The process can also be successfully carried out by adding a saturated solution of aluminum chloride dissolved in nitrobenzene. Anhydrous conditions (including freedom from atmospheric moisture), must be maintained to prevent hydrolysis of the salt.

Anal. Calcd. for $AlC_6H_9O_6$: Al, 13.21; C, 35.30; H, 4.45. Found: Al, 13.94; C, 35.50; H, 4.47.

In verifying the method of Lösch,[4] 3 g. of granular aluminum metal was added to a mixture of 60 g. of acetic acid and 3 g. of acetic anhydride and the solution refluxed. The white precipitate which formed was separated from the remaining aluminum, washed with dried acetone, and dried at 110°. A yield of 9.6 g. was obtained. A greater yield could undoubtedly have been obtained had the reaction been allowed to continue for a longer period.

The diffraction pattern of the aluminum triacetate prepared from aluminum chloride was quite diffuse, indicating very minute sized crystals.[7] A sharp pattern was not obtained until the crystals had been refluxed in acetic anhydride for a half hour. The pattern of the aluminum triacetate prepared by our method was identical with the pattern of the compound prepared according to the procedure of Lösch. The interplanar spacings are recorded in Table III.

TABLE III

INTERPLANAR SPACINGS OF ALUMINUM TRIACETATE

d	Intensity	*d*	Intensity
9.62	S	3.05	VW
8.08	S	2.92	VW
7.86	VS	2.85	VW
6.72	M	2.49	VW
6.23	W	2.39	VW
4.98	W	2.26	VW
4.60	W	2.17	VW
3.98	W	2.11	VW
3.85	M	2.02	VW
3.65	VS	1.89	VW
3.50	W	1.87	VW
3.37	W	1.52	VW
3.17	VW	1.49	VW

Aluminum Tripropionate $Al(CH_3CH_2CO_2)_3$.—To a refluxed mixture of 20 ml. of propionic acid and 20 ml. of propionic anhydride was added 3 g. of solid anhydrous aluminum chloride. Upon cooling, a white precipitate formed which was filtered, washed with dried acetone, and dried at 110°. A yield of 3.4 g. was obtained. The compound was also prepared with the aluminum chloride dissolved in nitrobenzene.

Anal. Calcd. for $AlC_9H_{15}O_6$: Al, 10.95; C, 43.90; H, 6.15. Found: Al, 11.84; C, 44.18; H, 6.24.

It was not found possible to prepare the tripropionate using the procedure suggested by Lösch.

The diffraction pattern of the aluminum tripropionate was also extremely diffuse. The crystals could not be refluxed in propionic anhydride since they were soluble. Hence, dried acetone was employed and a sharp pattern obtained. The interplanar spacings are recorded in Table IV.

TABLE IV

INTERPLANAR SPACINGS OF ALUMINUM TRIPROPIONATE

d	Intensity	*d*	Intensity
8.49	S	3.55	W
8.03	VS	3.27	W
6.67	VW	3.21	VW
6.37	VW	2.85	VW
6.06	VW	2.60	VW
5.53	VW	2.45	VW
5.25	VW	2.37	VW
4.86	M	2.22	VW
4.60	W	1.99	VW
4.27	VW	1.87	VW
3.79	M[a]		

[a] Very diffuse.

(7) C. W. Bunn, "Chemical Crystallography," Oxford University Press, London, 1948, p. 127.

Summary

A method of preparation for aluminum diacetate and dipropionate is given along with a verification of Sheĭnkman's procedure for the preparation of the diacetate.

A new method is described for the preparation of aluminum triacetate and tripropionate and Lösch's procedure for the preparation of the triacetate is verified.

The X-ray powder diffraction patterns of the above compounds were taken and the interplanar spacings reported.

MADISON, WISCONSIN RECEIVED SEPTEMBER 26, 1949

[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT, UNIVERSITY OF SOUTHERN CALIFORNIA]

Surface Areas of Proteins. II. Adsorption of Non-Polar Gases[1]

BY SIDNEY W. BENSON AND DAVID A. ELLIS

In an earlier paper[2] we reported on the initial results of a program of investigation of the surface properties of dry, lyophilized proteins through the methods of gas adsorption. In the present paper we wish to report the additional results which have been obtained through an extension of this work to a study of the adsorption of a series of non-polar gases of different molecular dimensions on dry, lyophilized proteins.

The purpose of the present investigation was to discover whether or not the protein particles contained any appreciable pore structure, by measuring the isotherms of gases of different molecular diameter; and to try to confirm the inherently particle dependent nature of the property of physical adsorption. To accomplish this the following series of non-polar gases was selected: nitrogen, oxygen, argon, methane, neopentane, sulfur hexafluoride and *n*-butane. For purposes of comparison the isotherms were measured for each gas at a set of nearly corresponding temperatures, in the vicinity of their normal boiling points.

(1) This work was carried out through the aid of a grant from the Research Corporation. This paper was presented at the High Polymer Forum of the American Chemical Society during the Fall Meeting, held at Atlantic City, N. J., September, 1949.

(2) Benson and Ellis, THIS JOURNAL, **70**, 3563 (1948).

Experimental

Preparation of Proteins.—The proteins used in the present work were egg albumin, bovine plasma albumin, and porcine plasma fraction II. The purification and lyophilization of these proteins is described in the previous paper.[2] Most of the adsorption studies were made on samples which had been spray-frozen from a 2% aqueous solution, sprayed into liquid nitrogen and vacuum dried first at low temperatures and then at room temperature.

As a check on the effect of the spray-freezing technique on the proteins, electrophoresis patterns were made of two samples of egg albumin, one lyophilized from 5.6% solution and the second a 2% solution spray-frozen in liquid nitrogen.[3] The runs were made for a period of 3 hours at *p*H 8.4. The *p*H chosen was that for which the graph of Longsworth, *et al.*,[4] of mobilities *vs.* *p*H for the various components of egg white shows the greatest separation. The patterns obtained for the two samples in 1% solution were identical with each other and indistinguishable from the patterns obtained by Longsworth.[4] This, together with the retention of solubility, seems to constitute fairly good evidence for the conclusion that the spray-freezing technique does not denature the proteins or change their composition.

An attempt was made to pick up evidence for crystal structure by means of X-ray diffraction techniques.[3] Powder patterns taken of lyophilized and spray-frozen samples of bovine plasma fraction V and of egg albumin showed no peaks except for 1 to 2 Å. spacings. Allowing the samples to adsorb water vapor to a relative moisture content of 20% did not change the X-ray diagram.

Purification of Gases.—(1) The methods used for preparing helium, nitrogen and oxygen are essentially those described in the earlier report.[2] (2) Argon was obtained in a steel cylinder from the Matheson Co. It was labeled C. P. grade, 99% pure. It was passed through a drying tube containing calcium chloride and phosphorus pentoxide, through activated charcoal at 77°K., and over copper at 400°. (3) The methane was also Matheson Co., C. P. grade, 99% pure. It was dried and distilled at liquid nitrogen temperature a few times. (4) The *n*-butane (Matheson Co.) was also C. P. grade, 99% pure. It was dried and then pumped at liquid nitrogen temperature to remove non-condensables. (5) The neopentane was research grade supplied through the courtesy of the Phillips Petroleum Corp. It was dried and pumped at 77°K. to remove non-condensables. (6) Sulfur hexafluoride (Matheson Co.) was C. P. grade, 99% pure. It was also dried and pumped to remove non-condensables.

All gases except helium were condensed and vapor pressures were measured at convenient temperatures and checked against values found in the literature. In Table I are shown the observed vapor pressures compared with values found in Landölt–Bornstein.

TABLE I

VAPOR PRESSURES OF GASES

Gas	Temp.	Vapor pressures, mm. Observed	From literature
CH_4	77.5°K.	14.0	11.3
n-Butane	0.0°C.	779.5	775
Neopentane	0.0°C.	539.3	540
SF_6	−78.5°C.	334.9	332[a]
A	90.3°K.	1031.7	1030.0
	77.5°K.	206.2	206.0

[a] This comparison is within the accuracy of the original data and the uncertainty in the absolute temperature of the Dry Ice-bath used for measurements.

Apparatus and Cryostat.—The adsorption apparatus was the same as the one described in the earlier paper.[2] A new feature was a low temperature cryostat which consisted of an outer aluminum cup containing a snug fitting block of aluminum. The inner block of aluminum had adjacent holes drilled to accommodate the sample tube and gas thermometer. It was wrapped with insulated, fine copper wire through which a heating current could be passed. The entire assembly was immersed in a large dewar containing an appropriate coolant such as liquid nitrogen or liquid oxygen. Heating current was controlled by a relay actuated by the gas thermometer. The temperature remained constant to ±0.1° and by following the slow cyclic variation, readings could be made to within ±0.01°. This cryostat can be used in the region from liquid nitrogen temperatures to Dry Ice temperatures.

For higher temperatures above Dry Ice temperatures, an assembly consisting of a pyrex outer shell and an inner copper can wound with insulated copper wire was used. The space between the pyrex cup and the copper can was filled with a heat conducting liquid such as alcohol or ether, and the interior of the copper can was filled with the same liquid stirred by a small motor. With this liquid, bath temperature fluctuations were within ±0.05 to ±0.02° and again readings could be consistently made to within ±0.01°. The outer cooling bath contained a slush of Dry Ice and alcohol or ether.

Both cryostats are shown in exploded view in Fig. 1. They are modifications of similar units described by Steacie and others.[5,6,7]

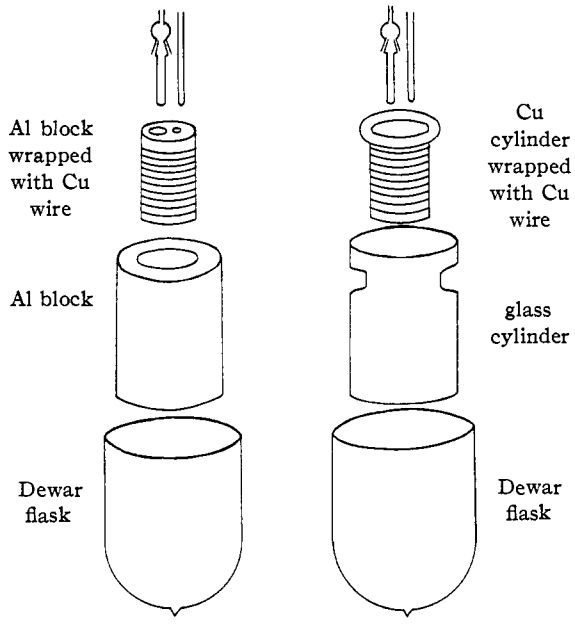

Fig. 1.—Cryostats.

Experimental Results

Adsorption isotherms were measured for each of the gases at a number of different temperatures using different proteins. In each case, adsorption as well as desorption points were measured. As a check for consistency, the total gas adsorbed was always compared with the amount desorbed.

(3) The authors wish to express their appreciation to Dr. R. D. Vold and Mr. J. D. Grandine of the Chemistry Department of the University of Southern California for their coöperation in making the powder patterns. We are also indebted to Dr. J. Mehl of the Biochemistry Department for his assistance with the electrophoresis patterns.

(4) Longsworth, Cannan and MacInnes, THIS JOURNAL, **62**, 2580 (1940).

(5) Shepherd, *J. Res. Natl. Bur. Stand.*, **2**, 1154 (1929).

(6) Savelli, *Ind. Eng. Chem., Anal. Ed.*, **13**, 868 (1941).

(7) Steacie, *J. Chem. Phys.*, **12**, 484 (1944).

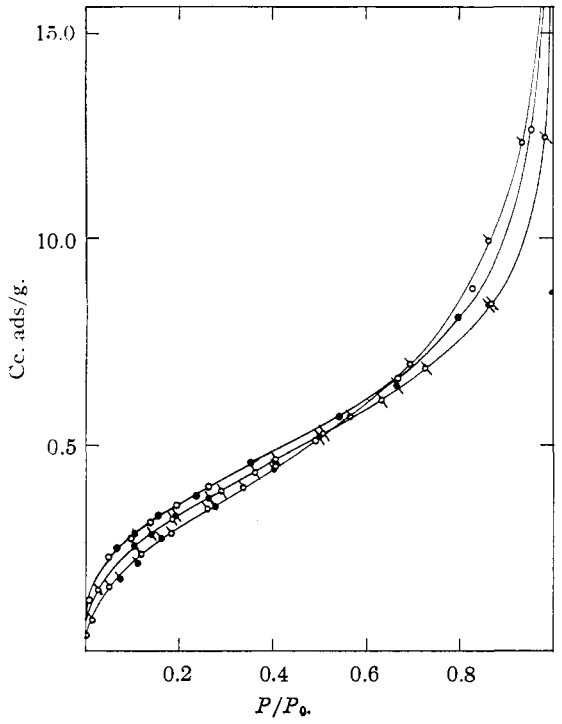

Fig. 2.—Egg albumin, 2% spray frozen, 0.8423 g.: nitrogen, 77.5° A., O; oxygen, 90.3° A. ◔; argon, 77.5° A. ◕.

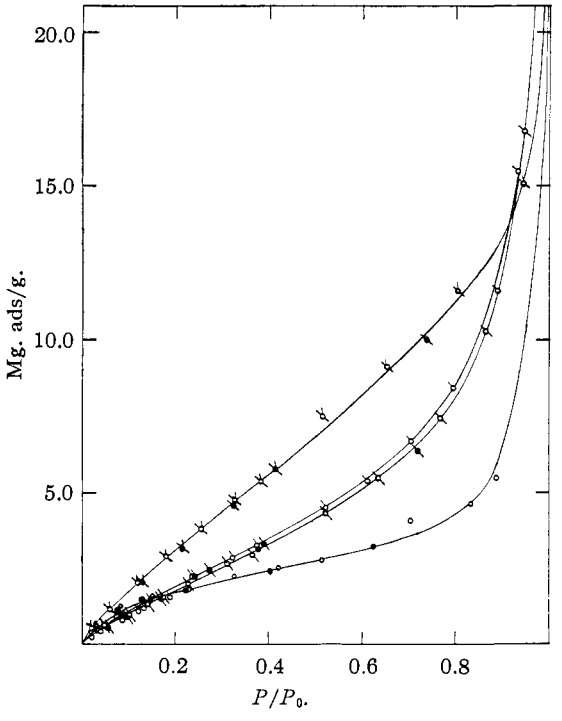

Fig. 3.—Egg albumin, 2% spray frozen, 0.8423 g.: methane, 117.2°A., O; *n*-butane, 0.0°, ◔; neopentane, 10.0°, ◕; SF_6, −64° ◕.

These were always found to agree to within 0.5%. The adsorption in all cases was found to be rapid and reversible with no evidence of hysteresis. Below 0.5 saturation equilibrium was always reached in less than five minutes. Near saturation equilibrium was slower, taking about ten to fifteen minutes.

Typical data are shown in Figs. 2 and 3. They are all BET, type II isotherms and in all cases it was observed that smooth curves were obtained over the entire region up to saturation. The data from these isotherms were plotted according to the simple BET equation and found to give good straight lines in the region up to about 0.4 saturation. Figures 4 to 10 represent the BET plots of the data shown in Figs. 2 and 3. From the slopes

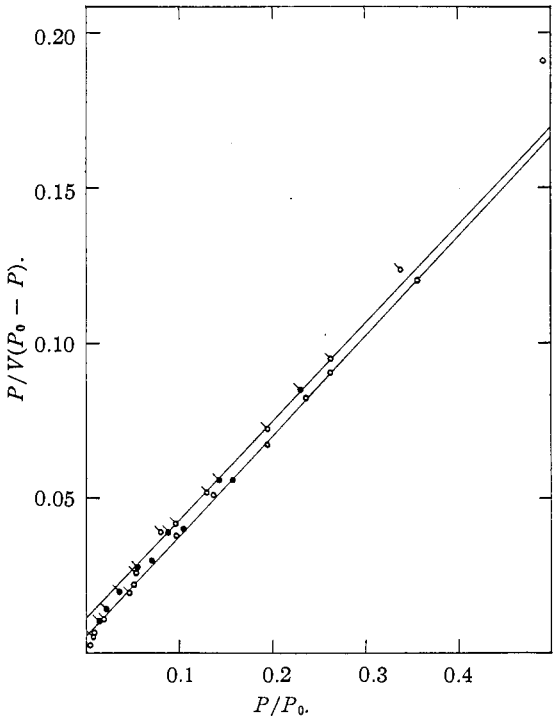

Fig. 4.—Egg albumin 2% spray frozen, 0.8423 g.: nitrogen at 77.5°A., O; 90.3°A., ◔.

and intercepts of these lines, values of the number of molecules of adsorbate required to form a surface monolayer and the average net heats of adsorption for the first layer were calculated. By multiplying the former by the area of an individual molecule, the surface area of the protein could be obtained. The surface areas of the molecules were calculated from the liquid densities of the adsorbates at the temperatures employed. In Table II are listed the different gases and their liquid densities. For purposes of computation it was assumed that the liquids have a simple cubic lattice and that the molecules are adsorbed on the protein surface in a square lattice. The areas so calculated may be converted to the customary areas based on the assumption of a hexagonal

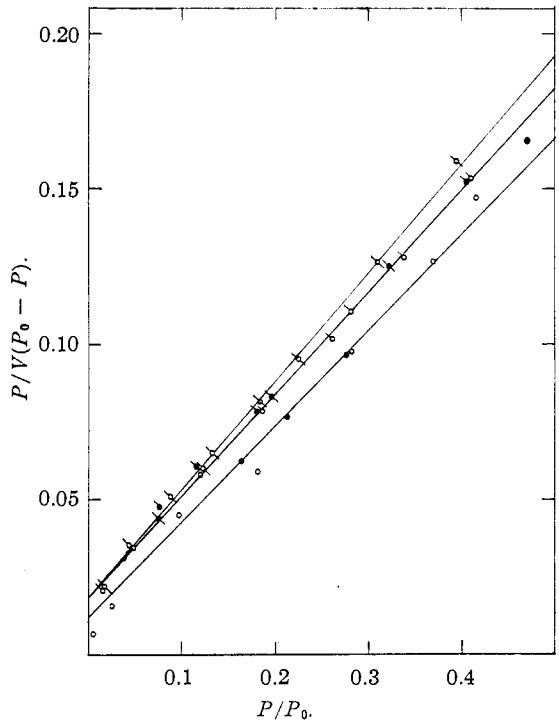

Fig. 5.—Egg albumin 2% spray frozen, 0.8423 g.: oxygen at 77.5°A., O; 90.3°A., ◔; 94.8°A., ◔.

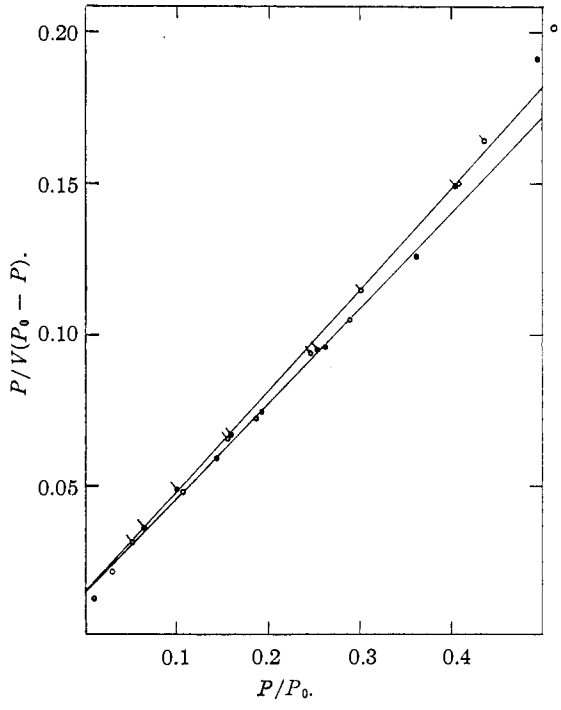

Fig. 6.—Egg albumin 2% spray frozen, 0.8423 g.: argon at 77.5°A., O; 90.4°A., ◔.

close-packed liquid lattice and a close-packed monolayer by multiplying the cubic areas calculated here by a factor of 1.09.

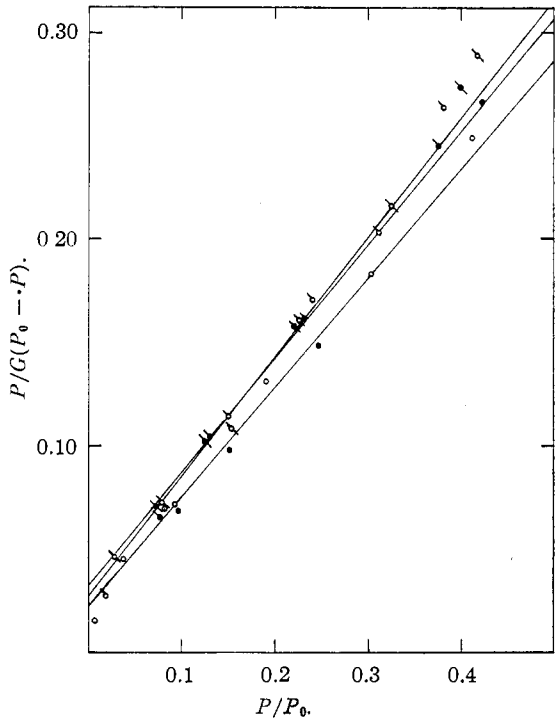

Fig. 7.—Egg albumin 2% spray frozen, 0.8423 g.: methane at 107.0°A., O; 112.2°A., ◔; 117.2°A., ◔.

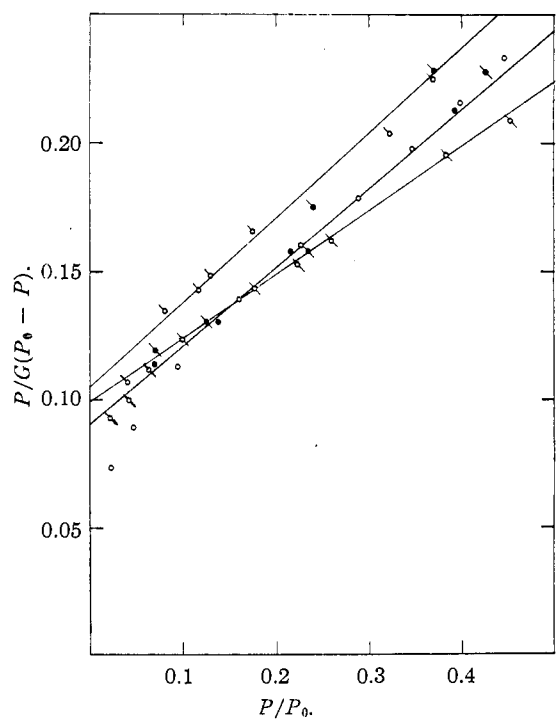

Fig. 8.—Egg albumin 2% spray frozen, 0.8423 g.: *n*-butane at −10.0°C., O; 0.0°C., ◔; 10.0°C., ◔.

Typical results obtained from isotherms measured on samples of egg albumin, bovine plasma

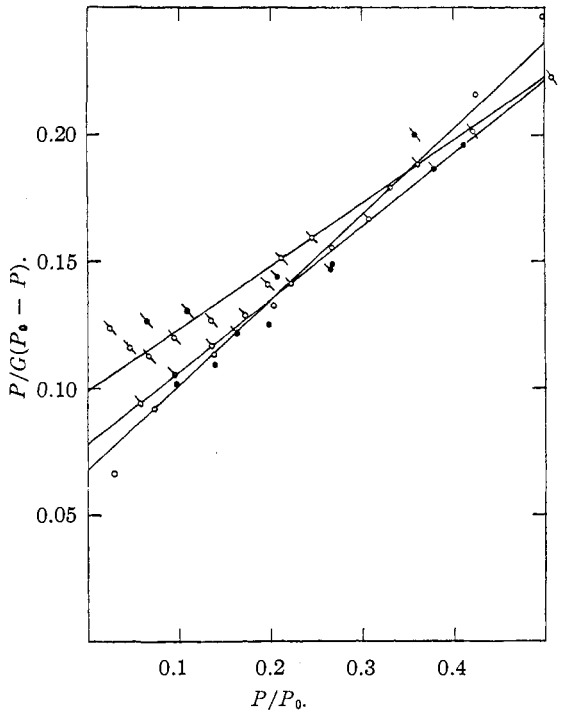

Fig. 9.—Egg albumin 2% spray frozen, 0.8423 g.: neopentane at 0.0°C., O; 10.0°C., ◔; 20.0°C., ◕.

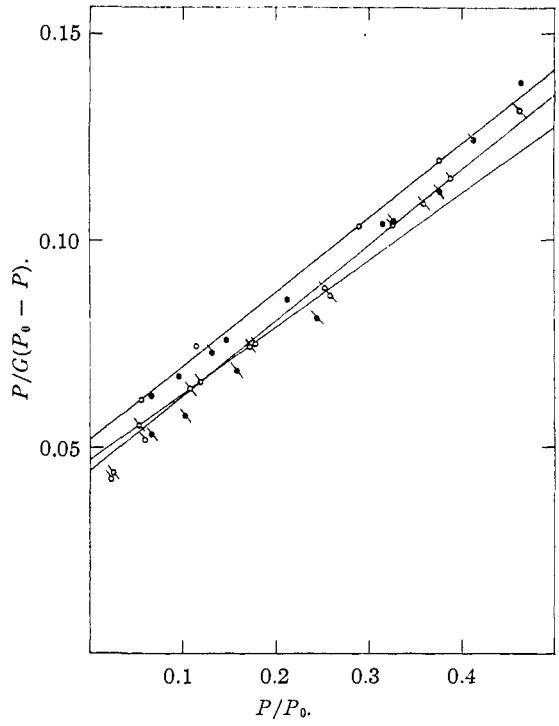

Fig. 10.—Egg albumin 2% spray frozen, 0.8423 g.: SF_6 at −70°C., O; −64°C., ◔; −58°C., ◕.

albumin and porcine plasma fraction II are summarized in Tables III–VI.

TABLE II

PHYSICAL PROPERTIES OF ADSORBATES

Gas	Molecular weight	Temp.	Liquid density g./cc.	Molar volume, cc./mole	Cubic cross section, sq. Å.	Cubic cross section sq. m./mmole
Nitrogen	28.00	77.4°K.	0.8116	34.52	14.86	89.6
		90.2°K.	0.7527	37.20	15.64	94.2
Oxygen	32.00	77.4°K.	1.175	27.25	12.70	76.5
		90.2°K.	1.120	28.57	13.10	79.0
		95.0°K.	1.099	29.12	13.27	80.0
Methane	16.03	107.0°K.	0.4324	37.08	15.60	94.0
		112.2°K.	0.4237	37.85	15.81	95.3
		117.2°K.	0.4177	38.40	15.96	96.2
Argon	39.94	77.52°K.	1.453	27.50	12.77	77.0
		90.35°K.	1.374	29.08	13.25	79.8
Sulfur hexafluoride	146.06	−70.0°C.	2.53[a]	57.8	21.00	126.5
		−58.0°C.	2.52[a]	58.0	21.02	126.8
Neopentane	72.10	0.0°C.	0.613	117.6	33.70	221.1
n-Butane	58.08	−10.0°C.	0.6115	95.0	29.20	176.0
		0.0°C.	0.6010	96.7	29.54	178.0
		+10.0°C.	0.5901	98.4	29.88	180.0

[a] Solid: extrapolated values for the supercooled liquid would give areas about 20% larger.

From the isotherms at different temperatures it was possible to calculate thermodynamic, isosteric, partial molal differential heats of adsorption for the different gases. Typical curves are shown for the data of Table VI in Figs. 11 and 12. The results for all gases were quite similar compared with each other and compared against different protein samples. They show heats which

TABLE III

ADSORPTION OF NITROGEN AND OXYGEN BY EGG ALBUMIN

Sample: 0.7445 g. of spray-frozen from 2% solution (prepn. no. 1)

Run	Gas	Temp., °K.	Mm.,[a] millimole/g.	Area (BET) sq. m./g.	ΔE_{net} (BET), cal./mole
33	N_2	77.5	0.1950	17.5	481
31	N_2	90.4	.1912	18.0	660
34	O_2	77.4	.1882	14.4	574
32	O_2	90.3	.1860	14.7	551

[a] Mm is the no. of millimoles of adsorbate per gram of adsorbent required to form a monolayer.

TABLE IV

ADSORPTION OF NITROGEN, OXYGEN AND *n*-BUTANE BY EGG ALBUMIN

Sample: 0.6620 g. spray-frozen from 2% solution (prepn. no. 3)

Run	Gas	Temp.	Mm. millimole/g.	Area (BET) sq. m./g.	E_{net} (BET), cal./mole
38	N_2	77.4°K.	0.1535	13.8	586
41		90.2°K.	.1758	16.6	538
39	O_2	77.4°K	.1669	12.8	438
40		90.2°K.	.1566	12.4	499
45	*n*-Butane	−10.0°C.	.0534	9.4	784
43		0.0°C.	.0575	10.0	758
44		+10.0°C.	.0510	9.2	851

are slightly greater than the heats of liquefaction, and diminish toward a constant value (the heat of vaporization) as the adsorption approaches the monolayer region. The fact that the constant

TABLE V

ADSORPTION OF NITROGEN, OXYGEN AND *n*-BUTANE ON DIFFERENT PROTEINS

Run	Protein	Sample, g.	Gas	Temp.	Mm., millimoles/g.	Area (BET) sq. m./g.	E_{net}(BET), cal./mole
51	Egg albumin	1.1599	N_2	77.5°K.	0.0675	6.0	627
59				90.4°K.	.0738	6.9	561
52	(Lyo. 5.6% sol.		O_2	77.5°K.	.0744	5.7	501
56	prepn. 2)			90.4°K.	.0733	5.8	574
54			*n*-Butane	−10.0°C.	.0266	4.0	899
53				0.0°C.	.0227	4.0	1060
55				+10.0°C.	.0257	4.6	794
68	Bovine albumin	0.7683	N_2	77.5°K.	.1304	11.7	612
71				90.4°K.	.1233	11.6	691
69	(Spray-frozen 2%		O_2	77.6°K.	.1389	10.6	496
70	soln., prepn. 2)			90.4°K.	.1345	10.6	546
81			*n*-Butane	−10.0°C.	.0449	7.9	775
80				0.0°C.	.0486	8.6	725
82				+10.0°C.	.0477	8.6	688
75	Porcine II	0.7688	N_2	77.5°K.	.1183	10.6	579
72				90.3°K.	.1170	11.0	652
76	(Spray-frozen 2%		O_2	77.5°K.	.1223	9.4	476
73	soln. prepn. 2)			90.3°K.	.1161	9.2	545
78			*n*-Butane	−10.0°C.	.0382	6.7	762
77				0.0°C.	.0374	6.7	752
79				+10.0°C.	.0415	7.5	670

TABLE VI

COMPARISON OF ADSORPTION OF DIFFERENT GASES ON EGG ALBUMIN

Sample: 0.8423 g. spray-frozen from 2% solution (prepn. no. 3)

Run	Gas	Temp.	Mm., millimoles/g.	Area (BET), sq. m./g.	E_{net} (BET), cal./mole
61	N_2	77.5°A.	0.1330	11.9	625
59		90.3°A.	.1325	12.5	604
62	O_2	77.5°A.	.1389	10.6	508
74		90.3°A.	.1250	9.9	528
89		94.8°A.	.1213	9.7	573
91	A	77.5°A.	.1365	10.5	484
90		90.4°A.	.1286	10.2	576
86	CH_4	107.0°A.	.1130	10.6	666
87		112.2°A.	.1081	10.3	631
88		117.2°A.	.1033	10.0	710
63	*n*-Butane	−10.0°C.	.0453	8.0	736
60		0.0°C.	.0475	8.5	754
64		+10.0°C.	.0494	8.9	703
67	Neopentane	0.0°C.	.0341	6.9	970
65		10.0°C.	.0378	7.7	870
66		20.0°C.	.0402	8.2	728
83	SF_6	−70.0°C.	.0297	3.74	598
84		−64.0°C.	.0306	3.85	669
85		−58.0°C.	.0332	4.18	634

values agree with the observed values of the heats of vaporization to within 5% is an excellent check on the self-consistency of the method. In the region below 0.15 saturation the estimated error is about 5–10% for the heat of adsorption calculated in this way. Although there are a number of discrepancies, average net heats for the first layer estimated in this way show good qualitative agreement (±10%) with the average net heats calculated from the simple BET equation.

In concluding this section it may be well to add a few words on the homogeneity, stability and reproducibility of the areas determined. The process of spray-freezing is such that the samples are not homogeneous. The reason for this is that a considerable part of the spray hits the walls of the dewar containing the liquid nitrogen and condenses in the liquid film. On evacuation and drying the resultant protein is a mixture of samples frozen under somewhat different conditions and so is not completely homogeneous. On transfer, storage and shaking there is a tendency for the more finely divided material to settle in the top of the bottle. It has been our experience that samples taken from the top and bottom of the same bottle may differ in specific area by as much as 20% (see for example, samples in Tables IV–VI).

However, individual samples seem quite stable and do not show any drifts in surface area, nor are these areas affected by adsorption measurements run on other gases. Finally repeat measurements on the same sample will give agreement in surface area to within 2–3% which is about the accuracy of the area measurements. As a typical example we can compare oxygen runs made on the sample shown in Table VI. Run no. 58 was the first made in this series using oxygen at 90.4°K. and it gave an area of 9.6 sq. m./g. (not listed in Table VI). Run 74 with oxygen at 90.3°K. was made after three weeks and also after nitrogen, *n*-butane and neopentane had been run and a value of 9.8 sq. m./g. was obtained. Finally a last run was made about three weeks later (Run 89) after sulfur hexafluoride and methane had been run on

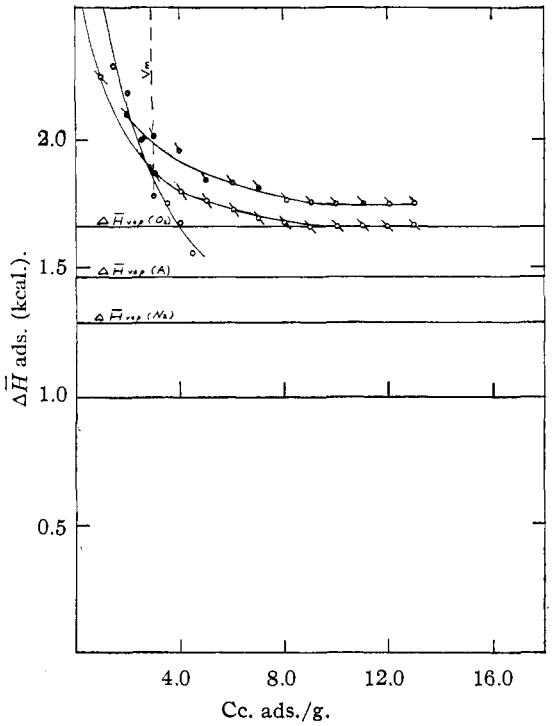

Fig. 11.—Egg albumin 2% spray frozen 0.8423 g.: nitrogen, 77.5 and 90.3°A., O; oxygen, 77.5 and 90.3°A., ◑; argon, 77.5 and 90.3°A., ◔.

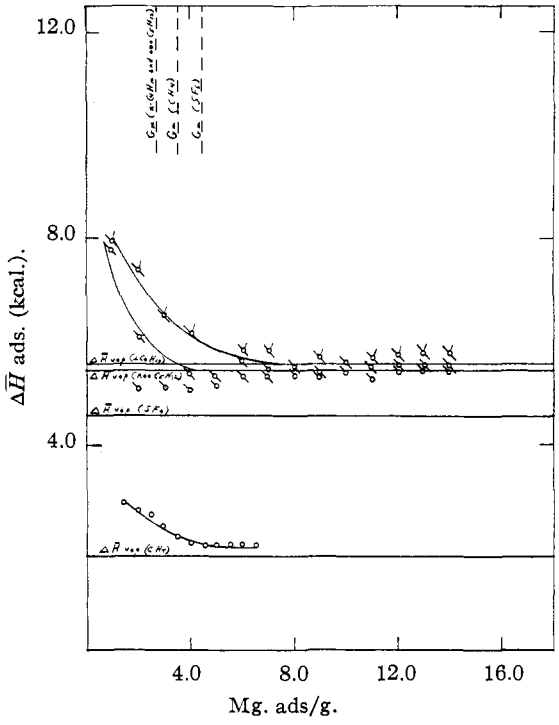

Fig. 12.—Egg albumin 2% spray frozen 0.8423 g.: methane, 112 and 117°A., O; SF_6 −64 and −70°C.; ◑; neopentane, 0.0 and 10.0°C., ◔; *n*-butane, 0.0 and 10.0°C., ◴.

the same sample and an area of 9.7 sq. m./g. was obtained at 94.8°K. This is a fairly typical example of the area stability of given samples.

Discussion

The data obtained from the various isotherms at different temperatures and for gases of quite different dimensions indicate that the adsorption of these gases represents a common process which is not specific and may be properly identified as physical adsorption. Furthermore, the absence of hysteresis and the rapidity with which equilibrium is reached also indicate that there is no fine pore structure to the protein particles. This is in qualitative agreement with the appearance of these solid proteins in the electron microscope, where they have the shape of thin sheets with cylindrical branches attached at angles to the sheets.[2]

The surface areas computed from oxygen, argon and methane adsorption are in excellent agreement with each other (Table VI) and may be construed as lending strong support to the simple multi-molecular mechanism of adsorption provided by the BET theory. The surface areas obtained with *n*-butane are generally 15–20% lower than the oxygen areas, whereas the nitrogen areas seem to be uniformly 10–15% higher than the latter. These differences are quite definite and outside the limit of experimental error. In the case of *n*-butane it has been remarked by other workers[8] that *n*-butane gives lower values for surface areas than either nitrogen or oxygen. This has been explained as being due to the unsymmetrical structure of the *n*-butane molecule and a possible ambiguity about its packing on the surface. While such argument seems quite reasonable, it cannot be used to account for the even greater discrepancies observed with the symmetrical molecules neopentane and sulfur hexafluoride. The surface areas calculated for neopentane are quite definitely smaller (Table VI) than the *n*-butane areas and the sulfur hexafluoride areas are unusually low. Since sulfur hexafluoride is the smallest of these three molecules, its behavior is certainly anomalous in this regard.

A further anomaly is to be observed in the temperature sensitivity of the calculated surface areas. For oxygen, argon and methane, there seems to be little effect of temperature on surface area, or perhaps a small expected decrease in area with increase in temperature. However, in the cases of the other gases and quite frequently with nitrogen, there is a rather large increase in surface area with increasing temperature. As may be seen, this increase may be as much as 10% or more for an interval of 20°. The odd behavior of sulfur hexafluoride is somewhat further complicated by the fact that the pure substance is a solid at the temperatures employed.

The odd behavior of these gases is quite real and

(8) Brunauer, "Physical Adsorption," Chap. 9, Princeton University Press, Princeton, N. J., 1943.

reproducible, and definitely beyond experimental error. It is, of course, possible that the BET theory provides too simple a picture from which to draw conclusions based on such relatively small differences. In support of this it is certainly probable that the idealization of the solid protein surface by an infinite, homogeneous plane cannot be correct.

A further point is the uncertainty as to the areas to be ascribed to the adsorbed molecules. A change in packing on the surface, as for example from hexagonal to cubic, can be made to account for differences in area (for symmetrical molecules) of 15%. This can be magnified if we envisage an extremely hypothetical case in which a cubic liquid forms a hexagonal close-packed layer or vice-versa. Such extreme cases may provide a factor of 45% in measured surface areas.

If we represent by S the surface area (square meters) covered by a quantity of gas V_m (cc. STP), then S is given by the equation $S = \gamma(M/d)^{2/3}Vm$, in which M = molecular weight, d = density of the liquid, and γ is a packing factor. Table VII shows the values of γ corresponding to different packing in the bulk liquid and on the surface.

TABLE VII

PACKING FACTORS FOR DIFFERENT LIQUID AND SURFACE GEOMETRY

	Liquid structure	
Surface structure	Cubic lattice	Hexagonal close-packed lattice
Square lattice	0.377	0.475
Close-packed lattice	.327	.411

Finally, it is quite possible that the adsorption may be localized in one case and non-localized in another. A change either abrupt or gradual from localized to non-localized adsorption which may be obtained with increased temperature will allow for more efficient packing of adsorbent molecules on the surface at the higher temperature and therefore increased surface area.

While the present results cannot be used to discriminate between the above conditions, they certainly indicate a sufficient lack of sophistication in our understanding of the processes of physical adsorption to merit further study.

Summary

1. Comparative studies have been made of the adsorption isotherms of N_2, O_2, CH_4, A, *n*-butane, SF_6 and neopentane at a series of corresponding temperatures on several samples of dry, lyophilized proteins.

2. Electrophoresis patterns and solubility behavior indicate that the spray-freezing technique does not change the composition of the proteins.

3. The isotherms were treated by the simple BET theory, and resonable and fairly consistent values of the surface areas and net heats of adsorption were obtained. Only SF_6 showed markedly anomalous values of the surface area.

4. The data are such as to indicate that non-polar gases are physically adsorbed by the proteins in a manner which is independent of the specific protein and depends principally on the state of subdivision.

5. The total lack of hysteresis and speed of attainment of equilibrium over a range of molecular symmetries and sizes, together with the small net heats of adsorption past the monolayer region, indicates that the proteins have no fine pore structure.

6. There seem to be definite anomalies in the behavior of the calculated BET surface areas with temperature for certain gases which cannot be understood in terms of the BET model.

LOS ANGELES 7, CAL. RECEIVED AUGUST 3, 1949

[CONTRIBUTION FROM CHEMISTRY DEPARTMENT, UNIVERSITY OF SOUTHERN CALIFORNIA]

Surface Areas of Proteins. III. Adsorption of Water[1]

BY SIDNEY W. BENSON, DAVID A. ELLIS AND ROBERT W. ZWANZIG

The surface areas of a number of dry, lyophilized proteins have been measured by the technique of nitrogen gas adsorption interpreted by the BET theory.[2,3,4,5] The results obtained have been reasonable and self-consistent but are several orders of magnitude smaller than the surface areas obtained from water adsorption isotherms which can also be fitted to the BET theory.[6] Several explanations have been offered for this discrepancy[4,7] but as yet there is a paucity of evidence on which to base conclusions.

It is the purpose of the present paper to describe some recent work on the adsorption of water by proteins and to report some very important differences which have been established between the nature of adsorption processes with respect to such non-polar gases as nitrogen and oxygen on the one hand and polar substances such as water on the other.

The experiments to be reported involve two

(1) This work has been carried out with the aid of two grants, one from the Research Corporation and one from the Office of Naval Research, Contract N6-onr-67900. This paper was presented at the High Polymer Forum of the American Chemical Society during the Fall Meeting at Atlantic City, N. J., September, 1949.

(2) Benson and Ellis, THIS JOURNAL, **70**, 3563 (1948).

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

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

(5) Benson and Ellis, article in preparation.

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

(7) Pauling, *ibid.*, **67**, 555 (1945).