88° (0.5 mm.), $n^{25}D$ 1.4637, d^{25}_4 1.0485; Σ *MR* 47.11, *MR* calcd. 47.14.

Anal. Calcd. for $C_{10}H_{13}NO_2$: C, 67.02; H, 7.31. Found: C, 66.99; H, 7.03.

α-Cyano-2-cyclopentene-1-acethydrazide (V).—A solution of 17.9 g. (0.1 mole) of ethyl α-cyano-2-cyclopentene-1-acetate and 3.3 g. (0.1 mole) of 95% hydrazine in 55 g. of ethanol was heated to reflux for one hour. On removal of the solvent there was obtained 10.5 g. of solid (64% yield), m.p. 90–91°.

Anal. Calcd. for $C_8H_{11}N_3O$: C, 58.16; H, 6.71; N, 25.44. Found: C, 58.10; H, 6.42; N, 25.60.

Curtius Reaction with α-Cyano-2-cyclopentene-1-acethydrazide.—A reaction mixture containing 5 g. (0.03 mole) of α-cyano-2-cyclopentene-1-acethydrazide, 20 ml. of water, 2.5 ml. of concentrated hydrochloric acid and 100 ml. of ether was cooled to 0° in an ice–salt-bath. To this cold mixture 2.1 g. (0.03 mole) of sodium nitrite in 20 ml. of water was added slowly while the reaction mixture was kept below 5°. After completion of addition, the ether phase was recovered and dried with sodium sulfate overnight.

After addition of 50 ml. of ethanol, the ether was removed by evaporation, and the alcoholic solution heated to reflux for two hours. The alcohol was then removed to yield a brown sirupy residue which was heated to reflux for two hours with a saturated aqueous solution of barium hydroxide containing 0.03 mole of base. The reaction mixture was filtered, and the excess barium ions were removed by careful precipitation with sulfuric acid. The clear filtrate was evaporated to a small volume and yielded 1.5 g. of solid material[8] which formed long white needles, recrystallized from water, m.p. 197–200° dec.

Anal. Calcd. for $C_8H_{12}N_2O_3$: C, 52.16; H, 6.57; N, 15.21. Found: C, 52.33; H, 6.24; N, 15.43.

A comparison of the X-ray diffraction pattern of this compound with a sample of 2-(2-cyclopentyl)-hydantoic acid (III), prepared from the amino acid using the potassium cyanate–glacial acetic acid procedure, proved to be identical.[9] Further, this compound was degraded, by heating with alkali under pressure, to a ninhydrin-active material which was identical with cyclopentene glycine as indicated by paper chromatography.

2-(2-Cyclopentyl)-hydantoic Acid (III).—To an ice-cold solution of 350 mg. (0.0025 mole) of 2-cyclopentene-1-glycine and 284 mg. (0.0035 mole) of potassium cyanate in 20 ml. of water, 210 mg. (0.0035 mole) of glacial acetic acid was added over a period of about 20 minutes. The resulting solution was warmed on a steam-bath for 45 minutes and then allowed to cool to room temperature. After neutralization to a congo red end-point, the solution was placed in a refrigerator overnight. A precipitate, 200 mg., was obtained and recrystallized from water to yield white needles, m.p. 197–198° dec.

Anal. Calcd. for $C_8H_{12}N_2O_3$: N, 15.21. Found: N, 15.20.

(8) The residue possessed a small amount of ninhydrin-active material which on paper chromatography corresponded to 2-cyclopentene-1-glycine; however, no crystalline material could be isolated.

(9) These data were furnished by Dr. S. H. Simonsen and Mr. J. L. Ogilvie of the Department of Chemistry.

AUSTIN, TEXAS

[CONTRIBUTION FROM THE RESEARCH DIVISION, ARMOUR & CO.]

The Degradation of Collagen. II. The Solubilization Process in the Acid *p*H Range

BY ARTHUR VEIS AND JEROME COHEN

RECEIVED OCTOBER 6, 1954

The partial dissolution of intact collagen has been followed as a function of *p*H and the duration of extraction at 60°. At this temperature collagen does not undergo heat shrinkage. A certain portion of the collagen dissolves readily at each *p*H and the amount of this primary solubilization at a given *p*H approaches a limiting upper value. After the primary solubilization has become extensive at *p*H 2.0 the breakdown of the collagen structure proceeds at a significantly greater rate. Dye binding data indicate an opening up of the structure of the insoluble residues but no increase in the number of basic groups on the protein (<1 mmole/100 g.). Carboxyl groups are exposed during the degradations, but only up to the amount expected to be available based upon the analysis for glutamic and aspartic acids. The dissolution process also brings about a change in the effective *pK* of some of the titratable groups. While there is thus little evidence for extensive peptide bond hydrolysis in the residues, the total nitrogen content of the insoluble residues decreases with increasing degradation whereas the soluble extracts, lower in nitrogen than the original collagen when the fraction solubilized is small, gradually increase in total nitrogen. These data support the view that intact collagen may be composed of a series of related proteins of not quite identical composition. The initial phases of the collagen–soluble collagen (gelatin) transition appear to take place with a very limited amount of peptide bond hydrolysis.

The collagen–gelatin transition and the accompanying or subsequent degradation of the soluble material has been studied most extensively through the examination of the thermal degradation of soluble commercial gelatins and extrapolation back to the "parent gelatin" molecule.[1,2] However, since these studies involved the use of a pretreated starting "undegraded" gelatin preparation their results cannot be readily carried over to a discussion of the initial collagen–gelatin transformation. As Kanagy[3] has pointed out, the usual prolonged liming pretreatment will result in the formation of gelatin without the application of heat. Acid-precursor gelatins, on the other hand, seem to resemble collagen in several respects and are quite different from the alkali-precursor gelatins.[4] In order to help bridge this gap in our understanding of the collagen–gelatin conversion we have examined the dissolution of an essentially untreated purified bovine hide collagen in the acid *p*H range.

(1) G. Scatchard, J. L. Oncley, J. W. Williams and A. Brown, THIS JOURNAL, **66**, 1980 (1944).

(2) J. Pouradier and A. M. Venet, *J. chim. phys.*, **49**, 238 (1952).

(3) J. R. Kanagy, "Chemistry of Collagen," National Bureau of Standards Circular, C458, 1947.

Experimental

The collagen containing corium of a fresh steer hide, mechanically split from the dermal and fleshy layers, was purified as described previously.[5] The repeated washings with organic acids used in this treatment essentially remove all of the low temperature acid-soluble collagenous protein, variously called "procollagen" or "extracted-skin collagen." The intact, low-temperature acid-insoluble collagen residue was used in all subsequent investigations.

Solubilization studies were carried out in a direct fashion. Weighed samples of collagen, in the form of very small fibrous cubes, were placed in contact with ten times their weight of water, or the equivalent volume of salt or buffer solution. The collagen was allowed to become thoroughly

(4) W. M. Ames, *J. Sci. Food Agriculture*, **3**, 454 (1952).

(5) A. Veis and J. Cohen, THIS JOURNAL, **76**, 2476 (1954).

wetted by the solution at room temperature. When the more acid extractions were made, *i.e.*, *p*H 2.5 and 2.0, hydrochloric acid was added to the slurry, in amounts determined from titration data, to give the collagen the appropriate *p*H. These slurrys were allowed to equilibrate overnight at 4°. The equilibrated slurry was deaerated, capped and heated at 60.0 ± 0.05° for periods ranging from one to four hours. At the end of the heating periods, the slurrys were immediately filtered through a coarse sintered glass filter and chilled. The insoluble residue was suspended in cold water and dialyzed against cold tap and then distilled water to remove all added salt. The filtrate was likewise dialyzed. After dialysis, the protein was recovered by lyophilization and weighed. Recoveries ranged between 95–100% of the original starting weight, the losses being due primarily to insoluble material which was trapped in the filter and not to losses from the soluble portion. There was always a detectable trace of soluble nitrogen in the dialysate water.

The extraction experiments were duplicated with collagens prepared at different times from three different hides. No discrepancies were noted in the final results.

Nitrogen analyses were made according to the recommendations of McKenzie and Wallace[6] with a mercury catalyst. The precision of the measurements was not as had been hoped owing particularly to the hygroscopic nature of the protein and the difficulty of sampling the insoluble residues. We are indebted to Mr. R. Hubata for his care in making these analyses.

The dye-precipitation experiments were carried out exactly as described earlier.[5]

Titration curves for the soluble filtrates and insoluble residues of the extractions were constructed essentially following the method described by Ames.[7] The soluble protein was directly titrated under an inert atmosphere; the insoluble protein suspensions, and blanks, were shaken for 18 hours to equilibrate. In every case the ionic strength was adjusted to 0.10 *N* with potassium chloride. The *p*H measurements were made at 25.0 ± 0.5°.

Results and Discussion

I. **The Rate of Solubilization.**—The solubilization data, expressed in terms of *s*, the fraction of the original starting weight recovered in the soluble fraction, are given in Fig. 1A. From *p*H 5.75, the isoelectric point of the untreated collagen (determined by titration), to *p*H 4.0 the collagen did not swell and there was no difference in the rate of solubilization. At *p*H 3.0 and lower swelling was evident and the solubilization tendency increased. This relation between swelling and ease of solubilization has been described by many others. Highberger[8] in particular found that at 25° about 3% of hide collagen was dissolved at *p*H 2 in 48 hours whereas less than 0.5% was dissolved at *p*H 6 (figures estimated from dissolved nitrogen data).

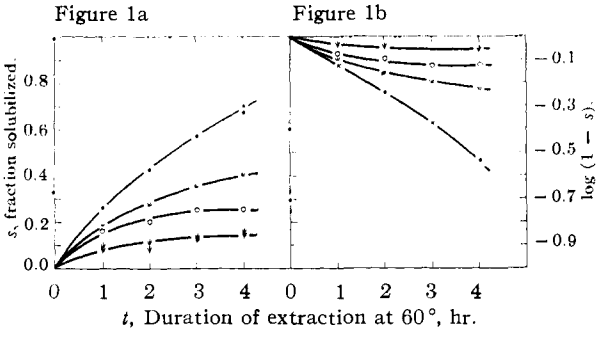

Fig. 1.—The solubilization of bovine hide collagen as a function of the extraction *p*H and the duration of extraction at 60°: ψ, *p*H 5.75, 5.0, 4.0; O, *p*H 3.0; ω, *p*H 2.5; κ, *p*H 2.0. (Fig. 1a, left; fig. 1b, right).

The point of special interest in the present study lies in the fact that each iso-*p*H curve except at *p*H 2.0 tends to approach a limiting upper value. This fact is demonstrated most clearly in the plot of log $(1 - s)$ *vs.* *t*, Fig. 1B. After about 2.5 hours at *p*H 2.0 there is an inflection point in the logarithmic plot of the solubilization curve and disintegration appears to proceed more rapidly. Such a change in the solubilization curve is not evident at any higher *p*H in the time interval examined. As shown earlier,[5] similar solubilization runs at *p*H 5.75 (zero added ionic strength) over a 16-hour period yielded only 20% of soluble material.

Nitrogen analyses, Fig. 2, of both residues and soluble material indicate some variation of the nitrogen content of each portion with the extent of extraction. The first extracts contain less nitrogen than the residues whereas more complete degradations give a mixture of soluble proteins which approach the original collagen in nitrogen content. Commercial acid-process gelatins, which represent almost total extracts of the original collagen, contain 17.5–18.5% N.[3,4,7] Other, less complete, collagen extracts have shown variations in nitrogen content from that of intact collagen, for example, published values range from 17.0% N[9] to 17.7% N[10] for bovine procollagens. In addition, slightly different amino acid composition figures have been noted for collagen, procollagen and other collagen extracts.[9] Harkness and his co-workers[11] have shown that low temperature citrate-soluble collagen has a higher content of hydroxyproline than insoluble collagen whereas the insoluble portion is higher in tyrosine.

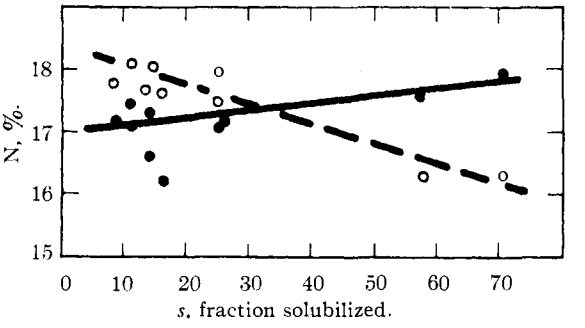

Fig. 2.—The nitrogen content of extracted collagens as a function of the fraction solubilized: O, insoluble residues; ●, soluble extracts.

One may tentatively conclude from the nitrogen analyses and the shape of the solubilization–time curves that, similar to the low temperature acid extraction of procollagen, high temperature extraction involves primarily the separation of segments of fairly uniform composition at a given value of *s* and the *p*H. That is, a reasonably well defined

(6) H. A. McKenzie and H. S. Wallace, *Australian J. Chem.*, **7**, 55 (1954).

(7) W. M. Ames, *J. Sci. Food Agriculture*, **3**, 579 (1952).

(8) J. H. Highberger, *J. Am. Leather Chem. Assn.*, **31**, 345 (1936).

(9) B. N. Orekhovitch, *Ind. Congr. Biochem. (Paris)*, **2**, 106 (1952).

(10) J. H. Bowes, R. G. Elliot and J. A. Moss, "Nature and Structure of Collagen," Academic Press, Inc., New York, N. Y., 1953, p. 199–207.

(11) R. D. Harkness, A. M. Marko, H. M. Muir and A. Neuberger, "Nature and Structure of Collagen," Academic Press, Inc., New York, N. Y., 1953, pp. 208–212.

distribution of molecular species rather than a random distribution will result from a given mild treatment. A secondary breakdown process which leads to the progressively more rapid disintegration of the insoluble structure is probably taking place at all *p*H's, but the process does not appear to become significant except at low *p*H and when the primary disruption of the collagen has become extensive.

II. Ionic Properties of the Insoluble Residues.—The insoluble collagen residue of each extraction was examined by the dye precipitation method.[5] The anion binding method is capable of yielding the number of free cationic groups on the protein with a precision of about 1 millimole of such groups per 100 g. of protein. Thus it should be possible to detect the presence of residues resulting from the appearance of soluble particles of about 100,000 molecular weight or smaller, the typical molecular weight range of standard commercial gelatins, if, when these fragments split off new terminal α-amino groups are created on the still insoluble structure.

The Orange-G binding data, Fig. 3, exactly duplicated earlier data[5] except that in every case where the extraction *p*H was lower than the isoelectric point of the untreated collagen the functional groups were rendered maximally accessible to the dye within one hour whereas over four hours at 60° were required to effect the maximum reorientation of isoelectric collagen. There was no detectable increase in maximum Orange-G binding at *p*H 2.2. Even the most drastic dissolution conditions, where 70% of the original solid material was removed, did not result in the appearance of any significant number of new terminal amino groups. One may argue from this that the residues of each extraction represent nearly intact collagen structures chemically and do not contain any large numbers of hydrolyzed or fractional polypeptide chains held in the structure by non-peptide bonds. There is, however, a physical change related to the increased availability of side chain functional groups.

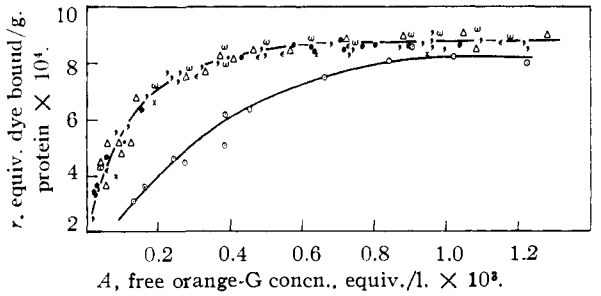

Fig. 3.—The binding of orange-G by extracted collagen residues in citrate buffer at *p*H 2.2, 25°. The solid line through the points labeled "original" is that determined earlier.[5] The points that curve are those determined for unheated, acid-swollen collagen: ⊙, original; ε, *p*H 2, 1 hr.; X, *p*H 2. 2 hr.; Δ, *p*H 3, 1 hr.; ω, *p*H 4, 1 hr.; ●, *p*H 5, 1 hr.

Bowes and Moss[12] recently have reported some more sensitive α-amino end group determinations on collagen and modified collagens by the fluorodinitrobenzene method. No α-amino groups are detectable in untreated collagen. The α-amino groups of aspartic acid and glutamic acid are found in heat-shrunk collagen that has been in water at 65–88° for 15 minutes, a treatment nearly analogous to the isoelectric extraction at 60° for 1 hour (at 60° collagen does not undergo heat-shrinkage). Only 0.15 mmole of aspartic acid and 0.03 mmole of glutamic acid are found per 100 g. of collagen, amounts well below the sensitivity of our experiments. These quantities correspond to molecular weights on the order of 1 to 3 × 10^6 for the soluble fragments which are obtained by this treatment. However, these new terminal amino groups may only be the consequence of the reorientation in configuration which occurs rather than the products of peptide hydrolysis.

Treatment with acid alone, though it swells the collagen structure, does not cause the above noted molecular reorientation. The Orange-G binding curve of collagen swollen with HCl at *p*H 2.0 and held for 1 hour at room temperature or for 48 hours at 4° is identical with the binding curve of the untreated native collagen (Fig. 3, points on curve labeled original). It would be interesting to examine the reaction of unheated acid-swollen collagen with fluorodinitrobenzene.

Cation binding data offer much the same information as the anion binding study. A reorientation of the carboxyl groups occurs on the extraction residues and renders more of the carboxyl groups available to the dye. As illustrated in Fig. 4, 38 mmoles of carboxyl groups per 100 g. of collagen are originally exposed to the dye. After extraction, whether at *p*H 5.75 to $s = 0.10$ or at *p*H 2.0 to $s = 0.45$, about 40 mmoles of additional anionic groups are exposed per 100 g. of collagen. Since 100 g. of intact collagen contains 124 mmoles of carboxyl groups including 47 which are tied up as acid-stable terminal amide linkages,[13] the ~78 mmoles of carboxyl groups found after extraction is thus approximately equivalent to the theoretical number of such groups which originally should have been available to the dye. These observations provide no evidence for extensive hydrolysis. The r/A *vs.* r curve is shifted to still higher values of r after extraction at

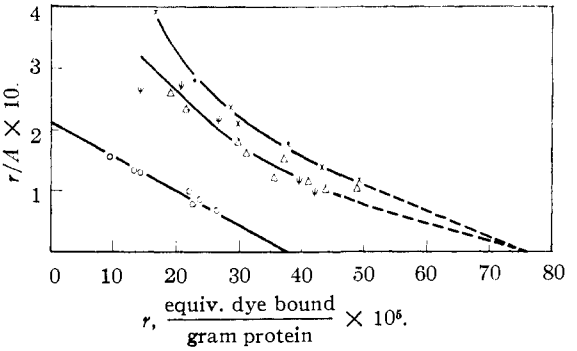

Fig. 4.—The binding of Safranin-O by extracted collagen residues in phosphate buffer at *p*H 11.5, 25°: O, original collagen; X, *p*H 2, 4 hr. extracted, 60°; Δ, *p*H 6, 2 hr. extracted, 60°; ψ, *p*H 2, 2 hr. extracted, 60°.

(12) J. H. Bowes and J. A. Moss, *Biochem. J.*, **55**, 735 (1953).

(13) J. H. Bowes and R. H. Kenton, *ibid.*, **43**, 358 (1948).

*p*H 2.0 to $S = 0.70$. The "secondary" solubilization is significantly large in this case and the data may indicate that hydrolysis is occurring, but it must be pointed out that there was no equivalent increase in anion binding.

Titrations were made in order to get another estimate of the functional group availability. The central portions of the titration curves of two extracts and their insoluble residues are shown in Fig. 5. The titration curve of the *p*H 2.0 extract ($s = 0.70$) (C, Fig. 5) exactly duplicates that of the acid-prepared gelatin described by Ames,[7] whereas the *p*H 5.0 extract ($s = 0.14$) titration data (D, Fig. 5) are intermediate between that of the acid-prepared gelatin and an exhaustively alkaline prepared gelatin. The lower nitrogen and increased free carboxyl content (Table I) of the *p*H 5.0 extract may thus be partially attributed to the loss of amide nitrogen.[7,14] However, the extraction conditions are not favorable to terminal amide hydrolysis. The difference in N content is also too large to be accounted for entirely in this way. Further, a comparison of the titration data on the residues of the *p*H 2.0 and 5.0 extractions (A and B, respectively, Fig. 5), where the same amide nitrogen relationship would logically be expected, yields opposite results. The *p*H 5.0 residue has both a large number of titratable carboxyl groups and a higher nitrogen content than the *p*H 2.0 residue. In the binding study the *p*H 2.0 residue showed a slightly higher affinity and capacity for the cationic dye, but both residues had the same capacity and affinity for the anionic dye.

TABLE I

ANALYSIS OF TITRATION DATA

Protein and origin	Carboxyl groups, mmoles/100 g., *p*H 1.5–6.0[a]		Apparent α-amino + imidazole, mmoles/100 g. *p*H 6.0–8.5[a]		N, %
		(Theoret.)		(Theoret. imidazole)	
*p*H 2.0, extract, $S = 0.70$	94	124	9	5.0	17.9
*p*H 5.0, extract, $S = 0.14$	100		20		16.9
*p*H 2.0, residue	92		29		17.6
*p*H 5.0, residue	100		23		18.0

[a] The titration ranges are those suggested by Cannan.[15]

Cassel, McKenna and Glime[16] have shown that commercial gelatin, degraded hide powder and purified hide collagen all have the same quantitative content of the polar amino acids: arginine, histidine, lysine, glutamic acid and aspartic acid. These are the groups primarily responsible for the dye-binding and titration behavior. As the conditions of the experiments described here did not favor terminal amide hydrolysis, and as the dye-binding experiments did not demonstrate any substantial increase in the number of titratable carboxyl groups for any of the residues, this reaction must be relatively insignificant. There also appears to be no detectable increase in the number of free α-amino groups on any of the residues, certainly not to the extent that the titration curve would be significantly altered. Evidently, the shape of the titration curves in the central *p*H 4–9 range depends upon the spatial arrangement of the polar groups and consequently on their interaction and effective pK values. Thus, the differences between curves A and B of Fig. 5 and between those of the soluble and insoluble portions from a given extraction probably reflect the opening up of the collagen structure indicated by the dye binding studies. This structural reorientation may be the first step in the release of unhydrolyzed collagen or gelatin to the solution phase.

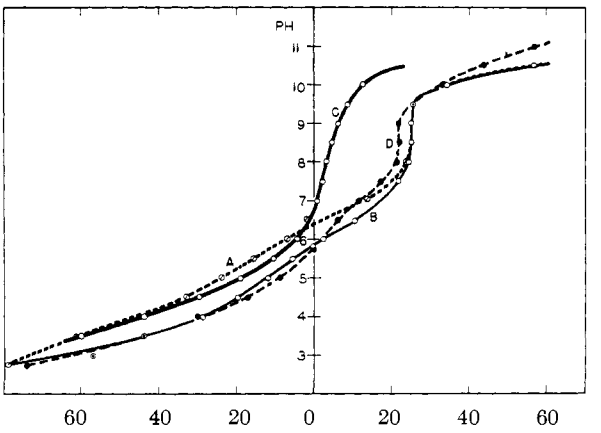

Fig. 5.—Acid-base titration curves for collagen residues and their soluble extracts: curve A, residue from *p*H 2.0, 60°, 4 hr. extraction; curve C, the soluble extract from A; curve B, residue from *p*H 5.0, 60°, 2 hr. extraction; curve D, the soluble extract from B.

Summary and Conclusions

1. Acid dissolution data suggest that collagen fibers break apart into more or less discrete units depending upon the *p*H of extraction. Fewer than 1.0 mmole of new free terminal amino groups per 100 g. of residue protein are created by the dissolution process. Thus, the soluble fragments must initially, on the average, have a molecular weight above 100,000 if the dissolution process involves hydrolysis of peptide bonds.

2. Nitrogen analyses of both the soluble and insoluble protein moieties indicate that the soluble material obtained under the least drastic conditions has a different amino acid content than the residue, and is characterized by a lower total nitrogen content. Since the nitrogen content of both residues and soluble fragments changes regularly with extent of solubilization these data support the view[9] that intact collagen may be composed of a series of related proteins of not quite identical composition.

3. Some of the nitrogen lost during acid extraction may be due to hydrolysis of the asparagine and glutamine terminal amide residues though this explanation is unlikely. Carboxyl groups would be exposed by this process.

4. Along with the slight chemical changes, the heat treatment, quantitatively independent of *p*H, causes a reorientation in the configuration of the

(14) J. H. Bowes and R. H. Kenton, *Biochem. J.*, **43**, 365 (1948).
(15) R. K. Cannan, *Chem. Revs.*, **30**, 395 (1942).
(16) J. Cassel, E. McKenna and A. Glime, *J. Am. Leather Chem. Assn.*, **48**, 277 (1953).

functional groups on the fibrous structure. Such a change manifests itself in the increased affinity of the protein for dye ions. Further, the effective pK of some of the titratable groups is altered during the heating process. The structural reorientation of the fibrous network may be the first step in the solubilization of collagen in acid media.

CHICAGO, ILLINOIS

[CONTRIBUTION FROM THE RESEARCH DIVISION, ARMOUR AND COMPANY]

The Degradation of Collagen. III. Characterization of Soluble Products of Mild Acid Degradation

BY ARTHUR VEIS, DELBERT N. EGGENBERGER AND JEROME COHEN

RECEIVED OCTOBER 6, 1954

The soluble protein obtained during the mild thermal and acid dissolution of purified bovine hide collagen which has had no previous pretreatment does not behave as a typical gelatin but is more closely related to native collagen and "procollagen." The unfractionated soluble collagen is exceedingly polydisperse and consists of molecules ranging in molecular weight from 20×10^6 to less than 1×10^6. The largest fragments are highly asymmetric and have radii of gyration on the order of 6000 Å., deduced from light scattering and viscosity measurements at 40°. The soluble material obtained under a variety of conditions has been fractionated and the molecular parameters of each fraction have been determined. These parameters have been related to the initial phases of the collagen–gelatin transformation process. The stability of collagen solutions at 40° is discussed

The mild thermal and acid degradation of previously untreated bovine hide collagen under conditions where only a limited portion of the protein is dissolved yields soluble fragments which do not behave as typical commercial gelatins. In the previous paper of this series[1] it was argued, from a consideration of the properties of the insoluble residues of each extraction, that these soluble fragments might be related more closely to collagen than to gelatin. The work discussed here was undertaken as a test of the validity of the above argument. Particular emphasis is placed upon the question of the existence of thermally solubilized collagen as opposed to the necessary immediate appearance of a "parent" gelatin of molecular weight near 100,000 to 200,000.[2]

The literature on collagen is filled with conflicting terminology relating to "native" collagen and its various forms and degradation products, ranging from Nageottes' early Collagen A and B through Scatchard's "parent gelatin," Orekhovitch's procollagen, the English School's "extracted skin" collagen, on to the new "parent gelatin" described by Gallop and Bear. This profusion of names, each with unfortunate semantic overtones, makes absolutely necessary some clarification of the particular language used in the preceding paragraph, and in those that follow.

The confusion and diversity of results reported is akin to a separate experiment, in itself testifying to the fact that collagens originating in different tissues are not identical in physical fine structure, degree of cross linking, or even in amino acid content. Indeed, collagenous proteins originating in the same tissue, *e.g.*, insoluble skin collagen and "extracted skin" collagen, are not identical on several counts, both physical and chemical. In this discussion we are concerned with the degradation of mature, insoluble and intact skin collagen only into water soluble or water dispersible fragments. Because of our previous conclusions[1] that extensive hydrolysis is not a factor in the mild acid extraction of this soluble protein and because of the experiments to be described below we have chosen to term these extracts "soluble collagen" with the implication that much of the chemical and physical structure characteristic of the three-dimensional network which comprises mature, intact skin collagen may be retained in at least part of the soluble protein. The term "acid-precursor gelatin" is not applicable, though of names now in common usage it is the closest, because it implies a longer history of pretreatment for the insoluble collagen than we have used. The term "parent gelatin" is discussed in the sense in which it was introduced by Scatchard and his co-workers[2] and not in the sense now used by Gallop and Bear. The relationships between "soluble collagen," "extracted skin collagen," Gallop's "parent gelatin" and the usual gelatins are to be the subjects for further research.

Experimental

Materials.—Bovine hide collagen was prepared as described previously[3] by a method similar to that of Highberger.[4] All of the low temperature acid-extractable collagen (procollagen) is removed by this treatment. The soluble collagens were obtained by thermal extraction of native collagen at 0.1 ionic strength and 60° for varying lengths of time at several pH's.[1] Each soluble extract was filtered and then dialyzed against cold tap water to remove the buffer salts and finally against cold distilled water until salt free. The collagen was recovered by lyophilization.

The gelatin used for occasional comparison studies was the Pharmaceutical Grade Gelatin of the Grayslake Gelatin Company, an acid pretreated pig skin gelatin.

Five times recrystallized bovine serum albumin, very generously supplied by Dr. K. C. Robbins of the Armour Laboratories Division Research Department, was used in light-scattering calibration check determinations.

Light Scattering.—The light-scattering photometer has been described previously.[5] Scattering measurements were taken at angles of 30 to 135° from the incident beam in 15° intervals. The cell temperature was constant at 40° for all

(1) A. Veis and J. Cohen, THIS JOURNAL, **77**, 2364 (1955).

(2) G. Scatchard, J. L. Oncley, J. W. Williams and A. Brown, *ibid.*, **66**, 1980 (1944).

(3) A. Veis and J. Cohen, *ibid.*, **76**, 2476 (1954).

(4) J. H. Highberger, *J. Am. Leather Chem. Assoc.*, **31**, 345 (1936).

(5) A. Veis and D. N. Eggenberger, THIS JOURNAL, **76**, 1560 (1954).