Surface Potentials of Protein Solutions*

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Film potentials on protein solutions both at air-solution and at *n*-octadecane–solution surfaces have been measured with a polonium air electrode. The rapid-flow technique has been used to investigate the film potentials at the air-solution surface as a function of the age of the surface. The film potentials of quiescent solutions are marked functions of the *p*H of the solution, emphasizing the contribution of the ionic double layer, and the potentials of the isoelectric films of all the proteins are approximately the same. The initial rate of change of the potential with time is markedly different for the various proteins. Whereas the rate of change of the potential of bovine serum albumin films appears to be diffusion controlled, this is not true of the films of other proteins, for which rate of adsorption and rate of surface spreading are rate determining.

Protein molecules in solution accumulate at surfaces and interfaces to form distinct films characterized by many interesting features. The extent of adsorption on glass (Bull, 1957) and at hydrocarbon-water interfaces (Ghosh and Bull, 1962) has been measured by direct analytical procedures. The ellipsometer (Trurnit, 1954) has been employed to study the rate and extent of adsorption of chymotrypsin on treated metal slides. At very low protein concentrations, the adsorbed films have the characteristics of spread monolayers of surface-denatured protein; with increasing protein concentration, the thickness of the surface films progressively increases to a limiting value which approaches that to be expected of a layer of native molecules.

The present paper deals with the change in electrostatic potentials at *n*-octadecane–water interfaces and at air-solution surfaces resulting from the formation of films of adsorbed protein. Both static and flowing surfaces have been investigated along with the influence of *p*H and other factors on the potentials.

EXPERIMENTAL

Proteins having a wide range of isoelectric points were used. Crystalline porcine pepsin was obtained from Armour Laboratories and used without further purification. Bovine serum albumin was also from Armour; solutions of this protein were passed through mixed ion-exchange resin columns before use. Egg albumin from hens' eggs was prepared by the method of Kekwick and Cannan (1936) followed by exhaustive dialysis. Fresh bovine erythrocytes were washed with physiological saline, hemolyzed with toluene, and centrifuged. The hemoglobin (HbO_2) was crystallized and recrystallized twice by the addition of cold ethanol in the cold followed by dialysis against water. Ribonuclease was from Armour and used without further purification. Salmine was from Delta Chemical Works.

The surface potential was measured by use of a silver disk about 1 cm in diameter upon which a small amount of polonium had been deposited (Canadian Radium and Uranium Corp., N. Y.). The buffer solution with or without protein was introduced into a 250-ml beaker and the surface of the solution cleaned with a small nozzle attached to a vacuum line. The beaker was placed in a screened grounded cage, a Ag AgCl wire electrode was dipped into the solution, and the polonium electrode was lowered to about 1 mm above the solution surface. Both electrodes were manipulated from outside the screened cage. The potential was measured on the sensitive scale of a Beckman Model GS *p*H meter. The observed potential was independent of the distance between the surface and the electrode provided the distance did not exceed about 3 mm. Readings of the potential could be made to less than 1 mv; the reproducibility of the potential readings was about 5 mv. The difference between the potential observed at surfaces with and without protein was taken as the potential change (ΔV) due to the adsorbed film of protein. Experiments were also done with a thin layer of liquid *n*-octadecane at the surface of the protein solutions to obtain the ΔV at this interface. Both with the air-solution surface and with the *n*-octadecane solution interface, the addition of protein rendered the air electrode more positive. This means that the dipoles across the protein film are orientated with their positive poles directed away from the solution. Experiments on air-solution surfaces were conducted at room temperature (25–26°). Those involving *n*-octadecane were done at temperatures slightly above the m.p. of this substance (28.17°).

Readings of the potential of the static surface could be taken after one minute, but it seemed desirable to obtain information on freshly formed surfaces, and to this end a rapid-flow technique was used; this apparatus is diagrammed in Figure 1. The method is essentially the same as the channel method of Posner and Alexander (1949) with several modifications. The protein solution was allowed to flow out of a slit in a tube along a lucite channel which was 0.1 cm deep, 2 cm wide, and 15 cm long. The flow rate was regulated by the supply of solution to the tube, which in turn was regulated by a constant pressure head. A reference Ag.AgCl electrode dipped into the solution in the tube and the polonium electrode was placed about 1 mm above the flowing surface. The entire flow system was placed inside a grounded screened cage, and a well-shielded lead for the air electrode was necessary for steady readings. The air electrode could be moved

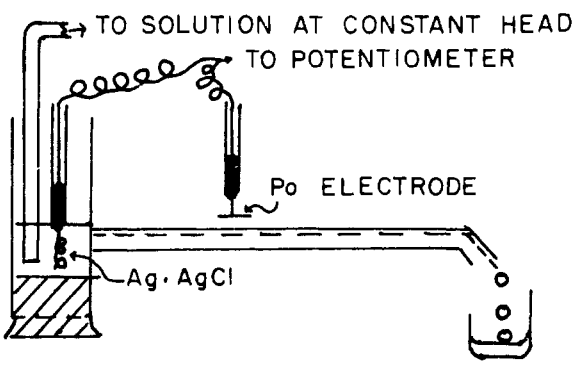

FIG. 1.—Rapid-flow apparatus for surface potentials.

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along the flow channel and the age of the surface estimated from the rate of flow. The rate of movement of the surface was obtained from observations of talc sprinkled on the moving surface; it was noted that there was no turbulence in the motion of the surface. The buffers were acetate in the acidic range and Tris above *p*H 6. The ionic strength was 0.05.

Results

Potential readings on quiescent solutions in beakers, as noted above, could be taken after about one minute of the formation of the surface and changes in potential followed after this interval. At the air-solution surface and for the more concentrated protein solutions, the greatest part of the potential change occurred within one minute after formation of the surface. Thus on 0.06% protein solutions, slow changes in the potential were observed with pepsin solutions (about 14 mv in 10 minutes), HbO_2 solutions at *p*H 4.15 (6 mv in 10 minutes), ribonuclease solutions (about 25 mv in 10 minutes), and salmine solutions (about 40 mv in 10 minutes at *p*H 5.87). At the same concentration no change in ΔV could be detected for films of bovine serum albumin or egg albumin beyond one minute. There was also little change in ΔV with these two proteins at the liquid *n*-octadecane–solution interface with time. The static values of ΔV for 0.06% protein solutions as a function of *p*H are plotted in Figure 2 for the air-solution surfaces and in Figure 3 for *n*-octadecane–solution interfaces.

The influence of HbO_2 concentration on the static values of ΔV at *p*H 5.9, 6.6 and 8.1 is shown in Figure 4. It will be observed that the ΔV reaches a high value at relatively low protein concentration (about 0.001%). At concentrations greater than this there were only slight increases.

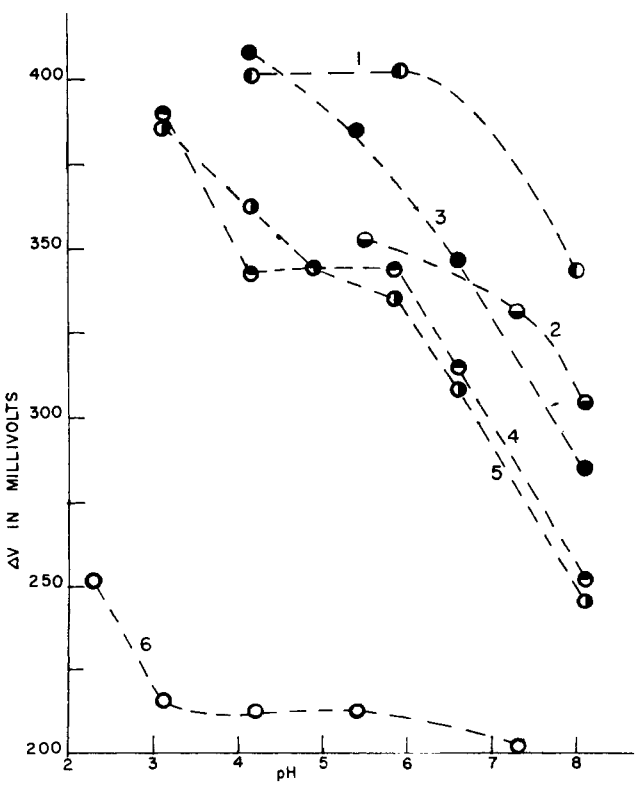

FIG. 2.—Static potentials at air-solution surfaces as a function of *p*H. Protein, 0.06%. Ionic strength 0.05. Curve 1, salmine; curve 2, ribonuclease; curve 3, HbO_2; curve 4, egg albumin; curve 5, bovine serum albumin; curve 6, pepsin.

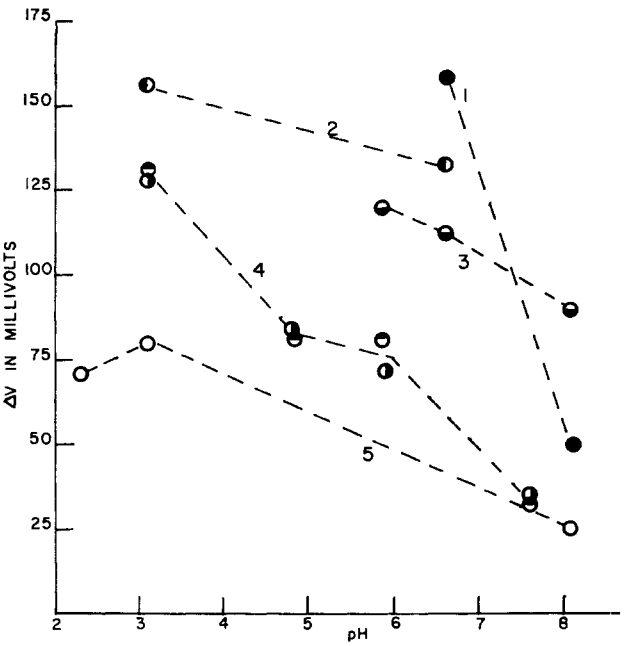

FIG. 3.—Static potentials at liquid *n*-octadecane–solution interfaces as a function of *p*H. Protein, 0.06%. Ionic strength 0.05. Curve 1, ribonuclease; curve 2, salmine; curve 3, HbO_2; curve 4, egg albumin and bovine serum albumin; curve 5, pepsin.

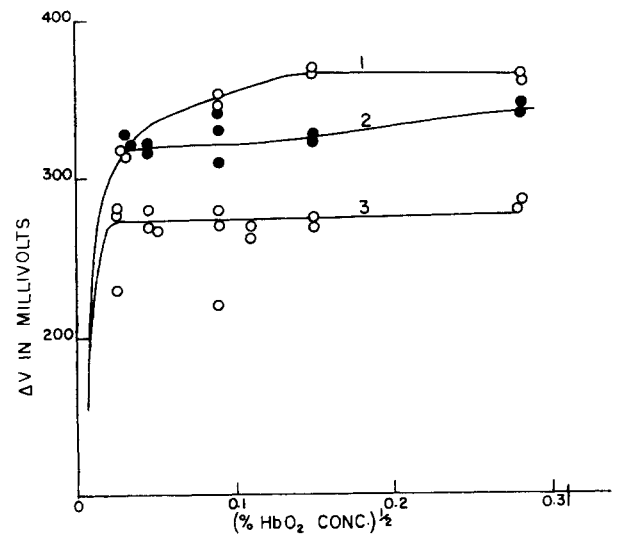

FIG. 4.—Static surface potentials at air-solution surfaces as a function of the square root of the HbO_2 concentration. Ionic strength 0.05. Curve 1, *p*H 5.87; curve 2, *p*H 6.59; curve 3, *p*H 8.08.

Figure 5 shows the static ΔV values for unbuffered solutions of isoionic egg albumin as a function of protein concentrations.

Table I shows the initial rate of change of ΔV with time as measured in the rapid flow apparatus.

Figure 6 shows the change of ΔV with time in the rapid-flow apparatus as exhibited by air-solution surfaces of egg albumin. It will be noted that the ΔV time curves for 0.0080 and 0.016% egg albumin solutions are S-shaped.

The flow pattern of the talc on the surface showed gelling of the egg albumin film, with a marked retardation of the flow rate of the surface for films whose ΔV approached 250 mv, and it is difficult to estimate the surface age beyond this point owing to this irregularity.

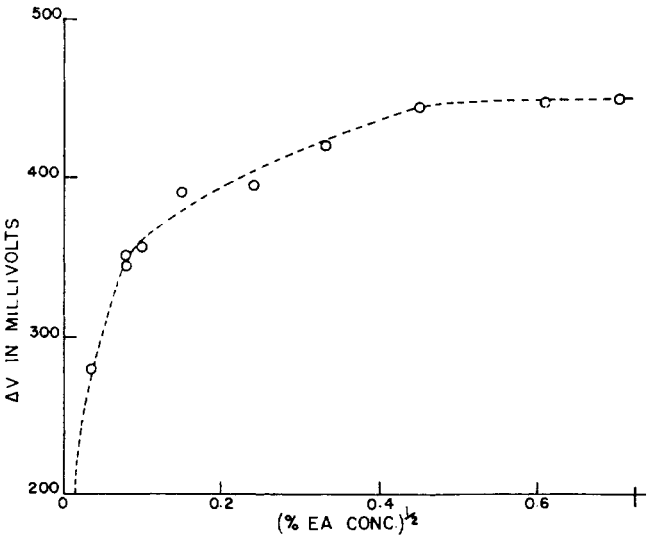

FIG. 5.—Static values of ΔV at an air-solution surface as a function of the square root of the egg albumin concentration. Unbuffered solutions.

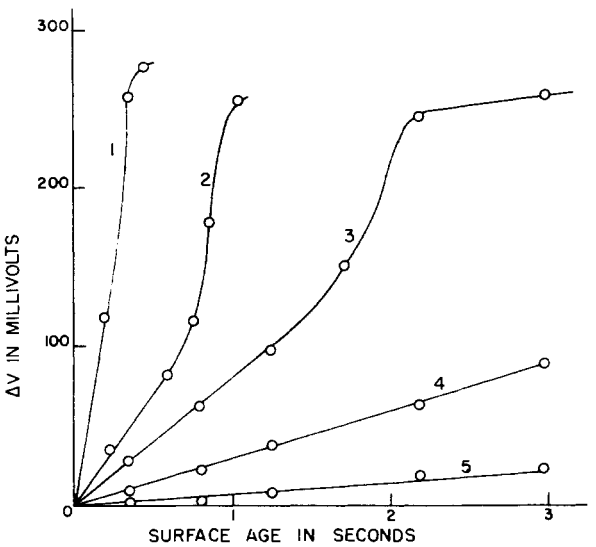

FIG. 6.—Variation of ΔV as a function of the age of the air-solution surface of egg albumin solutions. Isoionic egg albumin in 0.001 M NaCl. Egg albumin concentrations: curve 1, 0.05%; curve 2, 0.016%; curve 3, 0.008%; curve 4, 0.004%; curve 5, 0.001%.

TABLE I

INITIAL RATES OF CHANGE FOR 0.001% PROTEIN SOLUTIONS IN 0.05 IONIC STRENGTH BUFFERS (EXCEPT EGG ALBUMIN)

Solute	*p*H	ΔV (mv/sec.)
Bovine serum albumin	4–6	20
Egg albumin	Dist. H_2O as well as 0.001 M NaCl	10
HbO_2	6.6	4
HbO_2	4.1	15
Ribonuclease	7.9	1.5
Ribonuclease (oxidized)	7.9	3.5
Catalase	4.1	0.15
Poly-L-glutamic acid	7.3	0.011
Poly-L-glutamic acid	4.4	0.008

Measurements of ΔV as a function of time were made on a quiescent air-solution surface of a 0.001% egg albumin solution over more extended periods, and these results are shown in Figure 7. Here again an S-shaped curve between ΔV and time was found even for this low protein concentration.

Figure 8 shows the surface potential of bovine serum albumin films at the air-solution surface for several protein concentrations as functions of the age of the surface. Acetate buffers of *p*H 3.87, 4.87, and 5.90, all at an ionic strength of 0.05, were used.

It will be observed that there is a very rapid change of the surface potential with time for a 0.016% protein solution at a freshly formed surface followed by a much lower rate of change after about a half second. The observed ΔV of about 250 mv after 2 seconds is much lower than the equilibrium value of about 350 mv on a static surface. It is also to be noted that the observed potentials at a given bovine serum albumin concentration are independent of *p*H in the range reported in

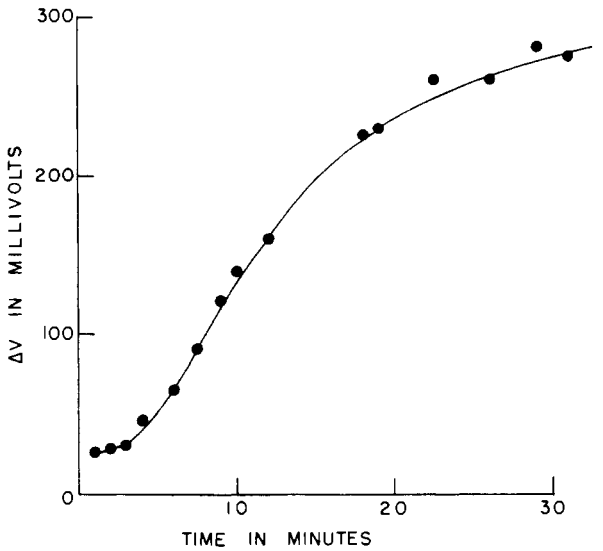

FIG. 7.—Variation of ΔV as a function of age of a quiescent air-solution surface of a 0.001% egg albumin isoionic solution in 0.001 M NaCl.

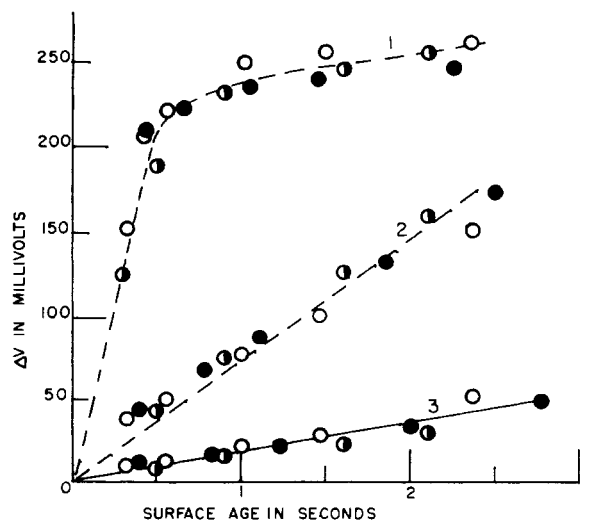

FIG. 8.—Surface potentials at air-solution surfaces of bovine serum albumin solutions as function of age of surface. Bovine serum albumin concentrations: curve 1, 0.016%; curve 2, 0.004%; curve 3, 0.001%. Open circles *p*H 5.9; half-filled circles *p*H 3.87; filled circles *p*H 4.87.

Figure 8, although Figure 2 shows the static potentials for this protein and in this *p*H range to be a fairly critical function of *p*H.

Discussion

It has been found that a monolayer of egg albumin spread on pure water by the technique of Trurnit (1960) has a potential of about 280 mv at the point of minimum compressibility; this corresponds to a film pressure of about 10 dynes per cm. This value for ΔV is reached by a solution of egg albumin at very low protein concentration (see Fig. 5). The rise in ΔV beyond this value at higher protein concentrations probably reflects the accumulation at the surface of the solution of protein in excess of that achieved by surface spreading. Bull (1938) observed, using the rotating-drum technique, that up to a concentration of 0.10% egg albumin the amount of protein adsorbed at the air-solution surface corresponded to that expected for a spread monolayer of protein. Greater adsorption was obtained at higher protein concentrations. In the present experiment, however, potentials in excess of that for a spread monolayer are achieved at concentrations significantly less than 0.10% protein. Perhaps this divergence in the results obtained by the two techniques is due to the fact that in the present experiments the surface of the solution is quiescent. We consider it likely that the rapid increase of ΔV as a function of time for egg albumin solution surfaces (Figs. 6 and 7) above about 100 mv is due to the interaction of the egg albumin molecules with each other at the surface with a consequent change in the orientation of the side chains of this protein.

The marked influence of *p*H on the static values of ΔV (Figs. 2 and 3) indicate an important contribution of the electrical double layer in the solution below the surface to ΔV. The role of the double layer in the phase potentials of proteins spread on buffers of various compositions and *p*H values has been commented on by Glazer and Dogan (1953). Although the shapes of ΔV-*p*H curves for spread monolayers resemble those shown in Figure 2 of this paper, Glazer and Dogan report significantly lower potentials from *p*H 5 to 8 than we have found for films on protein solutions. It should also be mentioned that their buffer systems differed from our own.

At the same *p*H, the ΔV values are larger, the higher the isoelectric points of the proteins, both for the air-solution surface and for the *n*-octadecane–solution interface. If the potentials are compared at the respective isoelectric points of the proteins, it turns out that the potential at the air-solution surface is about 350 mv and at the *n*-octadecane–solution interface about 85 mv; at the isoelectric point there is no double layer. The role of the double layer potential can also be estimated by comparing the phase potentials at the *n*-octadecane–solution interface with the ζ potentials of *n*-octadecane emulsion particles covered with protein. The ζ potentials have been calculated from the electrophoretic mobilities of such particles; the mobilities were measured microelectrophoretically (Chattoraj and Bull, 1959). These results are shown in Table II.

If the film is assumed to be built up of n dipoles per unit area μ the vertical component of each dipole then equation (1) holds (Davies and Rideal, 1961), where

$$\Delta V = 4\pi n\mu + \psi_0 \qquad (1)$$

ψ_0 is the potential of the ion atmosphere and is a function of the number of molecules adsorbed in the film as well as of the ionic strength. For low values of ζ, ψ_0 is nearly equal to ζ (Haydon, 1960). The constancy

Table II

Comparison of the Phase Potential ΔV with the ζ Potential

Both expressed in ordinary millivolts of 0.06% protein solutions in contact with *n*-octadecane, 0.05 ionic strength.

Protein	*p*H	ΔV	ζ	$\Delta V - \zeta$
Bovine serum albumin	3.45	125	+38.0	87.0
	4.30	105	+19.3	85.7
	4.90	85	0	85.0
	5.90	80	−12.5	92.5
	6.35	55	−17.5	82.5
	7.17	47	−23.0	70.0
Pepsin	2.40	75	−10.0	85.0
	4.50	68	−13.0	81.0
HbO_2	6.0	120	+12.8	107.2
	7.0	107	− 3.8	110.8
	8.0	90	−22.4	112.4

of (ΔV-ζ) (see Table II) suggests that the protein films have similar structures in the *p*H range studied. The potential of the protein film without the double layer potential (isoelectric films) no doubt arises from a reorientation of the water molecules at the surface as well as from electrical vectors in the protein. Without more knowledge of the structure of protein films than is now available, it is not possible to interpret either the sign of the film potential or its magnitude. Davies (1954) has offered some suggestions regarding the relationship between the structure of spread protein films and phase potentials, but to what extent these ideas can be applied to the present study is unclear.

Trurnit (1954) has discussed the equations for the diffusion of protein molecules to a plane surface with and without stirring of the solution. For the sake of clarity these equations are presented here. With stirring of the bulk solution equation (2) holds, where

$$n = \frac{DCt}{\delta} \qquad (2)$$

n is the number of molecules adsorbed per unit area, D is the diffusion coefficient of the molecules being adsorbed, C is the concentration in moles per cubic centimeter, t is the time in seconds, and δ is a small distance from the surface through which the concentration is not constant. For an unstirred solution equation (3) holds. For small values of the double layer po-

$$n = 2C\left(\frac{Dt}{\pi}\right)^{1/2} \qquad (3)$$

tential equation (4) is applicable, where κ is the

$$\psi_0 = \frac{4\pi nz}{D\kappa} \qquad (4)$$

Debye-Huckel reciprocal distance and z is the net charge on the protein molecules. Substituting equation (4) into equation (1) yields equation (5), or, at a

$$\Delta V = 4\pi n\mu + \frac{4\pi nz}{D\kappa} \qquad (5)$$

given charge and ionic strength, equation (6). Com-

$$\Delta V = \text{const} \times n \qquad (6)$$

bining equations (6) and (2) gives equation (7).

$$\Delta V = \text{const } C{\cdot}t \qquad (7)$$

The lineality of ΔV with time as exhibited by bovine serum albumin films (see Fig. 8) shows that the system

in the flow channel is perhaps equivalent to a stirred system, the movement of the solution underlying the surface affecting the concentration of the surface layer.

A consequence of equation (7) is that plots of ΔV at selected surface ages against the protein concentration should be linear. Such plots are shown in Figure 9 for air-solution surfaces of bovine serum albumin solutions at *p*H 4.87 and ionic strength of 0.05.

The linearity of the plots in Figure 9 indicates that for bovine serum albumin diffusion continues to govern the extent of adsorption until the surface has adsorbed sufficient protein to yield a potential of about 250 mv.

The times in seconds for a surface of bovine serum albumin solutions to reach 250 mv at different protein concentrations are shown in Table III, and as anticipated from equation (7), the product $C \cdot t$ is nearly constant for different protein concentrations.

TABLE III

TIME REQUIRED TO REACH 250 MV SURFACE POTENTIAL AT DIFFERENT CONCENTRATIONS OF BOVINE SERUM ALBUMIN (BSA)

*p*H 4.87. Ionic strength 0.05.

% BSA × 10^3 (C)	Time (sec) (t)	$C \cdot t$
5.0	2.6	0.0130
6.0	2.1	0.0126
8.2	1.6	0.0131
11.2	1.1	0.0123

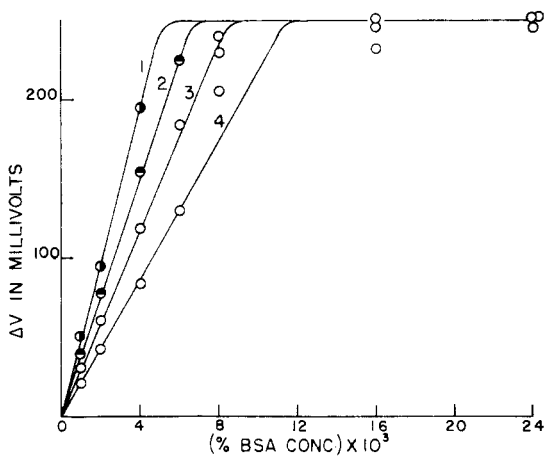

FIG. 9.—ΔV as a function of bovine serum albumin concentration, *p*H 4.87, ionic strength 0.05. Curve 1, 2.6 seconds; curve 2, 2.1 seconds; curve 3, 1.6 seconds; curve 4, 1.1 seconds.

The process of surface denaturation of proteins in solution may be divided into three steps: (1) diffusion of the protein to the surface; (2) adsorption of the protein on the surface; and (3) unfolding of the protein molecules on the surface. For bovine serum albumin, it appears that the first step is rate determining; however, for the other proteins the rate of change of ΔV with time appears to be independent of diffusion coefficient. Thus ribonuclease and catalase, which have the largest and smallest diffusion coefficients, respectively, of the proteins examined, are both characterized by slow changes of ΔV with time; the equilibrium values of ΔV for both of these proteins were in the same range as for the other proteins. The slow change of ΔV with time probably means a slower adsorption or a slower unfolding or both. The contrast between the behavior of ribonuclease and oxidized ribonuclease illustrates the influence of a change in molecular structure on the rate of change of ΔV with time. The influence of molecular structure is further substantiated by the considerable differences in the initial rate of change of ΔV with time between *p*H 4.1 and 6.6 for hemoglobin solutions; in the acid region this protein dissociates into subunits, with the release of heme. Haurowitz *et al.* (1955) have shown the heme groups of hemoglobin to exert a stabilizing influence on this protein at surfaces. The somewhat greater rate of change of ΔV with time at *p*H 7.3 as compared with that at *p*H 4.4 for poly-L-glutamic acid could be related to the transformation of this polypeptide from the helical configuration at *p*H 4.5 to a random coil as the *p*H is raised (Idelson and Blout, 1958).

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