

SURFACE EFFECTS ON BLOOD-CLOTTING COMPONENTS AS DETERMINED BY ZETA-POTENTIALS. I

Francis E. Horan, ... , Lloyd A. Wood, Irving S. Wright

J Clin Invest. 1950;29(2):202-211. <https://doi.org/10.1172/JCI102247>.

Research Article

Find the latest version:

<https://jci.me/102247/pdf>



SURFACE EFFECTS ON BLOOD-CLOTTING COMPONENTS AS DETERMINED BY ZETA-POTENTIALS.¹ I.

By FRANCIS E. HORAN, FREDERIC G. HIRSCH, LLOYD A. WOOD,
AND IRVING S. WRIGHT

(From the Department of Medicine, Vascular Research Laboratory, Cornell University Medical College, New York City, and The Department of Chemistry, Cornell University, Ithaca, New York)

(Received for publication August 22, 1949)

INTRODUCTION

The fact that different types of surfaces affect the clotting time of blood has been known for many years, and this information has been extended from its early position of a mere laboratory curiosity to one of practical clinical importance today. As early as 1886 Freund (1) discovered that the coating of vessels with vaseline or paraffin markedly delayed the clotting time of blood. Johlin (2) in 1929 performed experiments which indicated that interfacial adsorption was a factor in the clotting of blood plasma; he showed that clotting can be induced by the contact of plasma with substances which produce an adsorbing interface between the plasma and these substances, and he interpreted this effect in terms of the adsorption of an antiprothrombin at the interface, thereby liberating in the plasma a substance which induces clotting. The retardation of blood coagulation in collodion-lined vessels was investigated by Hirschboeck (3) in 1940, and he attempted unsuccessfully to explain his results in terms of the force of adhesion between blood and collodion. The noteworthy work of Jaques and associates (4) in 1946 demonstrated that it was possible to preserve blood from clotting for several hours by storing it in vessels coated with a silicone preparation. This discovery is important in research because it permits the study of native blood without the addition of anticoagulants such as heparin or sodium oxalate. One significant clinical aspect has been reported by Barker and co-workers (5), in which they showed that a much better correlation between prothrombin times and clotting times

is obtained if the clotting times are measured in silicone-coated vessels in place of glass.

A clear explanation for the effect of these surfaces on the blood-clotting mechanism does not exist at present. Gortner and Briggs (6) have postulated that the underlying factor is very likely connected with a difference in surface potentials (zeta-potentials) of the different materials. As evidence, they cited the zeta-potential for bare glass against water as -30 mv. while that for paraffin-coated glass was zero. They believed that the high zeta-potential of glass would favor adsorption of positively charged colloids at the glass-blood interface, thereby concentrating an essential material which initiates the clotting process. Since they found the zeta-potential of paraffin in contact with water to be zero they reasoned that blood should not clot in paraffin-lined vessels. Lozner and Taylor (7) have also supported the idea that the surface effect is due to a physicochemical modification of one or more of the constituents of cell-free plasma.

In addition to the surface electrical effects which may play an important role in blood coagulation, it is conceivable that the function of anticoagulants is partly of an electrical nature, since Chargaff and co-workers (8) and also Wolfrom (9) have reported that heparin behaves in solution as a highly charged anion and migrates in an electric field toward the anode.

In view of the many problems which exist in blood coagulation studies and in recognition of the fact that adsorption and flocculation of a colloid may be partially explained in terms of the electrical potential difference (the zeta-potential) existing at its interface, the present work was undertaken to investigate the electrokinetic effects of blood, blood components and certain anticoagulants in contact with silica and silicone-coated surfaces.

¹ This work was supported by the Office of Naval Research, U. S. Navy, Task Order 10, Contract N6onr-264 and by grants from the Kress, Lillia Babbitt Hyde and Lasker Foundations.

APPARATUS AND MATERIALS

Apparatus

The method used to determine the zeta-potentials consisted of measuring the streaming potential, which is the potential difference that develops between the ends of a capillary tube when a liquid is forced through the tube by an applied pressure. If the flow is streamlined, so that Poiseuille's law is applicable, this potential is related to the zeta-potential at the wall of the tube extending perpendicularly from the wall towards the center of the capillary by the equation of Helmholtz (10),

$$\zeta = 4\pi\eta KE/DP,$$

where η is the viscosity coefficient, K is the specific conductance of the liquid in the capillary, E is the streaming potential in mv. when the liquid is forced through under pressure P (nitrogen was used here to apply the pressure and was measured in cm. of mercury), and D is the average dielectric constant of the liquid in the ionic double layer. From this equation it is evident that the apparatus required must include devices for measuring the streaming potential, the pressure, and the electrical conductivity of the liquid in the capillary. In this work the viscosity and dielectric constant were assumed to be the same as that of water, so that $\zeta = 9.662 \times 10^4 KE/P$ mv. at 25.0° C.. No attempt was made to control the temperature rigidly, since it has been shown (11) that the

temperature coefficient of the zeta-potential of aqueous systems can be ignored in streaming potential measurements made under ordinary laboratory conditions (approximately 25° C.).

The apparatus used was essentially that as described by Jones and Wood (12) and consisted of two principal units, namely, one for the measurement of the streaming potential at a known pressure, and the other for the determination of the electrical conductivity of the solution in the capillary. The circuit diagram for the apparatus having positions for two capillary tubes is given in Figure 1. A general view of the complete streaming potential apparatus is shown in Figure 2 (the more sensitive L and N, Type E galvanometer, however, was substituted for the one shown in the photograph).

The potential difference between the electrodes was measured with an L and N, Type K potentiometer. The proper choice of the null instrument is critical here, because the electrical resistance of the instrument must be large compared with the resistance of the cell being measured. For ordinary potential measurements an instrument with a higher internal resistance is necessary. This requirement is satisfied by the L and N thermionic amplifier (Cat. No. 7673) which has a resistance of at least 10^{13} ohms, and is sensitive to a potential difference of about 0.2 mv. The entire apparatus was shielded with a copper screen and the connecting wires were shielded to prevent induced charges and electrical leakage. For

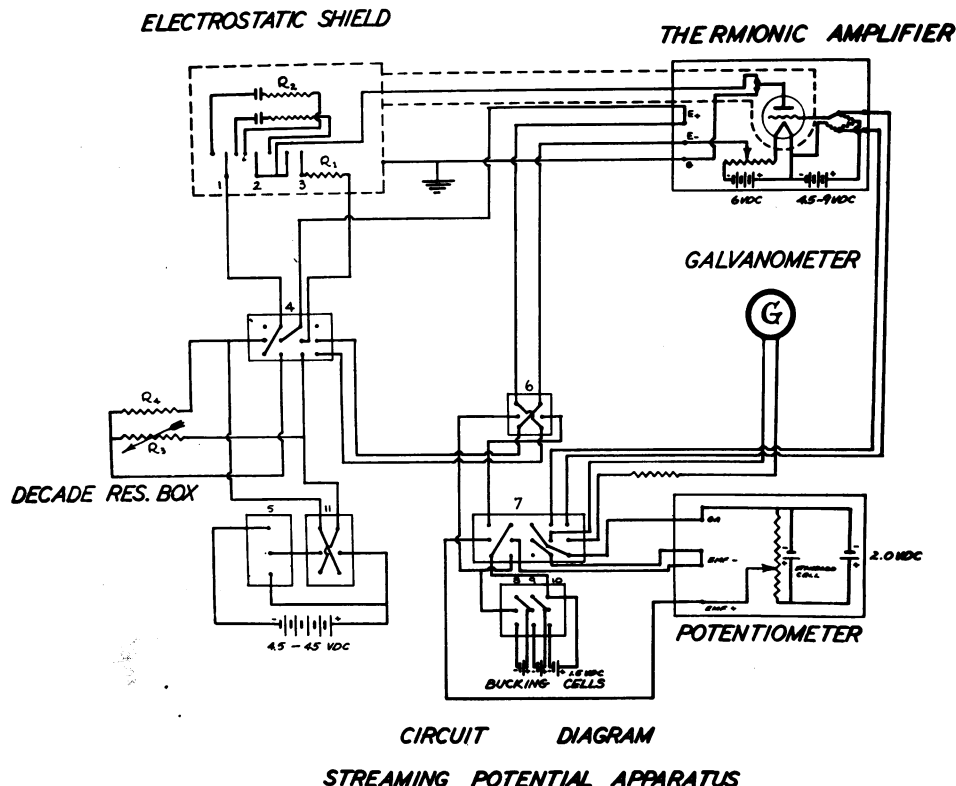


FIG. 1. CIRCUIT DIAGRAM FOR THE APPARATUS USED IN DETERMINING ZETA-POTENTIALS

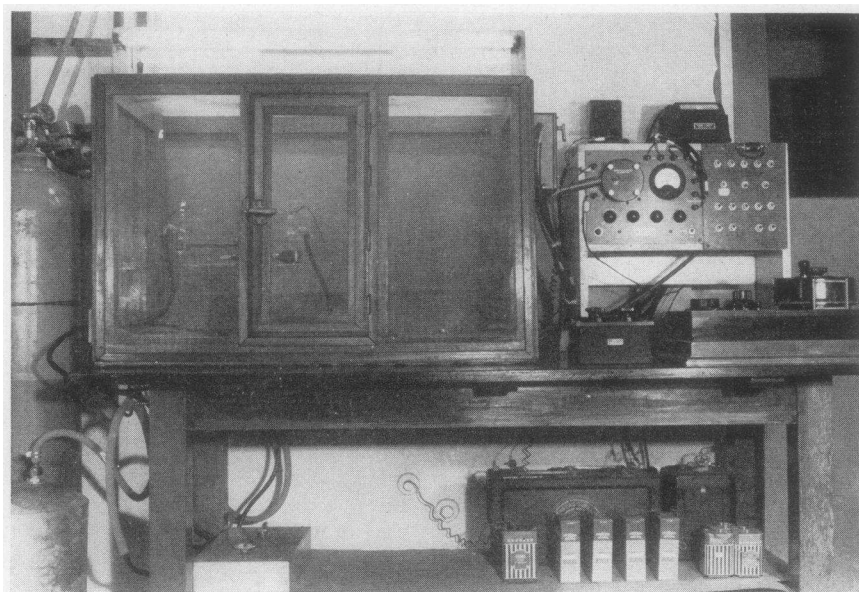


FIG. 2. A GENERAL VIEW OF THE APPARATUS USED IN DETERMINING ZETA-POTENTIALS

the measurement of the pressure of the nitrogen, a mercury manometer was used. A 15-gallon tank was employed as a ballast reservoir for the high pressure, while the other side was open to the air when flow was taking place. A pressure of about 40 cm. of mercury was used for the measurements reported in this work. Stop-cock and electrical-switch arrangements permitted measurements to be made when the liquid flowed in either direction.

For the determination of the specific conductance, it was necessary to measure the resistance of the solution in the capillary by means of a high-resistance direct-current bridge built into the streaming-potential apparatus. This bridge was capable of measuring resistance in the range of 10^6 to 10^{10} ohms with a precision of 0.5% or better. A decade resistance box adjustable in steps of 1 ohm from 1 to 10,000 ohms was used as the variable resistor. The capillary, a precision resistor of 1 megohm, and a precision resistor of 0.1 megohm constituted the

other three resistances of the bridge network. Thus, the specific conductance of the capillary solution could be determined after having determined the cell constant of the capillary (using a standard solution such as 0.01 N KCl) according to the usual equation, $K = L/\pi r^2 \cdot 1/R$, where K is the specific conductance at a given temperature, $L/\pi r^2$ is the cell constant, and R is the measured capillary resistance.

The cell consisted of two glass reservoirs, each of about 20-ml. capacity, connected by the capillary tube as illustrated in Figure 3. Pressure applied by nitrogen through either side-arm of the reservoir forced the solution from one side to the other. On the top of each reservoir was a glass cap through which was sealed a platinum wire which extended to the bottom of the reservoir. On the lower end of wire was deposited a porous mixture of silver and silver chloride, which is a nearly insoluble, reversible electrode. The vitreous silica capillary (1) was 6.9 cm. in length and 0.0204 cm. in bore, and its cell

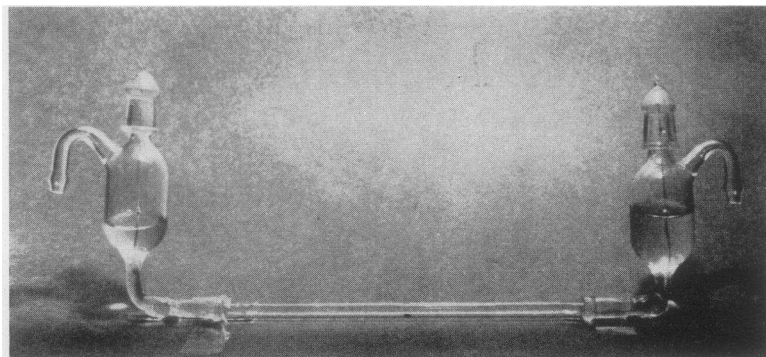


FIG. 3. A STREAMING CELL USED IN DETERMINING ZETA-POTENTIALS

constant was 21,000 cm⁻¹. The silicone-coated capillary (II) was prepared from Pyrex capillary tubing by treating with G.E. Dri-film (9987) several times before using; it was 14.6 cm. in length and 0.0372 cm. in bore, and its cell constant was 13,400 cm⁻¹.

In determining the zeta-potential, the usual procedure was to apply a pressure difference between the reservoirs and then to measure the electrical resistance of the solution while flowing in the capillary, according to the method outlined above; then the streaming potential E and pressure P were measured. The direction of flow was reversed under approximately the same pressure difference and the steps above were repeated.

Generally, the streaming potentials drifted at the start of an experiment, but it was found that constant values could be obtained by allowing the solution to stand in the capillary for 12 to 24 hours. The zeta-potentials for both directions of flow were averaged, and are precise to at least 10%.

Materials

Potassium chloride solution. 0.01 M and 0.001 M solutions were used.

Potassium phosphate solution. This solution was prepared by mixing 50.00 ml. of 0.1 M KOH with 50.00 ml. of 0.1 M KH₂PO₄. Then 10.00 ml. of this solution were diluted to 1000 ml. with distilled water (the potassium concentration was 0.001 M).

Blood, plasma and serum. Supplied by normal human sources.

Heparin. Supplied by Roche-Organon, Inc., Nutley, N. J. (1 ml. = 10 mg.)

Bovine plasma fractions. Supplied by Armour and Company, Chicago, Illinois.

A brief description of these samples follows:

Fraction I: bovine fibrinogen (40% sodium citrate, 42-48% clottable protein)

Fraction II: 100% gamma-globulin

Fraction III-1 (C155): 88% beta-globulin, 10% alpha-globulin, 2% albumin

Fraction IV (C143): 20% beta-globulin, 25% alpha-globulin, 55% albumin

Crystalline albumin—Armour crystalline bovine albumin.

Paritol. Supplied by Wyeth Inc., Philadelphia, Pa. (1 ml. = 10 mg.)

"Phosphatide Inhibitor." Supplied by Dr. R. S. Overman of these laboratories.

1,5 Pentanediol. Supplied by E. I. du Pont de Nemours & Co., Electrochemicals Department, Wilmington, Delaware.

Thrombin. Supplied by Parke, Davis and Co., Detroit, Michigan.

Thromboplastin. Dr. R. S. Overman of these laboratories kindly supplied this material, which was prepared by adding 2 ml. of distilled water to 50 mg. of rabbit lung thromboplastin and incubating at 37° C. for 10 minutes. The suspension was then centrifuged and the supernatant liquid was added to 200 ml. of phosphate solution. (The

concentration was approximately 5 mg. of thromboplastin per 100 ml. of phosphate solution.)

RESULTS

Solutions in vitreous silica and silicone-coated capillaries

The streaming potential varies inversely as the specific conductance of the streaming liquid, and it was found that with the apparatus described previously a potential difference of the order of only a few millivolts was developed with a plasma solution. (However, an amplifier suitable for measuring streaming potentials of the order of microvolts is now being constructed²). To increase the precision of measurement, solutions of much lower conductivity were employed, namely, 0.001 M KCl and also KH₂PO₄—KOH (approximately 0.001 M with respect to potassium ion). Both of these solutions were used as standards to which different proteins were added in an effort to determine their effects on the zeta-potentials set up at the surfaces of the two capillaries. Typical experimental results are given in Table I, showing that zeta-po-

TABLE I
Zeta-potentials of solutions in vitreous silica and silicone-coated capillaries

Solution	Zeta-potential (mv.)	
	Silica capillary	Silicone-coated capillary
0.001 M KCl	-68	-44
0.01 M KCl	-52	-25
KH ₂ PO ₄ —KOH (approx. 0.001 M)	-80	-61

tentials of the surfaces in contact with the solutions are negative and that the potential of the vitreous silica surface is more negative than that at the silicone. Increasing the concentration of the potassium chloride decreased the zeta-potential, as has been previously reported by Jones and Wood (12). The phosphate solution gave a more negative potential than the potassium chloride which is in agreement with work previously published by Bull and Gortner (13).

The effect of pH on the zeta-potential

Surfaces in contact with a protein solution generally become coated with the protein and there-

² This work is under the supervision of Professor W. C. Ballard, Jr., Cornell University School of Electrical Engineering, Ithaca, New York.

TABLE II

The effect of pH on fibrinogen and heparin in KCl solutions

Additive	Vitreous silica capillary		Silicone-coated capillary	
	pH*	Zeta-potential (mv.)	pH*	Zeta-potential (mv.)
None	5.5	-68	5.5	-44
None	6.2	-69	6.5	-62
5 mg. fibrinogen	6.0	-1	6.0	-9
5 mg. fibrinogen	7.0	-13	7.3	-26
5 mg. heparin	6.2	-60	6.2	-52
5 mg. heparin	6.7	-69	7.1	-58
5 mg. heparin	8.3	-82	9.0	-65

* To increase the pH above 5.5 (standard solution) small amounts of dilute KOH were added to the 0.001 N KCl solution.

after exhibit properties characteristic of the protein. Since the present work is primarily concerned with the zeta-potential of plasma proteins and anticoagulants, such as heparin, it is necessary to have some knowledge about the effect of pH on the potentials. The results in Table II indicate that a pH change has but little effect on the standard KCl solution, but does play an important role when fibrinogen or heparin is dissolved in the KCl solution (see also Figure 4). The somewhat erratic values obtained with the silicone-coated capillary are due probably to difficulties in preparing identical silicone surfaces. A more complete de-

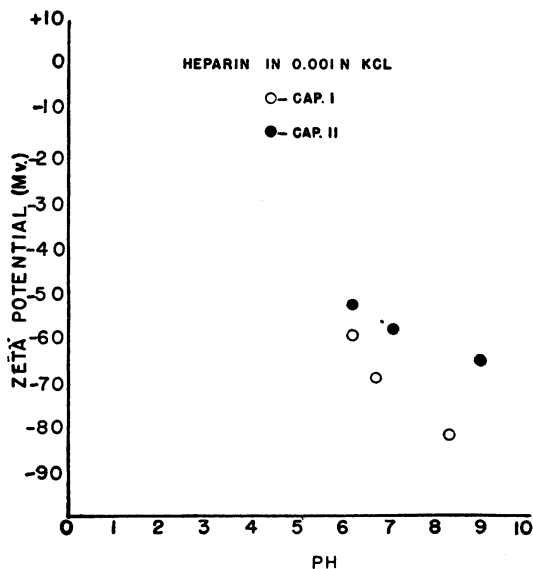


FIG. 4. EFFECT OF pH ON THE ZETA-POTENTIALS OF HEPARIN SOLUTIONS

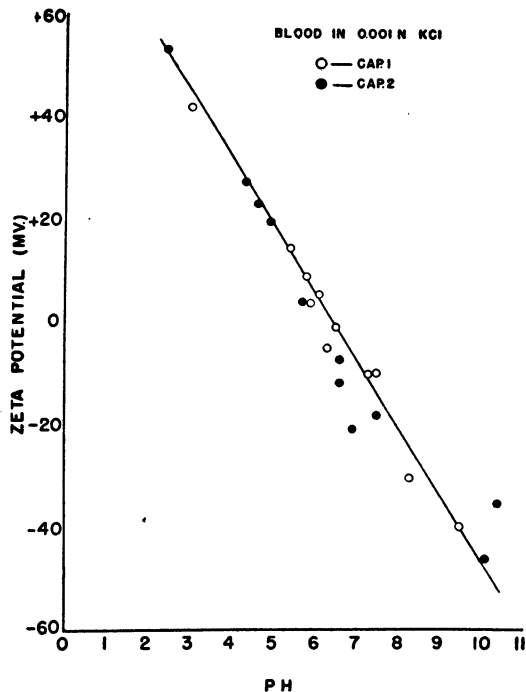


FIG. 5. EFFECT OF pH ON THE ZETA-POTENTIALS OF BLOOD SOLUTIONS

scription of the influence of pH on very dilute solutions of blood is given in Table III and Figure 5. It is recognized that the measurements made at the different pH values were not carried out at constant ionic strength, but as will be seen in the next section the changes in zeta-potential due to a pH change outweighs that due to a small variation in ionic strength. Thus, the pH must be fixed

TABLE III

The effect of pH on dilute solutions of blood in 0.001 N KCl*

Vitreous silica capillary (I)		Silicone-coated capillary (II)	
pH	Zeta-potential (mv.)	pH	Zeta-potential (mv.)
3.0	+42	2.9	+54
5.4	+14	4.3	+27
5.8	+9	4.6	+23
5.9	+2	4.9	+20
6.1	+6	5.7	+4
6.3	-5	6.6	-7
6.5	-1	6.6	-12
7.3	-10	6.9	-21
7.5	-10	7.5	-18
8.3	-36	10.1	-46
9.5	-41	10.4	-36

* 0.03 ml. of blood per 100 ml. of 0.001 M KCl; small amounts of dilute KOH and HCl solutions were added to change the pH of the standard KCl solution (pH 5.5).

TABLE IV
Effect of ionic strength on the zeta-potential

Solution	Vitreous silica capillary			Silicone-coated capillary		
	pH	Zeta-potential (mv.)	Sp. cond. (mhos $\text{cm}^{-1} \times 10^{-4}$) (25.0° C.)	pH	Zeta-potential (mv.)	Sp. cond. (mhos $\text{cm}^{-1} \times 10^{-4}$) (25.0° C.)
0.03 ml. blood per 100 ml. 0.001 M KCl	5.5	+ 7	148	5.5	+ 2	147
0.03 ml. blood per 100 ml. 0.01 M KCl	5.5	+ 1	1450	5.5	- 4	1450
0.05 ml. oxalated plasma per 100 ml. phosphate	6.9	-10	122	6.8	-10	120
0.05 ml. oxalated plasma +5 mg. heparin per 100 ml. phosphate	6.8	-23	217	6.8	-23	215
1.0 ml. oxalated plasma per 100 ml. phosphate	6.8	-12	299	6.8	- 9	289

in order to be able to detect significant changes in zeta-potentials brought about by factors other than the hydrogen-ion concentration.

The effect of ionic strength on the zeta-potential

The literature contains several works (14, 15) which demonstrate that the isoelectric point of a protein can be shifted by changing the ionic strength of its solution. If the isoelectric point were lowered, *i.e.*, shifted to the acid side, the zeta-potential would become more negative at a given pH on the alkaline side. To investigate the magnitude of this ionic effect dilute solutions of blood in 0.001 M KCl were studied. The data summarized in Table IV reveal that the stronger (tenfold) KCl solution made the zeta-potential somewhat less positive at a pH of 5.5, probably because of a slight shift of the isoelectric point to the acid side. However, in none of the experiments discussed in this work was the ionic concentration (specific conductance) changed as much as tenfold. The results of experiments with oxalated plasma and heparin, also listed in Table IV, indicate that the increase in negative zeta-potential is due primarily to the heparin and not to an increase in ionic strength, as judged by a comparison of the conductivities of the solutions.

The effect of heparin on the zeta-potential of blood

Several experiments were performed using a small amount of whole blood dissolved in the phosphate solution, and the zeta-potentials were determined before and after the addition of heparin. The data in Table V show that a small negative potential results in both capillaries when the dilute

blood solution is measured at a pH of 6.9, and that the potential is made decidedly more negative by the addition of heparin. Moreover, it is seen that the heparin-blood potential, which is intermediate between that of the blood and heparin alone, is approximately the same irrespective of whether the blood is first added to the capillary followed with heparin or the heparin is used first followed with blood.

The potential-determining material coating the surface of the capillaries may be due to one or more of the plasma proteins and also the red cells themselves. It is realized that since the solution is not isotonic, the red cells probably underwent lysis, liberating hemoglobin which could be the essential factor in developing the zeta-potential. To investigate this point, red cells were washed free from plasma with physiological saline. The red cells were then added to the phosphate solution, and the zeta-potential was determined. The results indicate that the zeta-potential with the red cells is somewhat more negative than that obtained with whole blood.

TABLE V
Effect of heparin on the zeta-potential of blood

Additive	Zeta-potential (mv.)			
	pH	Vitreous silica capillary	pH	Silicone-coated capillary
Amt./100 ml. phosphate				
0.05 ml. blood	6.9	- 6	6.9	- 8
0.05 ml. blood +5 mg. heparin	6.6	-45	6.6	-41
5 mg. heparin	6.9	-89	6.9	-84
5 mg. heparin +0.05 ml. blood	6.7	-43	6.7	-51
0.05 ml. washed red cells	7.1	-11	—	—

The effect of heparin on the zeta-potentials of plasma and serum

In addition to a study of whole blood and red cells, plasma and serum were also investigated. Non-clotting plasma (oxalated) and clotting plasma (prepared by centrifuging the venous blood in a silicone-coated tube) were used. A portion of the clotting plasma was allowed to clot by transferring it from a silicone-coated container to glass, the fibrin was removed, and the resulting serum was also investigated. The zeta-potentials of these different systems are recorded in Table VI. The non-clotting plasma gave a zeta-potential of about -10 mv. in both capillaries using two different concentrations of plasma. Heparin increased the negative potential of the non-clotting plasma to approximately the same degree in both capillaries and at two different concentrations of heparin. The zeta-potentials of the clotting plasma in the two capillaries were slightly more negative than in the case of the nonclotting plasma. Likewise, heparin gave a more negative effect in the case of the clotting plasma. The zeta-potentials of the serum were somewhat more negative than those of the plasma in both capillaries and heparin similarly made these potentials more negative.

The effect of heparin on the zeta-potentials of plasma fractions

The zeta-potentials of several bovine plasma fractions and the effect of heparin on these potentials were determined. The results given in Table VII agree qualitatively with those obtained by

TABLE VI

Effect of heparin on the zeta-potentials of plasma and serum

Additive Amt./100 ml. phosphate	Zeta-potential (mv.)			
	pH	Vitreous silica capillary	pH	Silicone-coated capillary
0.05 ml. plasma (oxalated)	6.9	+10	6.8	-10
0.05 ml. plasma (oxalated) plus 5 mg. heparin	6.8	-23	6.8	-23
1.0 ml. plasma (oxalated)	6.8	-12	6.8	-9
1.0 ml. plasma (oxalated) plus 1 mg. heparin	6.8	-22	6.8	-15
0.05 ml. plasma (clotting)	6.8	-14	6.7	-13
0.05 ml. plasma (clotting) plus 5 mg. heparin	6.8	-35	6.7	-33
0.05 ml. serum (no oxalate)	6.6	-17	6.6	-17
0.05 ml. serum (no oxalate) plus 5 mg. heparin	6.4	-29	6.3	-24

TABLE VII

Effect of heparin on the zeta-potential of plasma fractions

Additive Amt./100 ml. phosphate	Zeta-potential (mv.)			
	pH	Vitreous silica capillary	pH	Silicone-coated capillary
5 mg. Fraction II (C188A)	6.9	+10	7.1	+2
5 mg. Fraction II (C188A) +5 mg. heparin	6.9	-24	7.0	-24
5 mg. Fraction III-1 (C155)	7.0	-14	7.0	-12
5 mg. Fraction III-1 (C155)+5 mg. heparin	6.9	-29	6.9	-25
5 mg. fibrinogen	6.7	-20	7.1	-24
5 mg. fibrinogen+5 mg. heparin	6.6	-37	6.8	-40
5 mg. fibrinogen	7.0	-16	7.1	-16
5 mg. fibrinogen+8 mg. heparin	6.9	-41	7.0	-25
5 mg. cryst. albumin	6.8	-53	6.8	-45
5 mg. cryst. albumin+5 mg. heparin	6.9	-52	7.0	-49
5 mg. fibrinogen+5 mg. cryst. albumin	6.8	-15	6.8	-12
5 mg. fibrinogen+5 mg. cryst. albumin+5 mg. heparin	6.7	-36	6.6	-28
5 mg. Fraction IV (C143)	7.1	-58	6.8	-43
5 mg. Fraction IV (C143) +5 mg. heparin	7.0	-51	6.9	-49

electrophoresis of plasma (16), namely, the albumin boundary moves the fastest in an electric field and is therefore charged the most highly negative, whereas the mobility of Fraction II (C188A) (gamma globulins) is the lowest and consequently is the least negatively charged.

It is of interest to note that heparin makes the zeta-potentials of Fraction II (C188A), Fraction III-1 (C155) and fibrinogen more negative, whereas it has little or no effect on the crystalline albumin and Fraction IV (C143). The synthetic mixture of fibrinogen and albumin shows that fibrinogen probably has a greater influence on the surface than does albumin. Fraction IV (C143), which contains 55% albumin, gives zeta-potentials which are practically identical with those developed by the crystalline albumin.

Effect of other anticoagulants on the zeta-potentials

Heparin is the most potent anticoagulant known at the present time. Recently, however, a synthetic material called paritol has been investigated clinically by Sorenson, Seifter, and Wright (17) and has been found to have anticoagulant properties. Overman (18) of these laboratories has likewise isolated a phosphatide material from beef brain

thromboplastin which inhibits the blood-clotting process. A comparison of paritol and the "phosphatide inhibitor" with heparin is made in Table VIII, showing that the three anticoagulants behave similarly with fibrinogen in that they cause the zeta-potential to become more negative.

Ferry (19) has shown that many alcohols, particularly 1,5-pentanediol, will inhibit the formation of a fibrin clot by the action of thrombin on fibrinogen. The effect of this glycol on the zeta-potential of blood was investigated, and the results in Table VIII indicate that 1,5-pentanediol reacts differently from heparin, since the latter increases the negative potential of blood, whereas the former appears to have no electrical effect.

Effect of heparin on the zeta-potentials of other blood-clotting substances

The production of thrombin by the action of thromboplastin on prothrombin is considered to be one of the first steps in the blood-clotting mechanism. From the data in Table IX it is seen that the zeta-potentials of thrombin and thromboplastin are made more negative by the addition of heparin, the latter being affected more markedly than the former. The data also show that protamine, which counteracts the effect of heparin physiologically, gives a highly positive zeta-potential which becomes negative on the addition of heparin.

TABLE VIII
Effect of other anticoagulants on the zeta-potentials

Additive	Zeta-potential (mv.)			
	pH	Vitreous silica capillary	pH	Silicone-coated capillary
Amt./100 ml. phosphate				
5 mg. fibrinogen	6.7	-20	7.1	-24
5 mg. heparin	6.7	-64	6.6	-44
5 mg. heparin +5 mg. fibrinogen	6.6	-37	6.8	-40
5 mg. paritol	6.7	-63	6.7	-54
5 mg. paritol +5 mg. fibrinogen	6.7	-32	6.7	-29
5 mg. "phosphatide inhibitor"	6.7	-69	6.8	-85
5 mg. "phosphatide inhibitor" +5 mg. fibrinogen	6.7	-39	6.7	-53
5 ml. 1,5-pentanediol*	4.2	-11	4.2	-14
5 ml. 1,5-pentanediol* + 0.03 ml. blood	4.4	+18	4.4	+19

* 100 ml. of 0.001 N KCl solution was used in place of the 100 ml. of phosphate solution. It is important to note that the 1,5-pentanediol-blood solution was measured at pH 4.4, at which pH blood itself gives a zeta-potential of about +20 mv.

TABLE IX

Effect of heparin on the zeta-potentials of other blood-clotting substances

Additive	Zeta-potential (mv.)			
	pH	Vitreous silica capillary	pH	Silicone-coated capillary
Amt./100 ml. phosphate				
5 mg. thrombin	6.7	-39	5.8	-20
5 mg. thrombin +5 mg. heparin	6.7	-44	6.4	-45
5 mg. thromboplastin	6.7	-29	6.7	-28
5 mg. thromboplastin +5 mg. heparin	6.6	-56	6.6	-35
5 mg. protamine	7.0	+30	6.6	+32
5 mg. protamine +5 mg. heparin	7.0	-46	7.0	-44
5 mg. heparin	6.7	-64	6.6	-44

DISCUSSION

No striking difference in zeta-potentials appears when a very dilute solution of blood is streamed through vitreous silica and silicone-coated capillary tubes. Apparently the same protein (s) coats both of these surfaces equally well, and the zeta-potential does not offer a clear explanation of the different clotting times of blood in glass and silicone. However, it is possible that the pH of 6.9 used here might be in the critical region where the surface coatings become identical; for the work of Moyer and Moyer (20) on the electric mobilities of quartz and collodion particles in human serum indicate that the two types of particles take on different protein coatings at higher pH values, but that these mobilities become identical under conditions of lower pH. It is also possible that different results would be obtained if more concentrated solutions were used, since it is known that the differences in clotting times of blood in glass and silicone tubes becomes less marked as the blood is diluted. Work is now in progress to determine whether a higher pH and higher protein concentration will give significant differences in the zeta-potentials of glass and silicone surfaces.

Similarly the zeta-potentials of oxalated plasma in vitreous silica and silicone-coated capillaries were practically identical. At the present time it is not understood why the native plasma (no oxalate) gives a slightly more negative potential than the oxalated plasma; this difference is considered not to be due to a change in ionic strength, and it is possible that the addition of oxalate ion or the removal of calcium ions may change the zeta-po-

tentials. The zeta-potential of the non-oxalated serum likewise is more negative than that of the parent plasma, a change which is apparently brought about by the removal of fibrinogen.

In view of the fact that the whole blood gave a lower zeta-potential (-6 mv.) than the plasma (-10 mv.) it was felt that this difference must be due to the red cells or to a product(s) formed by lysis of them, probably hemoglobin. The zeta-potential of a very dilute solution of red cells, which had been washed free of plasma with physiological saline, was -11 mv., which is compatible with the isoelectric point of 6.78 for hemoglobin (21). Thus, it seems that the capillary surface is influenced by a factor in the whole blood resulting in a lower zeta-potential (-6 mv.), and that this factor disappears when the washed red cells and plasma are measured separately. The possibility remains, however, that the present apparatus may not be sensitive enough to detect significant differences in zeta-potentials at these relatively low values.

At present it is not known definitely which of proteins in the plasma coats the capillary surface and determines the zeta-potential. However, since Fraction III-1 (C155) of bovine plasma (88% beta-globulin) gives a zeta-potential of -14 mv. and bovine fibrinogen produces a potential of -16 mv., it is quite likely that either beta-globulin or fibrinogen are the essential surface-coating proteins, since the zeta-potential of native, human plasma (no oxalate) is -14 mv. The synthetic mixture of equal amounts of fibrinogen and crystalline albumin likewise indicate that fibrinogen has a greater affinity for the capillary wall than does albumin. These results do not agree with those of Moyer and Gorin (22) who investigated serum proteins in phosphate buffers, pH 7.6, and found that when quartz particles were placed in serum they became coated with albumin, whereas collodion particles preferentially adsorbed globulins. As mentioned previously, it is possible that greater differences between silica and silicone capillaries would become evident at a pH as high as 7.6.

Perhaps the most striking feature of this work is the manner in which heparin affects the zeta-potentials of components involved in blood-clotting systems. In general, heparin causes the zeta-potentials of very dilute solutions of whole blood,

plasma, serum, gamma-globulins, beta-globulins, fibrinogen, thrombin, and thromboplastin to become more negative. Heparin has little or no effect on bovine crystalline albumin or on bovine Fraction IV (C143) which contains 55% albumin, 25% alpha-globulins and 20% beta-globulins. These increases in the zeta-potential are considered not to be due to a change in ionic strength brought about by the addition of heparin, but more to the formation of a complex of the plasma component with heparin, thus substantiating the electrophoretic work of Chargaff (8). Fischer (23) has shown that heparin shifts the isoelectric point of some proteins to the acid side of the normal isoelectric pH. This shift would undoubtedly cause the zeta-potential to become more negative, but the reason for the shift is probably due to the formation of a heparin complex. If the heparin were desorbing the protein from the surface, one would expect a high negative potential corresponding to that of heparin alone; however, the zeta-potentials were intermediate between those of the plasma or plasma component and the heparin, with the exception of the crystalline albumin. Additional evidence for the heparin complex is furnished by the precipitate formed when protamine and heparin are mixed in suitable concentrations, which parallels the change of the positive zeta-potential for protamine to a negative one after the addition of heparin.

Of interest also is the similar action of two other anticoagulants, namely, paritol and "phosphatide inhibitor," which likewise cause the zeta-potential of fibrinogen to become more negative, presumably by complex formation. However, 1,5-pentanediol, which inhibits the action of thrombin on fibrinogen probably has no effect on the zeta-potential of blood.

SUMMARY

The application of zeta-potential measurements to study factors contained in the blood or affecting the clotting mechanism has been undertaken.

The zeta-potentials of very dilute solutions of blood, serum, plasma, plasma fractions, and three anticoagulants were determined by measuring the streaming potentials of the solutions in vitreous silica and silicone-coated capillaries.

The zeta-potentials for the protein solutions in vitreous silica and silicone were practically identi-

cal at pH 6.9, but it is possible that differences would appear at higher pH values.

A serum gave a more negative zeta-potential than its parent native plasma (no anticoagulant), which in turn was more negative than an oxalated plasma.

The capillary surface appeared to be influenced by a factor in the whole blood resulting in a lower zeta-potential than that obtained when the washed red cells and plasma were measured separately.

A comparison of the zeta-potential of the plasma with zeta-potentials of plasma fractions indicates that either the beta-globulins or fibrinogen or both were the essential surface-coating proteins in plasma.

In general, heparin caused the zeta-potentials of whole blood, serum, plasma, gamma-globulins, beta-globulins, fibrinogen, thrombin and thromboplastin to become more negative but had no effect on bovine crystalline albumin. These results are interpreted in terms of a heparin complex.

Two other anticoagulants, paritol and "phosphate inhibitor," made the zeta-potential of fibrinogen more negative in a manner similar to heparin, whereas 1,5-pentanediol, which inhibits the actions of thrombin on fibrinogen, probably had no effect on the zeta-potential of blood.

BIBLIOGRAPHY

1. Freund, E., as reported by Bordet, J., and Gengow, O., *Recherches sur la coagulation du sang*. Ann. de l'Inst. Pasteur, 1903, 17, 822.
2. Johlin, J. M., Interfacial adsorption as a factor in the clotting of blood plasma. *J. Biol. Chem.*, 1929, 81, 99.
3. Hirschboeck, J. S., Delayed blood coagulation and absence of clot retraction in collodion lined vessels. *Proc. Soc. Exper. Biol. & Med.*, 1940, 45, 122.
4. Jaques, L. B., Fidler, E., Feldsted, E. T., and Macdonald, A. G., Silicones and blood coagulation. *Canad. M. A. J.*, 1946, 55, 26.
5. Margulies, H., and Barker, N. W., The coagulation time of blood in silicone tubes in patients receiving dicumarol. *Am. J. M. Sc.*, 1949, 218, 52.
6. Gortner, R. A., and Briggs, D. R., Glass surfaces versus paraffin surfaces in blood-clotting phenomena; hypothesis. *Proc. Soc. Exper. Biol. & Med.*, 1928, 25, 820.
7. Lozner, E. L., and Taylor, F. H. L., The effect of foreign surfaces on blood coagulation. *J. Clin. Invest.*, 1942, 21, 241.
8. Chargaff, E., Ziff, M., and Moore, D. H., Studies on the chemistry of blood coagulation. XII. An electrophoretic study of the effect of anticoagulants on human plasma proteins, with remarks on the separation of the heparin complement. *J. Biol. Chem.*, 1941, 139, 383.
9. Wolfrom, M. L., and Rice, F. A. H., Electrophoretic resolution of heparin and related polysaccharides. *J. Am. Chem. Soc.*, 1947, 69, 2918.
10. Abramson, H. A., *Electrokinetic Phenomena and Their Application to Biology and Medicine*. The Chemical Catalogue Company, New York, N. Y., 1934, Chaps. II and IV.
11. Bull, H. B., and Gortner, R. A., Studies on electrokinetic potentials. VII. The temperature coefficient of the ζ -potential. *J. Phys. Chem.*, 1931, 35, 456.
12. Jones, G., and Wood, L. A., The measurement of potentials at the interface between vitreous silica and solutions of potassium chloride by the streaming-potential method. *J. Chem. Phys.*, 1945, 13, 106.
13. Bull, H. B., and Gortner, R. A., Studies on electrokinetic potentials. VI. Electrical phenomena at interfaces. *J. Phys. Chem.*, 1931, 35, 309.
14. Moyer, L. S., Electrokinetic aspects of surface chemistry. VIII. The composition of the surface film on the fat droplets in cream. *J. Biol. Chem.*, 1940, 133, 29.
15. Alberty, R. A., A study of the variation of the average isoelectric points of several plasma proteins with ionic strength. *J. Phys. Colloid Chem.*, 1949, 53, 114.
16. Stenhagen, E., Electrophoresis of human blood plasma. Electrophoretic properties of fibrinogen. *Biochem. J.*, 1938, 32, 714.
17. Sorenson, C. W., Seifter, J., and Wright, I. S., A new synthetic anticoagulant (heparinoid): preliminary report of its actions in humans. *Bull. New York Acad. Med.*, 1949, 24, 448.
18. Overman, R. S., and Wright, I. S., A new blood clotting inhibitor. *J. Biol. Chem.*, 1948, 174, 759.
19. Ferry, J. D., and Morrison, P. R., Preparation and properties of serum and plasma proteins. VIII. The conversion of fibrinogen to fibrin under various conditions. *J. Am. Chem. Soc.*, 1947, 69, 388.
20. Moyer, L. S., and Moyer, E. Z., Electrokinetic aspects of surface chemistry. VII. The electrophoretic behavior of microscopic particles in the presence of horse, human, or rabbit serum. *J. Biol. Chem.*, 1940, 132, 373.
21. Biörck, Gunnar, On myoglobin and its occurrence in man. *Acta. med. Scandinav.*, 1949, Suppl. 226, 34.
22. Moyer, L. S., and Gorin, M. H., Electrokinetic aspects of surface chemistry. IX. The electric mobilities of quartz and collodion particles in mixtures of horse serum and serum proteins in relation to the mechanism of film formation. *J. Biol. Chem.*, 1940, 133, 605.
23. Fischer, A., Antiprotrombin und Globuline. *Biochem. Ztschr.*, 1932, 244, 464. Also Schmitz, A., and Fischer, A., Ueber die Wirkung von Heparin auf Serumalbumin. *Ibid.*, 1933, 259, 53.