disintegrating cells (Table 3) could be found 3 h after irradiation by the direct method (incubation of erythrocytes at 37°C for 30 min without exposure to additional chemical factors). By contrast with this, after incubation of erythrocytes from control and irradiated animals with Triton X-100 or with ethanol a significant increase was observed in the number of cells with altered membranes after irradiation in doses of between 200 and 500 R (Fig. 3). The degree of increase in the number of damaged cells depended on the dose of irradiation. Changes after irradiation in doses of 200 and 500 R, revealed after treatment of the cells with Triton X-100, differed significantly ($P < 0.05$) from the normal level and from one another. A similar result could be obtained by treatment of the erythrocytes with other chemical agents. For example, after treatment of the erythrocytes of intact rats with 0.3% Tween at 37°C for 30 min the number of erythrocytes with damaged membranes was 44.96 ± 4.20 million cells, compared with 126.86 ± 30.80 million cells in rats irradiated in a dose of 400 R. The number of irradiated erythrocytes in zone 1, disintegrating under the influence of the chemical agents, did not depend on the dose of irradiation.

The degree of damage to the cells following exposure to ionizing radiation in doses of up to 500 R can thus be assessed by the number of erythrocytes passing through urografin during centrifugation.

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STUDY OF IMMUNOLOGIC REACTIONS ON A SILICON SURFACE BY ELLIPSOMETRY

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UDC 612.017.1-08

KEY WORDS: adsorption; proteins; ellipsometry; immunologic reactions.

Ellipsometry is an optical method whereby changes in the state of polarization of light reflected from the phase boundary between two media can be used to measure the thickness and optical constants of thin films located on the boundary and also to study the kinetics of surface processes leading to film formation (such as adsorption, electrochemical reactions on electrodes, and so on) [1].

Rothen [7] showed by the use of ellipsometry that immersion of glass and metal plates covered with a layer of antigen in homologous antiserum leads to specific adsorption of antibodies on them. The thickness of the adsorbing layer under these circumstances is measured in tens or even hundreds of Angström units, whereas during nonspecific adsorption from heterologous antiserum it measures about 5-20 Å. The reason why these processes can take place is because determinant groups of the adsorbed antigen remain accessible for the active centers of the antibodies. The sensitivity of this method, according to our own observations and data obtained in other countries [9], is about 10^{-6} g antibodies/ml.

In 1969 Rothen and Mathot [8] developed an even more sensitive (down to 10^{-9} g/ml) variant of this method, known as immunoelectroadsorption (IEA). An appropriate electron po-

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(Presented by Academician of the Academy of Medical Sciences of the USSR B. A. Lapin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 90, No. 9, pp. 378-380, September, 1980. Original article submitted December 2, 1979.

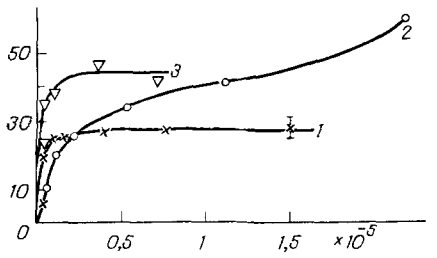

Fig. 1

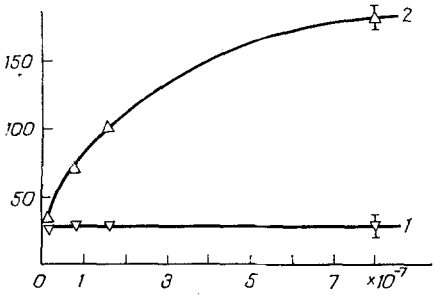

Fig. 2.

Fig. 1. Isotherms of nonspecific adsorption of proteins on silicon surface. 1) HSA; 2) OVA; 3) IgG. Here and in Figs. 2 and 3: abscissa, protein concentration (C), in moles/liter; ordinate, thickness of adsorption layer (d_2), in Å.

Fig. 2. Isotherm of specific adsorption of IgG from rabbit antiserum against HSA on an HSA monolayer formed from a 0.75×10^{-6} M solution. 1) HSA; 2) IgG.

tential was applied to the metal plate coated with antigen, and in that way they carried out electrophoretic fractionation of the proteins in the antiserum additionally. The whole process of performance of the immunologic reaction could be counted in minutes. These authors suggested that their method could be used for routine clinical tests for quantitative determination of antibodies against viruses, parasitic agents, and growth hormones circulating in the patient's blood stream. However, other workers reported [6] that during immunoelectroadsorption of antibodies on a metal surface electrochemical reactions take place at the same time and interfere with interpretation of the ellipsometric results. Accordingly the IEA method has not yet been accepted for routine serologic diagnosis.

The object of this investigation was to study adsorption isotherms of proteins on a solid body and to determine the optimal conditions for ellipsometric recording of immunologic reactions, taking into account facilities actually available in clinical laboratories.

The following proteins were used in the work: human serum albumin (HSA), mol. wt. = 67,000, from Reanal (Hungary); ovalbumin (OVA), mol. wt. = 45,000, from Calbiochem (USA); rabbit immunoglobulin G (IgG), mol. wt. = 150,000, from Calbiochem; immunoglobulin fractions of rabbit antisera against HSA and OVA, obtained by precipitation with 35% ammonium sulfate and dialyzed against 0.15 M NaCl. The concentration of antibodies against HSA in the rabbit antiserum was determined in Heidelberger's quantitative precipitation test [12] with an accuracy of ±5%.

All proteins were dissolved in 0.02 M buffer solutions with pH approximately equal to the isoelectric point of the protein: for HSA and OVA in citrate buffer with pH 4.8; for IgG and the antiserum in Tris-buffer with pH 7.2.

As adsorbent, optically polished plates of monocrystalline silicone (Si), coated with a thin layer of the oxide (SiO_2), were used. The surface of the plates was cleaned by successive washing in hot solutions of detergent, chromate mixture, and distilled water. Adsorption on them from the protein solutions took place at 20°C for 20 h, after which the samples were washed under a stream of distilled water and dried in air, protected against dust.

All measurements were made on the instrument described previously [1], with an angle of incidence of 65°.

The optical constants of the silicon support were determined by Drude's exact equations. In light of wavelength $\lambda = 6328$ Å they were:

$$n_{Si} - ik_{Si} = 3{,}86 - i \cdot 0{,}02 \text{ and } n_{SiO_2} = 1{,}45,\ d_{SiO_2} = 15\ \text{Å}.$$

The thickness of the adsorption films (d_2) was calculated by a linearized Drude's first order equation: $\bar{\Delta} - \Delta = \alpha d_2$ [6, 8]; since independent solution of the refractive index (n_2) and thickness (d_2) in very thin (about 50 Å) adsorption layers is difficult and requires high-precision measurements, it was assumed in the calculations of α that n_2 is about 1.8, as other workers also have done [3, 4]. In that case the values obtained for d_2 will be the "mean

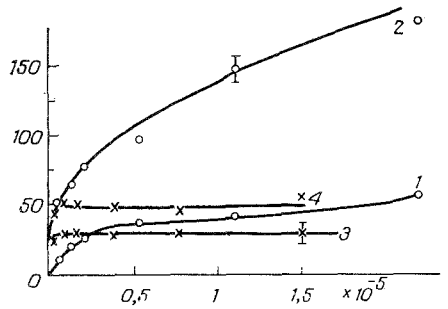

Fig. 3. Comparison of specific and nonspecific adsorption of IgG from antiserum against OVA (antibody concentration 1.5×10^{-7} M) on monolayers of OVA and HSA. 1) OVA; 2) specific adsorption on OVA layer; 3) HSA; 4) nonspecific adsorption on HSA layer.

equivalent" values. That this method of interpreting the results is correct was confirmed experimentally by Smith [10]. This approach creates an inaccuracy of about 20% in absolute values, but comparative relationships correctly reflect the principles of the adsorption process. The problem of conformation of the adsorbed molecule is solved on the basis of calculations of the area per molecule (along the isotherm) and of its crystallographic dimensions or on the basis of a combination of ellipsometry with spectroscopy, by which it is possible to determine the number of contacts between the protein molecule and the surface of the adsorbent [5].

The results of the determinations are given in Figs. 1-3. Isotherms of adsorption of IgG and HSA on silicon and of antiserum on a layer of antigen are monomolecular and are described by a Langmuir equation [2]. This was not found for OVA, possibly because of the increased tendency of this protein to aggregate in aqueous solutions. The limiting areas on the molecule (from the Langmuir equation) and thicknesses of the adsorption layers were: for HSA 28 Å and 3.2×10^3 Å^2; for IgG 45 Å and 4.5×10^3 Å^2, and 150 Å and 1.5×10^3 Å^2 (for adsorption on antigen), which correlate well with their dimensions determined by crystallographic methods (for HSA 106 × 50 × 21 Å, for IgG 80 × 50 × 40 Å [11]). This indicates absence of surface denaturation of these proteins and also differences in the orientation of IgG in the adsorption layer: in the case of specific adsorption on antigen — "perpendicularly" to the surface, and in the case of nonspecific adsorption on a silicon surface — depending on the greatest area of contact, i.e., "sideways" and, evidently, with "loss" of immunologic activity, since adsorption of antigen on them did not take place. The constants of adsorption equilibrium were 4.5×10^9 and 4.4×10^9 cm^3/g-mole, respectively, evidence of the closely similar energies of interaction with the substrate in both cases and, consequently, evidence of physical adsorption.

Having compared the limiting areas on the molecule for HSA and IgG in the case of its specific adsorption on HSA, the largest accessible number of adsorption centers on the surface of the antigen (HSA) molecule can be estimated, i.e., its "valence" is about $(3.2 \times 10^3 / 1.5 \times 10^3) \times 2 = 4$. (The surface of the HSA molecule which takes part in nonspecific adsorption on silicon is allowed for here by the coefficient 2.)

Certain practical conclusions regarding the usefulness of ellipsometry as a diagnostic method can also be drawn from these results. To obtain a full adsorption monolayer of antigen it is sufficient to use solutions with concentrations of about 10^{-4} g/ml. The data given in Figs. 2 and 3 demonstrate the sufficiently high specificity and sensitivity (10^{-5} g/ml) of the method, and since the quantity of the adsorbed antibodies depends on their concentration in solution, it is possible in principle to construct a calibration curve and to carry out quantitative estimation of the concentration of antibodies in a test serum. A study of the kinetics of adsorption of antigens and antibodies which the writers are currently undertaking (by means of special cuvette) indicates that the duration of the adsorption process of proteins can be reduced to 1-2 h.

The output of measurements even on the simplest ellipsometer with manual control, which we ourselves developed and which, from the point of view of cost, is within the scope of any laboratory, is about 50-100 specimens per hour. On instruments with automatic control, the number of measurements may amount to 750 specimens per hour. It is evident that with certain modifications the method can be successfully used for mass rapid immunologic diagnosis. Prospects for the use of the IEA method, however, are not yet quite clear.

The authors are grateful to A. Ya. Kul'berg and I. O. Tarkhanova for generously providing the proteins and antisera.

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