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- CONTROLS
- INSTRUMENTATION
- OXYGENATORS
- EMERGENCY SYSTEM
- TEMPORARY SYSTEM
- PERMANENT SYSTEM
- TOTAL REPLACEMENT
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Interaction of Blood Proteins With Solid Surfaces

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Polymer Science and Standards Division
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TABLE OF CONTENTS

FIGURES

TABLES

NOTICE

This is a report prepared as an account of progress of work sponsored by the National Heart, Lung and Blood Institute. It is not to be construed as a report, opinion, or recommendation of the National Heart, Lung and Blood Institute. When produced in final form it will be available from the National Technical Information Service, 5285 Port Royal Road, Springfield, Virginia 22151.

Certain commercial materials and equipment is identified in this report in order to adequately specify the experimental procedure. In no case does such identification imply recommentation or endorsement by the National Bureau of Standards nor does it imply that the equipment identified is the best available.

ABSTRACT

The purpose of this investigation is to help develop methods of characterizing the surface properties of implant materials for use in the cardiovascular system. These properties are related to the ultimate success or failure of an implant in vivo. The investigation during the current reporting period has been chiefly concerned with the amount of protein deposited on implant material surfaces. Ion exchange treatment of radiolabeled proteins has been found to lead to much greater accuracy and reliability of adsorption measurements. Results are reported for the quantitative adsorption of labeled proteins thus purified onto well characterized test surfaces, and onto the surfaces of materials supplied by contractors to the NHLBI Biomaterials Program. The effect of the label (^{125}I vs. ^{14}C) on adsorption of proteins was studied. Hydrophobic and hydrophilic oxidized silicon surfaces were used to demonstrate that the adsorption of γ-globulin, measured by ellipsometry, and serum albumin, measured by radiolabeled protein, both are significantly affected by charge interactions between the surface and the protein.

SUMMARY

This investigation is designed to help develop surface characterization methods for implant materials to be used in the cardiovascular system. Since surface properties play a major role in determining the success or failure of implants, these studies can form the basis for *in vitro* test methods used to design and select suitable implant materials.

The first in a complex series of events that occurs when blood comes into contact with an implanted device is the adsorption of proteins on the surface. The detailed characteristics of this adsorbed protein layer can affect subsequent events, such as attachment of formed elements, which may lead to failure of the implant. Factors which are potentially important in determining the response of a protein-covered surface to formed elements in the blood are the amount of each protein deposited from the mixture, the lateral distribution of the protein on the surface, the depth of penetration of the protein into the material, and any changes in the conformation of the proteins resulting from their interaction with the surface.

Accomplishments during the past year are described below.

Improved Protein Purification Method

Using human serum albumin (HSA) labeled with ^{125}I, which has been purified by ion exchange chromatography as well as our usual gel permeation chromatographic separation, we have obtained results for the amount of protein absorbed from solution which are consistent with

monolayer coverage.

Adsorption and Penetration of Proteins on NHLBI Contractor Biomaterials

Using HSA (^{125}I) purified according to our new procedure, we have obtained results for the adsorption of HSA from solution onto six substrates obtained from NHLBI contractors. Hydrogel and heparin coated samples have been found to adsorb much more protein than uncoated surfaces, indicating possible penetration of protein into the coatings Another HSA sample, labeled with ^{14}C, was also adsorbed onto six surfaces, including three which had been studied with the ^{125}I labeled HSA. Generally good agreement was found between the adsorbance of the two labeled proteins.

Protein Adsorption on Model Surfaces

The adsorption isotherm and rate of adsorption of purified HSA (^{125}I) onto a well characterized linear polyethylene surface, NBS Standard Reference 1475, have been determined. Silicon oxide surfaces having different surface charges have been found to adsorb greatly different amounts of HSA (^{125}I). Platinum as observed in previous studies (1) exhibits an unusually high adsorption of iodine-labeled protein.

The effect of surface charge on the adsorption of γ-globulin onto silicon oxide has been investigated, and it has been found that both surface charge and intermolecular repulsion contribute to the net adsorption of protein.

INTRODUCTION

The adsorption of plasma proteins is the first in a complex series of events that occurs when a synthetic material is placed in contact with blood. It is known that this protein adsorption can alter the course of subsequent reactions at the implant surface but the mechanism by which this occurs is not well understood. If the detailed behavior of proteins absorbed at the solid surface were known and capable of control, the fate of artificial materials implanted in the body could be predicted with greater certainty and more successful materials perhaps could be designed. This investigation is directed toward the identification of factors capable of affecting protein adsorption and the development of a description of the changes that occur in the protein upon interaction with a surface.

Using an ion exchange treatment which we have recently developed for further purifying radiolabeled proteins, we have been able to obtain accurate quantitative information about the adsorption of human serum albumin onto a number of candidate synthetic implant materials provided by contractors to the NHLBI Biomaterials Program. Specially prepared oxidized silicon surfaces, in the hydrophobic and hydrophilic states, have also been used to test the effects of surface free energy and electric charge interactions on γ-globulin adsorption by ellipsometry and on radiolabeled human serum albumin adsorption by radiotracer counting.

EXPERIMENTAL

Protein Purity

All proteins used in the experiments reported here were dissolved in 0.15 M potassium phosphate buffer solution, at pH 7.4 unless otherwise noted. Gel permeation chromatography separations were performed at room temperature on a Sephadex G-200 column, 2.5 cm in diameter and 100 cm long. Flow rate through the column was approximately 0.1-0.2 ml/min. The column packing was tested by passing blue dextran (MW 40,000) solution through the column. Approximately 10 ml of protein solution, at a concentration of about 0.5 mg/ml, were used in each chromatographic separation. Specific activities of radiolabeled protein solutions used in the adsorption experiments were typically of order 10^6-10^7 counts/min/mg protein.

Fibrinogen

Four grams of purified human fibrinogen were obtained for use in studying adsorption onto NHLBI contractor biomaterials. The fibrinogen, distributed by AVCO Everett Research Laboratory, Inc., has been stored at -80°C since it was received. According to the supplier, the protein is 96 ± 0.5% clottable. It was supplied frozen in 0.3 M NaCl. Electrophoretic analysis of the reduced protein showed the characteristic 3 band patterns. Traces of Factor XIII and plasminogen were found, together with 2.5% "cold-insoluble globulin".

γ-Globulin

The purity of our bovine γ-globulin (Cohn Fraction II) from ICN Pharmaceuticals, Inc. has been tested by gel permeation chromatography

fractionation on a Sephadex G-200 column, as reported in our last Annual Report (1977). Although the elution chromatogram showed the presence of some high molecular weight aggregates, the amount of protein deposited on a test surface was the same from a solution of the chromatographed monomer as from the unfractionated protein. Therefore, we have concluded that it is not necessary to further purify our "as-received" γ-globulin in order to obtain meaningful quantitative adsorption data.

Human Serum Albumin

Human serum albumin (HSA) obtained from commercial sources, even though stated to be of high purity, has usually been found by us to contain considerable amounts of dimer and higher molecular weight aggregates, as well as polypeptide fragments. Sodium dodecyl sulfate gel electrophoresis and gel permeation chromatography both indicated that commercial samples of HSA were quite impure. Each sample of ^{125}I labeled and unlabeled albumin which we have received has been chromatographically fractionated to separate the desired protein monomer from the other components. Further details of the purification procedure and their effect on the behavior of the albumin solutions in quantitative adsorption experiments appear in a later section of this Report. The ^{14}C labeled HSA was prepared by Dr. I. Miller at the Weizmann Institute, Rehovot, Israel.

The ^{14}C label was introduced by acetylation of cold, freeze-dried HSA with labeled acetic anbydride. No change in enzymic activity of other proteins, such as trypsin and RNAse was found by Dr. Miller after acetylation by this procedure. An electrophoretic analysis of the

labeled HSA, performed by Dr. Miller, revealed essentially pure HSA.

Ellipsometry

All ellipsometry experiments reported here were performed with a commercially available ellipsometer, which employs a He-Ne laser light source (λ = 632.8 nm) with an incident angle of 70°. Further details of the ellipsometry instrumentation, the cells used to study adsorption from solution, and experimental procedures may be found in previous Annual Reports (1-5).

Analysis of the ellipsometry data to determine protein film thickness, refractive index, and amount adsorbed on the substrate was performed with a computer program developed at NBS by Dr. F.L. McCrackin (6).

Radiotracer Counting

A highly efficient γ-counting system which we assembled with commercially available components was described in our last Annual Report (5). All radiotracer protein adsorption studies were carried out at 37°C in pH 7.4 phosphate buffer solution under static conditions. The very important rinsing procedures required to remove radioactive solution carry-out from adsorbates have been described in previous Annual Reports (1-3). Also described in the earlier reports are the method of determining specific activity of the labeled protein and the tests for self-absorption of radiation by the solution and its container. Most experiments were carried out in duplicate, to test for reproducibility. Our current procedures usually permit us to replicate a given adsorption result within ± 2%.

Control Surfaces

For comparison of results obtained by radiotracer methods and ellipsometry, three control surfaces were used. Linear polyethylene, a well characterized polymer (Standard Reference 1475) was obtained from the Office of Standard Reference Materials at NBS. It was molded into 1 mm thick sheets, from which discs were punched for the adsorption experiments. Oxidized silicon surfaces, obtained from the semiconductor processing group at NBS, were prepared by controlled oxidation of polished silicon wafers. Freshly prepared surfaces, which were hydrophobic, could easily be hydrolyzed by refluxing in boiling water overnight. Thus, the effect of surface free energy on protein adsorption could be studied, with minimal variation of surface chemistry.

NHLBI Contractor Biomaterials

Most of the NHLBI contractor samples were shipped to us in sterile packages. All were packaged dry, except for the Polysciences hydrogel-coated polyetherurethane, which was packed under water. Samples were rinsed in distilled water before each protein adsorption experiment.

Secondary Ion Mass Spectrometry (SIMS)

The SIMS technique was described in detail in our last Annual Report (5). At that time, we reported that instrumental breakdowns had prevented us from exploiting the technique in a productive way. These difficulties have been resolved, and spectra are currently being obtained. Following a recent discussion with Dr. Dale Newbury (NBS),

we have prepared platinum and chromium slides coated with human serum albumin for exploratory studies of protein SIMS spectra. The information thus obtained will enable us to proceed with SIMS investigations of protein adsorbed on NHLBI contractors' samples.

RESULTS AND DISCUSSIONS

PURIFICATION OF LABELED AND UNLABELED SERUM ALBUMIN

Separation of Aggregates by Fractionation

A major problem in the application of radiotracer methods to the study of protein adsorption is the presence of high and low molecular weight impurities. These impurities compete with the monomer for adsorption sites. Most of the available commercial suppliers of radiolabeled and unlabeled human serum albumin (HSA) quote a high degree of purity for their samples, in some cases as high as 100% as measured by electrophoresis. After using some of these samples for our adsorption studies, we have found that the commercially supplied proteins needed additional purification. In Figures 1-9 we show GPC separations of both labeled and unlabeled HSA obtained from commercial sources. Most of these commercial samples are either contaminated with low molecular weight polypeptides or high molecular weight aggregates (mostly dimer) or in some cases both. We have therefore continued to use a gel permeation chromatography (GPC) column packed with Sephadex G-200 to separate the various molecular weight components which might affect the adsorption of the monomeric protein. As discussed in a later section under Ion Exchange Purification, we have now shown that the GPC separation does not remove all of the impurities which can affect the adsorption measurement.

Figure 1 is a Standard Reference Material sample of bovine serum albumin, obtained from the Office of Standard Reference Materials at the National Bureau of Standards. This sample with its small amount of

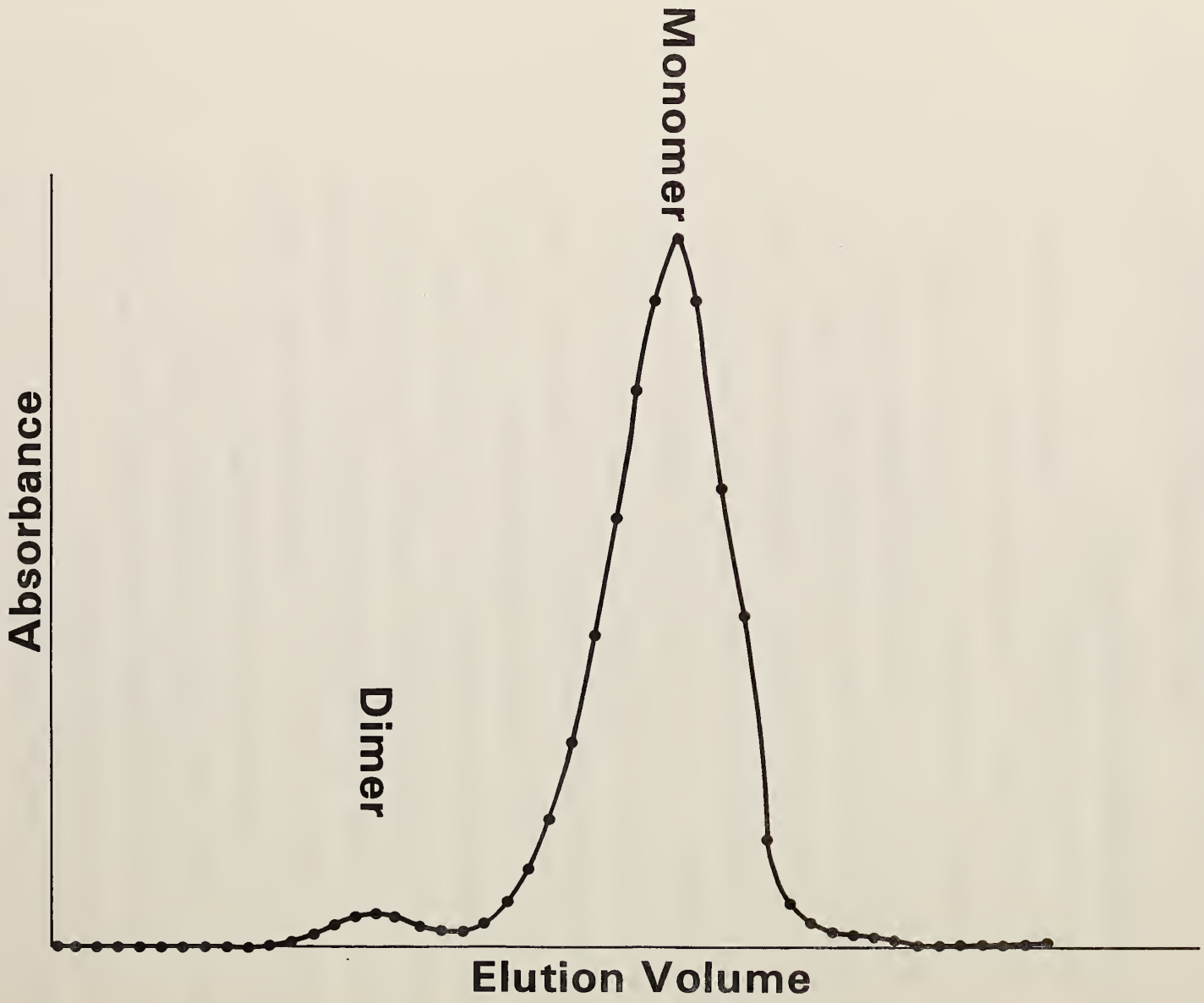

Fig. 1. Gel permeation chromatogram of Standard Reference Bovine Serum Albumin.

dimer provides us with a reference point for determining which fractions correspond to the HSA monomer. In this way, we discovered that one commercial sample of HSA (^{3}H) had a large amount of dimer but little or no monomer was present. Without the reference material GPC, we might have mistakenly used the dimer for our adsorption studies. In Figures 2-4, we observe that the unlabeled HSA preparations contain a large amount of dimer and higher aggregates as well as monomer. No low molecular weight polypetides were found in the unlabeled protein, indicating that these impurities in the labeled HSA probably resulted from the labeling procedure.

In Figures 5-9, we show GPC chromatograms of four different samples of ^{125}I labeled HSA and one sample of ^{131}I labeled HSA, all obtained from the same commercial labeling laboratory. Samples shown in Figures 5 and 6 display good molecular weight separations, with a small amount of both dimer and polypeptides in one sample, and no observable dimer in the other. However, Figure 7 shows another HSA (^{125}I) sample which is clearly quite contaminated with dimer and polypeptides. Figure 8 is a sample of HSA labeled with ^{131}I, which shows a small amount of dimer and no polypeptides.

Figure 9 is a plot of the radioactivity of each GPC fraction, for another ^{125}I labeled HSA sample. The specific activities of the aggregated (dimer and higher) molecular weight fractions are shown to be essentially the same as the monomer fractions, while the polypeptide fractions appear to have a much lower specific activity. Assuming that the concentration of protein in these fractions was correctly determined, using the same UV extinction coefficient as the whole

protein, we conclude that a high percentage of the low molecular weight fractions is unlabeled.

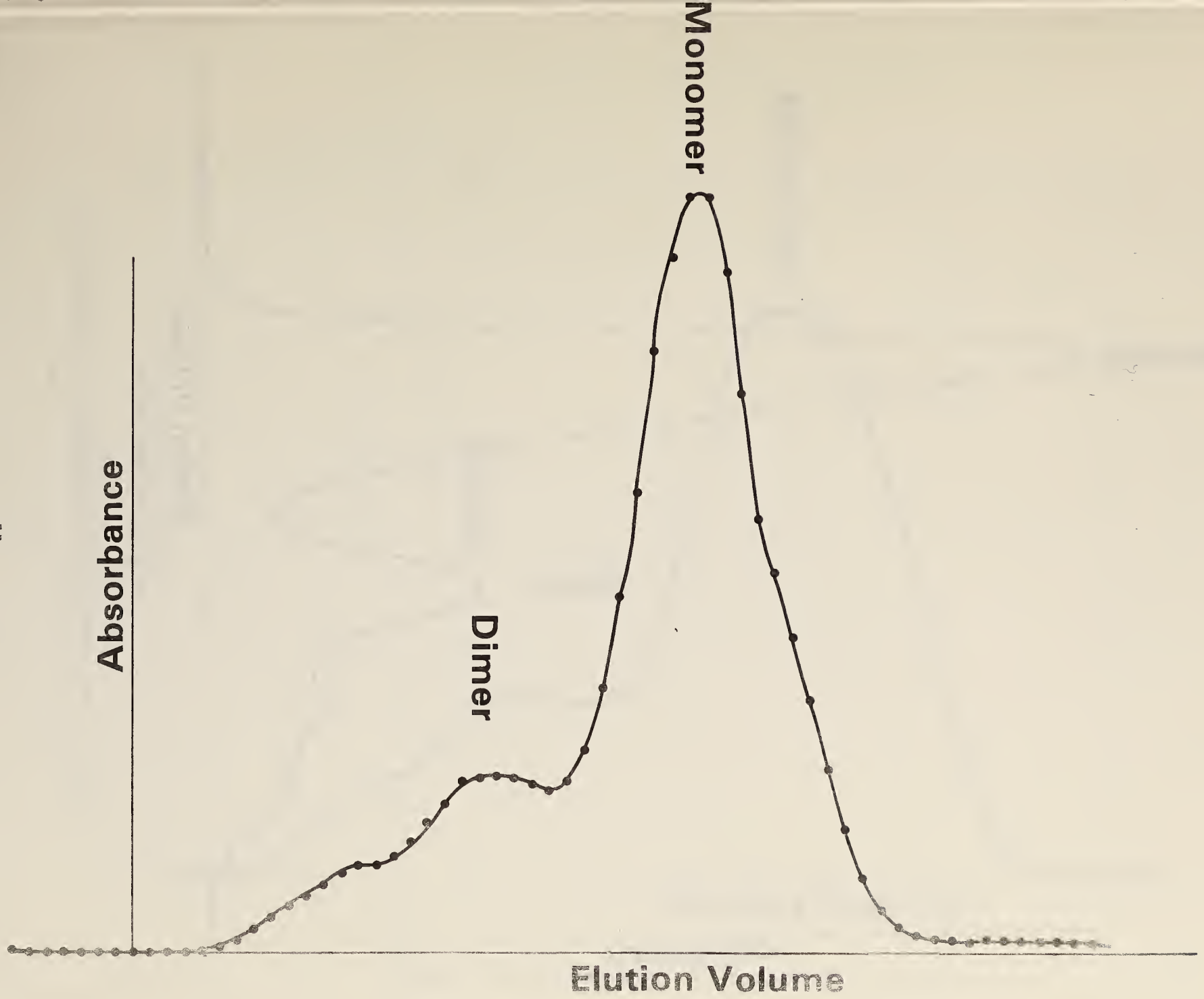

Fig. 2 Gel permeation chromatogram of unlabeled H.S.A.

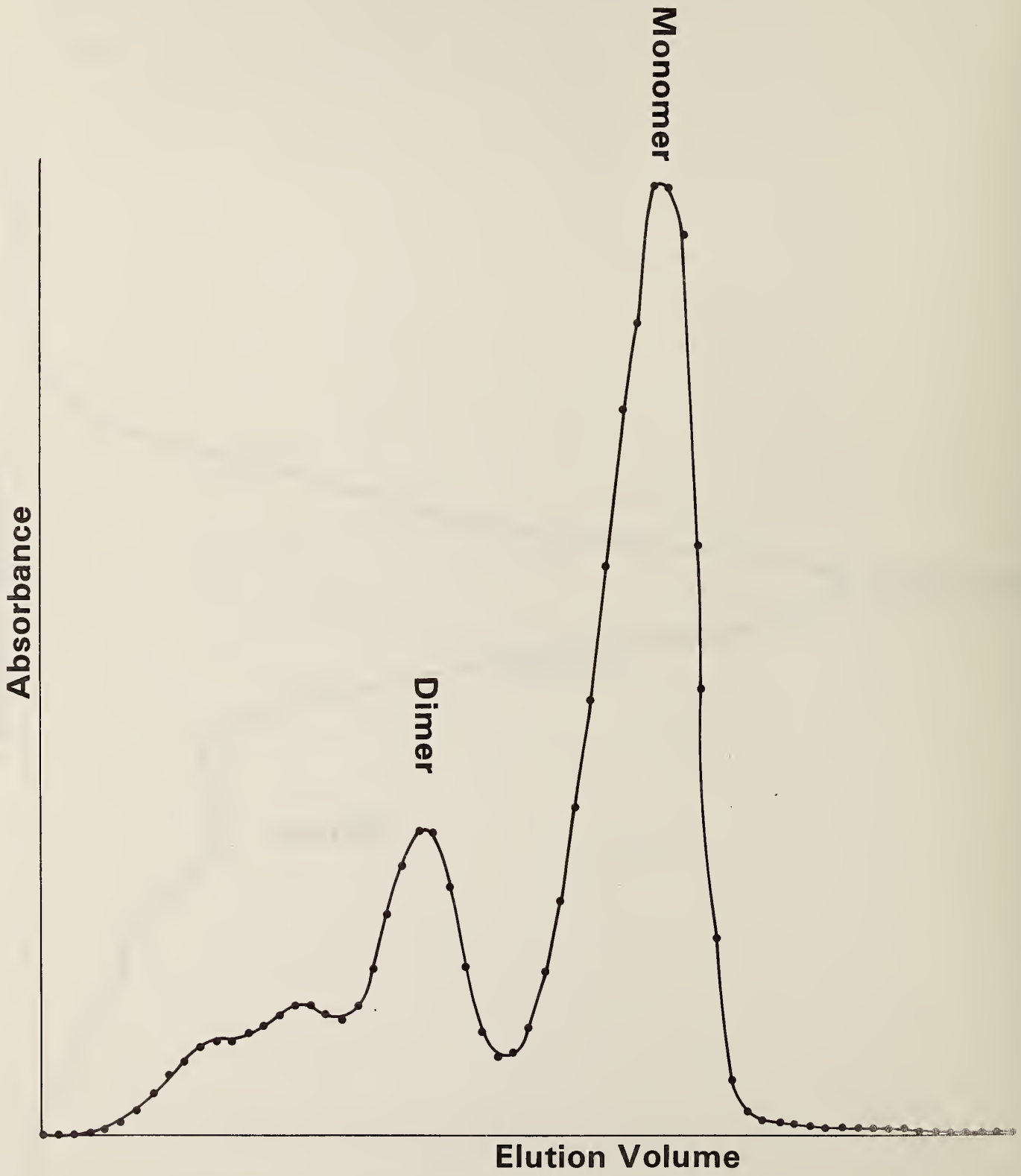

Fig. 3 . Gel permeation chromatogram of unlabeled H.S.A.

Fig. 4. Gel permeation chromatogram of unlabeled H.S.A.

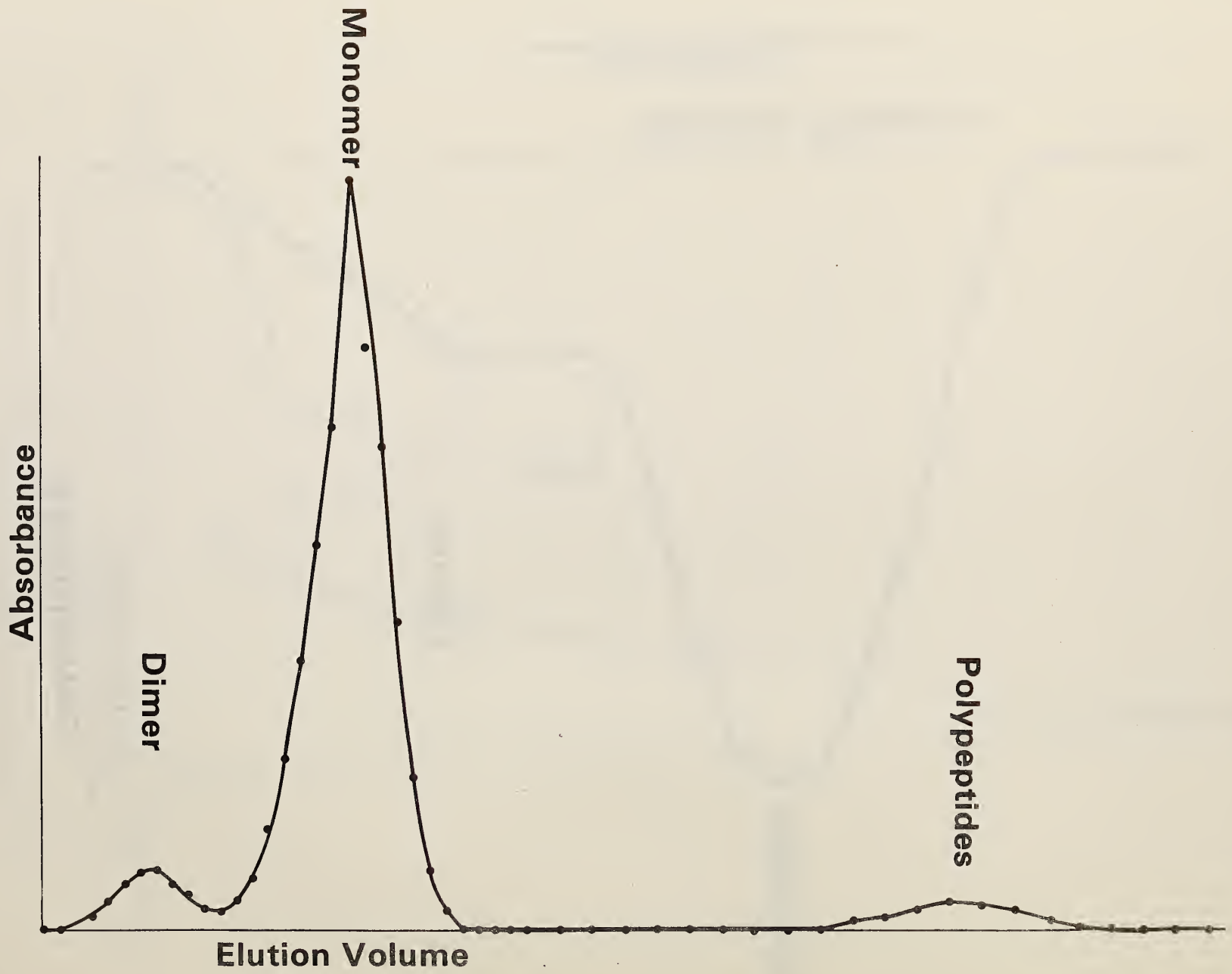

Fig. 5. Gel permeation chromatogram of ^{125}I H.S.A.

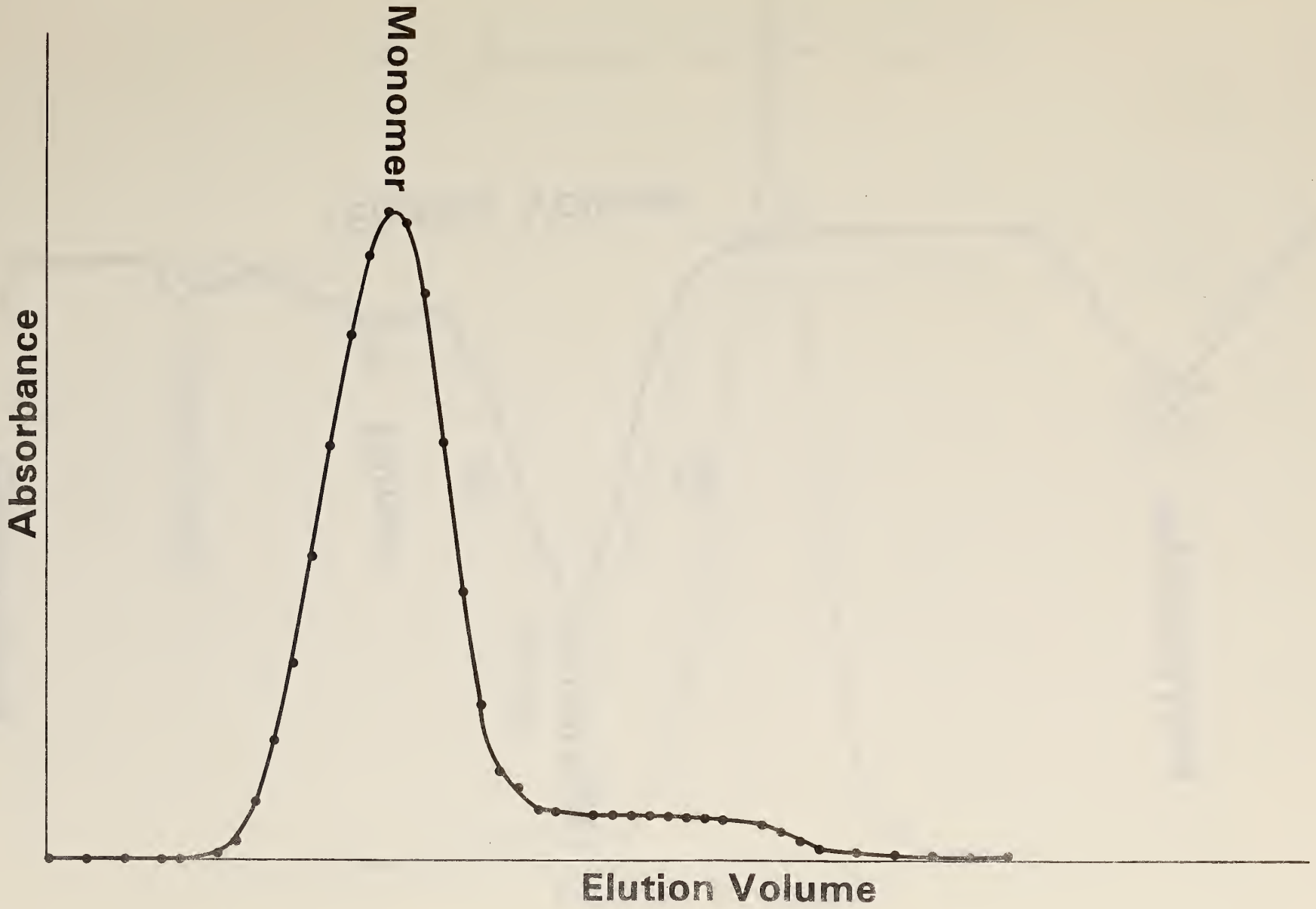

Fig. 6. Gel permeation chromatogram of ^{125}I H.S.A.

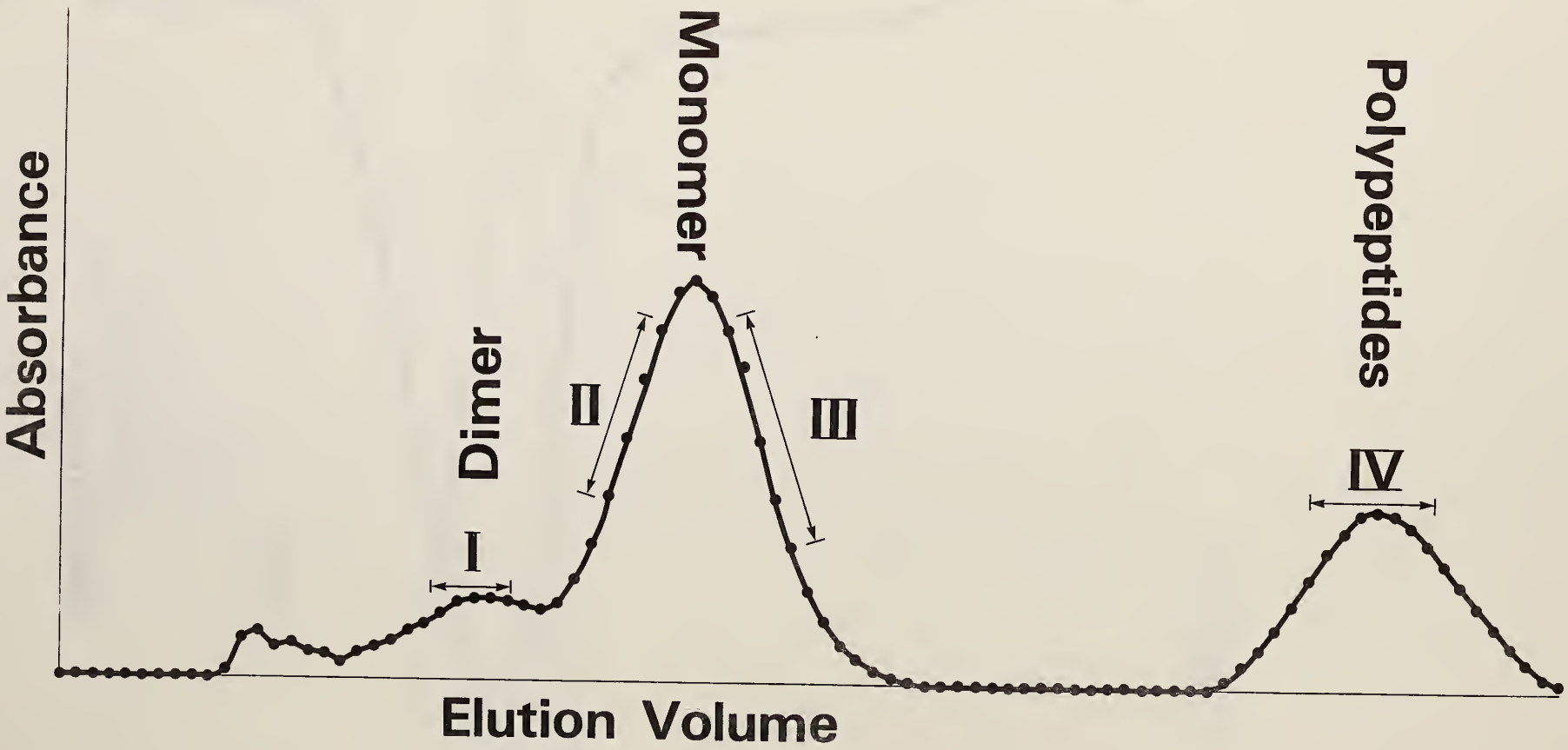

Fig. 7. Gel permeation chromatogram of ^{125}I H.S.A.

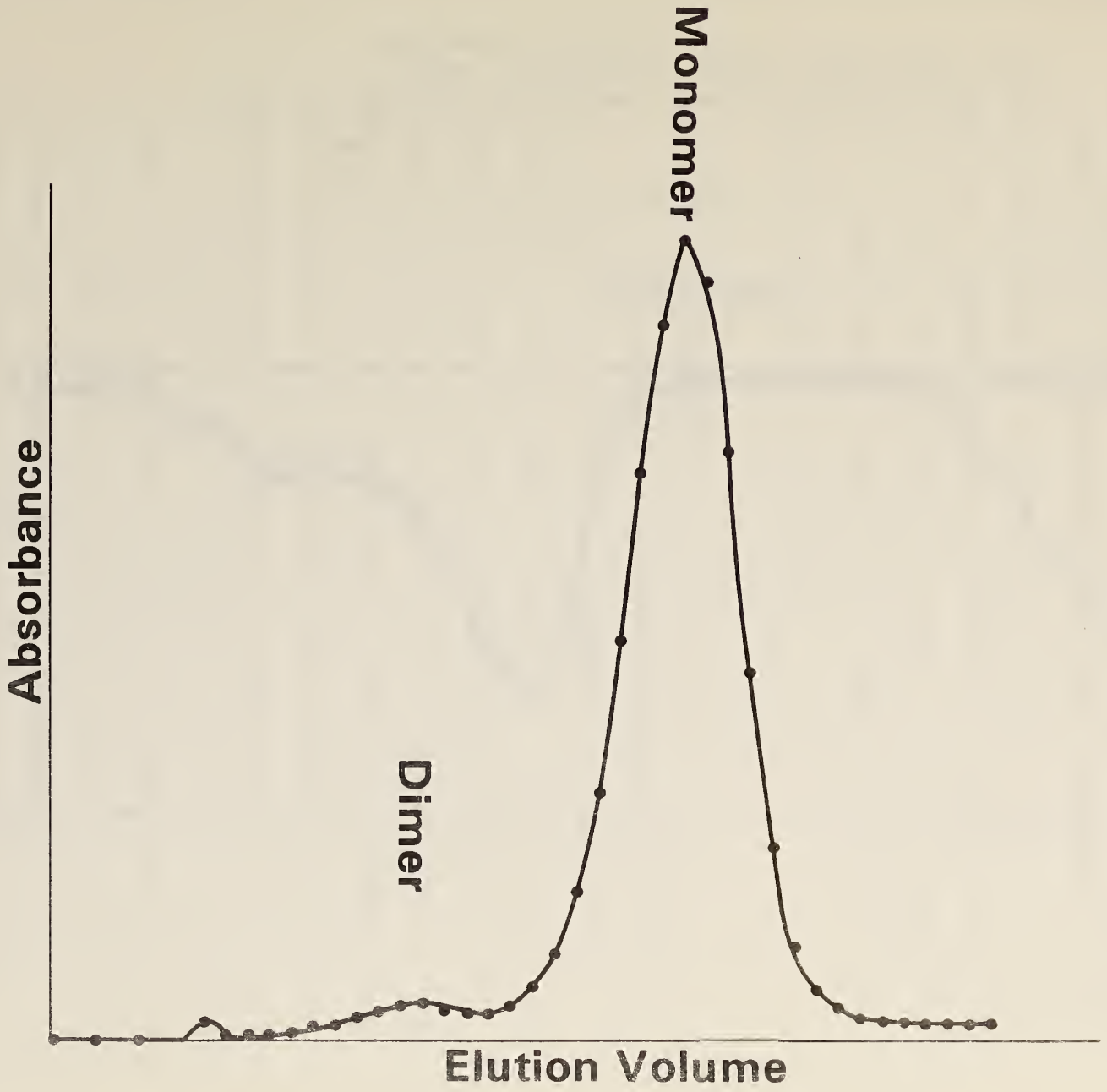

Fig. 8. Gel permeation chromatogram of ^{131}I H.S.A.

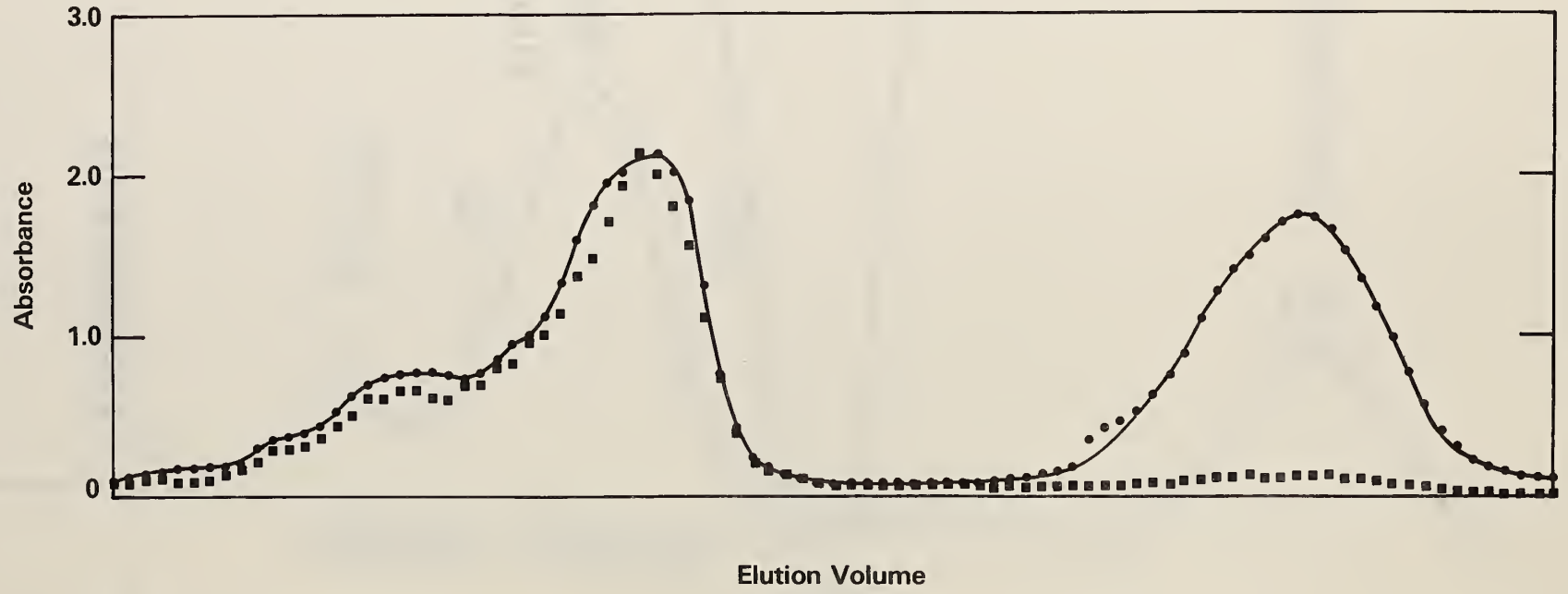

Fig. 9. Gel permeation chromatogram and specific activity of ^{125}I HSA. Solid line, elution chromatogram; broken line, specific activity.

Electrophoretic Analysis of Fractionated and Unfractionated HSA

SDS gel electrophoresis was performed upon a number of labeled and unlabeled HSA solutions, including fractionated and unfractionated samples. In each case, the results were consistent with the GPC data for that sample. Unfractionated HSA displayed several bands in its electrophoretic pattern, corresponding to monomer, dimer, and higher aggregates. Monomer and dimer fractions each displayed a single band, with the dimer trailing behind the monomer, as in the unfractionated HSA. Autoradiographs of unfractionated labeled HSA indicated the presence of activity in each fraction.

Ion Exchange Purification

The commercially labeled and nonlabeled proteins were further purified by passing these materials through an ion exchange column packed with 50-100 mesh Dowex 1-X4 resin (7). This additional purification was necessary to prevent any confusing and potentially misleading experimental artifacts due to the presence of impurities still remaining in the fractionated monomeric protein after passage through the GPC column. Two passes of the protein through 2 cc of the resin, which had been previously washed with 1 N HCL and with 20% and 0.85% NaCl, were sufficient to give reproducible results for the adsorbance of HSA (^{125}I) on polyethylene. A third pass through the column gave essentially the same result as the second, indicating that the column had removed the interfering impurities. Following the above experiments, we subjected an unchromatographed labeled protein solution to the same ion exchange treatment and found that the resulting solution

gave adsorbance values on polyethylene which were identical to those using ion exchange treated protein monomer fractions. These results suggest that fractionation may not be necessary in order to obtain adsorbance results consistent with monolayer surface coverage of monomeric protein. Rather, it may only be important to remove unreacted iodine and/or iodide ion, species which could be preferentially adsorbed to the substrate and yield high apparent adsorbances of labeled protein.

ADSORPTION OF HSA (^{125}I) AND (^{14}C) ON CONTROL SURFACES

Adsorption of HSA (^{125}I) Fractions

Various molecular weight components of HSA (^{125}I), indicated as I, II, III, IV on the elution pattern in Fig. 6, were used in adsorbance measurements on polyethylene. The results were obtained before the ion exchange treatment of the protein, described above, was found to lower the adsorbance of HSA to a value consistent with monolayer surface coverage. Nevertheless, the relative values obtained from fractions I-IV may be indicative of the adsorption behavior expected for fractionated HSA. The dimer fraction (I) showed an adsorbance slightly smaller than did the monomer fractions (II and III) which were both about the same. If small quantities of dimer were present in monomer fraction II, they apparently had little or no effect on the measured adsorbance, as compared with monomer fraction III, which is assumed to have less dimer content than II. The apparent adsorbance of the polypeptide fraction (IV) was much less than that of the higher molecular weight components, which is consistent with the observation that the adsorbance of proteins increases with molecular weight (1).

Adsorption of HSA (^{125}I) and (^{14}C) on Polyethylene

Figure 10 shows the adsorbance from different concentrations of monomeric HSA (^{125}I), purified by ion exchange treatment, on polyethylene. Each point is an average of two samples. The overall isotherm shape suggests Langmuir adsorption and is consistent with the formation of a close-packed monomolecular layer based on nominal surface area. This general behavior has been observed (1) with other

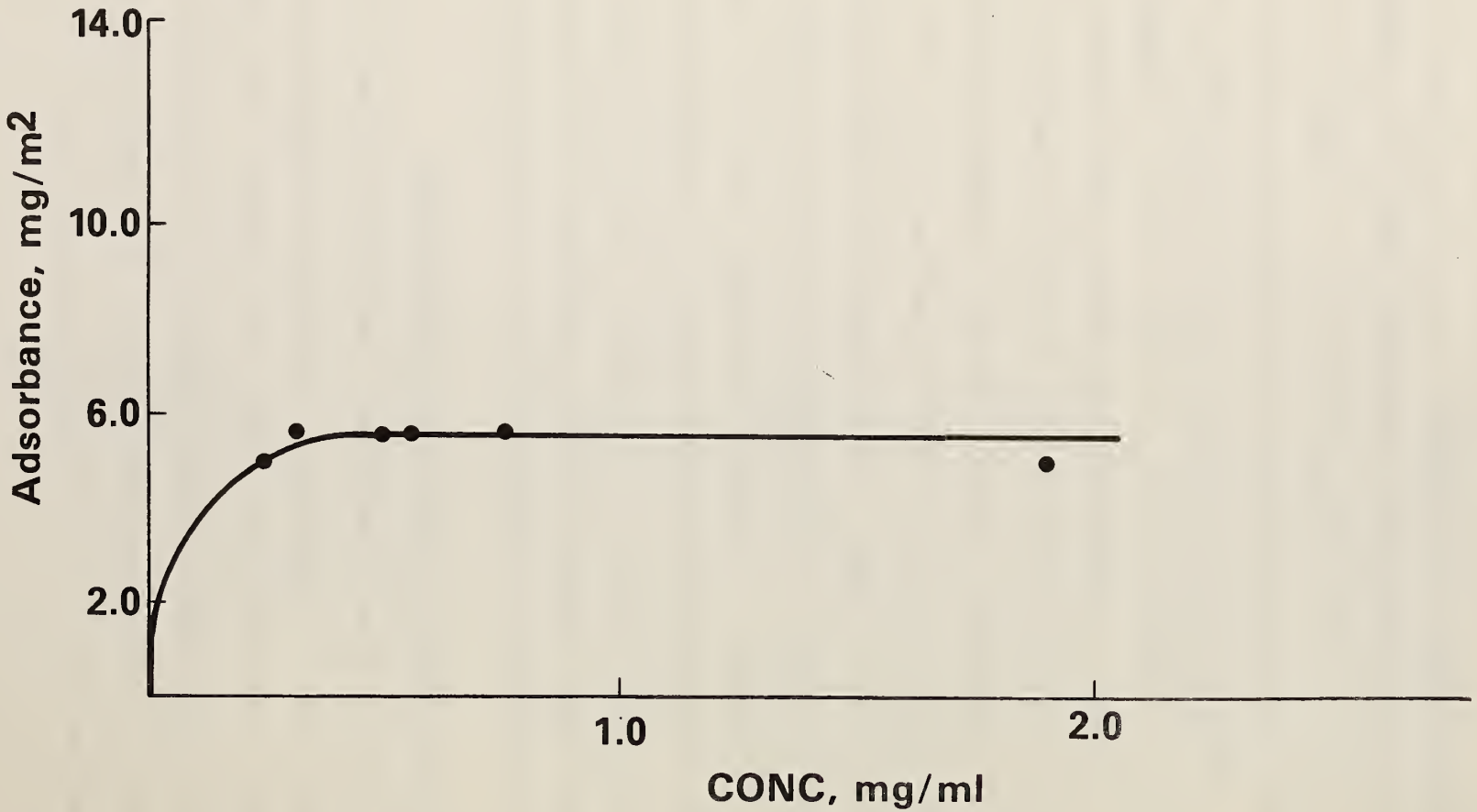

Fig. 10. Adsorption isotherm for the monomer of ^{125}I H.S.A. on polyethylene.

types of macromolecules at the solid-solution interface and on hydrophobic polymer surfaces (2,3).

Figure 11 is a plot of the adsorbance of HSA (^{125}I) on polyethylene as a function of time. As observed, the adsorbed protein concentration on the surface increases with time until a plateau value of approximately 5 mg/m^2 is reached. A little more than five minutes is required for the plateau value to be achieved.

Adsorption of ^{14}C labeled HSA on polyethylene was found to yield 5.44 mg/m^2, from a solution concentration of 4 mg/ml. This agrees well with the adsorbance of the iodine labeled compound and is consistent with monolayer adsorption.

The rate of desorption of the ^{14}C labeled HSA is shown in Figure 12. About one third of the protein is desorbed within the first hour, and the amount remaining after 17 h was found to be about half of that initially adsorbed. These results are compared with desorption from a polyetherurethane substrate, Biomer, in the following section.

Adsorption on Silicon Oxide and Platinum

HSA (^{125}I) monomer fraction, purified by 2 passes through an ion exchange column as described above, was used to measure the adsorption from solution onto three different test surfaces, which we have used in previous studies of protein adsorption. The results are tabulated in Table I. The theoretical monolayer coverage for HSA, assuming close-packed side-on ellipsoids (4 nm in diameter and 12 nm long), is about 2.5 mg/m^2, and for end on ellipsoids the value rises to 8.2 mg/m^2. One of the values in Table I lies within these limits, while the others are

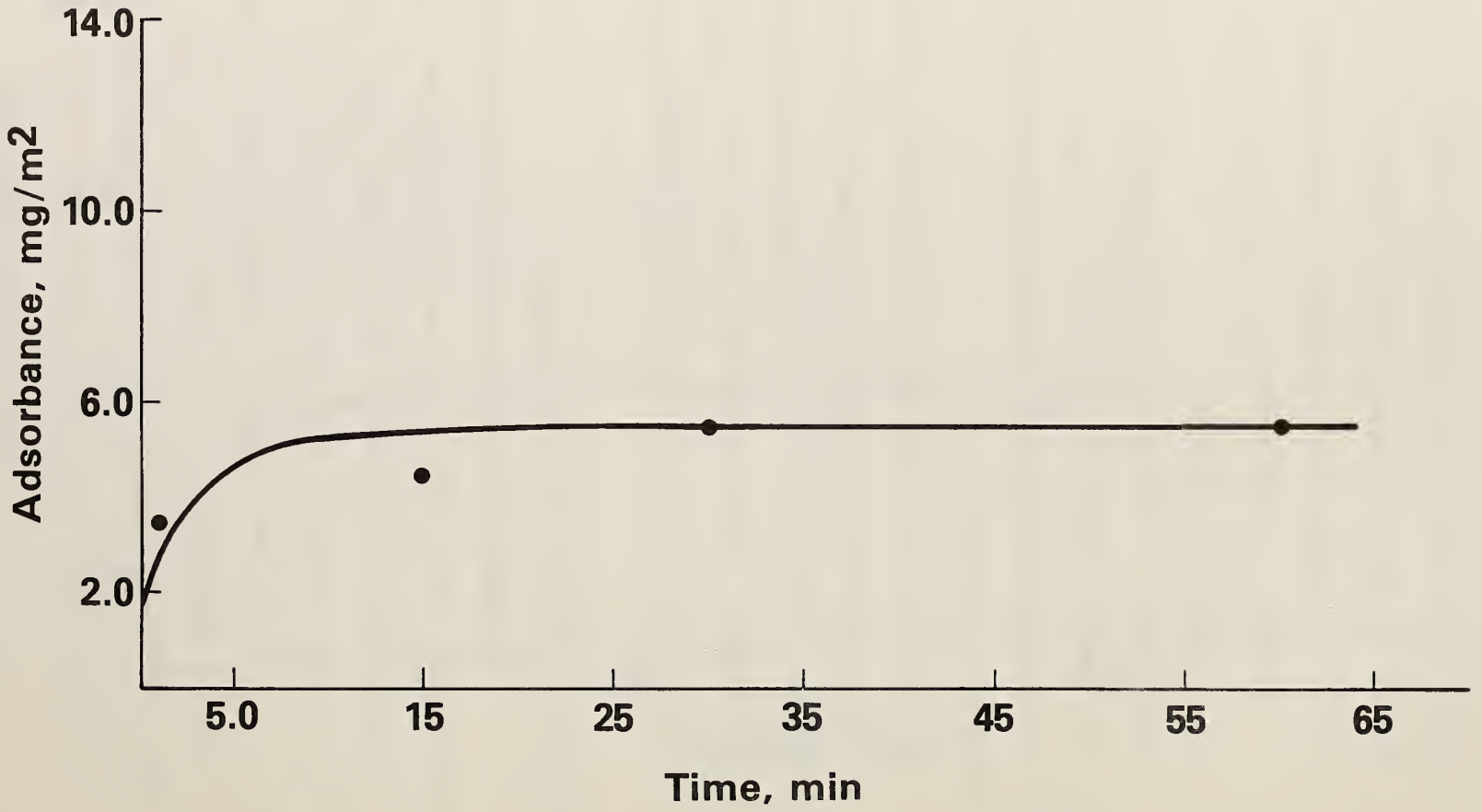

Fig. 11. Rate of adsorption of the monomer of ^{125}I H.S.A. on polyethylene. Solution concentration 0.560 mg/ml.

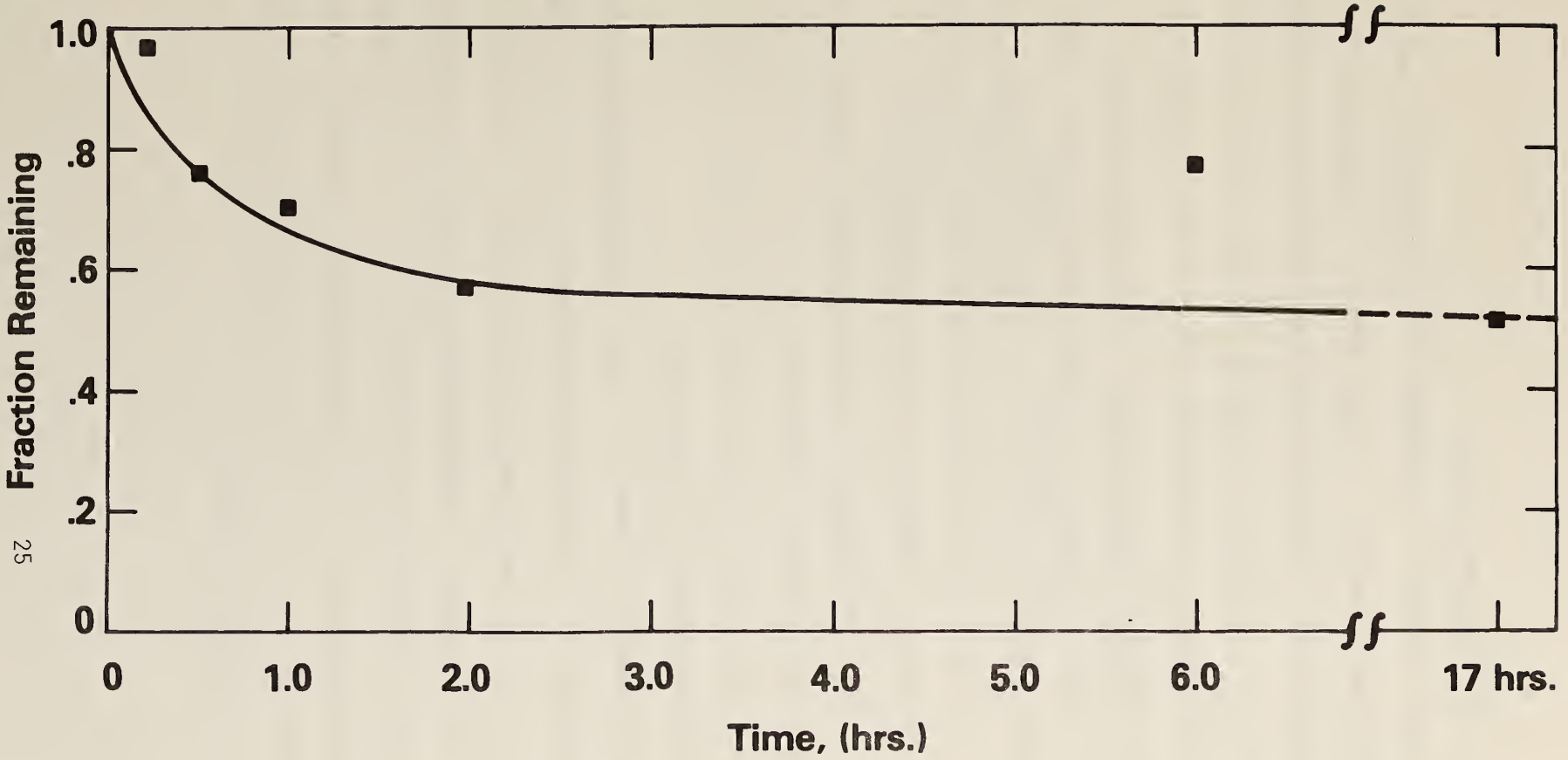

Fig. 12. Rate of desorption of ^{14}C H.S.A. from polyethylene into buffer solution.

well outside the monolayer limits.

The large difference in adsorbance on the hydrophobic and hydrophilic silicon oxide surfaces is qualitatively in accord with the results of MacRitchie (8) for γ-globulin on powdered silica surfaces of high and low free energy, and with our results for γ-globulin on the same two surfaces, as reported herein. At pH 7.4, albumin has a high negative charge (isoelectric point 4.9), and the hydrophilic surface is also negatively charged. Charge repulsion between protein and surface therefore leads to a relatively low amount adsorbed. On the hydrophobic surface, where the charge is very small, the adsorbance is within the approximate limits for monolayer coverage.

One adsorption experiment was performed using ^{14}C labeled HSA on the hydrophobic SiO_2 surface. The adsorbance, from a solution concentration of 2.5 mg/ml, was 5.3 mg/m^2, which agrees fairly well with the 3.2 mg/m^2 measured using ^{125}I labeled HSA.

We have previously reported (9) a very high adsorbance on platinum for HSA labeled with ^{131}I, which was attributed to a specific effect of the labeling atom. The value which we report here is even higher than that of the earlier report. Autoradiographs of platinum slides showing a very non-uniform distribution of adsorbed HSA (^{125}I) were reported in our last Annual Report (5). It is not surprising therefore that large differences in protein adsorption on platinum between different samples are found by radiotracer counting.

TABLE I

ADSORPTION OF ^{125}I HSA ON CONTROL SURFACES

Sample	Adsorbance (mg/m^2)
Oxidized Silicon (Hydrophobic)	3.2
Oxidized Silicon (Hydrophilic)	0.69
Platinum	150

CANDIDATE BIOMATERIAL SURFACES

HSA (^{125}I) purified by the ion exchange method described above was used to measure the adsorption from solution onto six different NHLBI contractor samples, as shown in Table II. In each case, the adsorption time was one hour. The protein solution concentration was 0.56 mg/ml for all samples except for the heparin on silastic, for which it was 1.9 mg/ml.

HSA (^{14}C) was adsorbed for one hour, at a solution concentration of 4 mg/ml.

Ethylcellulose Perfluorobutyrate and Glow-Discharge Coatings

The two hydrophobic substrates, ethylcellulose perfluorobutyrate and glow-discharge tetramethyldisiloxane coated polyethylene, both adsorb essentially the same amount of HSA as does polyethylene from the same solution concentration. In our last Annual Report (5), we reported the quantitative adsorption of fibrinogen onto these same surfaces, measured by ellipsometry. The adsorption of fibrinogen on both surfaces was essentially the same as on polyethylene, which is in agreement with our present findings for serum albumin.

Polyacrylamide Hydrogel

The large apparent adsorbance of HSA on the Polysciences hydrogel-grafted polyethylene substrate is probably due to entrainment of protein solution by the hydrogel. When the sample reported in Table II was soaked in water for 18 minutes, the apparent adsorbance was

TABLE II

ADSORPTION OF ^{125}I HSA AND ^{14}C HSA ON CANDIDATE BIOMATERIALS

Sample	Adsorbance (mg/m^2)	
	^{125}I	^{14}C
Ethylcellulose Perfluorobutyrate (Midwest Research Institute)	5.03	
Glow-Discharge Tetramethyldisiloxane Coated Polyethylene (Research Triangle Institute)	5.04	
Polyacrylamide Hydrogel Grafted to Polyetherurethane (Polysciences, Inc.)	35.52	
Polyetherurethane 3-2000-1-E (Stanford Research Institute)	8.49	7.64
Polyetherurethane 3-1000/425 (7/3)-1-X (Stanford Research Institute)	7.11	4.32
Heparin Bonded to Silastic (Carnegie-Mellon University)	11.50	
Biomer (Ethicon, Inc.)		3.08
Carbon Coated Stainless Steel (General Atomic Co.)		7.87

reduced to 25.4 mg/m^2. Thus, although the protein may not be tightly bound to the hydrogel, it does not quickly diffuse out of the hydrogel. These results are highly tentative, and further experiments would be required to quantitate the rate of diffusion into and out of the hydrogel coating.

Polyetherurethanes

In our last Annual Report (5), we reported very high apparent adsorbances of HSA (^{125}I) on the two polyetherurethanes from Stanford Research Institute from a solution concentration of 7 mg/ml, corresponding to many monolayers of protein, while polyethylene adsorbed about the same (5 mg/m^2) as it does from a much lower solution concentration. In our present experiments, reported in Table II, the adsorbances on the polyetherurethanes are only 40-50% higher than on polyethylene from a solution concentration of 0.56 mg/ml. Since the earlier experiments were performed with labeled HSA which had not been purified by the ion exchange method, it seems likely that the high results were due to adsorption of labeled contaminants (possibly iodide ions) by the substrates.

While we did not have enough purified ^{125}I labeled protein to repeat the adsorption experiments at higher solution concentrations, we have adsorbed ^{14}C labeled HSA on the polyurethanes, at a solution concentration of 4 mg/ml. The adsorbances, as shown in Table II, agree fairly well with the ^{125}I labeled HSA. For both labeled proteins the adsorbance is somewhat higher on the 3-2000-1-E sample. Since this sample is also more permeable to water, the results may indicate some

penetration of protein solution into the more porous substrate.

Heparin-Coated Silicone Rubber

Ellipsometry measurements on the heparin-coated silicone rubber samples from Carnegie-Mellon University were attempted. as reported in our last Annual Report (5). However, they were unsuccessful due to light scattering from the rough surface. The HSA (^{125}I) adsorbance reported in Table II is higher than expected for monolayer coverage, which suggests some permeation of solution into the heparin coating.

Biomer

The adsorbance of ^{14}C labeled HSA on Biomer was similar to the other polyetherurethanes reported in Table II. While the adsorbance was somewhat lower than on polyethylene (5.44 mg/m^2), there was no significant desorption of the protein from Biomer into buffer solution after 96 h, as shown in Figure 13. The amount retained on the two surfaces, however, was about the same after prolonged desorption.

Carbon Coated Steel

Samples of "ion plated" carbon on stainless steel were obtained from General Atomic Co. The adsorbance of ^{14}C labeled HSA on this substrate, as shown in Table II, was somewhat higher than on polyethylene or the polyetherurethane substrates.

Ellipsometry was used to determine the adsorbance of fibrinogen on the carbon coated steel. The value thus obtained, 5 mg/m^2, is

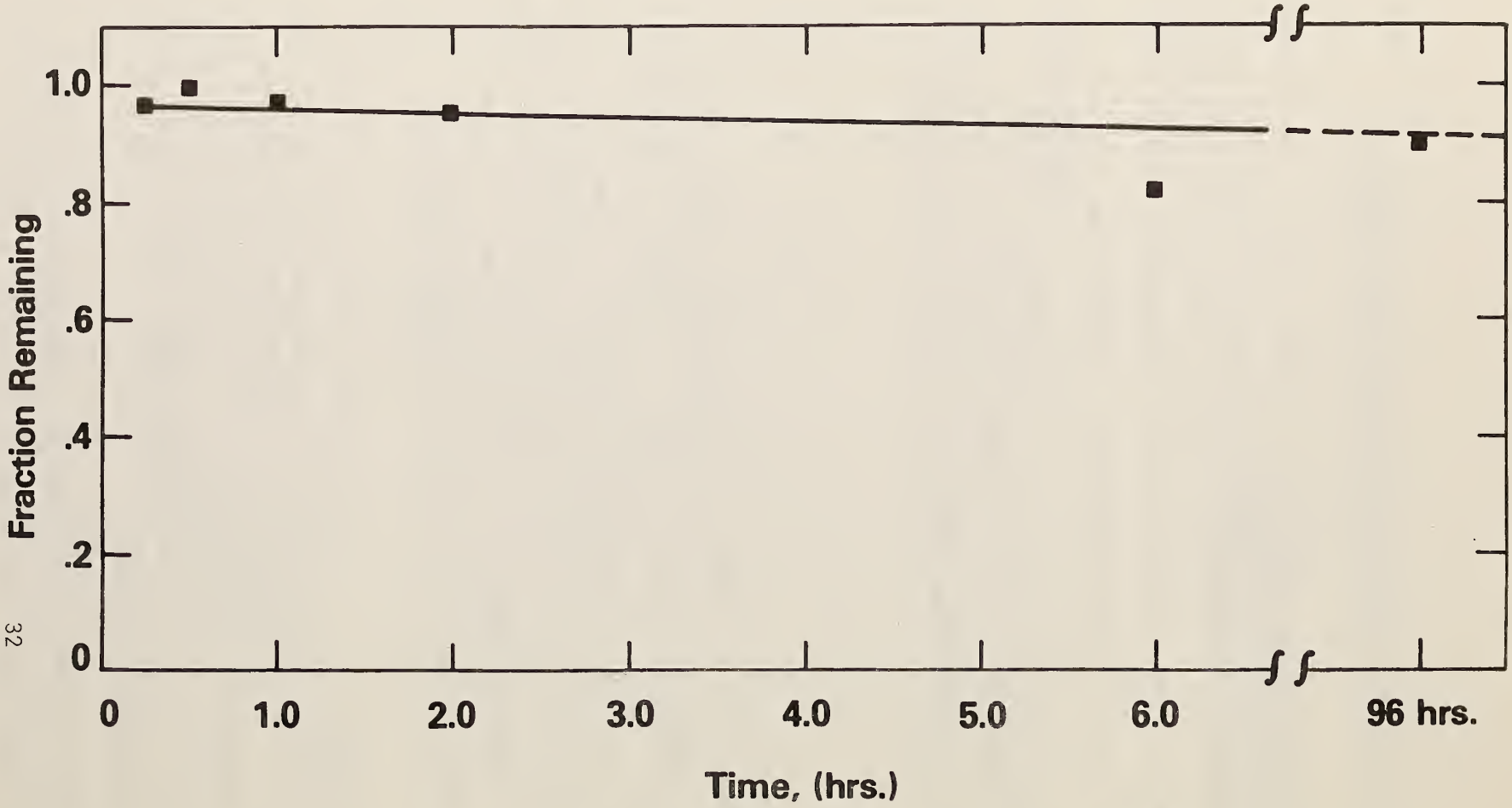

Fig. 13. Rate of desorption of ^{14}C H.S.A. from Biomer into buffer solution.

comparable to the adsorbance of fibrinogen on polyethylene and polyetherurethanes. Thus, the reason for the somewhat high adsorbance of the ^{14}C labeled HSA is not readily apparent.

ADSORPTION OF γ-GLOBULIN ON OXIDIZED SILICON

In our last Annual Report (5), we discussed the ellipometric measurement of adsorption of bovine γ-globulin on a specially prepared oxidized silicon surface in its "native" (hydrophobic) and hydrolyzed (hydrophilic) states, as a function of surface concentration. Preliminary results were also given for the adsorbance and molecular extension of the protein at three different pH's. These studies have been extended to a wider range of pH, as shown in Figure 14. At the highest pH used in this study (9.2), the adsorbance on hydrophobic silicon oxide is about four times that on the hydrophilic substrate. Near the isoelectric point (IP) of the γ-globulin (6.4), the adsorbance is about the same on the two surfaces, and at lower pH both are somewhat lower than at the IP.

Qualitatively, these phenomena may be explained in the following way. The hydrophilic surface has ionizable SiOH groups, which cause the surface to become progressively more negative above the point of zero charge. This point is unknown for the particular silicon oxide used in this study, but from studies of other forms of SiO_2 it probably lies between pH 1 and 4 (10). On the hydrophobic surface, the decrease in adsorbance above and below the IP is probably due to charge repulsion between the adsorbed protein molecules. On the hydrophilic surface, this effect is added to charge repulsion between the protein and the surface at high pH. At low pH, where the surface charge of both substrates is very small, the adsorbance on both surfaces is about the same.

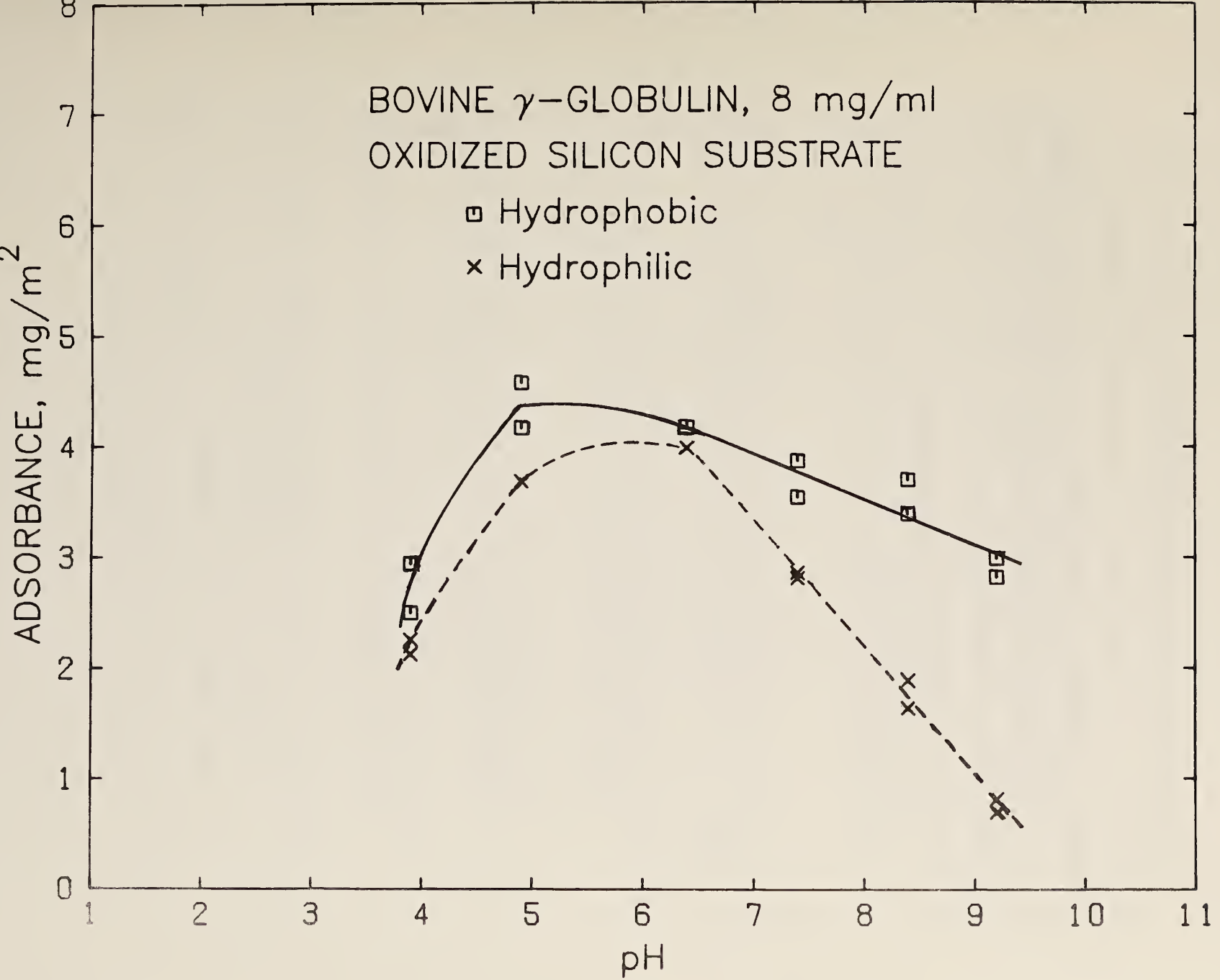

Fig. 14. Adsorbance vs pH for bovine γ-Globulin on hydrophobic and hydrophilic silicon oxide.

A qualitatively similar effect of pH on the adsorption of bovine serum albumin on high surface area silica powders was reported by MacRitchie (13), and by us (1) using an IR solution depletion method. The present ellipsometry study, performed on a flat surface and without appreciable solution depletion of protein, is a more realistic model of the behavior of an implant material in contact with blood proteins.

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15. Supplementary Notes

16. Abstracts

The purpose of this investigation is to help develop methods of characterizing the surface properties of implant materials for use in the cardiovascular system. These properties are related to the ultimate success or failure of an implant in vivo. The investigation during the current reporting period has been chiefly concerned with the amount of protein deposited on implant material surfaces. Ion exchange treatment of radiolabeled proteins has been found to lead to much greater accuracy and reliability of adsorption measurements. Results are reported for the quantitative adsorption of labeled proteins thus purified onto well characterized test surfaces, and onto the surfaces of materials supplied by contractors to the NHLBI Biomaterials Program. The effect of the label (^{125}I vs. ^{14}C) on adsorption of proteins was studied. Hydrophobic and hydrophilic oxidized silicon surfaces were used to demonstrate that the adsorption of γ-globulin, measured by ellipsometry, and serum albumin, measured by radiolabeled protein, both are significantly affected by charge interactions between the surface and the protein.

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