JOURNAL OF APPLIED POLYMER SCIENCE VOL. 8, PP. 1813–1824 (1964)

Hydration and Density of Collagen and Gelatin*

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Synopsis

The water sorption by adult and embryonic native and insoluble collagen was investigated, along with that of calfskin gelatin. Greater sorption was obtainable with the collagen fractions than with gelatin, but the technique could not distinguish the fractions themselves. The suggestion is made that the greater sorption by collagen over gelatin and differences in apparent wettability between the adult and embryonic insoluble collagen might be explained by the formation of helical grooves with aging. Density measurements of the collagen fractions indicate that collagen becomes more crystalline with aging. This also applied to the insoluble collagen. These results are consistent with the view that differences in collagen solubility are due to differences in molecular ordering. The density of the insoluble collagens and gelatin passes through a maximum at 3–4% sorbed water. The phenomenon is explained on the basis of water bridges comprised of a single water molecule doubly hydrogen bonded to closely aligned chains. A discussion is presented in this connection. A calculation based on the density yields a molecular volume of 4.32×10^{-19} cm.^{3} for tropocollagen. This is in agreement with the molecular dimensions and consistent with the idea that collagen is formed from an aggregation of the monomer.

I. Introduction

The prevailing thought concerning the formation of collagen is that the soluble monomer tropocollagen, aggregates end to end[1] to form three helically entwined polypeptide chains stabilized by interaction of the side chains.[2] This view, referred to as the tropocollagen hypothesis by those who formulated it,[1] has been summarized by Schmitt[3] and Crick and Rich.[4] A variation of this view is held by Huggins,[5] who postulates the formation of a single strand folded back upon itself to form the helical structure. Regardless of either interpretation, it has been known for a long time that collagen may be extracted to yield soluble fractions in neutral salts, dilute alkaline and acid buffers, in addition to an insoluble residue. The variation in solubility has been interpreted on the basis of differences in molecular organization or crystallinity[6,7] and chemical crosslinkages[8] which accrue with aging. On the other hand, other workers[9–12] maintain that collagen is not chemically identical throughout, and that its solubility characteristics are a result of its formation from an acid-soluble "procollagen" and an insoluble "metacollagen," each of which has properties different from

* Supported by a contribution from the Old Dominion Foundation.

those of native collagen, such as swelling, shrinkage, and electron microscopical appearance.

With these different interpretations in mind, the water sorption of native and insoluble collagen of the adult and embryo was undertaken to investigate possible surface differences. Precision density measurements were also made of these fractions as a measure of crystallinity. A similar parallel study was made with calfskin gelatin.

II. Experimental

The collagen was prepared from the hides of a 15-year-old bull and a 4-month embryo. The same area (right front leg) was chosen for each to eliminate differences due to location. The hides were washed with ice water immediately after obtaining them from the animal, the major part of the hair was clipped off, and the hides trimmed with a currier knife to obtain the corium layer. The latter was cut into small rectangular pieces which served as the starting material for the preparation. The method of Veis, Anesey, and Cohen[8] was used because it caused minimal alteration of the initial material. This was evidenced by the physical similarity of

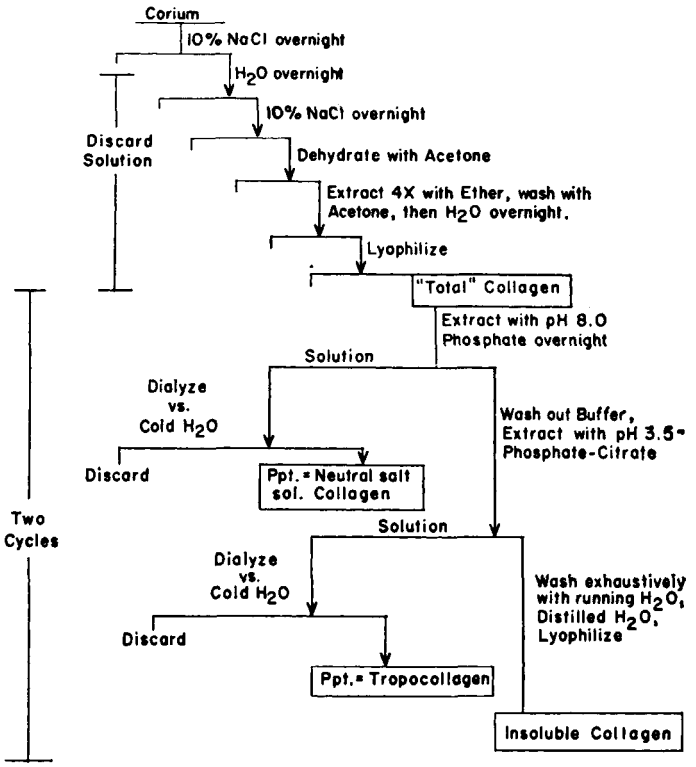

Fig. 1. Preparation of collagen.

TABLE I
Summary of Data

Substance	N, %	Hypro., %	Tyr., %	Density, g./cm.^{3}	cm.^{3}	
					V_m	C
"Total" adult collagen	18.3	14.1	1.2	1.343	10.5	50.6
Insol. adult collagen	18.3	14.3	1.0	1.343, 1.345	10.7, 11.2	21.0, 19.9
"Total" embryonic collagen	18.1	13.4	1.4	1.336–1.341	8.38	37.2
Insol. embryonic collagen	18.1	13.9	1.3	1.333, 1.335	9.11	28.1
					8.84	37.2
Calfskin gelatin	17.1	13.2	—	1.345	8.39	17.8
Bull's Data[14] at 25°C.						
"Hide Collagen"	—	—	—	—	9.52	17.8
Gelatin	—	—	—	—	8.73	17.4

the starting material and final product, despite the fact that soluble proteins had been extracted. A flow sheet of the procedure is given in Figure 1. Minor changes from the original procedure, which does not mention quantities or volumes used, might result from the following: a 200–500 g. portion of corium was extracted in 10 liters of 10% NaCl. Buffer extractions were made with 250 ml. All operations were conducted in the cold, as mentioned in the procedure.

Two fractions are used in the following experiments. The first is the native collagen, called total collagen in this paper because it consists of the sum total of all the fractions. This is the residue prior to the first pH 8.0 extraction. The second is insoluble collagen, which has had all the soluble collagen fractions removed. In the original procedure,[8] the insoluble collagen is designated as purified intact collagen. These fractions were dried after lyophilization in vacuo over P_2O_5 for three weeks prior to use. During this time they were outgassed at room temperature by intermittently making and breaking the vacuum.

A chemical characterization of these fractions as well as that for a commercially purified calfskin gelatin is given in Table I. Nitrogen was determined by micro-Kjeldahl, hydroxyproline by the method of Woessner,[13] and tyrosine by the method of Folin and Ciocalteu.[14]

The sorption experiments were carried out in the manner devised by Bull,[15] whose investigation, conducted almost twenty years ago, on the water sorption of proteins, including collagen and gelatin, is still the most referred-to study.

Dried, outgassed samples were weighed in preweighed small vials which were then placed in slightly larger vials, and partially immersed in H_2SO_4 solutions of varying concentrations. The latter were contained in screw-capped jars which were sealed with grease, and the entire assembly submerged in a waterbath at 20.1°C. for 30 days. This was sufficient time for equilibrium to be established.[15] At the end of this period, the jars were removed from the bath and the water sorbed by the samples obtained

from the weight change. This procedure yielded identical values for water sorption as the procedure used by Bull. In the latter instance, the water sorbed was obtained by difference after heating the sample to 105°C. for 24 hr. The equilibrium vapor pressure was obtained from the International Critical Tables[16] from the concentration of the H_2SO_4 solution at equilibrium. This was determined by titrating a diluted aliquot with standard alkali.

The density measurements were made by the gradient density method of Linderstrom-Lang.[17] The method which is one of three precision methods for the density determination of solids[18] has been reviewed recently.[19] For the present system, mixtures of CCl_4 and xylene were used (5:2 overlaid by 5:3, v/v). The gradient tube was immersed in a 33-gallon waterbath held at 20.1 ± 0.001°C. Glass floats, standardized against standard KBr solutions whose densities were determined pycnometrically, were used to establish the position of the gradient.* Using a cathetometer to determine the position of the floats to the nearest 0.001 cm., the gradient was found to be linear in the range of 1.324–1.356.

The density determination of the collagen samples required the sample size to be 4–8 mg. to give reproducible results. In spite of this precaution, variation persisted among the density values for a particular sample. Thus, although the gradient column was capable of reporting six significant figures, the density for gelatin is good to 1 part in the fourth decimal, adult collagen, 4 parts in the fourth; and embryonic collagen, 1 part in the third. All densities will be reported to four significant figures for consistency.

Considerable differences were encountered in the time required for particular samples to become wetted and submerge to a final position in the column. Gelatin samples reached their final position in a few minutes; adult collagen, in 30–45 min.; and embryonic collagen, in the neighborhood of 6 hr. During this time, volume increases were detected in the case of the embryonic collagen affecting the density to the extent of about two parts in the third decimal. No correction for this change was made. No changes were detected in gelatin and adult collagen.

III. Results and Discussion

The sorption isotherms for adult and embryonic insoluble collagen (AIC and EIC, respectively) are combined with that of calfskin gelatin to Figure 2. When a BET plot[20] is made of the data, the resulting curves (Fig. 3) exhibit linearity up to $P/P_0 = 0.5$. At greater vapor pressures, the water sorption values depart markedly from linearity toward higher values. Similar observations have been made previously by Bull.[15] The volume of H_2O, V_m, necessary to cover a monolayer of surface and the constant, C, related to the heat of sorption, have been collected for the various

* The author is indebted and grateful to Dr. Henry Leidheiser for the preparation of these floats. They are now available from the Scientific Glass Apparatus Co., Bloomfield, New Jersey.

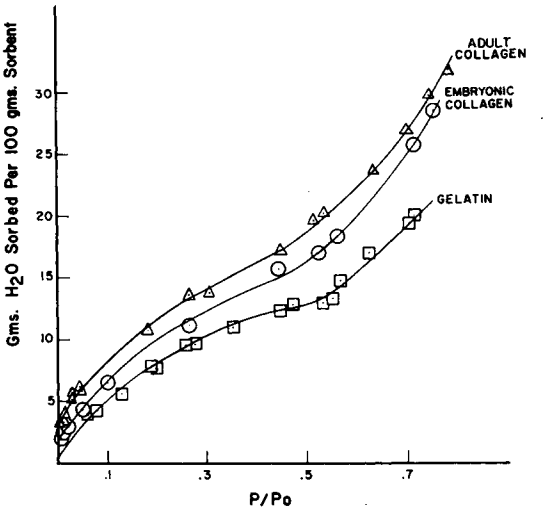

Fig. 2. Water sorption by insoluble collagen and gelatin.

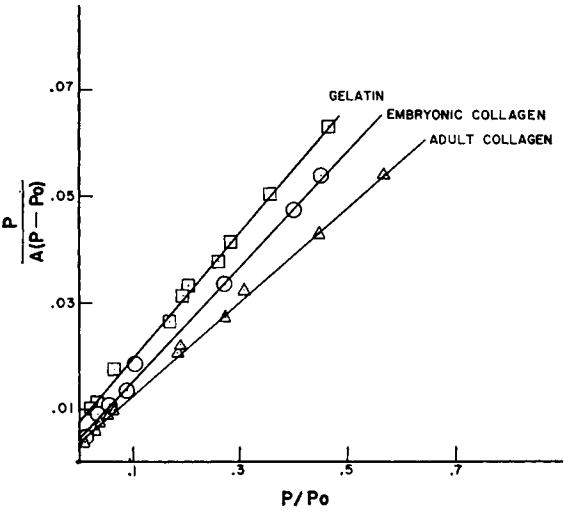

Fig. 3. BET plot of insoluble collagen and gelatin.

sorbents in Table I. Included are values to indicate the extent of reproducibility of a sorption experiment with a particular sorbent. In these instances, the entire preparation procedure for AIC and EIC was repeated. A satisfactory agreement is achieved with the AIC and the results compare favorably with Bull's data (Table I). Excellent agreement with Bull's data is present in the case of gelatin (Table I). On the other hand, sorptions with all the embryonic collagen, insoluble and total, were characterized by a considerable scattering of points. The sorption data re-

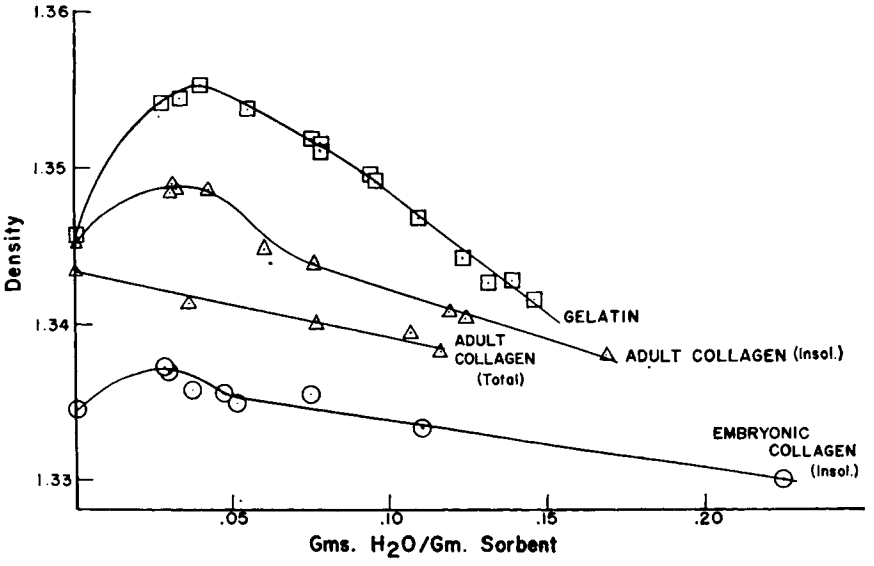

Fig. 4. Effect of H_2O content upon density of collagen and gelatin.

corded here for embryonic collagen are given with reservation because of this, and no significant difference from the adult collagen is implied.

The density data for the anhydrous collagen fractions and gelatin are given in Table I. The influence of sorbed water on the density is shown in Figure 4. With the exception of the adult total collagen and corresponding embryonic collagen (not shown), a definite density maximum occurs at 3–4% sorbed water. Similar behavior has been recorded for such diverse substances as cotton,[21] rayon,[22a] silk,[23] wool,[24] leather,[25] nylon,[26] β-lactoglobulin,[27] and ovalbumin.[2] The possible significance of this density maximum will be discussed shortly.

Returning to the density of anhydrous collagen, an appreciable difference is apparent between the insoluble collagen of the adult and the embryo. The increased density of the older collagen suggests that age changes associated with molecular ordering persist even after the formation of the insoluble collagen. The data favor the views of Jackson[6] and Gross[7] who emphasize the variation of crystallinity with aging of collagen. The soluble fractions of the adult collagen do not appear to influence the density perceptibly. This is probably representative of a very old collagen, and would probably be different for a younger adult. The total embryonic collagen appears to be characterized by its disorganization. No representative density could be determined, only a range which included the embryonic and adult insoluble collagen The same type of behavior was evidenced in sorption isotherms with this material.

Previous determinations of the density of collagen are those of Pomeroy and Milton[25] using nitrogen as displacement medium. They obtained 1.41 for hide collagen. Rougvie and Baer,[29] using kangaroo tail tendon with

bromoheptane and CCl_4, obtained 1.34. No chemical analysis accompanied either preparation.

If it is assumed that the rodlike molecules of tropocollagen have the shape of a right cylinder, 3100 A. long and 13 A. in diameter,[30] the volume of such a cylinder will be 4.12×10^{-19} cm.^{3} Assuming an aggregation of the monomer to form the fibrous collagen, the volume of the molecule as it exists in a hypothetical cell lattice will be V/n, where

$$V/n = M/\rho N$$

and V is the volume of the unit cell, n is the number of molecules per unit cell, ρ is the density (1.343 g./cm.^{3} for adult collagen), N is Avogadro's number (6.025×10^{23}), and M is the molecular weight (350,000).[30] V/n comes out to be 4.32×10^{-19} cm.,^{3} which is in good agreement with the value obtained geometrically, and supports the validity of the above assumptions.

The high density obtained for gelatin is surprising. Fessler and Hodge,[31] in an ultracentrifugation study using a density gradient, found parent gelatin more dense than collagen fibrils. The reason for this is obscure at present.

The density maximum observed when various substances sorb small amounts of water has received several interpretations. Hermans and co-workers[22a] believe, in the case of cellulosic materials, that the water penetrates into pores inaccessible to organic solvents causing contraction of the fibers. They further maintain that this is the region where the first hydrate (cellulose hydrate I) is formed. Their data are interpreted in terms of the apparent density for the sorbed water. In the case of cotton, the apparent density of the sorbed water was calculated to be greater than 2. This high value which is acceptable to them in this instance was rejected by them as an explanation for the high density values for cellulose in water generally.[22b] A maximum in the density has been obtained for cotton system with helium as the displacement medium.[32]

Alexander and Hudson[33] believe that the volume contraction following the chemisorption of water to form definite hydrates is sufficient to explain the phenomenon of maximum density in the low moisture region. The concept of hydrate formation for fiber–water systems was proposed initially by Katz,[34] who pointed up the similarity of these systems with those of H_2SO_4 and H_2O. Stamm and Loughborough[35] made a similar comparison. One might add additional similarity, that the density of the H_2SO_4–H_2O system passes through a maximum at 3% H_2O.[36]

Consistent with the hydrate concept is the high differential heat of wetting (differential heat of absorption) for the fibrous material, including collagen.[37]

McMeekin and co-workers[27] found the maximum density occurring at about 14% H_2O for β-lactoglobulin. They explained this on the basis of a combined effect of the apparent packing of the protein and the electrostriction of the water.

TABLE II
Calculated Interatomic Distances Between Carbonyl Oxygens

Atom or group	Distance, A.	Model
Glycine–Proline	3.48	Rich and Crick,[44] Collagen II
Glycine–Hypro	3.06	" " " "
O_1–O_2	3.47	Ramachandran et al.,[45] Two-bonded
O_2–O_3	2.73	" " "
O_1–O_3	4.87	" " "

It would seem that the hydrate concept neglects one important aspect, and that is that a stoichiometric hydrate invariably has a lower density than the anhydrous parent substance. Hence, it is difficult to see how the formation of a less dense material would produce an increase in the density without bringing in a concomitant event. More plausible, it seems, is the postulation of the formation of water bridges, doubly hydrogen-bonded structures joining accessible chains in closer alignment. Previous workers have provided evidence that water molecules will fit into the geometry of the collagen chains forming hydrogen-bonded bridges between adjacent carbonyl groups.[38–42] The innovation suggested at present is that in the region of the density maximum, the water exists primarily as single molecules.[43] These then can fit into an available space, drawing the already close chains closer together. At the higher vapor pressures, the water exists as chains and aggregates. These, upon entering a void, will expand the structure. In the light of this explanation, the insoluble adult collagen, in becoming more dense, creates more closely aligned chains for the single molecules to bridge. The steeper maximum in this case as compared to the embryonic counterpart is consistent with the explanation. Similarly, the closely aligned chains of gelatin, as reflected by its high density, are capable of forming numerous water bridges because of the absence of the restrictive internal architecture possessed by collagen. On the other hand, the total collagen of the adult and embryo, both of which do not form the maximum, possess a greatly expandable structure of the soluble collagen over an insoluble core. In the case of the embryonic total collagen, as little as 0.03 g. H_2O per gram caused the sample to float in the density column, indicating a density change from about 1.33 to 1.31.

In an often quoted paper, Esipova and co-workers[40] visualized water bridges taking place between adjacent free carbonyls on the same chain. This view has also been espoused by Harrington and von Hippel.[42] The positioning of a water bridge between two adjacent carbonyls on the same chain necessitates a span of distance in excess of that usually attributed to a hydrogen bond, and one which could not be achieved by a single water molecule. Given in Table II are the calculated distances for the carbonyl oxygens taken from the coordinates of the atoms given by Rich and Crick[44] for their collagen II model and from those of Ramachandran et al.[45] for their two-bonded standard structure. In the Rich and Crick model, the distances given are too great to be spanned by a single water molecule.

The 3.06 A. between carbonyls of glycine and hydroxyproline was not mentioned by the authors[44] as a possible water position For the two-bonded standard structure, only the distance O_2–O_3 is favorable for hydrogen bond formation. However, this distance represents an interchain distance and a hydrogen bond is postulated between O_2 and a neighboring N.[45] From their x-ray data of rat tail tendon, Burge et al.[39] suggested two possible positions for water molecules, between two carbonyls on neighboring chains, and a particular pair on the same chain. Both were considered not entirely satisfactory by them and untenable by others.[44] Esipova et al.[40] also mentioned the possibility of interchain bridging if the chains are 5–6 A. apart. The main difficulty at present is due to the lack of a universally acceptable model for collagen. The interpretation of hydration effects, which must rest upon such a model, must therefore dwell in uncertainty. A certain amount of confusion, however, may be forestalled by eliminating the all too common practice of equating tendon to collagen. Another complication which may be surmised from the present study is that collagen may be viewed as a system whose spacial atomic positions change with time, tending toward an increase in density. Crystallographic changes indicating greater crystallinity with aging have also been observed.[46] In the light of this, results of structural studies will be dependent upon the age of the animal, and it is conceivable that changes concomitant with aging may produce changes in interpretation.

The present work points up two apparent paradoxes. One might ask with good reason why a crystalline substance like collagen is capable of sorbing more water than an amorphous material like gelatin, each having almost identical chemical composition. The question is appropriate since it is generally accepted that the reactive regions of a structure are amorphous. Almost identical results as recorded here have been obtained by Bull,[15] although no comment was made concerning it. It seems conceivable that in the molecular ordering which proceeds with the aging of collagen, helical grooves are formed to accommodate more water. Evidence for the presence of such grooves has recently been submitted.[47]

The second apparent paradox concerns the wettability of the embryonic and adult insoluble collagen. From the long time required for the embryonic insoluble collagen to submerge in the organic medium, one would surmise that it is much more lyophilic toward H_2O than the adult counterpart. The explanation cannot be due to their differences in density, since one would expect the solvent to penetrate the less dense material, in this case the embryonic insoluble collagen, more readily. If one assumes a more lyophilic surface for EIC than AIC, one would expect to obtain a greater water sorption in the former instance. This is not the case. The answer again may be the helical grooves. Although differences in the lyophilic nature of the surfaces may exist, the presence of the grooves in the older collagen may permit a more rapid penetration of the solvent.

While it is possible to achieve reproducible results, it does not appear that sorption experiments are sufficiently sensitive to differentiate between

closely related chemical species such as the various collagen fractions. This has been the case with ovalbumin and plakalbumin[48] and collagen as a film and a fiber.[38] On the other hand, the precision density measurements offer concrete evidence that the molecular ordering of the collagen changes with aging, presumably toward greater crystallinity. Evidence for this has also been obtained from x-ray data.[46] This would tend to favor the opinions of Jackson[6] and Gross[7] who claim that the variations of solubility of collagen are due to variations in the molecular organization or crystallinity. Although the present work does not eliminate the procollagen–metacollagen hypothesis as a possibility, it makes it less plausible, for the calculation of the molecular volume is consistent with the idea that collagen is formed from an aggregation of the monomer.

Added in proof: Electron micrographic evidence of this has recently been published; E. Borysko, "Collagen," in *Ultrastructure of Protein Fibers*, Academic Press, N. Y., 1963, p. 19.

The author is grateful to Dr. Henry Leidheiser and Dr. Melvin Hobson for reading the manuscript, and for their suggestions.

References

1. Schmitt, F. O., J. Gross, and J. H. Highberger, *Symposia Soc. Exptl. Biol.*, **9**, 148 (1955).
2. Crick, F. H. C., and A. Rich, *Nature*, **176**, 780 (1955).
3. Schmitt, F. O., in *Connective Tissue, Thrombosis and Atherosclerosis*, I. H. Page, Ed., Academic Press, New York, 1959, 43.
4. Crick, F. H. C., and A. Rich, in *Recent Advances in Gelatin and Glue Research*, G. Stainsby, Ed., Pergamon Press, London, 1958, p. 20.
5. Huggins, M. L., *Proc. Natl. Acad. Sci., U. S.*, **43**, 209 (1957); *J. Am. Chem. Soc.*, **76**, 4045 (1954).
6. Jackson, D. S., in *Connective Tissue, Thrombosis and Atherosclerosis*, I. H. Page, Ed., Academic Press, New York, 1959, p. 67.
7. Gross, J., in *Connective Tissue, Thrombosis and Atherosclerosis*, I. H. Page, Ed., Academic Press, New York, 1954, p. 77.
8. Veis, A., J. Anesey, and J. Cohen, *J. Am. Leather Chemists' Assoc.*, **55**, 548 (1960).
9. Barga, J., J. Balo, and D. Sazbo, *Acta Physiol. Hung.*, **9**, 61 (1956).
10. Tustanovskiĭ, A. A., A. L. Zaides, G. V. Orlowskaya, and A. N. Mikhailov, *Dokl. Akad. Nauk SSSR*, **97**, 121 (1954).
11. Zaides, A. L., *Biofizika*, **1**, 279 (1956).
12. Mazurov, V. I., and V. N. Orekhovich, *Biokhimiya*, **24**, 33 (1959).
13. Woessner, J. F., Jr., *Arch. Biochem. Biophys.*, **93**, 440 (1961).
14. Folin, O., and V. Ciocalteu, *J. Biol. Chem.*, **73**, 627 (1927).
15. Bull, H. B., *J. Am. Chem. Soc.*, **66**, 1499 (1944).
16. Blake, F. C., and C. H. Greenewalt, *International Critical Tables*, III, McGraw-Hill, New York, 1928, p. 303.
17. Linderstrøm-Lang, K., *Nature*, **139**, 713 (1937).
18. Smakula, A., in *Methods of Experimental Physics*, Vol. 6, Pt. A, Academic Press, New York, 1959, p. 283.
19. Oster, G., and M. Yamamoto, *Chem. Revs.*, **63**, 257 (1963).
20. Brunauer, S., P. H. Emmett, and E. Teller, *J. Am. Chem. Soc.*, **60**, 309 (1938).
21. Davidson, G. F., *J. Textile Inst.*, **18**, T175 (1927).
22. Hermans, P. H., *The Physics and Chemistry of Cellulose Fibers*, Elsevier, Amsterdam, 1949, (a) p. 206; (b) p. 201.

23. Goodings, A. C., and Turl, L. H., *J. Textile Inst.*, **31**, T69 (1940).
24. King, A. T., *J. Textile Inst.*, **17**, T53 (1926).
25. Pomeroy, C. D., and R. G. Mitton, *J. Soc. Leather Trades Chemists*, **35**, 360 (1951).
26. Speakman, J. B., and K. Saville, *J. Textile Inst.*, **37**, P271 (1946).
27. McMeekin, T. L., M. L. Groves, and N. J. Hipp, *J. Polymer Sci.*, **12**, 310 (1954).
28. Neurath, H., and H. B. Bull, *J. Biol. Chem.*, **115**, 519 (1936).
29. Rougvie, M. A., and R. S. Baer, *J. Am. Leather Chemists Assoc.*, **48**, 735 (1953).
30. Boedtker, H., and P. Doty, *J. Am. Chem. Soc.*, **78**, 4267 (1956).
31. Fessler, J. H., and A. J. Hodge, *J. Mol. Biol.*, **5**, 446 (1962).
32. Filby, E., and O. Maass, *Can. J. Res.*, **7**, 131 (1932).
33. Alexander, P., and R. F. Hudson, *Wool, its Chemistry and Physics*, Reinhold, New York, 1954, p. 91.
34. Katz, J. R., *Ergebn. Exakt. Naturwiss.*, **3**, 372 (1924); *Trans. Faraday Soc.*, **29**, 279 (1933).
35. Stamm, A. J., and W. K. Loughborough, *J. Phys. Chem.*, **39**, 121 (1935).
36. *Handbook of Chemistry and Physics*, 43rd Ed., Chem. Rubber Publ. Co., Cleveland, 1961, p. 2093.
37. McLaren, A. D., and J. Rowen, *J. Polymer Sci.*, **7**, 27 (1951).
38. Bradbury, E. M., R. E. Bunge, J. T. Randall, and G. R. Wilkinson, *Discussions Faraday Soc.*, **25**, 173 (1958).
39. Burge, R. E., P. M. Cowan, and S. McGavin, in *Recent Advances in Gelatin and Glue Research*, G. Stainsby, Ed., Pergamon Press, London, 1958, p. 25.
40. Esipova, N. G., N. S. Andreeva, and T. V. Gatovskaia, *Biofizika*, **3**, 529 (1958).
41. Frazer, R. D. B., and T. P. MacRae, *Nature*, **183**, 179 (1959).
42. Harrington, W. F., and P. H. von Hippel, *Arch. Biochem. Biophys.*, **92**, 100 (1961).
43. Starkweather, H. W., *J. Polymer Sci.*, **B1**, 133 (1963).
44. Rich, A., and F. H. C. Crick, *J. Mol. Biol.*, **3**, 483 (1961).
45. Ramachandran, G. N., V. Sasisekharan, and Y. T. Thathachani, in *Collagen*, Madras Symposium, Interscience, New York, 1962, p. 81.
46. Kratky, O., M. Lauer, M. Ratzenholer, and A. Sekora, in *Collagen*, Madras Symposium, Interscience, 1962, p. 227.
47. Haisa, M., *Bull. Chem. Soc. Japan*, **35**, 765 (1962).
48. McLaren, A. D., and M. Ottsen, *Compte Rend. Trav. Lab. Carlsberg*, **27**, 325 (1950).

Résumé

On a étudié la sorption d'eau par le collagène insoluble provenant d'adultes et d'embryons, de même que celui de la gélatine de la peau de veau. On a obtenu une plus grande sorption avec les fractions de collagène qu'avec la gélatine, mais la technique ne peut distinguer les fractions elles-mêmes. On suggère une plus grande sorption par le collagène vis-à-vis de la gélatine, et les différences dans le mouillage apparent entre le collagène insoluble adulte et embryonnaire s'explique par la formation de rainures hélicoïdales avec l'âge. Les mesures de densité du fraction de collagène indiquent que le collagène devient plus cristallin avec l'âge. Ceci s'applique aussi au collagène insoluble. Ces résultats concordent avec le fait que les différences dans la solubilité du collagène sont dues aux différences dans l'ordre moléculaire. La densité du collagène insoluble et de gélatine passe par un maximum à 3–4% d'eau sorbée. Le phénomène s'explique sur la base de ponts d'eau d'un simple molécule d'eau doublement liée à l'hydrogène pour aligner fermement les chaînes. On présente une discussion dans ce sens. Un calcul utilisant la densité conduit à un volume moléculaire de 4.32×10^{-19} cm^3 dans le tropocollagène. Ceci est en accord avec les dimensions moléculaires, et avec l'idée que le collagène est formé à partir d'une aggregation de monomère.

Zusammenfassung

Die Wassersorption von natürlichem und unlöslichem Erwachsenen- und Embryonenkollagen sowie von Kalbshautgelatine wurde untersucht. Die Sorption der Kollagenfraktionen war grösser als diejenige von Gelatine, doch war die Methode nicht zur Unterscheidung der Fraktionen selbst geeignet. Man nimmet an, dass die grössere Sorption von Kollagen im Vergleich zu Gelatine sowie die verschiedene Benetzbarkeit von unlöslichem Erwachsenen- und Embryonenkollagen durch die Bildung helixförmiger Rinnen beim Altern erklärt werden kann. Wie aus Dichtemessungen an den Kollagenfraktionen hervorgeht, nimmt die Kristallinität des Kollagens beim Altern zu. Dies gilt auch für unlösliches Kollagen. Dies Befunde stimmen mit der Ansicht überein, dass die Löslichkeitsunterschiede der Kollagene auf Unterschiede in der molekularen Ordnung zurückgehen. Die Dichte von unlöslichem Kollagen und Gelatine durchläuft bei einem Gehalt von 3–4% sorbierten Wassers ein Maximum. Diese Erscheinung wird auf Grund von Wasserbrücken erklärt, die aus einem einzigen, durch zweifache Wasserstoffbrückenbindung an nahe aneinander gelagerte Ketten gebundenen Wassermolekül bestehen. Diese Annahme wird eingehend diskutiert. Aus der Dichte von Tropokollagen wurde ein Molekülvolumen von $4{,}32 \times 10^{-19}$ cm^3 berechnet. Dies stimmt mit den Molekülдimensionen überein und entspricht der Ansicht, dass Kollagen durch Zusammenlagerund des Monomeren gebildet wird.

Received September 18, 1963