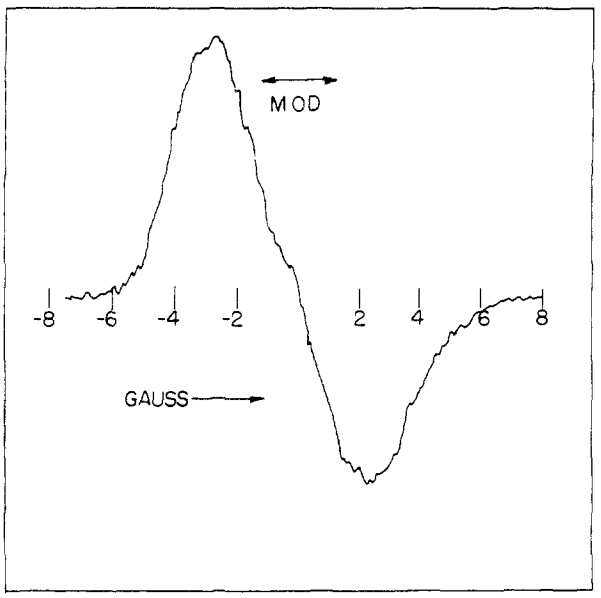


Fig. 2.—The first derivative of the proton magnetic resonance absorption in DOOCCH(D)C(OD)HCOOD at −196°, plotted as a function of applied magnetic field at a fixed frequency of 25.27 Mc. The arrow indicates the amplitude of the 30 c.p.s. modulation of the magnetic field.

The final correction is for the incomplete exchange of protons in the $CO_2H$ and OH groups. These protons have no near magnetic neighbors and thus they have a much smaller second moment than protons in the CHD–CH group. A value of 0.3 ± 0.1 gauss$^2$ is estimated. About 5% of the protons in the $CO_2H$ and OH groups were not exchanged. This reduces the net second moment by 7.5% of the difference between 0.3 ± 0.1 gauss$^2$ and the value computed assuming complete exchange. The various terms are summarized in Table I. The net values predicted for $\Delta H_2^2$ are 2.25 ± 0.5 gauss$^2$ for the *trans* and 3.65 ± 0.6 gauss$^2$ for the gauche configuration.

A typical recording of the first derivative of the proton absorption in the monodeutero-L-malic acid sample at −196° is reproduced in Fig. 2. Numerical integration of this curve gave a value of 3.43 gauss$^2$ for one side and 3.63 gauss$^2$ for the other. A second curve gave values of 3.28 and 3.71 gauss.$^2$ The resulting best experimental $\Delta H_2^2$ is 3.5 ± 0.2 gauss.$^2$ Line shapes plotted at room temperature had the same widths as those at low temperature so motional narrowing does not appear to be important. Comparison of the experimental value for the second moment with the calculated values shows conclusively that the protons are in the gauche configuration.

In addition, line shapes were plotted at −196° for a sample of $DOOCH_2CH(OD)COOD$. The second moment for this sample was 11.2 ± 0.6 gauss.$^2$ A detailed comparison of this value with the intramolecular value of 8.9 gauss$^2$ computed for $CH_2$–CH confirms the estimates of the intermolecular contributions to the broadening. The experimental second moments were corrected for the modulation broadening[18] produced by the relatively large modulation used.

In the preceding article,[4] the deuterium labelling experiments and the effect of *p*H on the kinetics of the enzymatic hydration of fumarate indicated that the reaction occurs in the *cis* manner represented by the structures of the enzyme–fumarate and enzyme–L-malate complexes given in Fig. 1. The finding here that the protons in crystalline DOOCCH(D)C(OD)HCOOD are in the gauche configuration proves that the addition is *cis*. In view of this mechanism it is perhaps not surprising that *meso*-tartrate is a much more powerful competitive inhibitor of pig heart fumarase than *d*- or *l*-tartrate.[19]

(18) E. R. Andrew, *Phys. Rev.*, **91**, 425 (1953).
(19) C. Frieden, Doctoral Thesis, University of Wisconsin, 1955.

URBANA, ILLINOIS
MADISON, WISCONSIN

---

[CONTRIBUTION FROM THE MOORE LABORATORY OF CHEMISTRY, AMHERST COLLEGE]

# Heats of Adsorption of Water Vapor on Bovine Serum Albumin[1]

BY C. H. AMBERG[2]

RECEIVED FEBRUARY 21, 1957

Heats of adsorption of water vapor on bovine serum albumin were measured calorimetrically at 20° and are presented together with the isotherms. The heat curves are discussed and critically compared with calculated values from the literature. Possible mechanisms for the adsorption process are considered, with special regard to the high initial heats and the appearance of a maximum in the heat-coverage curve near the B.E.T. value for a complete monolayer.

## Introduction

The interaction of proteins and water is a subject of considerable interest to the biochemist and biologist. An extensive amount of data has been accumulated over the past two or three decades, the bulk of the systems studied having been aqueous solutions of various proteins. While this has yielded important information regarding the extent of hydration as well as the size and shape of the hydrated molecules,[3] solution techniques tend to be insensitive to non-uniformity in properties over various regions of the interacting polymer molecule. For this reason, a number of workers

(1) Supported by the Office of Naval Research under contract N8-onr-66902. This paper was presented before the Colloid Division of the 128th National Meeting of the American Chemical Society in Minneapolis, Minn., September, 1955.

(2) Division of Applied Chemistry, National Research Council, Ottawa 2, Canada.

(3) J. T. Edsall in "The Proteins," Vol. I, part B, Academic Press, New York, N. Y., 1953, p. 549.

have studied the hydration of quasi-anhydrous proteins from the vapor phase. (A comprehensive review of this work was published by McLaren and Rowen.[4]) From the point of view of those interested in biological systems this method of attack has a disadvantage in that proteins such as the serum albumin discussed in this report occur naturally in solution or in an advanced state of hydration. Moreover, the adsorption of vapors is most conveniently studied in vacuum systems on the purest possible adsorbents whereas, of course, natural systems exist in the presence of both interacting and non-interacting gases and solutes. Nevertheless, there can be no doubt that, from the physicochemical point of view, the simplest systems are desirable provided they can be prepared without introducing permanently irreversible changes in the protein molecule.

Adsorption isotherms of water vapor on a variety of purified proteins were measured by Bull[5] at 25° and at 40°, in the presence of air. His data were discussed by Pauling[6] and an analysis of heat, free energy and entropy changes was undertaken by Dole and McLaren[7] and Davis and McLaren.[8] Although Bull did not measure desorption isotherms for all the systems studied by him, it is evident from his and also later work that many of these systems show marked hysteresis. In view of this the computation of thermodynamic equilibrium functions from either the adsorption or desorption branch of the hysteresis loop is of questionable significance and may actually result in grossly misleading values. This is demonstrated by the work done in this Laboratory which will be discussed later.

Pauling[6] concluded that in most cases the initial water uptake proceeded in such a manner that one water molecule would attach itself to a polar side chain. His view that internal hydrogen bonding of the peptide carbonyl and imido groups would largely preclude adsorption of water molecules by these groups was not substantiated by Mellon, Korn and Hoover.[9] These authors examined a series of polyglycine polymers with from two to six units per molecule and found definite correlation between the increase of water adsorption and increasing number of peptide links. By benzoylating successively higher numbers of free amino groups in casein these authors[10] demonstrated that amino groups are responsible for approximately one quarter of the total water uptake.

As part of their series of investigations on The Surface Areas of Proteins, Benson and his coworkers have given a detailed account of the adsorption of water vapor on a variety of proteins.[11-13] They showed conclusively that while the B.E.T. nitrogen area of egg albumin and bovine albumin could be increased substantially by spray freezing techniques, the analogous areas determined by application of the B.E.T. method to data on water adsorption were virtually independent of the state of subdivision of the protein. With nitrogen areas roughly in the range 1–5 m.$^2$/g. it was obvious from these data that water molecules would have to penetrate into the interstices of the protein structure much more extensively than nitrogen. It was held likely that water adsorption in contrast to nitrogen adsorption is a specific molecular property of the protein.

In view of the uncertainties connected with the calculation of heats of adsorption from isotherms exhibiting hysteresis it was felt that the calorimetric measurement of heats of adsorption of water vapor on a suitable protein would be of value. It seemed particularly interesting to check by direct measurement the anomalous negative "net" heats obtained by Seehof, *et al.*,[11] for the system $H_2O$–bovine serum albumin between 20 and 25°, which were also discussed by Benson and Richardson[13] in the light of more recent experiments. As a reliable calorimetric method had been developed in this Laboratory in connection with other adsorption problems[14-16] such measurements were carried out using bovine serum albumin, fraction V.

## Experimental

**Material.**—The adsorbent used was a sample of bovine serum albumin, fraction V, milled powder, lot No. N-12302, kindly provided by Dr. J. L. Oncley. Its nitrogen surface area was found to be 1.4 m.$^2$/g. 2.774 g. of the undried material was loaded into the calorimeter. Crystalline egg albumin (Armour, lot No. E-90115) was used for two preliminary runs, but it was abandoned in favor of the bovine albumin which has somewhat greater resistance to heat denaturation and is free from additives. It is not denatured at temperatures up to 70°.[17] The egg albumin had been spray frozen from an approximately 1% aqueous solution and had a nitrogen surface area of 26.4 m.$^2$/g. Poor reproducibility was experienced in the calorimetry with this adsorbent which may have been due to the necessarily low out-gassing temperature (two days at room temperature) or to inherent structural features of the crystallized material. Surface active agents frequently used in the crystallization process may also have influenced the behavior of the adsorbent.

Distilled water was used as the adsorbate; it was outgassed by boiling under reduced pressure and by further pumping down while the reservoir was kept in a Dry Ice–acetone bath. This procedure was repeated several times.

**Apparatus and Procedure.**—The apparatus used was described in detail by Beebe and Amberg.[18] Its essential features are the calorimeter, mercury U-tube manometer and water reservoir, all joined to a vacuum manifold and immersed in a constant temperature bath.

The calorimeter was the same in principle as the one used for previous work in this Laboratory.[14] A glass jacket surrounded the platinum cylinder (7 cm. long, 2 cm. diameter), the spacing between the two being about 1 cm. A copper–constantan thermocouple junction was soldered to the outside of the platinum cylinder and the leads joined to the external measuring system through Kovar-Pyrex seals de-

(4) A. D. McLaren and J. W. Rowen, *J. Polymer Sci.*, **7**, 289 (1951).
(5) H. B. Bull, THIS JOURNAL, **66**, 1499 (1944).
(6) L. Pauling, *ibid.*, **67**, 555 (1945).
(7) M. Dole and A. D. McLaren, *ibid.*, **69**, 651 (1947).
(8) S. Davis and A. D. McLaren, *J. Polymer Sci.*, **3**, 16 (1948).
(9) E. F. Mellon, A. H. Korn and S. R. Hoover, THIS JOURNAL, **70**, 3040 (1948).
(10) E. F. Mellon, A. H. Korn and S. R. Hoover, *ibid.*, **69**, 827 (1947).
(11) J. M. Seehof, B. Keilin and S. W. Benson, *ibid.*, **75**, 2427 (1953).
(12) S. W. Benson, D. A. Ellis and R. W. Zwanzig, *ibid.*, **72**, 2102 (1950).
(13) S. W. Benson and R. L. Richardson, *ibid.*, **77**, 2585 (1955).
(14) R. A. Beebe, B. Millard and J. Cynarski, *ibid.*, **75**, 839 (1953); R. A. Beebe and D. M. Young, *J. Phys. Chem.*, **58**, 93 (1954).
(15) C. H. Amberg, W. B. Spencer and R. A. Beebe, *Can. J. Chem.*, **33**, 305 (1955).
(16) R. A. Beebe and R. M. Dell, Office of Naval Research Contract N8-onr-66902, Technical Report No. 4.
(17) J. L. Oncley, private communication.
(18) R. A. Beebe and C. H. Amberg, Office of Naval Research Contract N8-onr-66902, Technical Report No. 7.

scribed in a previous publication.[15] The platinum cylinder contained a removable platinum bucket with a set of close-fitted iron fins and a threaded brass cap. Both the fins and cap had been successively nickel and chrome plated and the cap provided with two small holes for vapor admission. A 140 ohm heater wound from enamel covered Advance wire and wrapped in aluminum foil was housed axially in a 3 mm. well in the center of the fins.

Water vapor pressures were read on the mercury manometer by means of a Gaertner travelling microscope. The water reservoir, 30 ml. in volume, was about half filled with water. It was connected to the rest of the system through a two-way stopcock and ground glass joint and could be removed for weighing.

All runs were carried out at 20°. The constant temperature bath was controlled to ±0.01° but effective fluctuations in the calorimeter with the outer jacket evacuated were only ±0.003°.

The methods employed for the measurement of electrical energy input during calibrations and for amplification and recording of the thermal e.m.f. have been described in previous publications from this Laboratory. A Perkin–Elmer d.c. breaker-amplifier was used in conjunction with an Esterline–Angus automatic recorder. The procedure outlined by Beebe and Dell[16] was followed.

Before runs 1–3 the adsorbent was outgassed at room temperature for about 36 hr. and then the temperature was slowly raised to 50° where it remained for about 6 hr. A Dry Ice–acetone trap was used to collect the water removed. Before runs 4 and 5 the temperature was maintained at 100° for two hours each in order to test the possible effects of denaturing the adsorbent.

Helium dead-space calibrations were carried out using a conventional gas buret system. The variation in dead-space in the U-tube manometer with increasing pressure was negligible.

Before each run, a small quantity of helium (of the order of 0.3 mm. pressure) was admitted to the system to facilitate heat conduction inside the calorimeter. It had been found during the initial runs with egg albumin that in the absence of helium heat evolution became too slow to calculate heats of adsorption with any degree of confidence, particularly for the first vapor increment where the calculated heat was as much as 6 kcal./mole below that obtained in the presence of helium. With helium, the "adiabatic" method[16] resulted in constant reconstructed heat values 5–8 min. after vapor admission (±0.2% deviation from total heat of adsorption per increment) showing that if any further heat was evolved after that period, it was evolved too slowly to be detected by that method. The estimated maximum heat quantity that might thus have remained undetected would fall well within the expected precision stated below. Helium was admitted to the outer calorimeter jacket 30 minutes after vapor admission to speed up final temperature equilibration. After a further period of 30 min. no significant changes in vapor pressure were detected so that equilibrium was taken to have been reached one hour after vapor admission.

In order to calculate water vapor pressures, the partial pressure of helium in the system had to be subtracted from the total observed pressure. Table I shows a selection of pressure values from run 3 together with an estimate of the accuracy achieved in the final partial pressures of water vapor. Column 1 shows the total equilibrium pressures read on the manometer, column 2 the calculated partial pressures of helium, column 3 the partial pressures of water vapor, *i.e.*, the difference between columns 1 and 2, and column 4 the estimated per cent. accuracy of the values of column 3.

TABLE I

CALCULATION OF WATER VAPOR PRESSURES

| (1) Obsd. pressure, mm. | (2) Partial pressure of helium, mm. | (3) Partial pressure of water vapor, mm. | (4) % Accuracy |
|---|---|---|---|
| 0.699 | 0.319 | 0.380 | 1.3 |
| 2.097 | .267 | 1.83 | 0.7 |
| 6.026 | .205 | 5.82 | .3 |
| 10.617 | .145 | 10.47 | .2 |

Except for the somewhat smaller increments in the first half of run 1, the amount of water vapor admitted to the system ranged between 20 and 30 mg. per increment. The estimated precision is between 1 and 2% in the weighing of each increment, and no worse than 5% in the measurement of the heat evolved per increment.

## Results and Discussion

The results of runs 2 to 5 are shown in Figs. 1 and 2 (isotherms), and Figs. 3 and 4 (calorimetric heats of adsorption). Those for run 1 show too much scatter to be entirely reliable and have, therefore, been omitted.

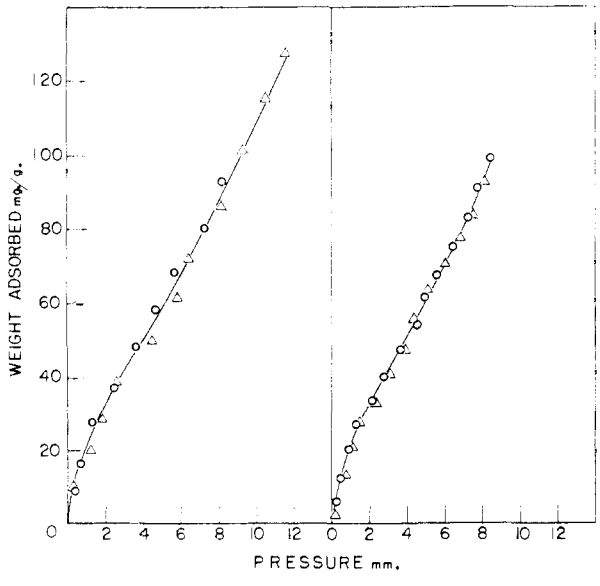


Fig. 1. (Left).—Adsorption of water vapor on bovine serum albumin, fraction V, at 20.0°: run 2, O; run 3, △; outgassed at 50°.

Fig. 2. (Right).—Adsorption of water vapor on bovine serum albumin, fraction V, at 20.0°: run 4, O; run 5, △; outgassed at 100°.

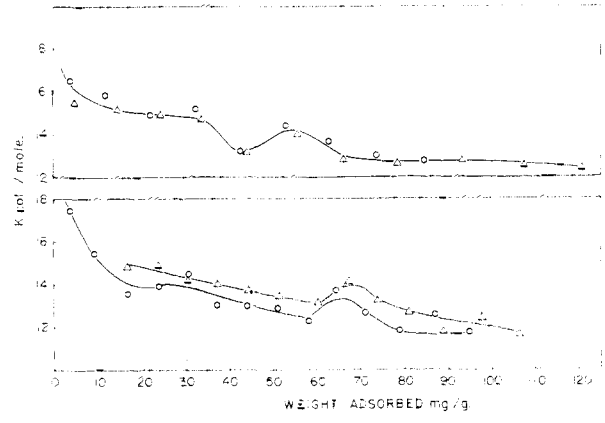


Fig. 3. (Top).—Heats of adsorption of water vapor on bovine serum albumin, fraction V, at 20.0°: run 2, O; run 3, △; outgassed at 50°.

Fig. 4. (Bottom).—Heats of adsorption of water vapor on bovine serum albumin, fraction V, at 20.0°: run 4, O; run 5, △; outgassed at 100°.

The two preliminary runs with crystalline egg albumin are not recorded because of very low reproducibility in both the isotherms and heat curves. It may, however, be stated that the heats of adsorption were of about the same magnitude as those for the bovine albumin.

The isotherms shown in Figs. 1 and 2 are of the S-shaped (Type II) variety. Amounts adsorbed are given on the basis of the initial protein weight. By duplicating as closely as possible the actual drying conditions, it was found in a separate experiment that at 50° the albumin was dehydrated to the extent of 5.07% of the initial weight. Nevertheless, as this was done in a separate adsorption system with the albumin in a simple adsorption bulb rather than the calorimeter, it was felt that the efficiency of dehydration may have differed somewhat in the two cases. Because of this uncertainty the initial weight of protein was not corrected to "dry weight." Low and Richards[19] have shown in the case of human serum mercaptalbumin mercury dimer that the extent of dehydration is relatively insensitive to the temperature of outgassing. While a total of 10% of water was removed by pumping to constant weight over $P_2O_5$ at temperatures up to 100°, all but about 1% of this was removed at 25°. The slight shift toward higher adsorption at a given vapor pressure when the present system was outgassed at 100° is very likely due to a similar small increase in the extent of dehydration. It was unfortunately not possible to determine whether the expected simultaneous degradative changes in the protein at 100° also affected the adsorption of water vapor. Both isotherms agree reasonably well with that of Seehof, Keilin and Benson[11]; their values were only slightly lower.

B.E.T. surface areas calculated from the isotherms shown in Figs. 1 and 2 were 204 m.$^2$/g. on the average, differences being within experimental error. This corresponds to an amount of 58.2 mg./g. of water vapor adsorbed at the B.E.T. monolayer. (Accepting the above correction to "dry weight" of protein, 61.2 mg./g. would be the resulting corrected value.) Here again it should be pointed out that the meaning of such figures is obscure. If calculated from the adsorption branch of an isotherm showing hysteresis they will represent a minimum area significant, at best, on a comparative basis. In view of the uncertainties involved in applying the B.E.T. equation, it is most surprising that the $c$-value computed by the B.E.T. method from Fig. 2 resulted in a heat of adsorption term only some 500–600 cal./mole below that measured calorimetrically at the "monolayer."[11] This may well be regarded as fortuitous; McLaren and Rowen[4] have examined in some detail the applicability of equations of the B.E.T. form to water sorption by proteins and compared B.E.T. heats with Clausius–Clapeyron heats. In general they found poor agreement between the two, as is to be expected from the simplifying assumptions involved in the B.E.T. function.

The heats of adsorption plotted against amount adsorbed are shown in Figs. 3 and 4. Initially they are in the region of 16 to 18 kcal./mole as measured. It was not possible by the method employed to determine heats down to very low coverages; the heats for such initial increments might well be higher. After a fairly rapid decrease the curves flatten out in the neighborhood of 15 kcal./mole. For runs 2 and 3 there occurs a minimum at 43 mg./g. of vapor sorbed, rising from about 13 kcal./mole by 1 kcal. to a maximum at 53 mg./g. and then decreasing fairly rapidly to flatten out again at 13 kcal./mole and 68 mg./g., and then decreasing very gradually. Run 4 follows the same pattern, except that the whole curve is shifted by some 15 mg./g. toward higher amounts adsorbed. A further small shift is indicated by run 5. These shifts may again be explained on the basis of improved dehydration, their order of magnitude being consistent with the data of Low and Richards[19] and with the percentage dehydration determined in this Laboratory; this was discussed above.

The molar heat of vaporization of liquid water at 20° is 10.54 kcal. All heats measured calorimetrically were larger than this, which clearly confirms the view of Benson and co-workers,[11,13] that their calculated negative "net" heats of adsorption were anomalous. On the other hand, the curves computed by Davis and McLaren[8] from Bull's data[5] do not show any evidence of negative "net" heats. They are, moreover, qualitatively similar to the curves obtained in this Laboratory, in that they have initially high heats and also a maximum in the B.E.T. monolayer region, notably in the case of egg albumin and lyophilized lactoglobulin. Their absolute heat values are somewhat lower, but in view of the fact that they were obtained on proteins other than bovine serum albumin, and in view of the irreversible nature of these systems, this has probably little significance.

Frey and Moore[20] have measured the adsorption of water vapor on the amino acids glycine and leucine and on the cyclic anhydride of glycine, diketopiperazine, at 15, 25 and 40°. They obtained reversible isotherms and calculated isosteric heats that agreed well with the values for bovine serum albumin at half the B.E.T. monolayer coverage, but then decreased slightly more rapidly. They estimated that at the monolayer there would be one molecule of $H_2O$ sorbed per polar group ($NH_3^+$ and $COO^-$) for glycine and leucine and one molecule of $H_2O$ per carbonyl group in the diketopiperazine.

Following Pauling[6] several workers have correlated the number of polar side chains in the protein with the number of $H_2O$ molecules adsorbed at the B.E.T. monolayer and have found an approximate one-to-one correspondence. Such data were collected and critically reviewed by McLaren and Rowen.[4] They report a water sorption value of 3.74 mmoles per gram of serum albumin and a polar group content of 4.24 mmoles per gram. The first figure was obtained by Bull[5] (using horse rather than bovine albumin[6]), the second was calculated from the amino acid analysis of Brand, Kassell and Saidell.[21] A summation of hydroxy and carboxyl side chains together with cationic nitrogens carried out by Klotz and Urquhart[22] on the basis of analytical data by Brand[23]

(19) B. W. Low and F. M. Richards, THIS JOURNAL, **76**, 2511 (1954).

(20) H. J. Frey and W. J. Moore, *ibid.*, **70**, 3644 (1948).

(21) E. Brand, B. Kassell and L. J. Saidell, *J. Clin. Invest.*, **23**, 437 (1944).

(22) I. M. Klotz and J. M. Urquhart, THIS JOURNAL, **71**, 1597 (1949).

(23) E. Brand, *Ann. N. Y. Acad. Sci.*, **47**, 187 (1946).

results in the slightly lower value of 4.06 mmoles/g. If one takes our monolayer value to be 61.2 mg./g. of dry protein, *i.e.*, 3.40 mmoles/g., a molar ratio of 0.84 is obtained for water adsorbed per polar group in the adsorbent. It is not surprising that this ratio differs somewhat from unity, even if one accepted the B.E.T. monolayer value as an exact measure of the coverage of all high energy sites. (Being probably a minimum value when determined from the *adsorption* branch of a hysteresis loop, one might expect a corrected ratio to be closer to unity.) It is very likely that not all polar groups are available for the binding of $H_2O$ molecules, either because of intramolecular and intermolecular hydrogen bonding or because of spatial restrictions. Furthermore it may be assumed from the evidence with other proteins[4] that the peptide linkage will take part in the adsorption of water before the monolayer is complete. One must therefore regard the above ratio as including a partial compensation of these two phenomena by each other.

Hydrogen bonding of the O–H . . . O and N–H . . . O type is often assumed to be largely responsible for the binding of water molecules to polar sites in the protein. It is difficult to assign energies to such bonds in a structure as complex as is that of a protein molecule. Undoubtedly the interaction energy of any polar group will be influenced by neighboring groups. Nevertheless the initial high differential heats of adsorption (up to 17 kcal./mole) are not likely to be due to the formation of only one hydrogen bond per molecule of water for which the highest values quoted[24] do not appear to be in excess of one-half of the above value. One is, therefore, faced with two possible explanations, not necessarily mutually exclusive: (1) Two hydrogen bonds may be formed per molecule of water. This may take place between suitably spaced native polar centers or between a polar group and a water molecule already present. One must not lose sight of the fact that even dried proteins contain a residual quantity of firmly held water. An acceptance of this type of mechanism would even further invalidate the 1:1 correlation of water molecules to polar groups mentioned above. (2) The second explanation would be the existence of bonds in which the hydrogen does not take part; these would be of the ion–dipole type, *i.e.*, they would constitute a hydration of ions. The heats would be of a plausible order of magnitude for such bonds, and again the existence of the residual fraction of water in the "dehydrated" protein is also compatible with this concept.

The minimum for the heats of adsorption and rise by 1 kcal./mole to a maximum observed near the monolayer is in qualitative agreement with the curves calculated by Davis and McLaren.[8] It is clear that previously bound water molecules must be involved in causing the heats to increase with further adsorption, otherwise these more stable bonds would have been formed at much lower coverage. One way in which this could take place is through adsorption on two polar side chains which protrude from one protein helix (or adjacent helices) with spacing appropriate for a third water molecule to wedge between two adsorbed molecules, thus forming two hydrogen bonds. As is indicated by Figs. 3 and 4 about one-sixth of the quantity of water needed to complete the "monolayer" would be bound in this manner. It is interesting to note that this quantity corresponds almost exactly to that needed to account for the 0.84:1 ratio of water molecules to polar groups estimated above. Whether this is significant is again subject to the qualifications expressed in connection with that estimate.

An involvement in some way of the peptide linkage at this stage of the adsorption process cannot be entirely discounted. Marshall and Moore[25] examined the adsorption of ammonia on silk fibroin, which contains only 2 mole % of polar side chains. Nevertheless, a maximum similar to ours in the heat curve was obtained in the region of monolayer coverage. It should be noted, however, that both the isosteric heats as well as the surface area were calculated from a system showing marked hysteresis and, as pointed out by the authors, are therefore only apparent values. Yet it is quite likely that such apparent values are at least a qualitative representation of reality.

Several of the isosteric heat curves calculated by Davis and McLaren[8] show a second maximum in the region of about one mole of water adsorbed per 100 g. of protein, notably so for egg albumin, $\beta$-lactoglobulin, gelatin and wool. Unfortunately, calorimetric measurements in this Laboratory were not carried out at that coverage because the rate of adsorption became too low. It is conceivable that a process similar to that postulated above occurs for a second time. Interestingly enough, Buchanan, Haggis, Hasted and Robinson[26] estimated that in an aqueous solution of bovine serum albumin the same amount of water, *i.e.*, one mole per 100 g. of protein, is irrotationally bound; this was on the basis of dielectric measurements in the 1–10 cm. region.

**Acknowledgments.**—The author wishes to express his gratitude to Dr. R. A. Beebe, in whose laboratory this work was carried out, for his continued interest and valuable advice, to Dr. J. L. Oncley for the gift of a sample of bovine serum albumin and to him and to Dr. B. W. Low for discussions. He is indebted to Dr. R. M. Dell for design of part of the apparatus and to Mr. W. B. Spencer for carrying out one of the runs.

AMHERST, MASS.

(24) F. W. Putnam in ref. 3, p. 885.

(25) P. A. Marshall and W. J. Moore, THIS JOURNAL, **74**, 4779 (1952).

(26) T. J. Buchanan, G. H. Haggis, J. B. Hasted and B. G. Robinson, *Proc. Roy. Soc.* (*London*), **A213**, 379 (1952).