[CONTRIBUTION FROM CHEMISTRY DEPARTMENT, UNIVERSITY OF SOUTHERN CALIFORNIA]

Surface Areas of Proteins. I. Surface Areas and Heats of Absorption[1]

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The success enjoyed by the Brunauer–Emmett–Teller Method for interpreting gas adsorption isotherms in terms of the surface area and structure of the solid adsorbent has encouraged the hope that it might be similarly employed in elucidating the structure of crystalline proteins. Attracted by this possibility, a number of workers in recent years have made investigations of the water adsorption isotherms of dry, lyophilized, crystalline proteins.[1a-8] Some of these[3,6] have also made studies of nitrogen adsorption and found a considerable difference between the two types of adsorption, extending to differences of the order of magnitude of 100 to 200 in the areas observed with these two vapors.

The present study on the adsorption isotherms of nitrogen and oxygen was begun in the hope that these relatively inert gases should be capable of measuring a true surface area for protein particles and therefore of showing quite conclusively the presence or absence of a large, internal, pore structure for the protein molecule. With definite information such as this might provide on the superficial area of protein particles, isotherms for water and other adsorbates might then be interpreted in terms of structure with considerably more certainty.

The present paper is a report on the method of preparation of various proteins, the measurements of their nitrogen and oxygen adsorption isotherms and the interpretation of these isotherms in terms of surface areas of the protein particles.

Experimental

Preparation of Proteins.—The proteins used in this study were the following:

(1) **Bovine Plasma Albumin.**—This was obtained in dry, powdered form from Armour and Co. One part of the commercial product was kept untreated and another part was dissolved in water to make a 2% weight solution. This latter was lyophilized and dried.

(2) **Bovine Serum Fraction V.**—This was an Armour and Co. product obtained from Dr. Chester Hyman of the School of Medicine. The crude powder and a powder obtained by lyophilizing from a 2% water solution were used for measurement.

(3) **Human Plasma Fraction V-A.**—This was obtained from the Harvard Medical School and both crude and re-lyophilized samples were used.

(4) **Ash-Free Gelatin.**—This was the Eastman Kodak Co. product No. 1099. It is obtained in amber flakes which yield an amber-colored 2% solution. Strangely enough the final relyophilized product was pure white.

(5) **Oxy-poly-gelatin.**—This is a product of the California Institute of Technology. It was made by oxidizing Eastman gelatin with potassium permanganate and then polymerizing the product with glyoxal. The sample obtained was an amber-colored solution containing 15% sodium chloride. One sample was made by lyophilizing and drying the original material (with salt) and then relyophilizing the mixture from a 2% solution. This product showed only a slight yellow tint. A second sample was prepared by dialyzing the original material against de-ionized water until it gave no test for sodium chloride. It was then lyophilized and the resulting powder relyophilized from a 2% solution. It had less tint than the sample containing salt.

(6) **Egg Albumin.**—This was prepared from eggs by crystallization from ammonium sulfate solution following the procedures of Hopkins,[9] Adair[10] and Taylor.[11] The initial material was recrystallized seven times, dialyzed against de-ionized water until salt free and then lyophilized. Two additional samples were made by relyophilizing from 8 and 2% solutions. A sample of denatured albumin was made by heating a water solution and filtering the resulting precipitate. This was washed well with water and a 2% suspension was lyophilized and dried. A sample containing 80% ammonium sulfate was made by lyophilizing a water solution made up to 2% protein content. The salt content was determined by dialyzing a known weight of the solution against a known volume of de-ionized water and vacuum drying this water afterwards to determine its salt content.

Spray Freezing.—A 2% solution of egg albumin was sprayed from an atomizer with a 1-mm. orifice directly into a liquid nitrogen bath. The diameter of the spray droplets was about 0.1 mm. This spray formed a white, massive precipitate on freezing from which the liquid nitrogen was evaporated. The resultant precipitate was then vacuum dried at about −30° to give a fine white powder.

The vacuum drying assembly was a variation of the apparatus used by Flosdorf and Mudd.[12] It consisted of a 500 cc. round-bottom flask with a ground joint which contained the frozen material. This was connected to a 500 cc. trap which was immersed in a Dry Ice–ether-bath to collect the water and was connected to a vacuum pump. Drying time was from eight to twelve hours.

Weighings made on the samples before and after lyophilization and subsequent vacuum drying to determine the efficiency of drying showed about a 6% loss in weight. The proteins so prepared are quite hygroscopic. Even though kept in tightly closed, screw-cap bottles they show an average increase in weight of about 2% in a two-week period.

Final drying was effected before each run by pumping on a weighed sample overnight at room temperature and continuing until the steady pressure shown on a McLeod gage was less than 10^{-4} mm. The loss in weight in this step was about 6–8% for most proteins. Any residual water after such treatment may be considered as part of the structure. All the proteins so treated retained their water solubility even after adsorption isotherms were run.

Adsorption Measurements.—A modification of the apparatus used by Pease[13,14] was employed for the gas ad-

(1) Presented at the Spring Meeting of the American Chemical Society, Division of Physical and Inorganic Chemistry, Chicago, Ill., April, 1948.

(1a) Briggs, *J. Phys. Chem.*, **35**, 2914 (1931); **36**, 367 (1932).

(2) Bull, THIS JOURNAL, **66**, 1499 (1944).

(3) Shaw, *J. Chem. Phys.*, **12**, 391 (1944).

(4) Pauling, THIS JOURNAL, **67**, 555 (1945).

(5) Cassie, *Trans. Faraday Soc.*, **41**, 450 (1945).

(6) Palmer, Shaw and Ballantyne, *J. Polymer. Sci.*, **2**, 318 (1947).

(7) Frey and Moore, Abstract, Fall Meeting American Chemical Society, 1947, Division of Physical and Inorganic Chemistry.

(8) Zettlemoyer, *Trans. N. Y. Acad. of Sci.*, **II, 10**, 56 (1947)

(9) Hopkins, *J. Physiol.*, **25**, 306 (1900).

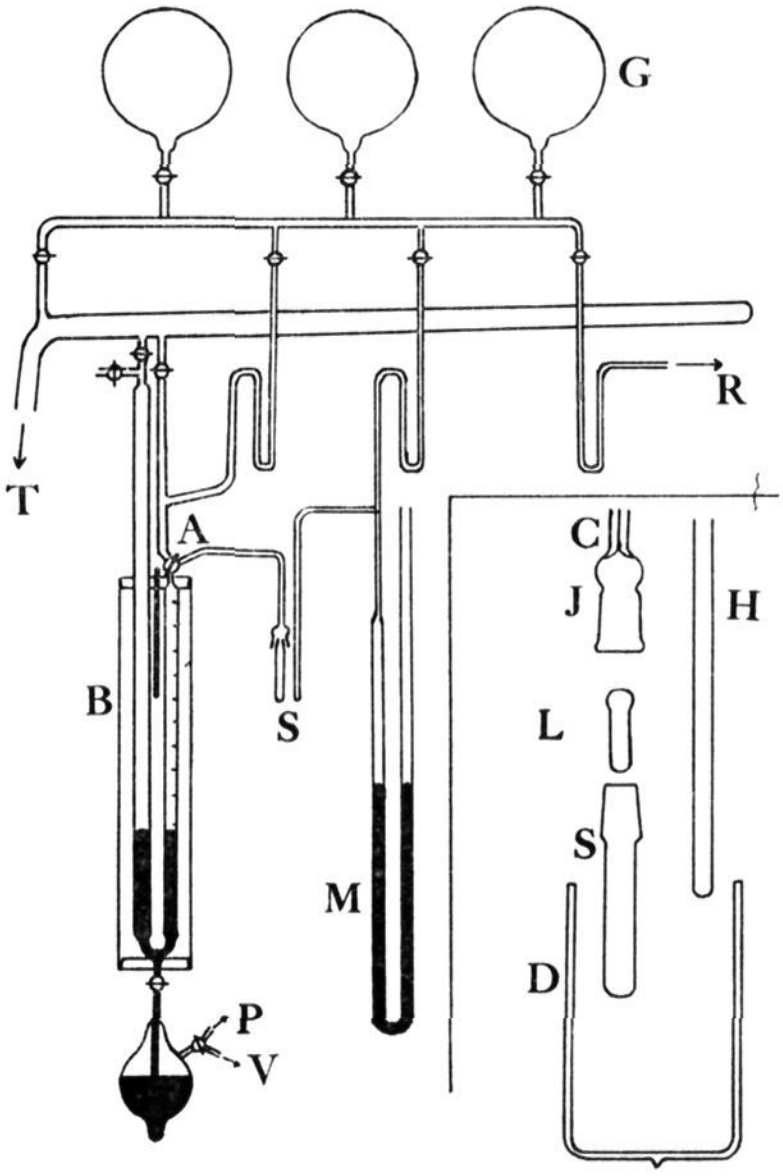
(10) Adair and Robinson, *Biochem. J.*, **24**, 993 (1930).

(11) Taylor, Adair and Adair, *J. Hyg.*, **32**, 345 (1932).

(12) Flosdorf and Mudd, *J. Immunology*, **29**, 389 (1935).

(13) Pease, THIS JOURNAL, **45**, 1176 (1923).

(14) Brunauer, "The Adsorption of Gases and Vapors," Vol. I, Physical Adsorption, Princeton University Press, Princeton, N. J., 1945



Absorption apparatus. Detail of sample tube.

Fig. 1.—Adsorption apparatus: A, valves; B, buret and manometer; G, gas storage bulbs; M, vapor pressure thermometer; P, to pressure; R, to gas purification train; S, sample tube; T, to trap and pumps; V, to vacuum. Detail of sample tube: C, capillary; D, Dewar flask; H, vapor pressure thermometer tube; J, ground joint; L, glass plug; S, sample tube.

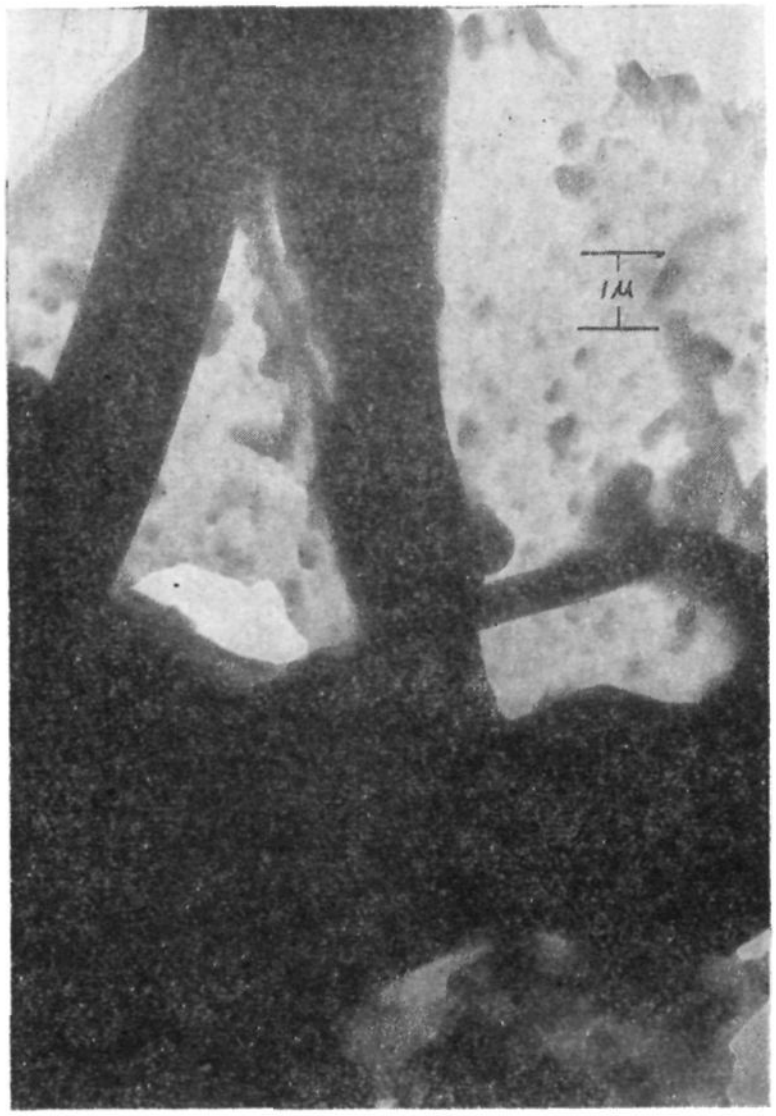


Fig. 2.—Photograph of bovine serum albumin prepared by lyophilizing 2% solution; dry particles spread on rubber cement film.

sorption isotherms. It is shown in Fig. 1. Pressures were read to 0.1 mm. with a cathetometer. Sample temperatures were measured to 0.02° with a gas thermometer immersed in a low temperature bath alongside the sample. The same gas used for the adsorption isotherms was used in this thermometer. This system is capable of covering a pressure range of 0–2 atmospheres. The small volume of gas in the bore of the capillary stopcock was allowed for in the volumes measured. All manometer readings were corrected to standard conditions.

The sample tube shown in the inset in Fig. 1 was filled with protein and a snug, hollow plug placed above the sample to reduce dead space. The standard taper joint was lubricated with Apiezon N high vacuum grease and showed no signs of leakage even though immersed in liquid nitrogen.

The dead space, which consists of two parts, that region at room temperature and that at bath temperature, was measured in the usual manner with dry helium gas. The dead space at bath temperature was about 10 cc. and that at room temperature 2 cc. These were checked very carefully to 0.01 cc. From these dead space measurements and a measurement of the dead space of the empty adsorption tube the sample densities could be calculated.

In all of the runs, equilibrium was attained within a few minutes and a complete adsorption–desorption cycle could be run in a few hours. There was no evidence of hysteresis in any of the runs. A blank run on an empty tube gave a calculated gas adsorption of less than 1% of the total gas volume at any point. In regular runs the deviations of individual points on a $V_{ads.}$ *vs.* $P/P_{sat.}$ plot were within 1% of the volume adsorbed for volumes above the BET point "B." Measurements made on different samples of the same protein gave areas checking to within 5%. The Berthelot equation and data on gases taken from Landolt–Börnstein were used to correct for non-ideality of nitrogen and oxygen.

The protein samples used varied from 0.500 to 2.000 g. and were weighed before and after runs to 1 mg. Specific areas are calculated on the basis of final, dry weight.

The helium, oxygen and nitrogen used were certified to 99% purity by the manufacturers. They were dried by passing over calcium chloride and phosphorus pentoxide and further treated before introduction into the vacuum system. The nitrogen was passed over copper at 400° to remove oxygen and then through a glass wool trap immersed in liquid nitrogen. The helium was passed through the same sequence preceded by a scrubbing over an activated charcoal trap immersed in liquid nitrogen to remove nitrogen.

Electron Microscope Studies.—Electron microscope studies were made of all the samples used and some pictures were taken of each. The crude, commercial proteins consisted of too large particles to be used under the electron microscope. The protein particles obtained by lyophilizing from 2% solutions were small enough to show structure. Samples were made by allowing the particles to adhere to a steel-mesh, supported film of rubber cement or

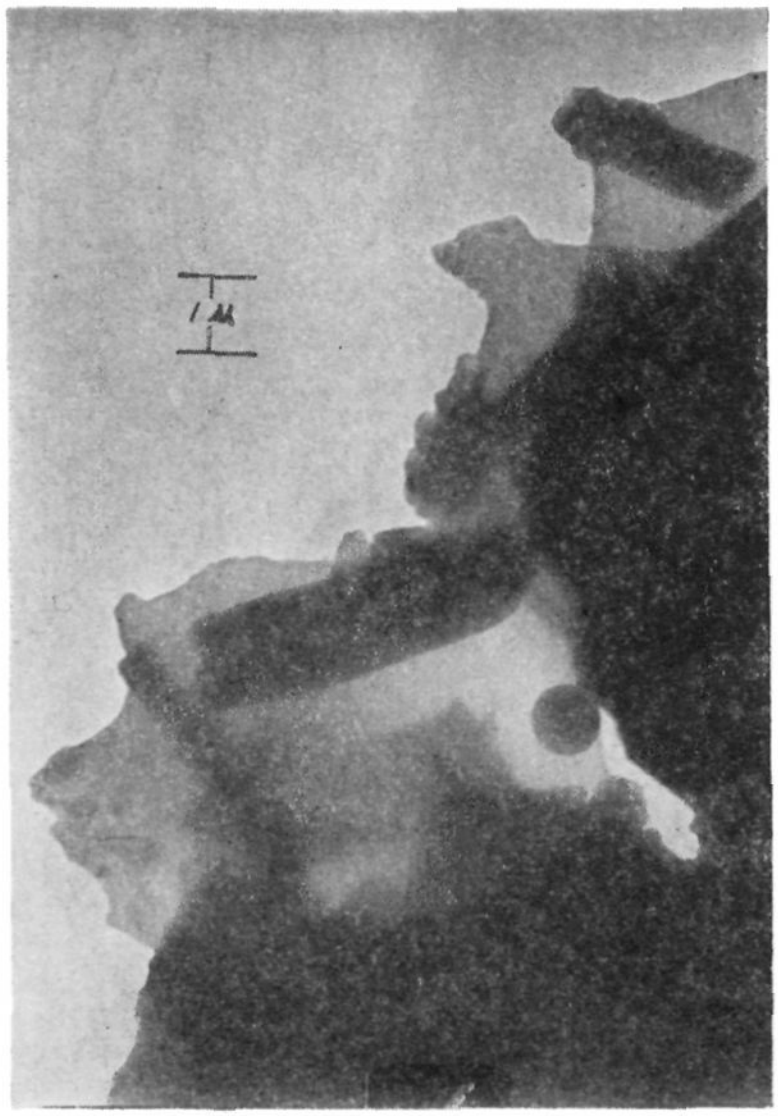


Fig. 3.—Photograph of egg albumin prepared by lyophilizing 2% solution; dry particles spread on rubber cement film.

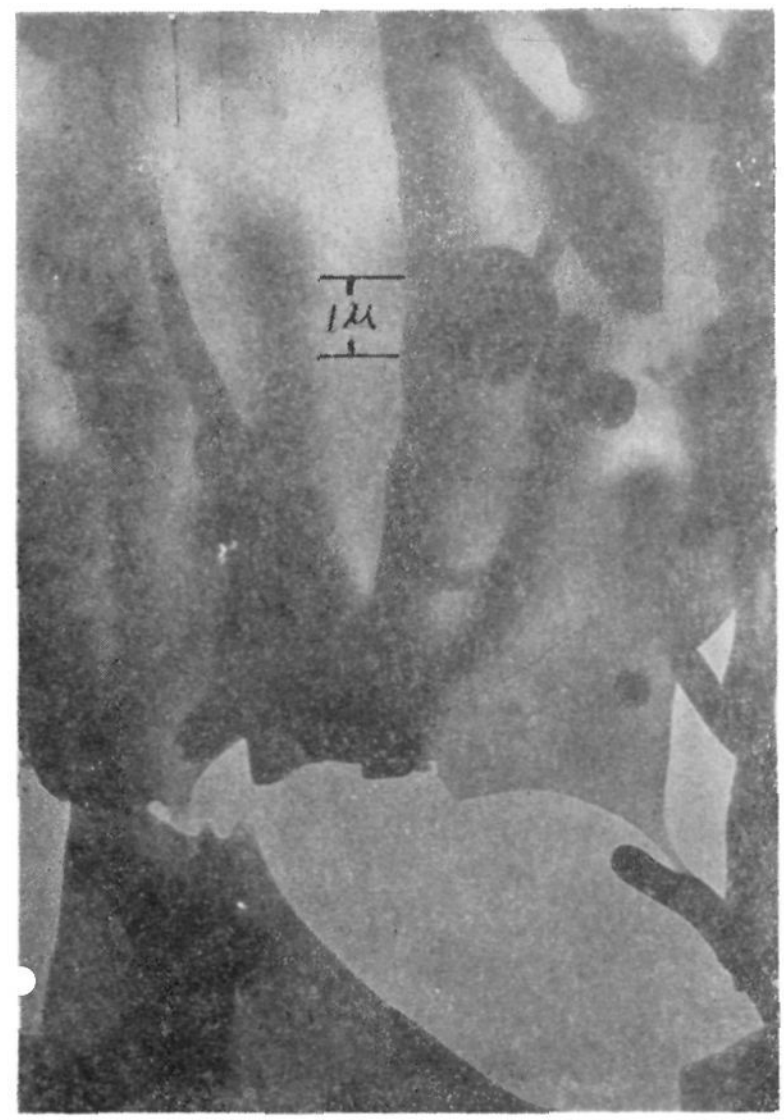


Fig. 4.—Photograph of egg albumin prepared by lyophilizing 8% solution; dry particles spread on rubber cement film.

collodion. Large particles showed no distinctive features but the smaller particles presented evidence of a fibrous, sheet structure with occasional holes and cylindrical fibers or branches extending from the sheets. These fibers had diameters of from 0.2 to almost 1 micron and were of irregular length. Evidence for sheet structure was obtained visually by changing the depth of focus to bring different sheets into individual prominence. Typical photographs are shown in Figs. 2, 3, and 4. These photographs were obtained through the courtesy of Dr. Daniel N. Pease and Dr. Richard F. Baker of the School of Medicine.

Results of Surface Area Measurements

The measured adsorption isotherms were plotted in terms of volume of gas adsorbed *vs.* relative pressure. Some typical isotherms plotted in this fashion are shown in Fig. 5. The same data are shown plotted according to the BET method in Fig. 5A. These are graphs of the quantity $P/V \cdot (P_0 - P)$ *vs.* P/P_0 and as can be seen from the figures they yield fairly good straight lines. From the slopes of these lines and their intercepts, the quantities V_m (the cc. STP of gas needed to cover the surface of the sample with a unimolecular layer) and $E_{(net)}$ (the average net heat of adsorption of the first layer) may be calculated. If it is assumed that the cross section of a nitrogen molecule is 16.2 sq. Å. (corresponding to a density of 0.808 g./cc. at 77.4°K.) and that for the oxygen molecule 12.8 sq. Å. (density of 1.14 g./cc. at 90.0°K.) it is possible to compute from V_m the surface area of the samples. Using the heats of liquefaction found in Landoldt–Börnstein at the appropriate temperatures and the quantity $E_{(net)}$ it is possible to compute $E_{(ads)}$ (the average heat of adsorption of the first gas layer). The results of the surface area measurements and these computations are shown in Table I. The values of the sample densities obtained from the helium dead space measurements are also given.

If the surface areas so calculated are interpreted in terms of uniform spherical particles, uniform rods or uniform sheets,[15] the dimensions corresponding to each of these shapes may be computed. Table II contains the results of such calculations.

Heats of Adsorption.—From the isotherms taken at 77.4 and at 90.0° K. with the same sample of egg albumin using both oxygen and nitrogen gases, it is possible by means of the Clausius–Clapeyron equation to calculate the partial molal heats of adsorption of these gases as a function of

(15) For the purpose of such calculations it is assumed that the areas of the edges may be neglected, *i. e.*, the rods and sheets are considered infinite in extent.

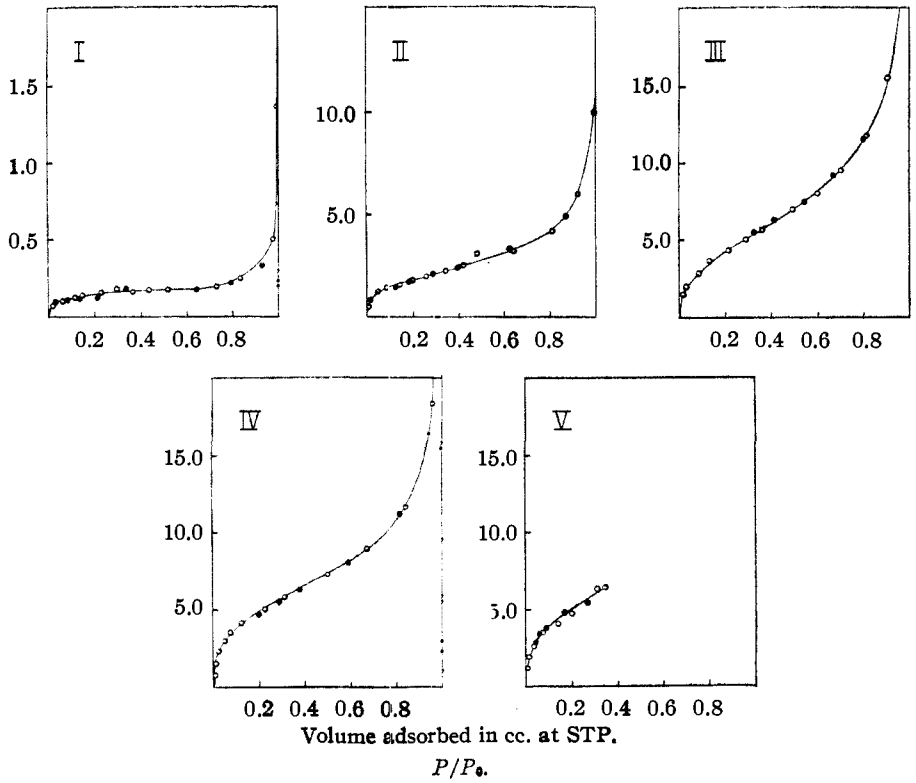


Fig. 5.—I, Nitrogen at 77.4°K. on bovine plasma albumin (crude sample); II, nitrogen at 77.4°K. on bovine serum Fraction V (sample lyophilized from 2% solution); III, oxygen at 77.4°K. on egg albumin (spray frozen from 2% solution); IV, nitrogen at 77.4°K. on egg albumin (spray frozen from 2% solution); V, nitrogen at 90.0°K. on egg albumin (spray frozen from 2% solution).

TABLE I

ADSORPTION OF NITROGEN AND OXYGEN ON PROTEINS

Material	Prepn.	Gas	Temp., °A.	Density, g./cc.	V_m[a] cc. STP	Area, sq. m./g.	E(net)[b] cal./mole	E(ads) cal./mole	"C" (from BET plot)
Bovine plasma albumin	Crude	N_2	77.4	1.33	0.123	0.54	660	2000	74
Bovine plasma albumin	Lyo-2%	N_2	77.4	1.46	2.07	9.06	564	1910	43
Bovine serum frac. V	Crude	N_2	77.4	1.35	0.058	0.26	675	2010	81
Bovine serum frac. V	Lyo-2%	N_2	77.4	1.27	1.57	6.88	596	1930	49
Human plasma frac. V-A	Crude	N_2	77.4	1.49	0.169	0.74	544	1880	35
Human plasma frac. V-A	Lyo-2%	N_2	77.4	1.32	2.53	11.1	642	1980	66
E-K ash-free gelatin	Lyo-2%	N_2	77.4	1.55	2.54	11.1	413	1750	15
Oxy-poly-gelatin	Lyo-2%	N_2	77.4	1.94	1.85	8.10	654	1990	71
Oxy-poly-gelatin with 15% NaCl	Lyo-2%	N_2	77.4	1.37	0.087	0.38	442	1780	11
Egg albumin	Lyo-8%	N_2	77.4	1.28	0.910	3.98	621	1960	58
Egg albumin	Lyo-2%	N_2	77.4	1.30	1.31	5.75	512	1850	28
Egg albumin (spray-frozen)	Lyo-2%	N_2	77.4	1.32	4.65	20.4	649	1990	67
Egg albumin (denatured)	Lyo	N_2	77.4	1.28	0.87	3.82	621	1960	57
Egg albumin with 80% ammonium sulfate	Lyo-2%	N_2	77.4	1.33	0.50	2.20	555	1890	37
Egg albumin (spray-frozen)	Lyo-2%	N_2	90.0	1.32	4.29	18.8	655	1990	39
Egg albumin (spray-frozen)	Lyo-2%	O_2	77.4	1.32	4.64	16.2	514	1850	28
Egg albumin (spray-frozen)	Lyo-2%	O_2	90.0	1.32	4.69	16.4	583	1920	25

[a] V_m is the volume (cc. at STP) of gas needed to cover the surface with a unimolecular layer. E(ads) is the average heat of adsorption over the first layer. Sample densities were computed from the helium dead space measurements. [b] E(net) is calculated from the BET equation: $E(\text{net}) = RT[\ln C + \ln (b_1a_2/a_1b_2)]$ "C" is obtained from the experimental BET plot and for the purposes of calculation it is assumed that the ratio $b_1a_2/b_2a_1 = 1$. $E(\text{net}) = E_{\text{ads. (1st layer)}} - E$ (liquefaction).

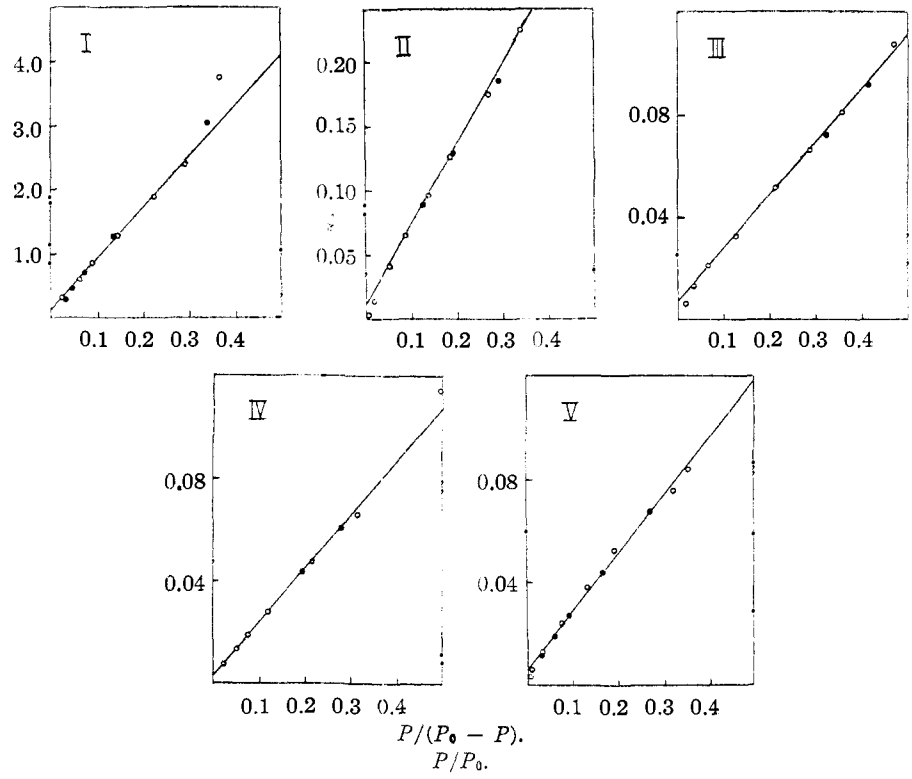


Fig. 5A.—BET plots: I, N_2 at 77.4°K. on bovine plasma albumin (crude sample); II, N_2 at 77.4°K. on bovine serum fraction V (sample lyophilized from 2% solution); III, O_2 at 77.4°K. on egg albumin (spray frozen from 2% solution); IV, N_2 at 77.4°K. on egg albumin (spray frozen from 2% solution); V, N_2 at 90.0°K. on egg albumin (spray frozen from 2% solution).

TABLE II

DIMENSIONS OF VARIOUS GEOMETRICAL SHAPES COMPUTED FROM SURFACE AREAS

Material	Gas	Diameters (in ångströms)		
	Gas temp., °A.	Uniform spheres	Infinite rods	Infinite sheets
Bovine plasma albumin—crude	N_2, 77.4	83,400	55,600	13,900
Bovine plasma albumin—lyo-2%	N_2, 77.4	6,660	4,440	1,110
Bovine serum frac. V—crude	N_2, 77.4	171,000	114,000	28,500
Bovine serum frac. V—lyo-2%	N_2, 77.4	6,840	4,560	1,140
Human plasma fraction V-A—crude	N_2, 77.4	54,420	36,280	9,070
Human plasma frac. V-A—lyo-2%	N_2, 77.4	4,086	2,624	681
E-K Ash-free gelatin—lyo-2%	N_2, 77.4	3,490	2,330	582
Oxy-poly-gelatin—lyo-2%	N_2, 77.4	4,860	3,240	810
Oxy-poly-gelatin—lyo-2% with 15% NaCl	N_2, 77.4	11,520	7,680	1,920
Egg albumin—lyo-8%	N_2, 77.4	11,760	7,840	1,960
Egg albumin—lyo-2%	N_2, 77.4	8,040	5,360	1,340
Egg albumin—(spray-frozen) lyo-2%	N_2, 77.4	2,230	1,488	372
Egg albumin denatured lyo	N_2, 77.4	12,300	8,200	2,050
Egg albumin with 80% ammonium sulfate—lyo-2%	N_2, 77.4	26,280	17,520	4,380
Egg albumin (spray-frozen)—lyo-2%	N_2, 90.0	1,730	1,150	288
Egg albumin (spray-frozen)—lyo-2%	O_2 77.4	2,808	1,870	468
Egg albumin (spray-frozen)—lyo-2%	O_2, 90.0	2,770	1,750	462

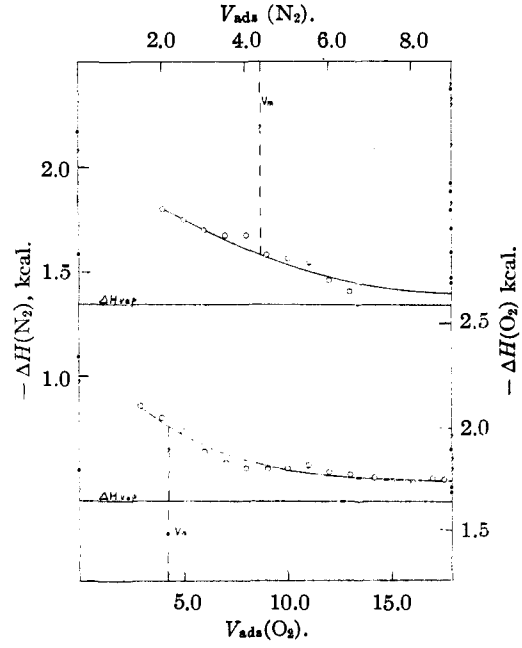


Fig. 6.—Plot of heat of adsorption *vs.* volume adsorbed per gram of sample computed from the Clausius–Clapeyron equation: egg albumin, 2% solution frozen in liquid nitrogen then lyophilized; at 77.4° and 90.0°K. top curve N_2, lower curve O_2.

the volume of gas adsorbed. Figure 6 shows the results of such calculation for both oxygen and nitrogen. The average heats of adsorption so computed agree to better than 5% with the same values obtained from the BET plots. It can be seen that the two gases behave similarly and in an expected fashion, the heat of adsorption being high for the first layer and decreasing rapidly to the value for the heat of liquefaction. This is a result of recent runs, earlier repeated runs with nitrogen having shown rather anomalous behavior.

Discussion

The low specific areas which the proteins display are in qualitative accord with the relatively large particles which have been observed under the electron microscope. The variation in these specific areas with method of preparation is similar to the type of behavior which may be expected of any soluble material precipitated from solutions of different concentrations. Dilute solutions produce small particles. These observations seem therefore to indicate that the areas measured with nitrogen are true particle areas. The only other nitrogen-protein isotherms reported are those of Shaw[15] for crystalline egg albumin. His value for the area (2.4 sq. m./g.) and average net heat of adsorption (648 cal./mole) are in good agreement with those of our own sample lyophilized from 8% solution. Although he does not report his concentrations, it would be reasonable to estimate that he used a solution of from 8–10% for his lyophilization.

The presence of salt seems to have a pronounced effect on the particle size, tending to decrease surface area and presumably increase particle size. This is not unexpected although there is at present insufficient evidence on which to speculate concerning a mechanism. Significant also is the difference for the two different samples used. In the case of oxy-poly-gelatin, 15% of sodium chloride decreased the specific area by a factor of over 20, whereas egg albumin showed a decrease of only a factor of 2 with 80% ammonium sulfate. Further experiments are now in progress to determine whether or not this is a specific effect of the particular salt or of the protein.

Denaturation, as was expected, reduced the specific surface area in the case of egg albumin. This is presumably due to the formation of larger particles in the system.

The generally low surface areas exhibited by all of these proteins is in quite marked contrast to the areas determined from water adsorption isotherms by other workers.[1,2,6,15a] The areas measured with water are from 40 to 250 times greater than those obtained in the present work on similar proteins. The conclusion drawn is that the protein particle presents no internal surface accessible to either the nitrogen or oxygen molecule. Such is not the case with water, a molecule whose diameter is not much smaller than either oxygen or nitrogen. It seems reasonable to conclude that the adsorption of water by proteins is a quite different process from the adsorption of oxygen or nitrogen. Further strong supporting evidence is provided by the very large hysteresis which is observed in the adsorption of water, a phenomenon singularly absent in the work with nitrogen and oxygen. It seems highly probable that water adsorption is closely related to hydration of specific polar regions in the individual protein molecules rather than non-specific adsorption on the exterior surfaces of large aggregates. Similar conclusions have been reached by other workers,[1a,4,5,6,15,16,17] drawing their conclusions from shifts in X-ray spacings accompanying water adsorption[4,16,17] and fairly close relations between the amounts of water required to make a unimolecular layer and the number of polar groups available for hydration.[18]

Hysteresis is to be expected in the case of water if these explanations are correct, since the water molecule must actually spread apart layers of protein to penetrate to the polar groups. Such a process would require considerable activation energy and plans are now under way to study the kinetics of this process in the hope of gaining further information about it.

Another consequence which may be expected to follow from the foregoing is that the water adsorption isotherms should be independent of the particle size whereas we have shown this is certainly not the case with nitrogen. Experiments are now in progress on samples of protein of every different nitrogen area to see if there are differences in their water isotherms.

Acknowledgments.—The authors wish to acknowledge their indebtedness to Dr. John W. Mehl of the School of Medicine for his advice in the preparation of the egg albumin samples, and to Dr. Daniel N. Pease and Dr. Richard F. Baker, also of the School of Medicine, through whose courtesy the electron microscope photographs were obtained.

Summary

The initial phase of a program of investigation of the molecular structures of crystalline proteins has been concerned with the measurements of surface areas by the BET method, using dry nitrogen and oxygen at different temperatures.

The proteins studied were egg albumin, bovine plasma albumin, bovine serum fraction V, human plasma fraction V-A, ash-free gelatin, oxy-poly-gelatin, heat-denatured egg albumin, and salt–

(15a) Shaw, *J. Chem. Phys.*, **12**, 391 (1944).

(16) Katz and Derksen, *Rec. trav. chim.*, **51**, 513 (1932).

(17) Boyes, Watson and Perutz, *Nature*, **151**, 714 (1943).

(18) E. F. Mellon, A. H. Korn and S. R. Hoover, "Water Absorption of Proteins." Paper presented at the High Polymer Forum, Meeting of the American Chemical Society, Chicago, Ill., April, 1948. These authors have presented very strong evidence for the specificity of water adsorption by peptide linkages through a study of the water adsorption isotherms of a series of synthetic, polyglycine polymers of from 44 to 59 monomer units.

protein mixtures. The proteins were fractionated, crystallized, dried and relyophilized from water solutions of different concentrations.

Specific areas ranged from about 0.20 to 4.00 sq. m./g. for proteins lyophilized from concentrated solutions, and 6.00 to 14.00 sq. m./g. for dilute solutions. Denatured egg albumin had a smaller area than the undenatured material. Salt–protein mixtures showed much smaller areas. Spray-freezing the dilute protein solutions into liquid nitrogen baths and then drying the resultant powder raised the areas four-fold. Electron microscope studies were made of all the proteins used.

The low specific surface areas are in qualitative accord with the large particles observed under the electron microscope. The areas obtained from water adsorption data of other workers are from 40–200-fold larger than the nitrogen areas. These comparisons lead to the conclusion that water and nitrogen adsorption are quite different phenomena. The former is more closely related to hydration of specific groups within the protein molecule with probable physical change in the protein dimensions.

Measurements on egg albumin were made with both nitrogen and oxygen at two different temperatures. From these isotherms, a calculation of the partial molal heats of adsorption as a function of volume adsorbed may be made. The net heats calculated in this way are in good agreement with the net heats obtained from the BET plots for the first layer.

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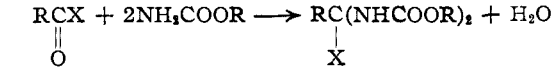
[CONTRIBUTION FROM THE LABORATORY OF THE U. S. CHEMICAL CORPORATION]

The Preparation of Indanone Derivatives by a Carbamate–Aldehyde[1] Reaction

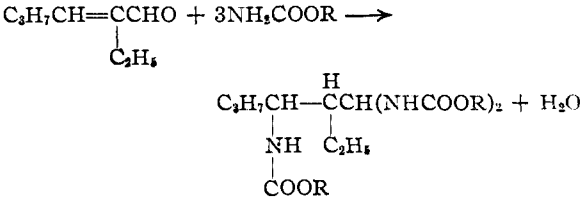
BY WILLIAM M. KRAFT

The recent publication of Kraft and Herbst[1a] of the research investigating the condensation of carbonyl compounds with carbamates described the reaction of one mole of saturated aliphatic aldehydes and pyruvic acid with two moles of aliphatic carbamate. In the case of the unsaturated



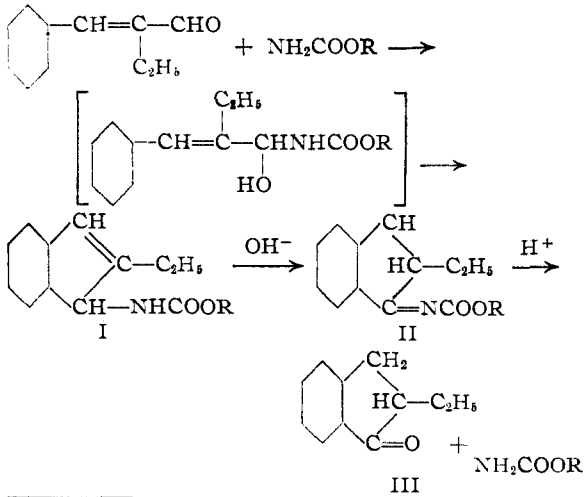
where X = H or COOH.

aldehyde, 2-ethylhexen-2-al-1, reaction was in a one to three ratio, two carbamate molecules adding at the carbonyl group, while the third added at the double bond, presumably at the β-position.



A study has been made to determine whether the latter type of reaction is a general one for α,β unsaturated aldehydes such as crotonaldehyde, cinnamaldehyde and α-ethylcinnamaldehyde in reactions with ethyl, isopropyl and benzyl carbamate. It was found that reactions with crotonaldehyde and cinnamaldehyde required two moles of carbamate to one of the aldehyde. Yields with cinnamaldehyde were low and were attended by resin formation (also noted by Martell[2]).

The α-ethylcinnamaldehyde-carbamate reaction was peculiar in that the reaction products all indicated a 1:1 addition. These products were unaffected by refluxing for several hours in dilute or concentrated mineral acids; consequently, it was considered unlikely that the usual addition reaction, at the carbonyl group, had occurred. However, when the product of the reaction was treated with dilute aqueous alkali, another crystalline material (II) was obtained, which on further treatment with dilute mineral acids regenerated the original carbamate and another compound, a ketone (III). Speculation as to the nature of this latter compound indicated the follow-



(1) This paper was presented before the Organic Division of the American Chemical Society, in Atlantic City, N. J., April, 1947.

(1a) W. M. Kraft and R. M. Herbst, *J. Org. Chem.*, **10**, 478 (1945).

(2) T. R. Lewis, Jr., F. R. Butler and A. E. Martell, *J. Org. Chem.*, **10**, 145 (1945).