[CONTRIBUTION FROM THE EASTERN REGIONAL RESEARCH LABORATORY[1]]

Water Absorption of Proteins. IV. Effect of Physical Structure[2]

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In our studies of the effects of the various groups in proteins on the vapor-phase sorption of water,[3,4,5] the effect of structure is an important consideration. There are two ways in which structure could affect sorption phenomena: (1) purely steric effects and (2) interaction or hydrogen bonding of sorptive groups. There is evidence that in other high-polymer systems both these phenomena occur. For example, Baker and Fuller showed that the introduction of methyl groups on nylon amide N increased the vapor-phase sorption by opening up the structure,[6] and Dole has given further evidence of such effects.[7] In the cellulose–water system, many investigators have contributed to the development of the present concept of an amorphous sorptive portion and of less sorptive areas of high lateral order held together by hydrogen bonds between the hydroxyl groups.[8,9,10] (In this paper "crystalline" refers to the truly crystalline state; "lateral order" designates the ordered regions as evidenced by X-ray analysis of high polymers.[11,12])

Crystalline native proteins contain 40 to 60% water and appear to be readily accessible to water vapor. The wet structure shrinks reversibly as the humidity is successively lowered, and there is a concurrent loss of definition in the X-ray pattern.[13] Perutz has shown that methemoglobin shrinks primarily in one dimension of the unit cell as the crystals are air-dried. Apparently this shrinkage is intermolecular, the rigid layers of protein being separated by water layers. On complete drying, this structure collapses further.[14] McMeekin and Warner[15] determined the water content of β-lactoglobulin crystals directly, and found that they contain 46% water, or 0.84 g./g. of dry protein. Senti and Warner[16] have recently reported the orthorhombic unit cell of wet β-lactoglobulin crystals to be 69.3 × 70.4 × 156.5 Å. The unit cell dimensions of the air-dried crystals are 60.7 × 61.0 × 112.4 Å. The shrinkage on partial drying is thus evident in all three axes.

It has been demonstrated that the conversion of globular proteins to the denatured state involves an increase in lateral ordering, and by mechanical action these ordered regions can be oriented.[17,18,19] The X-ray pattern thus produced is essentially identical with that of stretched keratin, the β-keratin pattern. The natural fibrous proteins can be disordered and disoriented by treatments which cause little or no hydrolysis. In the many studies of water absorption by proteins, the effect of these changes in structure has not been satisfactorily evaluated. We have investigated the effect of changes in structure on water sorption using three proteins: (1) ovalbumin, a native globular protein; (2) silk fibroin, the typical linear oriented fibrous protein; and (3) wool, which has the α-keratin coiled configuration. The data show that the sorptive groups of both ordered and disordered polypeptide chains are relatively accessible to water vapor. Pertinent earlier work is considered in the discussion of the results on each protein.

Experimental

Preparation of Samples.—Native ovalbumin was mixed with half its weight of distilled water, kneaded into a plastic mass, and extruded through a 0.013-inch diameter die (sample A). A portion of this fiber was denatured by boiling for twenty minutes in distilled water (sample B). A portion of sample B was oriented by stretching the denatured filaments 400% in steam (sample C).

Raw silk was dewaxed with alcohol and ether, and degummed by boiling for thirty minutes in 1% castile soap made neutral to phenolphthalein with oleic acid. The degummed sample was rinsed well, and the boil-off was repeated once. Then the sample was rinsed with water, with dilute hydrochloric acid, again repeatedly with water, and finally twice with 90% alcohol, and then dried in a vacuum oven (sample D). A sample of dewaxed raw silk was supercontracted by soaking it in saturated sodium thiocyanate solution for ninety minutes at room temperature. The resulting semi-transparent gelatinous but still fibrous mass was rinsed until free of thiocyanate and then degummed by autoclaving twice in water at 115°, rinsed well, washed twice with 90% alcohol, and dried in a vacuum oven (sample E). A portion of sample D was supercontracted by soaking in 98% formic acid for ten minutes, and then rinsed well and dried. The sorption results for this sample were the same as those for sample E, and therefore they are not given in the table. The sample of "dissolved silk" was prepared by heating a portion of sample D in saturated sodium thiocyanate until a clear sirupy solution was obtained at about 60°. The solution

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(2) This paper is part of a talk presented before the Meeting-in-Miniature of the Philadelphia Section of the American Chemical Society, January 20, 1949.

(3) Mellon, Korn and Hoover, THIS JOURNAL, **69**, 827 (1947).

(4) Mellon, Korn and Hoover, *ibid.*, **70**, 1144 (1948).

(5) Mellon, Korn and Hoover, *ibid.*, **70**, 3040 (1948).

(6) Baker and Fuller, *ibid.*, **65**, 1120 (1943); also in *Ann. N. Y. Acad. Sci.*, **44**, 329 (1943).

(7) Dole, Abstracts, 112th Meeting, American Chemical Society, September, 1947.

(8) Urquhart, *J. Textile Inst.*, **20**, 125T (1929).

(9) Mark, *J. Phys. Chem.*, **44**, 764 (1940).

(10) Hermans, "Contribution to the Physics of Cellulose Fibers," Elsevier. Amsterdam or New York, 1946.

(11) Albert F. Smith, paper presented at Symposium on Textile Fibers, Brooklyn Polytechnic Institute, November 24, 1945.

(12) Hermans and Weidinger, *J. Applied Phys.*, **19**, 491 (1948).

(13) Fankuchen, *Ann. N. Y. Acad. Sci.*, **41**, 157 (1941).

(14) Perutz, *Trans. Faraday Soc.*, **42B**, 187 (1946).

(15) McMeekin and Warner, THIS JOURNAL, **64**, 2393 (1942).

(16) Senti and Warner, *ibid.*, **70**, 3318 (1948).

(17) Astbury, Dickinson and Bailey, *Biochem. J.*, **29**, 2351 (1935).

(18) Lundgren and O'Connell, *Ind. Eng. Chem.*, **36**, 370 (1944).

(19) Senti, Copley and Nutting, *J. Phys. Chem.*, **49**, 192 (1945).

TABLE I

EFFECT OF CHANGES IN PHYSICAL STRUCTURE ON VAPOR PHASE WATER SORPTION OF OVALBUMIN, FIBROIN AND WOOL AT 30°

Sample	Description	Grams of water/1000 grams dry protein. Relative humidity, % 5.9	11.8	31.4	50.9	75.1	83.6	93.3
A	Native ovalbumin		35	74	106	161	194	263
A'[a]			47	85	115	172	198	
B	Denatured ovalbumin		34	68	97	143	167	212
B'			43	83	111	157	180	
C	Oriented ovalbumin		33	67	97	141	166	211
C'			45	84	111	156	178	
D	Fibroin	19	27	56	77	114	134	167
D'		28	37	67	88	120	138	
E	Supercontracted fibroin	18	26	53	75	114	137	178
E'		25	34	62	83	116	141	
F	Dissolved fibroin	19	27	55	78	118	142	185
G	Wool, washed	29	41	82	115	166	191	234
H	Wool, *p*H 3.5	25	34	69	97	140	161	197
I	Reduced wool, *p*H 4.5	25	34	70	99	145	162	199
J	Reduced wool, *p*H 3.5	25	34	70	97	141	160	194
K	Supercontracted wool, *p*H 3.5	21	30	63	90	131	148	180
L	Wool dissolved in thioglycolate	22	31	67	95	137	156	191
M	Wool insoluble in thioglycolate	23	32	68	98	139	162	205

[a] The prime (A') indicates data obtained on the desorption cycle.

was dialyzed for two days against distilled water. The precipitated fibroin was removed, broken up and washed with water until free of thiocyanate, then filtered off and air-dried (sample F).

A piece of fine worsted cloth was extracted twice with carbon tetrachloride, dried and washed in mild soap solution at room temperature, rinsed thoroughly and then air-dried (sample G). A control sample on the following treatments was prepared by soaking a portion of sample G in 0.05 *M* acid potassium phthalate–hydrochloric acid buffer (*p*H 3.5), rinsing until chloride-free and air-drying (sample H). The disulfide groups of a portion of sample G were reduced to thiol groups by soaking it for two days at 32° in 0.5 *M* thioglycolic acid which had been adjusted to *p*H 4.5 with sodium hydroxide, and then rinsed until the washings gave a negative nitroprusside test, soaked in 95% ethanol for two hours, rinsed once with alcohol and twice with water and air-dried (sample I). A control portion of sample I was soaked for three hours in phthalate buffer (*p*H 3.5), rinsed until chloride-free and air-dried (sample J). A portion of sample I was supercontracted by boiling it in the phthalate buffer (*p*H 3.5) for one hour, washed chloride-free and air-dried (sample K). A decrease in area of 35% was obtained by this supercontraction procedure.

Another portion of the original wool sample was reduced by soaking it in four successive portions of 0.25 *M* thioglycolate at *p*H 9.0. It was then dissolved by shaking it in a solution of 0.25 *M* thioglycolate in *N* sodium hydroxide for two and one-half hours, and filtered through a coarse fritted glass filter. A gelatinous undissolved residue remained on the filter. The two portions were acidified with acetic acid to *p*H 5.0; the precipitated protein was filtered off and washed repeatedly. They were then dialyzed against *p*H 3.5 phthalate buffer and then water, and washed repeatedly with water, 50% alcohol, 95% alcohol, and again with water. The washing finally resulted in almost complete removal of the odor of thioglycolic acid. The products were air-dried and ground through a 40-mesh screen. The dissolved portion (sample L) gave a recovery of 50%, and the insoluble portion (sample M) 15%. These two portions represent essentially all the original sample; the total recovery of 65% is undoubtedly low because of losses in the numerous manipulations in the procedure.

Sorption Measurements.—Approximately 2-g. samples of the proteins were dried at 70° to constant weight and then equilibrated at a series of relative humidities, obtained by saturated salt solutions, in evacuated desiccators at 30°. Full details of the techniques employed were presented in the first paper of this series.[3] Desorption measurements were carried out by approaching equilibrium from the "wet" side, as described in the second paper.[4] These measurements are indicated in Table I by primes (A').

Results and Discussion

Table I gives detailed data for the sorption of all the samples. The accuracy and reproducibility of the results are greater than can readily be shown in a figure, and we believe the data may be of value to others in the field. The results are discussed in the order in which they are presented in the table. In each case, three factors must be considered: the treatment imposed on the sample, the structural changes brought about, and the sorption results.

Ovalbumin.—The extent of the structural changes brought about by the ordering and stretching process is difficult to measure. Typical diffraction patterns of the same sample of protein treated in the same fashion were published by Nutting, Halwer, Copley and Senti,[20] and by Senti.[21] The native sample exhibits a diffuse two-ring pattern. The denatured sample has a relatively sharp three-ring pattern indicative of a fair degree of lateral order, and a random orientation of the diffraction regions.

Stretching the fiber produces a high degree of orientation parallel to the fiber axis, but there appears to be little further change in the degree of lateral order.

The absorption data show a decrease in water content, which is brought about by the ordering

(20) Nutting, Halwer, Copley and Senti, *Textile Research J.*, **16**, 599 (1946).

(21) Senti, *Amer. Dyestuff Reptr.*, **36**, 230 (1947).

process, but the magnitude is small. Moreover, on desorption the hysteresis is greater in the ordered samples B and C, and therefore the desorption curve of these samples lies close to that of the native sample below 50% relative humidity. The definite decrease in sorption brought about by an increase in order in the high humidity region, 75% and above, occurs where the binding of water is a low energy type. In fact, sorption in this region has often been considered to be a capillary condensation phenomenon. Bull has similarly compared the sorption of native and denatured ovalbumin.[22] His data are given as the average of the absorption and desorption values, so that they cannot be directly compared with ours, but the trend of his results is similar.

Silk Fibroin.—The degree of lateral order obtained by treating ovalbumin is about the best obtainable with the globular proteins, but still it does not approach that of silk fibroin. This protein is highly ordered and oriented. The orientation can be decreased readily by swelling it in various acids or lyophilic salts such as sodium thiocyanate. Fibroin dissolves in such solutions on heating and can be reprecipitated as a granular solid, which appears fibrous under the microscope. The X-ray diffraction pattern of these samples (D, E and F) indicates that the primary change is a disorientation, for the diffraction pattern of the sample precipitated from solution shows the same number of diffraction maxima as the supercontracted sample.

The accessibility to water vapor of the ordered regions, however, is a moot point. Senti[21] assumes that they are not accessible, since the wet and the air-dry fiber have the same equatorial spacings in the diffraction pattern. However, Matsunaga[23] showed that the unit cell of perfectly dried fibroin is penetrated by water and does swell. His data, which are in agreement with Senti's, show that there is no further swelling of the ordered regions between room humidity and the fully wet state. It appears that a part of the water is sorbed within the ordered regions and that the major portion sorbed at relatively high humidities does not affect the diffraction pattern. An additional piece of evidence comes from our previous work. We showed[5] that the sorption of silk, which is composed largely of the simple amino acids glycine and alanine and has few sorptive groups besides the peptide group, is almost identical with that of polyglycine polymers when calculated on the basis of nitrogen content.

The sorption data show that the two treatments of silk do not affect the water uptake except at the highest humidity studied. It must be remembered that although the silk had been disoriented and even put in solution, the diffraction patterns showed that the three samples had a comparable degree of lateral order. It would be surprising, however, if the reprecipitated sample recovered all the original order imposed by the natural spinning process of the silk worm.

Wool.—Wool occurs naturally in a folded configuration, the α-keratin structure, which has a low degree of lateral order. This structure can be disoriented by reducing the disulfide bonds to sulfhydryl and then heating the fiber in acid buffer. The fiber shrinks and becomes brittle and inelastic. There appears to be a definite loss of lateral order from the already low order of the α-keratin. The final pattern is much like that of an unordered globular protein.

The sorption data in the table show that the *p*H of the fiber must be considered, for sample G, which was washed with soap and therefore slightly alkaline, had a higher sorption than sample H, which was washed with buffer at *p*H 3.5. Briggs has demonstrated a similar effect in casein.[24] However, the control sample equilibrated with buffer (H), the reduced sample treated at *p*H 4.5 (I), and the sample (J) of the latter adjusted to *p*H 3.5, all gave essentially the same sorption curve. The supercontracted sample (K) was slightly but significantly less sorptive than the others.

Samples L and M were prepared as a further test of the effect of structure, for wool treated by the procedures described above is still in its cellular form. However, this structure is completely destroyed when the reduced wool is dissolved in alkali. The precipitated wool no longer has the α-keratin structure. Its absorption curve does not differ significantly from the control sample (H). The data of samples H, J, L and M show that reduction of the disulfide bond to the sulfhydryl does not affect the sorption isotherm. It appears most probable from this result that neither group contributes to the water absorption of wool.

The results on wool are consistent with those on ovalbumin and fibroin in showing that rather severe treatments do not alter the sorption markedly. Wool is of especial interest, for Cassie[25] has recently applied Barkas's treatment of swelling sorbents to this system. Cassie has calculated a stress-free isotherm and found it to be approximately three times the observed sorption. (Rather full discussions of sorption and swelling have recently been published in "Fibrous Proteins"[26] and a Faraday Society Symposium on Swelling and Shrinking.[27] White and Eyring[28] and White and Stam[29] have more recently summarized the results in this field.) Our data show no effect which can be attributed

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

(23) Matsunaga, *Mem. Coll. of Sci., Kyoto Imp. Univ.*, Series A, **20**, 157 (1937).

(24) Briggs, *J. Phys. Chem.*, **35**, 2914 (1931).

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

(26) "Fibrous Proteins," Soc. of Dyers and Colorists, May, 1946.

(27) Discussion on Swelling and Shrinking, *Trans. Faraday Soc.*, **42B**, Suppl. (1947).

(28) White and Eyring, *Textile Research J.*, **17**, 523 (1947).

(29) White and Stam, *ibid.*, **19**, 136 (1949).

to a restraint on swelling of the fiber by its physical structure, for complete destruction of that structure did not affect the sorption isotherm.

The complete absence of swelling pressures due to the intact fibrous structure cannot be concluded from these data, for in such a complex system a slight effect might be masked by other changes caused by the chemical treatment. Moreover, there are reasonable theoretical arguments for the existence of such pressures. We believe, however, that our experimental results show their effect is not large.

Wool *vs.* Casein.—A further point of comparison can be made between the sorption of wool and our previous data on casein, a completely amorphous protein. The complete sorption cycles for wool and casein are plotted in Fig. 1. The close similarity of all phases of the sorption cycle is consistent with our interpretation, advanced in previous papers, that sorption is on specific groups, for wool has a composition similar to that of casein,[30] except for its cystine content. The disulfide group would not be expected to be sorptive, and the comparison between wool and reduced wool supports this idea. It appears probable, therefore, that any proposed theoretical treatment of sorption by wool would also have to be applicable to amorphous proteins as well.

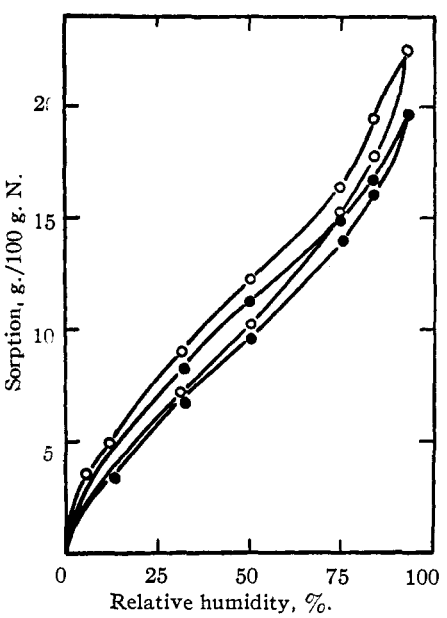

Fig. 1.—Sorption cycle of casein (O) and wool (●) at 30°.

A complete and quantitative analysis of the effect of internal structure on the sorption of water by proteins cannot be made at this time, for the degree of lateral order in proteins is difficult to assess. The X-ray diffraction patterns of the variously treated ovalbumin and wool samples do show qualitative changes in lateral order and orientation, but the extent and perfection of the order are probably not great. Silk and polyglycine appear to be more highly ordered, but even here the amount of ordered material may be less than, say, 50% of the total sample. The salient result of this study is that putting the protein in solution and then reprecipitating it, thus affecting the structure as much as possible, has little effect on its sorption isotherm. The data of this paper can best be interpreted as indicating that the groups responsible for sorption are always relatively accessible in proteins. If one considers the extreme heterogeneity of the side groups of proteins, it would seem surprising indeed if water were not able to penetrate the structure.

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Summary

Sorption isotherms have been measured on a series of wool, silk fibroin and ovalbumin fibers treated to alter the internal physical structure.

Wool was dissolved in alkaline thioglycolate and precipitated from solution. The product had essentially the same water absorption as the control sample. Reduction of the disulfide groups and supercontraction of wool also affected the sorption only slightly. There is no evidence of a restraint on sorption in these experiments which can be attributed to the physical structure of wool.

Alteration of the physical structure of silk fibroin was achieved by supercontracting it and by dissolving it in thiocyanate. There was little effect of these treatments on sorption.

Native ovalbumin was brought into a state of lateral order by denaturation, and the ordered regions were oriented by stretching the fibers. A slight but significant decrease in the absorption curve was produced by the increase in order, but this effect was much smaller on the succeeding desorption cycle.

Thus, severe treatments of these three typical proteins, which alter the internal structure as much as appears possible, have little effect on sorption of water. The concept that sorption is primarily related to the number of specific sorptive groups present appears to be applicable to proteins, probably because of the heterogeneity of the side chains present.

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(30) Hoover, Kokes and Peterson, *Textile Research J.*, **18**, 423 (1948).