Adsorptions of Purified Serum Proteins on Metalized Glass Slides and Their Significance for the Immunoelectroadsorption Method

CHRISTIAN MATHOT AND ALEXANDRE ROTHEN

The Rockefeller University, New York, New York, 10021

Received February 13, 1969

Human gamma globulins can be adsorbed from solution onto chromium plated slides. The rate of adsorption depends on the diffusion of this protein through a layer of immobilized water adjacent to the metal surface. Multilayers of human gamma globulins can be adsorbed under the influence of an electrical current (300μA for 30 sec). The building up of such multilayers is probably due in part to hydrophobic or hydrogen bonding or both. Implications regarding the immunoelectroadsorption test are reviewed.

INTRODUCTION

While the adsorption of gases or small molecules on solids has been extensively studied (1), there has been only scant interest in the adsorption of macromolecules until the late 1930's. Even much less work has been done to investigate the reactivity of such adsorbed layers. This lack of interest was possibly due in part to the fact that no instrument was available which could measure very thin films from a few Angströms up to about 400 Å with a suitable accuracy. With the development of the ellipsometer in the middle forties (2–4) a number of scientists began to study adsorbed films of proteins on solid surfaces and their reactions with antibodies (4–6) and enzymes (4, 7–14).

Recently a new method based on the measurement of the thickness of films adsorbed under the influence of an electric current has been developed in our laboratory to determine immunological reactions. This method has been named Immunoelectroadsorption (IEA) (15). It consists in electroadsorbing on a metalized glass slide first a layer from a carrier solution (usually 2% serum in water) containing some antigen and then a layer from an immune serum (16). For both adsorptions the slide acts as one of the electrodes and the current is of the order of 300μA. Under these conditions the layer electroadsorbed from an immune serum is thinner when there is no antigen present in the first solution. Thus a simple method, which proved very sensitive, is available for the quantitative assay of immunological reactions. The results obtained with this method (16–20), however, have indicated that more fundamental work on the mechanism of adsorption of protein layers would be needed for a clearer understanding of immunoelectroadsorption. With this in mind, we have made some kinetic studies of the adsorption of purified serum proteins under various conditions, with and without the help of an electric current, and the results obtained are reported in this article.

PREPARATIONS OF SERUM PROTEINS

All the proteins used in this investigation were obtained commercially except the gamma globulins (CM) which were prepared in our laboratories from whole human blood, type A, Rh negative. The isolation of the gamma globulins was made from the plasma by precipitation with 50% saturated

ammonium sulfate (AR) in distilled water (21). The temperature of the solutions was kept at 5°C. The 10th precipitate was redissolved in distilled water to $\frac{1}{20}$ of the original volume of serum. After a few minutes at 2°C, flocculation occurred and the suspension was then centrifuged at 2000*g* for 5 min at 5°C. After this step had been repeated 3 times, the final pellet was redissolved in veronal buffer 0.03*M* pH 8.60 to a final concentration in globulins of 1.054 mg/ml as determined by photometric adsorption at 2790 Å with the absorption coefficient taken as 0.0090 OD units/μg N/ml at 25°C.

The composition of the various fractions was determined by cellulose acetate electrophoresis (Gelman) at pH 8.6 with 0.01*M* veronal buffer using 2.0-μl samples. Electrophoresis was continued for 15 min at 10°C with 500V. Staining was done with Ponceau S. The stained proteins were eluted with 1*N* NaOH and their concentration was read photometrically at 5400 Å. Proteins in concentrations smaller than 5% could not be measured. The results obtained are shown in Table I.

TABLE I

Human protein fractions	Composition
Gamma globulins (I)	γG > 95%
Gamma globulins (CM)	γG = 92% β = 8%
β Globulins	β = 75% γG = 25%
Fraction F-IV	α_1 = 20% α_2 = 30%
	γG = 5% Alb = 45%
Fraction F 1-IV	α_1 = 50% α_2 = 20%
	β = 10% Alb = 20%
Fraction F 4-IV	α_1 = 20% α_2 = 35%
	Alb. = 45%
Albumin	Alb. > 95%
Hemoglobin	Hb > 95%
Fibrinogen	N.A.[a]
Glycoproteins	N.A.
Transferrin	Trf = 95% α_2(?) = 5%

[a] N.A.—Not assayed.

ADSORPTION OF PROTEINS ON METALLIC SURFACES

Langmuir (22) in the middle thirties was one of the first to determine by very simple optical means the thickness of adsorbed layers of large organic molecules on metalized surfaces. Trurnit (10, 11) using a recording ellipsometer studied the kinetics of adsorption of chymotrypsin and γ-globulins. Recently Vroman (23–25) investigated extensively the adsorption of plasma proteins involved in blood clotting, also with the help of a recording ellipsometer. The optical thickness as obtained by ellipsometry can now be expressed, if so desired, in real thickness with the use of computer programs such as those developed by McCrakin (26).

The rate of adsorption of proteins from aqueous solutions onto metallic surfaces are influenced in the main by at least three factors. (*1*) The nature of the metallic surfaces (*2*) the concentration of the solution and (*3*) the rate of diffusion of the protein through an immobilized layer of water adjacent to the slide (27–30).

The influence of the nature of the metal plays an important role in the adsorption although this effect was not investigated by Trurnit. For instance, the thickness adsorbed in 1 min from a solution of rabbit γ-globulins (95% pure), (0.125 mg/ml in veronal buffer 0.015*M*, pH 8.8) was 50 Å on nichrome, 40 Å on rhodium, 80 Å on gold, and very small on copper.[1]

Monomolecular layers of globulins directly adsorbed on a metallic surface adhere so strongly that they cannot be easily stripped off, (12). However when they are deposited on a slide coated with at least three monolayers of fatty acid deposited by the Blodgett–Langmuir method (22), the whole assembly, with the exception of the first deposited fatty acid layer, can easily be mechanically removed from the surface by stripping. Thus it is most probable that globulins adsorbed on slides coated with stearate layers do not penetrate the pile of layers and remain attached to the top layer.

The rate of adsorption of human γ-globulins (CM) without current on a chromium coated (4000Å) glass slide is shown in Fig. 1, where the square of the thickness (in A) is plotted against the time in seconds. In order to slow down the rate of adsorption, the concentration was kept small, 50 μg/ml

[1] The slides were prepared by Evaporated Metal Films Co., Ithaca, New York.

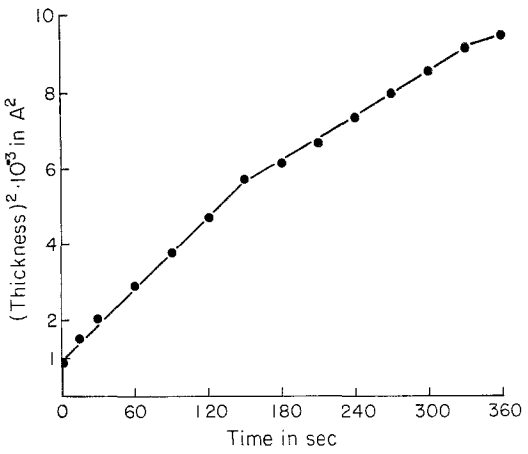

FIG. 1. Adsorption of gamma globulins on a chromium plated glass slide.

in veronal buffer 0.02M, pH 8.8. There was an immediate adsorption of approximately 30 Å, then the rate of adsorption became a linear function of the square root of time (t). After a layer ~75 Å thick had been adsorbed, a break occurred in the curve, but the linear relationship between adsorption and $\sqrt{t}$ remains for thicker adsorbed layers. This discontinuity observed in the adsorption rate could be explained by the completion of a first adsorbed monolayer. The rate of diffusion through an immobilized layer of water adjacent to the slide appears to be the factor controlling the rate of such reactions. Many authors, (10, 11, 31, 32) have attempted to calculate from experimental data, the thickness of this immobilized layer of water. The value found by Trurnit and others, 3.10^5 Å, appears much too large. However, in these calculations the diffusion coefficient in the immobilized layer was assumed equal to the diffusion coefficient as determined from measurements carried out in the bulk of the liquid. This assumption is probably incorrect since most of the physical properties of the liquid layer close to a solid surface are very different from those obtained for the bulk of the liquid itself (28–32) For instance, the viscosity of a film of water 200 Å thick is nearly fifteen times greater than that of water (30) and the rigidity of a film of water 350 Å thick is that of metallic lead (28). The coefficients of diffusion in films of ordered water must be much smaller than in bulk water, in the inverse ratio of the viscosities, and this would tend to reduce the calculated thickness of the immobilized layer of water.

If the rates of adsorption of the protein molecules were not controlled by the diffusion through the immobilized liquid layer, but were determined by the free area left on the slide, one could have expected the rate to be of the first order which is contrary to the experimental facts.

Once the globulin molecules have been adsorbed on the surface they cannot be desorbed, the process is irreversible. A metallic surface upon which a monomolecular layer of globulins has been adsorbed can adsorb more globulin molecules after washing and drying.

ELECTROPRECIPITATION OF γ-GLOBULINS AT A METAL-LIQUID INTERFACE

Figure 2 shows visual changes in concentration at the metal–liquid interface produced by the current. A chromium (4000 Å) coated glass slide was introduced along with a platinum electrode 0.75 mm in diameter in a small Pyrex test tube (8 × 75mm) containing 1.5 ml of a purified rabbit gamma globulins (γG > 95%) solution (3.40 mg/ml) in veronal buffer pH 7.54 0.03M.

The chromium plated slide was connected to the positive pole and the platinum electrode to the negative pole of a constant current power supply. In (A), the current was off and the slide appears clearly through the solution. In (B), the current has been on for 2 min with an intensity of 300 μA. A cloudy layer can be seen to have formed at the interface between the solution and the surface of the slide. Photographs (A) and (B) were taken under identical conditions of light and aperture. In (C), the current was turned off after 3 min and the solution locally disturbed to further visualize the precipitated proteins. These proteins could be centrifuged and isolated but could not be redissolved directly in veronal buffer pH 7.54, 0.03M. It is evident that some change in the tertiary structure of proteins must have been induced by the electroprecipitation. However, this precipitate could easily be dissolved in veronal buffer pH 8.6, 0.03M. This solution could be

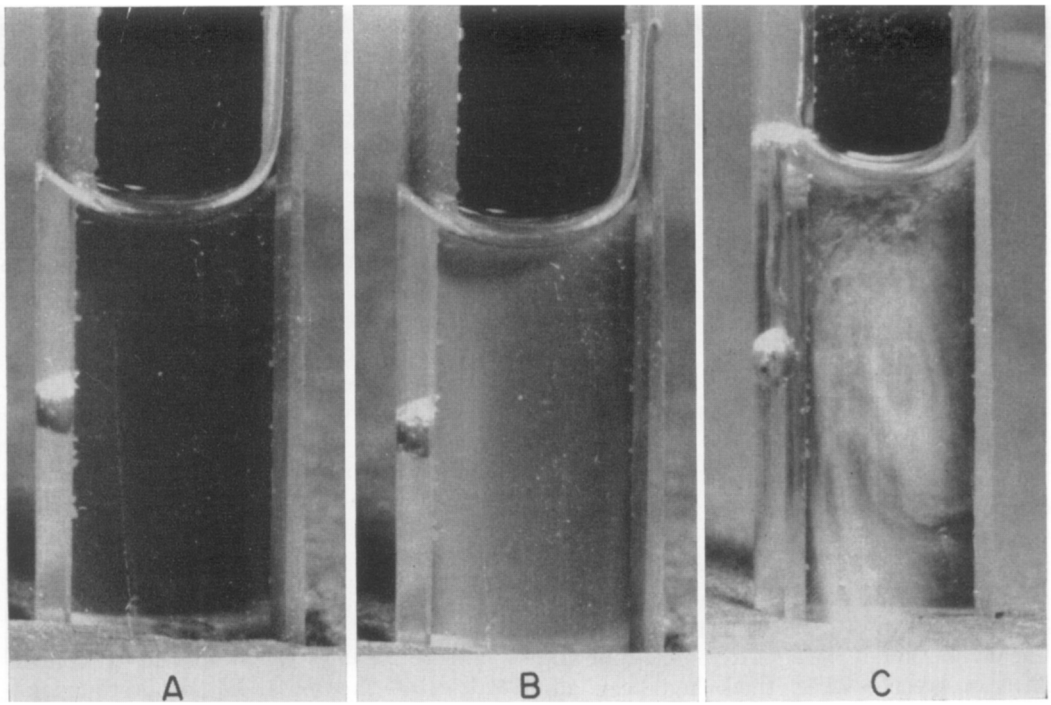

FIG. 2. Electroprecipitation of Human gamma globulins at a chromium interface

brought back to pH 7.6 with 0.1*N* hydrochloric acid and the proteins remained in solution. The nature of the precipitate IgG, IgM or both was not investigated. As one would have expected, no precipitate was formed at the other electrode. It is of interest to note that the protein layer appears to grow from the metal–liquid interface rather than from the bulk of the solution, the concentration gradient being the steepest near the metal surface.

This phenomena of electroprecipitation occurred only with concentrated solutions of gamma globulins (2.0 mg/ml or more) and was not observed with other serum protein fractions at similar concentrations. The thickness of the layer of protein adsorbed on the slide after 3′ at 300 μA was in excess of 400 Å.

ELECTROADSORPTION OF PROTEINS ON METALLIC SURFACES

The electroadsorption of serum proteins was conducted as described above for the electroprecipitation. After 30 sec at 300 μA, the slide was removed from the solution, washed and the thickness of the adsorbed layer measured with an ellipsometer. The reading on this instrument is linear with thickness up to about 300 Å;[2] for larger thicknesses a calibration curve made with transferred barium stearate layers can be used.

1. Electroadsorption of Human Gamma Globulins

Increasing amounts (0–12 mg/ml) of human gamma globulins (Pentex I) were added to 1 ml of normal human serum diluted $\frac{1}{20}$ in veronal buffer pH 7.6, 0.03*M*. The electroadsorption was carried out with 0.5 ml of solution for 30 sec at 300 μA. The results obtained are shown in Fig. 3 where the thickness increment of the adsorbed layer over that observed with the normal serum alone (about 140 Å) is plotted against the $\log_e$ of the amount of γG(I) added. It is apparent that there is a linear relationship between these two parameters from 40 ± 10 μg/ml to over 12,000 μg/ml. No increase in

[2] Scitronics 1500, Rudolph Inst. Eng. Co., Little Falls, New Jersey.

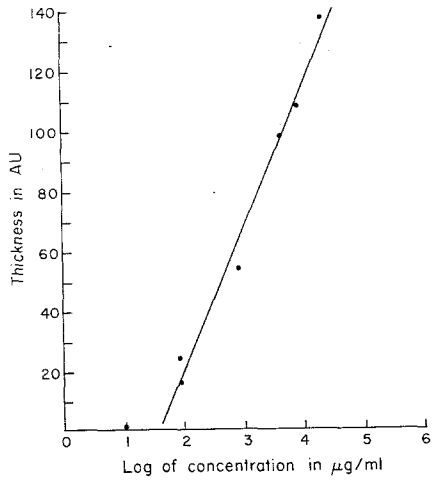

FIG. 3. Electroadsorption from a normal serum diluted $\frac{1}{20}$ in veronal buffer to which increasing amounts of human gamma globulins were added.

thickness was observed for concentrations below 40 μg/ml (intersect of the curve with the x-axis). The average concentration in γG of a normal serum is about 12 mg/ml (33). A serum diluted $\frac{1}{20}$, therefore, contains about 600 μg/ml of γG. Thus if we assume that γG are responsible for most of the adsorption, only concentrations greater than $(600 + 40) \simeq 640$ μg/ml would produce an adsorbed layer thicker than that observed with a normal serum.

To test this assumption, γG(I) solutions in veronal buffer pH 7.6, 0.03M, were prepared. The concentration range studied was 0.0–3.20 mg/ml and the results are shown in Fig. 4. The thickness of the layer adsorbed is constant (50 Å) up to a γG concentration of approximately 0.55 ± 0.05 mg/ml. Beyond 0.6 mg/ml the adsorption follows the same $\log_e$ dependence as the one observed in Fig. 3. It would thus appear that, at least in first approximation, gamma globulins are responsible for the larger part of the adsorption from a serum. The thickness of the layer of proteins electroadsorbed in 30 sec at 300 μA from a normal human serum diluted $\frac{1}{20}$ is approximately 140 Å. Since a gamma globulin monolayer is about 65 Å thick (37), at least two such layers must be adsorbed on the metalized surface. When the total γG concentration in the solution is increased to 12 mg/ml the thickness of the adsorbed layer after 30 sec at 300 μA is approximately 300 Å or almost 5 monolayers.

The resolution of the ellipsometer in the plane of reflection is only of the order of 0.5 mm, therefore, it is not possible to determine directly whether the γG molecules are adsorbed as monomers and form first a complete monolayer before a second layer is adsorbed or whether aggregates are formed around a single γG molecule. Two series of experiments were carried out to attempt to answer this question. In the first series of experiments a solution in 0.03M veronal buffer pH 7.6 containing 1.7 mg/ml of γG (Pentex I) was heated for 30 min at 61 ± 1°C. Under such conditions gamma globulins aggregate rather readily (34, 35). The results are shown in the dotted curve of Fig. 4. It is evident that the thickness of the layer adsorbed (100 Å) is larger than that observed with unheated γG (solid curve) up to a concentration of about 0.9 mg/ml. At higher concentrations no further increase in thickness is observed. It would thus appear that aggregated γG cannot form multilayers. In the second series of experiments an identical solution of γG (Pentex I) was heated in the presence of 50 mg/ml of urea (AR) for 30 min at 61°C. It is known that 6M urea or

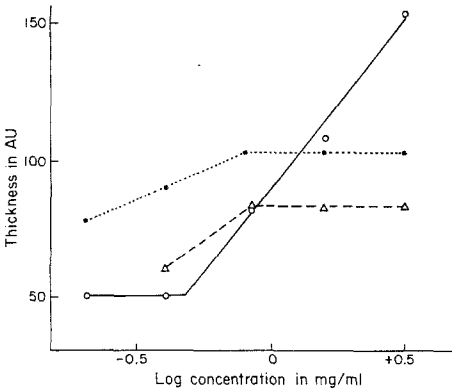

FIG. 4. Thickness of the layer of purified gamma globulins electroadsorbed as a function of the $\log_e$ of the concentration in mg/ml. O——O Native gamma globulins 0.0 to 3.20 mg/ml in veronal buffer 0.03M pH 7.6. ●·····● gamma globulins (1.7 mg/ml) heated for 30 min at 61°C. △ - - - -△ gamma globulins (1.7 mg/ml) heated for 30 min at 61°C in the presence of 50 mg/ml of urea.

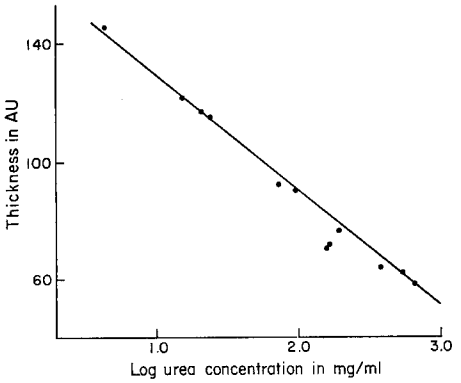

FIG. 5. Electroadsorption from a normal human serum diluted $\frac{1}{20}$ in veronal buffer 0.03M pH 7.6 as a function of the amount of urea added.

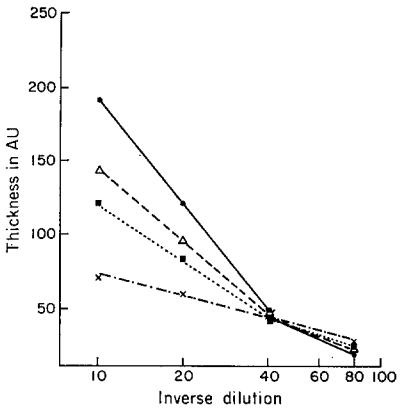

FIG. 6. Electroadsorption from a normal serum as a function of dilution and urea concentration.

3M guanidine HCl (34) will dissociate γG aggregates, probably by disrupting intermolecular hydrogen or hydrophobic bonds or both (HGHP).

The results are shown in the dashed curve of Fig. 4. At a concentration of 0.9 mg/ml, this curve gives an adsorption identical to that of nonheated gamma globulins. Beyonn this concentration no further increase ip thickness of the electroadsorbed layer (75 Å) is noted. These results can be explained if we assume that the building up of multilayers of γG is due to intermolecular HGHP bonds and that urea prevents their occurrence. This interpretation, was tested by adding urea in various amounts (0–100 mg/ml) to normal serum diluted $\frac{1}{20}$ in veronal buffer 0.03M pH 7.6. The results are shown in Fig. 5 where the thickness of the electroadsorbed layer is plotted against the $\log_e$ of urea concentration in milligrams per milliliter. The linear relationship observed thus supports the hypothesis of HGHP bonding for the formation of multilayers of gamma globulins. It must be pointed out that these results do not preclude the involvement of other types of bonds.

When the weight ratio (urea/γG) was increased from 200 to 800 the thickness of the adsorbed layer remains essentially constant: 60 Å or about one monolayer. This thickness decreases further to 30–40 Å upon increasing the urea globulins ratio to 1500. This was probably due to the denaturation of the protein.

The effect of urea on the decrease of the adsorption of γG on chromium is due primarily to the inhibition of HGHP bonding and not caused by denaturation. If this assumption is correct, increasing the concentration of urea should have no effect on the critical concentration of gamma globulins at which multilayers start to be formed, as long as the urea concentration is low enough to permit multilayers adsorption. To test this assumption 0.0, 25, 71 and 138 mg/ml of urea were added to a human serum diluted $\frac{1}{10}$ in veronal buffer 0.03M pH 7.6 and the thickness of the electroadsorbed layers was measured after 30 sec at 300 μA. Each serum–urea sample was further diluted to $\frac{1}{20}$, $\frac{1}{40}$, and $\frac{1}{80}$ and similarly tested. The results are shown in Fig. 6. The upper curve was obtained without urea, the next lower

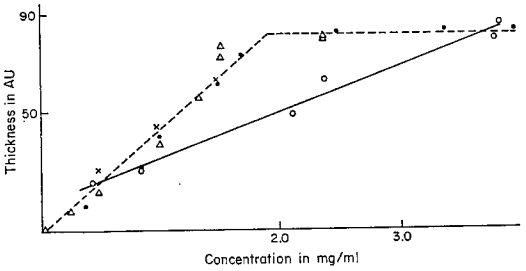

FIG. 7. Electroadsorption on chromium of serum proteins as a function of their concentration. ×- - - -× α-globulin (F1–IV); ●- - - -● hemoglobin; △- - - -△ β-globulins; ○——○ α-globulins (F4–IV).

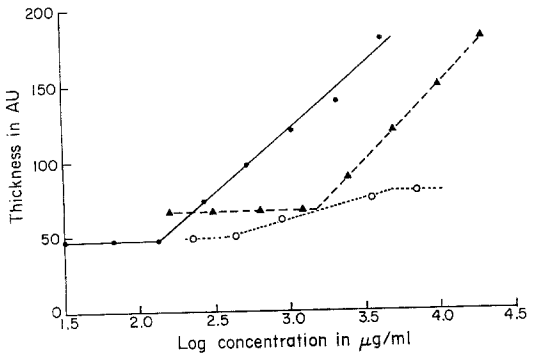

FIG. 8. Electroadsorption on chromium of serum proteins as a function of their concentration. ●——● Fibrinogen; ○- - - -○ Albumin; ▲- - - -▲ α-globulins (F1–IV).

ones with increased urea concentrations. Two points are worth noting: All 4 curves converge to the same thickness (45 Å) at a dilution of $\frac{1}{40}$, thus demonstrating the validity of the assumption. Secondly, the thickness of the layers electroadsorbed is a $\log_e$ function of the inverse of the dilution i.e. the concentration. Almost two layers of globulins were adsorbed at the dilution $\frac{1}{10}$ with 71 mg urea/ml, whereas no multilayer adsorption was observed at the same dilution with a concentration of urea increased to 138 mg/ml.

2. Electroadsorption of Other Serum Proteins

To determine whether other serum components could also form multilayers increasing amounts of β-globulins, hemoglobin, α-globulins (F1-IV), α-globulins (F4-IV) and albumin were added to normal human serum diluted $\frac{1}{20}$ in veronal buffer 0.03*M* pH 7.6. The electroadsorption was carried for 30 sec at 300 μA and the increase in thickness over that observed with normal serum is plotted in Fig. 7 against the concentration in milligrams per milliliter of the added proteins.

The same curve was obtained for β-globulins [△], hemoglobin [●] and α-globulins (F1-IV) [×]. It shows a linear increase in thickness with the concentration up to a thickness of about 80 Å then further increase in concentration does not produce any change in the thickness of the electroadsorbed layer. The addition of α-globulins (F4-IV) [○] did not produce as rapid an increase as the other fractions but the same limiting thickness (80 Å) was observed. Albumin added in concentrations ranging from 5 to 52 mg/ml did not produce any significant increase in the thickness of the adsorbed layer. In this respect, albumin is very different from other serum components.

The normal serum albumin concentration is about 40 ± 5 mg/ml (38) or about 55% of the total protein content. Since albumin added to a serum is unable to produce multilayers, the following solutions of proteins were tested for their ability to form multilayers: α-globulins (F1-IV): 0.15–9.91 mg/ml, α-globulins (F4-IV): 0.42–3.79 mg/ml, glycoproteins: 0.45–4.77 mg/ml, fibrinogen: 0.033–2.14 mg/ml, transferrin: 3.00–11.97 mg/ml and albumin 0.22–14.16 mg/ml were made in 0.03*M* pH 7.6 veronal buffer and electroadsorbed on chromium slides for 30 sec at 300 μA. Some of the results obtained are shown in Fig. 8 where the thickness of the layer is plotted against $\log_e$ of the concentration of protein in μg/ml. Under these conditions fibrinogen (solid curve), α-globulins (F1-IV) (dashed curve), appear to be able to form multilayers. The α-globulins (F4-IV) fraction followed a similar pattern.

Glycoproteins, transferrin and albumin (dotted curve), even at concentrations as high as 4.77 mg/ml, 11.97 mg/ml, and 14.16 mg/ml, respectively, did not form multilayers since the thickness of the adsorbed layers remained almost constant 40, 42, and 50 Å, respectively. The slight increase observed with albumin may possibly reflect a change in orientation of the adsorbed molecule.

3. pH Dependence of the Electroadsorption of Serum Proteins

The electrophoretic mobility of serum proteins at pH 8.6 ranges from 1.2 (39) for gamma globulins to 5.92 (40) for albumin. The isoelectric point of γG can be as high as 7.3 and that of α_1-glycoproteins as low as 2.7, thus one could have expected a significant difference in the adsorption of these proteins as a function of the pH. The results obtained are shown in Fig. 9. It is apparent that all the fractions tested (see legend of Fig. 9), except albumin, show a sharp maximum be-

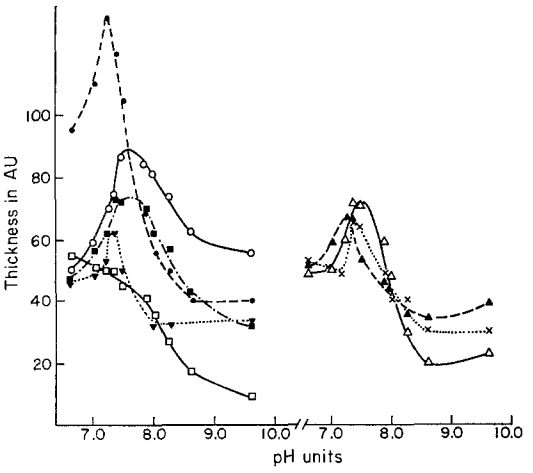

FIG. 9. pH Dependence of the Electroadsorption of Serum Proteins: ●- - - -● Normal human serum; ○——○ gamma globulins (Pentex I); ■·-·-■ "Reconstituted plasma" (41); ▼·····▼ α-globulins (F4–IV); □———□ Albumin; △———△ α-globulins (F–IV); ▲----▲ α-globulins (F1–IV); ×·····× β-globulins.

tween pH 7 and 7.5 and that this phenomenon bears no relation to the pI. This would indicate that electrostatic forces are not primarily involved in the adsorption of proteins on a metal surface. The reason for the different behavior of albumin is unknown.

DISCUSSION

The kinetics of adsorption of macromolecules such as proteins on a metallic surface is very complex because such relations involve a number of factors which usually cannot be studied separately. We have found that the thickness of adsorbed gamma globulins on chromium plated slides was a linear function of $t^{1/2}$. To explain this result we have postulated the presence of an oriented layer of water not easily disturbed adjacent to the solid surface. Such films have been proposed as way back as 1900 and play a major role in the kinetics of heterogeneous reactions as developed by Nernst. Derjaguin (32) has determined experimentally the viscosity of thin film of water at glass and quartz surfaces and has found values for the viscosity which are up to 15 times larger than the viscosity in the bulk of water. Consequently the diffusion coefficient in the film would be almost 15 times smaller than the diffusion coefficients determined in the bulk of water and for thinner films it could be even much smaller. Therefore, the thickness of the immobilized film of water could easily be smaller by one or two orders of magnitude than the value $\simeq 10^{-3}$ cm calculated from ordinary diffusion coefficients. Two types of forces are probably involved in the formation of the immobilized layer of water, Lifshitz dispersion forces (42–44) between the solid surface and the water and hydrogen bonding (45–48) between the water molecules. The mean displacement $\sqrt{\bar{x}^2}$ of a molecule diffusing through the immobilized layer could be given by Einstein's relation:

$$\sqrt{\bar{x}^2} = \sqrt{t} \cdot \sqrt{\frac{A}{\eta_d}}$$

where t is the time, η_d the viscosity of the film and A a constant.

As we have said in the introduction, the IEA test consists in carrying out two successive adsorptions on a metalized glass slide with the help of a weak current (300 μA) and the proper polarity. The antigen is deposited in the first adsorption, the serum to be tested is used in the second adsorption. Usually a thicker layer is adsorbed from an antiserum than from a normal serum on account of the specific binding of antibodies to the antigen molecules adsorbed on the slide. However, it is apparent from the data presented in this article that all of the serum proteins fractions the γ-globulins, if they are present above a certain limiting concentration, are capable of being electroadsorbed nonspecifically in thick multilayers, that is independently of the presence of an adsorbed antigen on the slide. It may happen that a significantly larger concentration of γ-globulins is present in a heterologous serum than in a normal or homologous (with respect to the antigen present on the slide) serum. In such a case the layer adsorbed from the heterologous serum may be thicker than the layer adsorbed from the homologous serum and thus the IEA test would fail to distinguish a true immunological reaction. This situation is likely to occur with sera of patients suffering from diseases involving the globulins producing system. This is the main reason

why the immune serum should be tested in two successive experiments. In one experiment the antigen used for the first electroadsorption is dissolved in a carrier solution, for example a 2% normal rabbit serum. In the second experiment the carrier solution is used without antigen for the first electroadsorption. The same immune serum is used in both cases for the second electroadsorption. In this way the non specific adsorption of gamma globulins is the same in both experiments and the difference in the thickness adsorbed from the immune serum, if any, is a true indication of a specific immunological interaction. It was shown in Fig. 6 that beyond a certain dilution ($\frac{1}{40}$) a normal serum did not give multilayers adsorption. Sera containing 25–30 mg/ml IgG must be diluted about 80–160 times before reaching this level (49). Sera should be diluted at least to the level at which no multilayer adsorption takes place if one wishes to determine an immunological reaction by comparing the thickness of the layer adsorbed from an antiserum on a given antigen to that adsorbed from a normal serum on the same antigen. It must be pointed out that in many pathological sera the gamma globulins level remains low enough so that sera diluted $\frac{1}{20}$ or so may be tested directly. Furthermore we have shown that the non specific adsorption of gamma globulins was very sensitive to pH, a sharp maximum being obtained between pH 7.0 and 7.2. If the specific adsorption is not too much dependent on pH, it might be preferable to carry out the second adsorption above pH 7.8 to decrease the nonspecific adsorption. This would increase the ratio of the specific to nonspecific adsorption and thereby improve the sensitivity of the IEA method.

CONCLUSION

The experimental results on the adsorption of various serum protein fractions presented in this article have permitted to postulate a partial mechanism for such reactions. Data on multilayers adsorption of gamma globulins have been helpful in improving our understanding of the immunoelectroadsorption method and in suggesting modifications which could make this test simpler to perform.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Lawrence D'Antonio of the University of Illinois, Urbana, Ill. for helpful discussions. This work was supported in part by Grant GM 08815 of U. S. Public Health Service.

REFERENCES

1. For instance;
   RIDEAL, E. K., "An Introduction to Surface Chemistry" Cambridge Univ. Press (1926).
   BRUNAUER, S., "The Adsorption of Gases and Vapors" Vol. I, Princeton Univ. Press (1943).
   RIDEAL, E. K., *in* "Surface Chemistry" (H. W. Melville and E. K. Rideal, eds.) Cambridge Univ. Press (1948).
   DAVIES, J. T. AND RIDEAL, E. K., "Interfacial Phenomena" Academic Press, New York (1961).
   YOUNG, D. M. AND CROWELL, A. D., "Physical Adsorption of Gases" Butterworths, London (1962).
   ADAMSON, A. W., "The Physical Chemistry of Surfaces" Interscience, N. Y. (1967).
   SALTSBURG, H., SMITH, J. N., AND ROGERS, M., (eds.) "Fundamentals of Gas-Surface Interactions" Academic Press, N. Y. (1967).
   FLOOD, E. A., (ed.) "The Solid Gas Interface" Vols. I and II Marcel Dekker, N. Y. (1967, 1968).
2. ROTHEN, A., *Rev. Sci. Instr.* **16,** 26 (1945).
3. ROTHEN, A., *Rev. Sci. Instr.* **28,** 283 (1957).
4. ROTHEN, A., *in* "Physical Techniques in Biological Research" (D. H. Moore, ed.) Vol. IIA. Academic Press, New York (1968).
5. ROTHEN, A., AND LANDSTEINER, K., *J. Exptl. Med.* **76,** (5) 437 (1942).
6. ROTHEN, A., *J. Biol. Chem.* **168,** 75 (1947).
7. MCGAVIN, S., AND I. IBALL, *Trans. Faraday Soc.* **49,** 984 (1953).
8. ROTHEN, A., *J. Am. Chem. Soc.* **70,** 2732 (1948).
9. SINGER, S., *J. Biol. Chem.* **182,** 189, (1950).
10. TRURNIT, H. J., *Arch. Biochem. Biophys.* **47,** 251 (1953).
11. TRURNIT, H. J., *Arch. Biochem. Biophys.* **51,** 176 (1954).
12. ROTHEN, A., *J. Phys. Chem.* **63,** 1929 (1959).
13. ROTHEN, A., *Helv. Chim. Acta.* **43,** 1873 (1960).
14. ROTHEN, A., *J. Colloid. Sci.* **17,** (2), 124, (1962).
15. MATHOT, C., ROTHEN, A., AND CASALS, J., *Nature* **202,** 1181 (1964).
16. MATHOT, C. AND ROTHEN, A., *Am. J. Trop. Med. Hyg.* **17,** 756 (1968).
17. MATHOT, C., D'ALESANDRO, P. A., SCHER, S., AND ROTHEN, A., *Am. J. Trop. Med. Hyg.* **16,** 443 (1967).

18. D'ANTONIO, L., ALGER, N., AND MATHOT, C., *Am. J. Trop. Med. Hyg.* (1969). (In press).
19. WILLIAMS, J., STECHSCHULTE, J., AND SADUN, E., *Am. J. Trop. Med. and Hyg.* **17,** 568 (1968).
20. MATHOT, C. AND ROTHEN, A., in "Recent Developments in Ellipsometry" Univ. of Nebraska, August 7–9, 1968. (In press).
21. DEUTSCH, H. F., *in* "Methods in Immunology and Immunochemistry" (C. A. Williams and M. W. Chase, eds.), Vol. I, Academic Press (1967).
22. BLODGETT, K. B. AND LANGMUIR, I., *Phys. Rev.* **51,** 964 (1937).
23. VROMAN, L., *Thromb. Diath. Haemorrhag.* **10,** 455 (1964).
24. VROMAN, L., *in* "Blood Clotting Enzymology" (Walter H. Seegers, ed.) p. 279–322. Academic Press (1967).
25. VROMAN, L., in "Recent Developments in Ellipsometry," Univ. of Nebraska, August 7–9 1968. (In press).
26. MCCRAKIN, F. L., "A Fortran Program for Analysis of Ellipsometer Measurements," NBS Publications (1969) (In press).
27. DAVIES, J. T., *Rec. Progr. Surface Sci.* **1,** 129 (1964).
28. HENNIKER, J. C., *Rev. Mod. Phys.* **21,** 322 (1949).
29. EHRLICH, G., *Ann. N. Y. Acad. Sci.* **101,** 722 (1963).
30. DERJAGUIN, B. V., *Discuss. Faraday Soc.* **42,** 109 (1967).
31. MOELWYN-HUGHES, E. A., "The Kinetics of Reactions in Solution," 2nd Ed. Oxford, (1947).
32. DERJAGUIN, B. V., FEDYAKIN AND TALAEV, *Compt. Rend. USSR* **167,** 376 (1966).
33. SCHULTZE, H. E. AND HEREMANS, J. F., "Molecular Biology of Human Proteins." Elsevier (1966).
34. HANNESTAD, K., *Clin. Exp. Immunol.* **2,** 511 (1967).
35. CHRISTIAN, C. L., *J. Exp. Med.* **108,** 139 (1958).
36. EDELMAN, G. M., KUNKEL, H. G., AND FRANKLIN, E. C., *J. Exptl. Med.* **108,** 105 (1958).
37. MATHOT, C. AND ROTHEN, A., "The Kinetics of Polysaccharide Type III—pneumococcus reaction with homologous rabbit antiserum" (in preparation).
38. SCHULTZE, H. E. AND HEIDE, K., *in* (K. Fr. Bauer ed.) "Medizinische Grundlagenforschung." Vol. III, Georg Thieme, Stuttgart, p. 351 (1960).
39. COHN, M. L., DEUTSCH, H. F., AND WETTER, L. R., *J. Immunol.* **64,** 381 (1950).
40. ARMSTRONG, S. H., BUDKA, M. J. E., AND MORRISON, K. C., *J. Am. Chem. Soc.* **69,** 416 (1947).
41. The reconstituted plasma was made from purified serum protein fractions in the following concentrations in mg/ml: Glycoproteins:3; transferrin:2; γ-globulins:23; α-globulins:12; β-globulins:9; albumin:35; hemoglobin:1; fibrinogen:3.
42. LIFSHITZ, E. M., *Doklady Akad. Nauk. SSSR* **97,** 643 (1954).
43. LIFSHITZ, E. M., *Doklady Akad. Nauk. SSSR* **100,** 879 (1955).
44. LIFSHITZ, E. M., *Zhur. Eks. Teor. Fiz.*, **29,** 94 (1955).
45. NEMETHY, G., *Fed. Proc.* **24,** III, 38 (1965).
46. KLOTZ, I. M., *Brookhaven Symp. Biol.* **13,** 25 (1960).
47. NEMETHY, G. AND SCHERAGA, H. A., *J. Chem. Phys.* **36,** 3382 (1962).
48. NEMETHY, G., *Angewandte Chemie* **6,** 195 (1967).
49. WINCHESTER, ROBERT, The Rockefeller University, (Personal communication to C. M.) (1969).