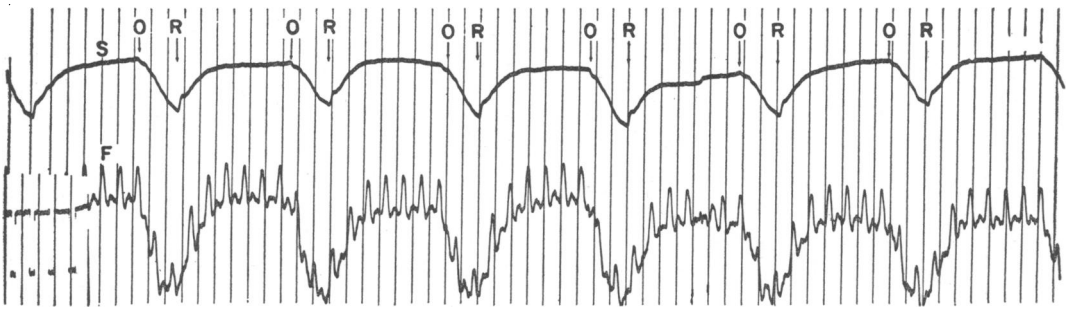

FIGURE 2 Rapid repeat partial occlusions, O, and release, R, of rabbit aorta during simultaneous recording of blood flow, F, and mean streaming potential, S. Calibration = 3 mv; and linear division = 0.4 sec. Decrease in aortic flow is approximately 4 ± 1 cc during partial occlusion.

Two problems of a mechanical and technical nature remain:

1. The microelectrodes introduced into the blood stream are likely to produce some turbulence which, when great, is known to increase the magnitude of the streaming potential sharply (14). That this factor must not be overly significant in these exepriments is shown by correlation between streaming potential–flow curves and linear regression of V_s to zero as flow approached zero (Fig. 3).

2. The possibility that the measured potentials were due to a change in position of the micropipettes produced by movement during mechanical occlusion was tested by inserting micropipettes into the vessel of the animal after which cardiac arrest was produced with no further electrode manipulations. These experiments indicated that the measured potential completely disappeared after cessation of blood flow, implying that the previously observed changes were not due to electrode position change (Fig. 3). Net V_s changes were of the same order of magnitude as those found during occlusion-opening experiments.

Electroosmosis (15) and streaming potential experiments both demonstrate that the pores and surfaces of the blood vessel wall are negatively charged at physiologi-

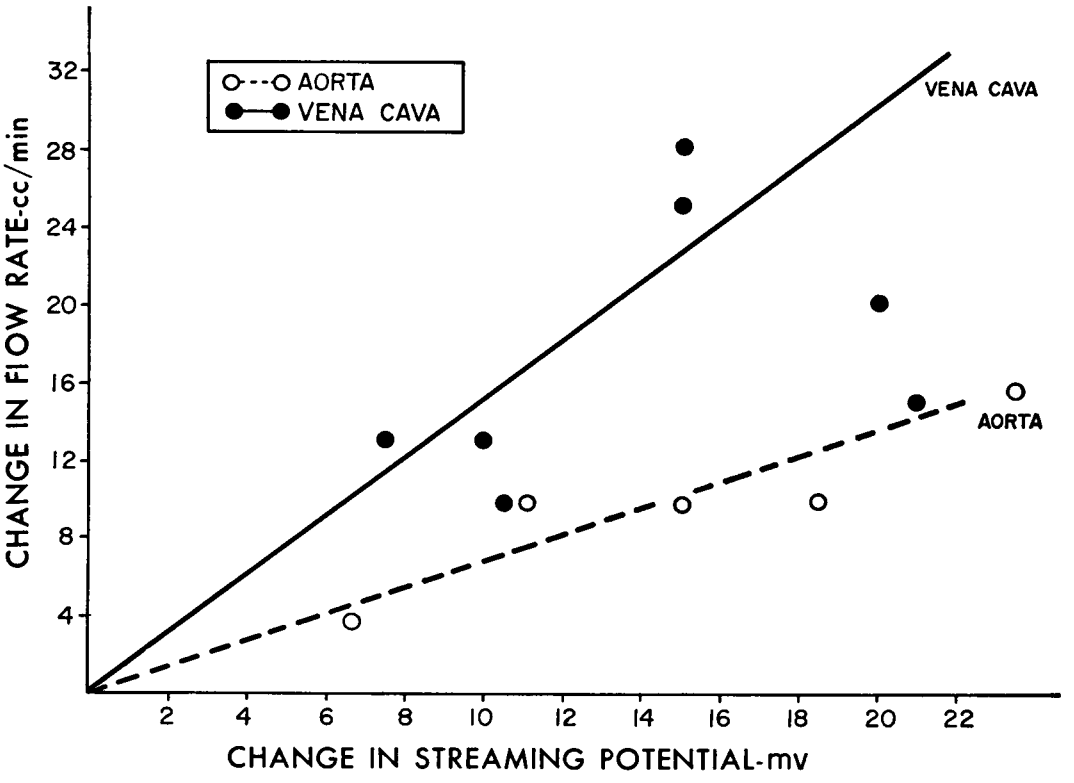

FIGURE 3 Change in flow rate versus change in streaming potential as measured in the aorta and vena cava of the dying rabbit.

cal pH. Since this polarization is partially lost upon injury to the blood vessel, a decrease in the negativity of interfacial potential will occur which tends to allow negatively charged formed blood elements to precipitate out of the stream. Thus a net decrease in the negativity of the interfacial potential may be one of the factors leading to thrombus formation in injured vasculature as previously suggested by a number of investigators (16–18).

SUMMARY

In vivo attempts to measure streaming potentials and to use the Helmholtz-Smoluchowski equation to derive approximate zeta potentials for the blood-intimal interface of mammalian blood vessels have been described. The results have shown that streaming potentials present in both aorta and vena cava are of the order of 5 to 10 mv. The effective "zeta" potentials calculated from the streaming potential were approximately 100 to 400 mv in both aorta and vena cava. This is probably the potential resulting from a large number of physical and metabolic factors inter-

acting at the blood-intimal interface. We conclude that the potential at the interface and its polarity deserve consideration as an important mechanism in the maintenance of vascular homeostasis in the living animal.

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