polarograph directly on the suspension. Apart from its use for amino-acids, the method should be applicable to peptides, provided authentic specimens of these exist for comparison. For the amino-acids, the value of the method lies principally in the small amount of material required for analysis, though at present the whole range of amino-acids have not been dealt with simultaneously. For certain amino-acids the microbiological methods, while using comparable amounts of material, are more accurate. For peptides it is improbable that it will be worth while developing the microbiological methods.

The results obtained in the analysis of gramicidin *S*, though intended primarily as a test of the method of analysis, may be considered to confirm the formula proposed by Syngé (1945). The material consumed in the analysis here (0.96 mg.) is about 10% of that which Syngé employed, using partition chromatography of acetamido-acids on silica columns.

SUMMARY

1. Various methods have been investigated in an attempt to render quantitative amino-acid analysis by partition chromatography on paper.

2. A polarographic modification of Kober's method for the estimation of amino-acids as their copper complexes is described. A few micrograms only are required.

3. Estimations of several amino-acids in a number of simple mixtures are presented. An analysis of gramicidin *S* using 1 mg. only, confirms the formula proposed by Syngé.

Thanks are due to the Council of the Wool Industries Research Association for permission to publish and to the Director of Research, Mr B. H. Wilsdon, for his interest and encouragement.

REFERENCES

- Consden, R., Gordon, A. H. & Martin, A. J. P. (1944). *Biochem. J.* **38**, 224.
Consden, R., Gordon, A. H. & Martin, A. J. P. (1947). *Biochem. J.* **41**, 590.
Folin, O. (1922). *J. biol. Chem.* **51**, 377, 393.
Frame, E. G., Russell, J. A. & Wilhelmi, A. E. (1943). *J. biol. Chem.* **149**, 255.
Harris, L. J. (1935). *Biochem. J.* **29**, 2820.
Hawes, R. C. & Stavinski, E. R. (1942). *Industr. Engng Chem. (Anal. Ed.)*, **14**, 917.
Kober, P. A. (1912). *Amer. chem. J.* **48**, 383.
Kolthoff, I. M. & Lingane, J. J. (1941). *Polarography*. New York: Interscience Publishers.
Nadeau, G. F. & Branchen, L. E. (1935). *J. Amer. chem. Soc.* **57**, 1363.
Pope, C. G. & Stevens, M. F. (1939). *Biochem. J.* **33**, 1070.
Syngé, R. L. M. (1945). *Biochem. J.* **39**, 363.

The Amino-acid Composition and Titration Curve of Collagen

BY JOANE H. BOWES AND R. H. KENTEN

The British Leather Manufacturers' Research Association, London, S.E. 1

(Received 11 February 1948)

The amino-acid composition of gelatin has been extensively studied, but until recently little attention has been paid to that of its precursor collagen, the natural protein of skin. Some of the analyses of collagen which have been reported were carried out by methods which are now considered unsatisfactory, and in no case have most of the major component amino-acids been determined in one sample of the protein. Further, most of the analyses have been made on collagens which had been given an alkaline or enzyme treatment, or both, to remove the keratins, elastic fibres and reticular tissue which occur in close association with it, and there is a risk

that such treatments will cause some modification of the collagen.

For the present study, a sample of collagen has been prepared with the minimum of chemical treatment, since it was considered that the presence of small amounts of elastic fibres and reticular tissue would lead to less error than the treatments necessary to bring about their removal. Also, it seems doubtful whether it is possible to remove these proteins completely by any of the methods which have been suggested. On this preparation Chibnall and his collaborators (Chibnall, 1946; Macpherson, 1946; Rees, 1946; Tristram, 1946) have determined

the basic and hydroxyamino acids, and the following neutral amino-acids: alanine, leucine, isoleucine, valine, phenylalanine and proline; the present authors have determined total N, amino N, amide N, glutamic and aspartic acids, and methionine.

The acid and basic amino-acid contents may also be deduced from the titration curve of a protein. This procedure has an advantage over analysis for the individual amino-acids in that it gives information regarding the reactive groups, including terminal groups, in the intact protein, and comparison of the results with those obtained by direct analysis can give information regarding the way in which the amino-acids are linked in the protein molecule (Chibnall, 1942; Cannan, Kibrick & Palmer, 1941, 1942).

It must be observed, however, that the titration curve of a fibrous protein only gives an exact indication of the titratable groups when it is determined in the presence of salts (Speakman & Hirst, 1933; Jordan Lloyd & Bidder, 1934; Steinhardt & Harris, 1940). This effect of salt is due in the first place to its influence on the Donnan equilibrium. The effective pH value for the equilibrium

is the pH value in the protein phase; in the absence of salts this pH value is higher than that in the external solution, except at very low pH values, and so the binding of hydrogen ions is less than that corresponding to the external pH value. It has also been suggested (Gilbert & Rideal, 1944) that stoichiometric binding of hydrogen ions by fibrous proteins only occurs when some anions are also bound in order to lessen the large potential which would otherwise exist between fibre and solution. The addition of salts, by increasing the concentration of anions, will facilitate this binding, and thus favour stoichiometric binding of hydrogen ions (see also Steinhardt & Harris, 1940). Similar arguments apply to titration with alkali. The concentration of salt necessary for stoichiometric binding depends on the protein; with collagen it has been shown to be 0.1 M or greater (Retterova, private communication).

In the present investigation the titration curve has been determined in the absence of salts, and also in the presence of 0.5 M-sodium chloride.

METHODS

Preparation of collagen. The back area of a freshly flayed ox hide was cut into pieces (c. 18 × 12 in.), which were washed in a revolving glass drum, first with water and then with 10% NaCl for 30 min. The pieces were left stationary overnight in a fresh NaCl solution, drummed for 30 min. in this solution, washed in several changes of distilled water, and dehydrated with acetone. The grain layer (containing the hair roots, and the greater part of the muscle and elastic fibres) and a thin layer from the flesh side were split off,

and the remaining material cut into 1 cm. cubes. The cubes were degreased with three changes of light petroleum (b.p. 40–60°) at room temperature for 6 days, washed with successive changes of distilled water, and dehydrated with acetone. The purified collagen contained ash 0.03, moisture 23.0 and grease <0.1%.

Analyses. Total N was determined by the method of Chibnall, Rees & Williams (1943), and amide N by the method of Bailey (1937) and Lugg (1938). The amino N was determined by the Van Slyke manometric procedure, using an auxiliary reaction vessel as described by Doherty & Ogg (1943). To ensure thorough wetting of the sample, the finely shaved collagen (0.15–0.20 g.) was placed in the extraction vessel with 2 ml. of water, the vessel evacuated while shaking, and left under vacuum overnight. The vessel was re-evacuated immediately before the determination.

Dicarboxylic acids were determined by the method of Consden, Gordon & Martin (1948), and methionine by the method of Baernstein (1932).

Titration curves. Collagen (0.75 g.) was placed in 75 ml. of solutions containing measured volumes of HCl and NaOH in resistance glass vessels at 20°. For the titration curve in the presence of salt, sufficient NaCl was added to give a final Cl⁻ concentration of 0.5 M in the acid solutions, and a final Na⁺ concentration of 0.5 M in the alkaline solutions. After 3 days, in which time equilibrium was known to have been reached, samples were withdrawn for pH determinations, and for titration with acid or alkali using bromocresol purple as indicator. When the final solution was too dilute for titration (between pH 3 and 11) the concentration of acid or alkali was obtained from the pH by the use of blank curves for water and for 0.5 M-NaCl; this avoided the necessity of choosing suitable activity coefficients for the two systems. In the more concentrated solutions, the amount of acid or base bound by the collagen is small compared with that remaining in solution, and larger samples of collagen (2 g. in 100 ml.) were used.

pH values were determined at 20° using a glass electrode assembly mounted in a thermostat (Coates, 1945). pH values above 9.0 were determined with a glass electrode of low sodium error (Dole, 1941).

In determining the amounts of alkali bound, precautions were taken to avoid contamination with CO₂. The collagen was placed in the vessel shown in Fig. 1a, and the air displaced with CO₂-free air passed in by the tube B. The solution of alkali was then added, the tube A closed with a small rubber bung, and B by a rubber cap. Samples for titration were taken by inserting a pipette at A, carrying a similar rubber bung, and forcing the solution into this pipette by passing CO₂-free air into the vessel at B. The titration was carried out in a vessel through which a stream of CO₂-free air was continuously blown. In the determination of pH, the solution was forced up into the electrode vessel in a similar way (see Fig. 1b), or, in the case of the electrode system mounted in the thermostat, a sample was transferred by means of a pipette.

The soluble N in some of the solutions below pH 2.0 and above pH 12 was determined by micro-Kjeldahl (Markham, 1942) to give an indication of the extent of decomposition, but no attempt was made to apply a correction, owing to lack of information concerning the origin of this N.

Estimate of errors involved in the determination. Errors may arise from the following causes: (1) Difference in the pH value between the solution inside the collagen and the

external solution. (2) Introduction of water with the collagen, thus increasing the total volume of solution. (3) Binding of water by the collagen, thus decreasing the amount of water available for solution of the acid or alkali. (4) Decomposition of the collagen, or liberation of groups not originally present in the native collagen.

Table 1. *Soluble nitrogen derived from collagen after 3 days' contact with acid or alkali*

In the absence of salt		In the presence of 0.5 M-NaCl	
pH	mg. N/g. collagen	pH	mg. N/g. collagen
1.58	0.14	1.64	0.95
1.68	0.12	1.73	0.85
1.80	0.15	1.85	0.77
—	—	2.00	0.69
—	—	2.10	0.70
12.38	1.54	12.46	1.04
12.59	1.72	12.59	1.28
—	—	12.66	1.21
12.78	1.95	12.71	1.08
12.92	2.22	12.86	1.44
13.16	3.86	13.12	3.08

0.03 mmol./g., and at pH 2.0, 0.003 mmol./g. The fact that the curves show well-defined maxima in the acid and alkaline ranges confirms that the net error must be small.

The soluble N present in the solutions (see Table 1) indicates that decomposition of the collagen was small even in the most acid and alkaline solutions. The chief source of this N is probably NH_3 from hydrolysis of amide groups. Under the conditions of the experiment the greater part of this NH_3 will be titrated with acid, thus compensating for the alkali bound by the freed carboxyl groups, and hence such decomposition will not affect the calculation of the amount of alkali bound by the original collagen.

RESULTS

Amino-acid composition

The collagen, prepared as described, may be considered to be a reasonably pure sample. Of the other proteins and tissues present in the fresh skin, albumins and globulins will be removed by treatment in sodium chloride solution (Kritzinger, 1948), and the greater part of the keratins, elastic fibres,

Fig. 1. Apparatus used for the determination of titration curves.

The first of these errors will be appreciable in the absence of salt, and may in part be the cause of the difference in the shape of the curves obtained with and without salt. In the presence of 0.5 M-NaCl it is negligible (Retterova, private communication). Since the second source of error partially compensates for the third, no correction was made for it. As the volume of solution is large compared with the amount of collagen, the net error must always be small. Assuming the collagen binds 50% of water (Cheshire & Holmes, 1942), the error at pH 1 would be of the order of

muscle fibres, sweat glands, fat cells, etc. will be removed with the grain layer (Dempsey, 1946). The remaining impurities are, therefore, a small amount of reticular tissue and some elastic fibres, the presence of which should not greatly affect the results obtained.

The total N of the present collagen preparation is rather higher than the value generally quoted for skin collagen, but is the same as that obtained by

Table 2. *Analyses of collagen*

	N as % protein N	g./100 g. collagen	mmol./g. collagen
Total N	—	18.6	—
Amide N	3.5	0.66	0.47
Amino N	2.5	0.46	0.33
Methionine	0.4	0.8	0.05
Aspartic acid	3.6	6.3	0.47
Glutamic acid	5.8	11.3	0.77

Bergmann & Stein (1939) for ox-hide tendon prepared in a similar manner. Earlier values for skin collagen have been determined on material which has had some treatment (alkaline or enzymic) likely to lead to loss of nitrogen from amide or guanidino

The values for the dicarboxylic acids are considerably higher than those obtained by Dakin (1920) for gelatin, and Schneider (1940) for collagen, and are of the same order as values recently obtained for gelatin by microbiological methods (Hac & Snell, 1945; Stokes & Gunness, 1945; Lewes & Olcott, 1945; Hac, Snell & Williams, 1945; Hier, Graham, Friedes & Klein, 1945). Gale (1945), using a specific decarboxylase, found an average value of 10% glutamic acid for this and other similar samples of collagen prepared by the present authors. This value is likely to be low owing to racemization of the glutamic acid during hydrolysis (Gale, 1945).

The methionine content of the present collagen is lower than that quoted by Baernstein (1932) for

Fig. 2. Titration curves of collagen with and without sodium chloride.

groups. The amide N is considerably higher than values quoted for gelatin (Dakin, 1920; Chibnall, 1942), presumably because gelatin is usually made under conditions of alkali treatment which favour the breakdown of the amide groups (Highberger & Stecker, 1941; Ames, 1944). The amino N is of the same order as values obtained by Rutherford, Harris & Smith (1937) and Kanagy & Harris (1935) for hide powder.

gelatin, but is in good agreement with recent microbiological determination on gelatin: Horn, Jones & Blum (1946), 0.78%; Lyman, Moseky, Butler, Wood & Hale (1946), 0.88 and 0.83%.

Titration curves

As in previous titration curves of collagen and other fibrous proteins, the curve in the absence of salt shows a broad isoelectric range in which no

combination with acid or alkali takes place, and the acid and alkaline portions of the curve are shifted to lower and higher pH values respectively (Fig. 2). In the presence of sodium chloride, however, there is a definite isoelectric point at pH 6.6–6.8, and the curve approaches that of a soluble protein. The curve shows clearly defined acid and base-binding maxima of 0.90 and 0.36 mmol./g. of collagen at pH 1.5 and 12.5 respectively.

In contrast to the findings of Theis & Jacoby (1941*c*; 1942*b*), who used collagen which had been given a preliminary alkaline treatment, the acid-binding maximum is greater in the presence of salt than in its absence, and there is little indication that the two curves would meet if extended to lower pH values. There is also an indication that maximum base binding is less in the absence of salt.

Previous titration curves of collagen, all of which were determined on collagen which had received either an alkaline or enzymic treatment, show isoelectric points between 5.4 and 7.9 (Theis & Jacoby, 1940, 1941*a, b, c*; 1942*a, b*; 1943*a, b*; Atkin, 1937), and in those curves where definite maxima can be distinguished (Theis & Jacoby, 1941*c*, 1942*a*, 1943*a, b*), an acid-binding capacity of 0.85–0.90 mmol./g., and a base-binding capacity of 0.38–0.45 mmol./g. The curves of alkali-treated collagen all show an appreciable base-binding capacity in the pH range 6–8, which is absent in the present curve; this is also characteristic of titration curves of gelatin, and may be attributed to increase in the number of carboxyl groups resulting from hydrolysis of amide groups. The present curve can be divided into three portions, pH 1.5–4.9, 4.9–9.6, and 9.6–12.5, and from consideration of the probable pK's of the groups involved, may be interpreted as indicated in Table 3.

It is not possible to distinguish separate sections of the curve due to titration of the imidazole and α -amino groups, as these are present in small amounts and do not differ greatly in pK (imidazole, pK 5.5–6.5; α -amino, pK 7.5–8.5 (Cohn & Edsall, 1943)). Since collagen contains a high proportion of proline and hydroxyproline, it is possible that there are some terminal imino as well as α -amino groups, and these will titrate in a similar pH range (imino of proline, pK 9.7; imino of hydroxyproline, pK 10.1 (Cohn & Edsall, 1943)). It was also not possible to assign any portion of the curve to titration of phenolic hydroxyl groups, and in view of the small amount of tyrosine in collagen and the doubt concerning the extent to which it titrates (Fruton & Lavin, 1939; Steinhardt, 1939; Neuberger, 1943) it has been omitted from the analysis of the curve.

There is no indication that the guanidino group is titrating in the pH range covered, and the pK of this group in collagen must be greater than 14. A similar observation has been made by Lichtenstein (1940) in connexion with the titration curve of gelatin.

DISCUSSION

As far as is known to the present authors, the figures for total N, amide N and amino N are the first to be reported on skin collagen which has received no alkaline or enzymic treatment, and it is not surprising, therefore, that the values for the first two are higher than those generally quoted for collagen.

The free amino N as determined by the Van Slyke method and the number of ϵ - and α -amino groups deduced from the titration curve (see Table 3) are in close agreement, but appreciably less than the value indicated by determination of the lysine and hydroxylysine in the basic amino-acid fraction of

Table 3. *Analysis of titration curve*

Groups titrating	Method of calculation	pK from curve	Amount present (mmol./g.)	
			From curve	From analysis
(a) Total basic groups	Titration from 1.5 to isoelectric point	—	0.90	0.94
(b) Imidazole	Titration from 4.9 to 9.6	7.5	0.07	0.05
Imino				—
α -Amino	Titration from 9.6 to 12.5	11.0	0.34	0.39
(c) ϵ -Amino*				—
(d) Free carboxyl	Titration from 1.5 to 4.9	3.5	0.87	0.79
(e) α -Amino + imino	(b) – 0.05 mmol. imidazole groups	—	0.02	—
(f) Guanidino	(a) – (b) – (c)	> 14	0.49	0.51
(g) Amide	From analysis	—	—	0.47
(h) Dicarboxylic acids	(d) + (g) – (e)†	—	1.32	1.26

* Including the side-chain NH_2 group of hydroxylysine. The pK of the amino group not α to the carboxyl group in hydroxylysine is 9.50 as compared with 10.3 for the ϵ groups of lysine under the same conditions (Van Slyke, Hiller, MacFadyen, Hastings & Klemperer, 1940), and it may be assumed to titrate in approximately the same range as the ϵ -amino groups of lysine.

† It is assumed that terminal carboxyl groups are equivalent to α -amino + imino groups.

the hydrolysate (Macpherson, 1946). It is possible that in the intact protein some of the ϵ -amino groups are bound into the polypeptide chain or for some reason not available for reaction, or alternatively the analytical figure may be too high. The lysine N was determined by difference (total N of basic fraction minus arginine, histidine and hydroxy-lysine N), and hence is subject to a cumulative error.

oxylic acids are bound, or that there is any appreciable number of terminal amino or carboxyl groups.

The analytical results obtained in this investigation, together with those previously obtained (Chibnall, 1946; Macpherson, 1946; Rees, 1946; Tristram, 1946) on the same sample of collagen, are given in Table 4.

Table 4. *Composition of collagen*

Amino-acid	(1) N as % protein N	(2) As g./100 g.	(3) As g. residues/ 100 g.	(4) As mmol./g.	(5) Minimum mol. wt. calc. from data in (1)	(6) Assumed number of residues	(7) Apparent minimum mol. wt. calc. from (5) and (6)
Total N	—	18.6 a	—	—	—	—	—
Amino N	2.5 a	0.46	—	0.33	—	—	—
Glycine	26.3 c	26.2	19.9	3.50	286	136	38,880
Alanine	8.0 b	9.5	7.6	1.06	941	41	38,580
Leucine } Isoleucine }	3.2 b	5.6	4.8	0.42	2,350	17	39,950
Valine	2.2 b	3.4	2.9	0.29	3,420	11	37,620
Phenylalanine	1.9 b	4.2	3.7	0.25	3,960	10	39,600
Tyrosine	0.6 b	1.4	1.3	0.08	12,540	3	37,620
Tryptophan	0.0 d	0.0	0.0	0.00	—	—	—
Serine	2.5 b	3.4	2.7	0.33	3,010	13	39,130
Threonine	1.5 b	2.4	2.0	0.20	5,020	8	40,160
Cystine	0.0 e	0.0	0.0	0.00	—	—	—
Methionine	0.4 a	0.8	0.7	0.05	18,820	2	37,640
Proline	9.9 b	15.1	12.7	1.32	760	51	38,760
Hydroxyproline	8.0 c	14.0	12.1	1.07	941	41	38,580
Lysine	4.7 b	4.5	4.0	0.31	3,200	12	38,400
Hydroxylysine	1.2 b	1.3	1.1	0.08	12,560	3	37,680
Arginine	15.3 b	8.8	7.9	0.51	1,969	20	39,380
Histidine	1.2 b	0.8	0.7	0.05	18,820	2	37,640
Aspartic acid	3.6 a	6.3	5.5	0.47	2,092	19	39,750
Glutamic acid	5.8 a	11.3	10.0	0.77	1,297	30	38,910
Amide N	3.5 a	0.66	—	0.47	2,157	18	38,740
Total found	99.8	119.00*	99.6*	10.76*	—	419	38,730 (mean)

* Excluding amide N.

(a) Determined by present authors.

(b) Determined by Chibnall and collaborators on the same sample of collagen.

(c) Bergmann & Stein (1939).

(d) Block & Bolling (1945).

(e) Baernstein (1930).

The values obtained by the present authors for the dicarboxylic acids are 50% higher than the earlier values of Dakin (1920), and, although probably still an underestimate, are the best values at present available for collagen. (Consden *et al.* (1948) report 91 and 96% recoveries of glutamic and aspartic acids, respectively, from synthetic mixtures simulating wool.) If a correction based on the results of Consden *et al.* (1948) on wool is applied, the total dicarboxylic acid content agrees well with that deduced from the titration curve.

With the exception of the ϵ -amino groups of lysine, the values deduced from the titration curve are in agreement with those obtained by analysis, and there is no indication that any of the other reactive side-chain groups of the basic or dicarb-

From a survey of the literature, it is evident that of the remaining amino-acids, tryptophan and cystine are absent, but that glycine and hydroxyproline are present in considerable amounts. Values for these last two have been selected from the literature to complete the attempted survey of the amino-acid composition of collagen given below. Of these, the figure for hydroxyproline is most likely to be in error; the method used is not entirely reliable and the determination was made on gelatin.

When the composition of collagen was considered by Braybrookes (1939) and by Bowes (1943) the analysis was incomplete. The higher figures now obtained for some of the amino-acids, notably phenylalanine and the dicarboxylic acids, and the addition of valine, threonine, serine, and hydroxy-

lysine to the list of amino-acids present in appreciable amounts, have now made up the deficiency. Using the values given in Table 4, it is now possible to account for over 99 % of the total N of collagen (see col. 1), and the sum of the amino-acid residues (col. 3) approaches 100. The analysis of collagen is, therefore, virtually complete. Although the values of some of the amino-acids may be adjusted slightly as a result of improved methods of analysis, it is unlikely that any great changes in the values will be made, or that other amino-acids will be found to be present in important amounts.

Calculation of the minimum molecular weight from the data in cols. 1, 5 and 6 gives a value of about 39,000. Earlier calculations from the composition of gelatin have given figures of 10,300 (Jordan Lloyd, 1920), 10,000–12,700 (Cohn, Hendry & Prentiss, 1925), and 34,500 (Atkin, 1933).

The mean residue weight calculated by summation from the data in Table 4

$$\frac{\Sigma \text{ wt. of amino-acid residues/100 g. protein}}{\Sigma \text{ g. residues/100 g. protein}}$$

is 92.6, and the same value is obtained by calculation from the N distribution (Chibnall, 1942). From X-ray data and the density of dry gelatin Astbury (1940) obtained a value of 94 for the average residue weight in gelatin. Taking 39,000 as the mean molecular weight and 92.6 as the average residue weight, the number of residues/molecule is 421, of which 419 have been identified (col. 6, Table 4).

SUMMARY

1. Collagen has been prepared from ox hide with the minimum of chemical treatment. The total N

and amide N contents of the collagen are higher than those previously reported. The glutamic and aspartic acid contents are nearly double the values quoted by Dakin (1920) for gelatin.

2. The titration curve of this collagen has been interpreted in terms of the titratable groups, and the values deduced are, in general, in agreement with those obtained by direct analysis, and indicate that the side-chain groups of the basic and dicarboxylic acids are free, and that the number of terminal groups is small.

3. It is now possible to account for over 99 % of the nitrogen in collagen, and it is considered that it is unlikely that any further amino-acids will be found to be present in important amounts.

4. The minimum molecular weight calculated from the analytical figures is 39,000, and the average residue weight calculated both by summation and from the nitrogen distribution is 92.6.

In the course of this and other work the authors have had occasion to refer to the various determinations of the amino-acids in gelatin and collagen which have been made at different times, and it has been thought useful to put these on record. Copies of this compilation may be obtained from the Librarian, British Leather Manufacturers' Research Association.

The authors wish to thank the Council of the British Leather Manufacturers' Research Association for permission to publish this paper, the Director of the Wool Industries Research Association, in whose laboratories the determination of the dicarboxylic acids was carried out, and Dr R. Consden for advice and assistance in these determinations. They also wish to thank Dr E. F. Gale for determinations of the glutamic acid content of various samples of collagen.

REFERENCES

- Ames, W. M. (1944). *J. Soc. chem. Ind., Lond.*, **63**, 303.
 Astbury, W. T. (1940). *J. Soc. Leath. Tr. Chem.* **24**, 69.
 Atkin, W. R. (1933). *J. Soc. Leath. Tr. Chem.* **17**, 757.
 Atkin, W. R. (1937). *Stiasnyfestschrift*, p. 13. Darmstadt: Roether.
 Baernstein, H. D. (1930). *J. biol. Chem.* **89**, 125.
 Baernstein, H. D. (1932). *J. biol. Chem.* **97**, 669.
 Bailey, K. (1937). *Biochem. J.* **31**, 1406.
 Bergmann, M. & Stein, W. A. (1939). *J. biol. Chem.* **128**, 217.
 Block, R. J. & Bolling, D. (1945). *The Amino-Acid Composition of Proteins and Foods*. Springfield: Thomas.
 Bowes, J. H. (1943). *Rep. Progr. appl. Chem.* **28**, 321.
 Braybrooks, W. E. (1939). *J. Soc. Leath. Tr. Chem.* **23**, 73.
 Cannan, R. T., Kibrick, A. C. & Palmer, A. H. (1941). *Ann. N.Y. Acad. Sci.* **41**, 243.
 Cannan, R. T., Kibrick, A. C. & Palmer, A. H. (1942). *J. biol. Chem.* **142**, 803.
 Cheshire, A. & Holmes, M. L. (1942). *J. Soc. Leath. Tr. Chem.* **26**, 237.
 Chibnall, A. C. (1942). *Proc. Roy. Soc. B*, **131**, 136.
 Chibnall, A. C. (1946). *J. Soc. Leath. Tr. Chem.* **30**, 1.
 Chibnall, A. C., Rees, M. W. & Williams, E. F. (1943). *Biochem. J.* **37**, 354.
 Coates, G. E. (1945). *J. chem. Soc.* p. 489.
 Cohn, E. J. & Edsall, J. T. (1943). *Proteins, Amino Acids and Peptides*, p. 84. New York: Reinhold.
 Cohn, E. J., Hendry, J. L. & Prentiss, A. M. (1925). *J. biol. Chem.* **63**, 721.
 Consden, R., Gordon, A. H. & Martin, A. J. P. (1948). *Biochem. J.* **42**, 443.
 Dakin, H. D. (1920). *J. biol. Chem.* **44**, 499.
 Dempsey, M. (1946). *Progress in Leather Science*, 1920–45, p. 3. London: British Leather Manufacturers' Research Association.
 Doherty, D. G. & Ogg, C. L. (1943). *Industr. Engng Chem. (Anal. ed.)*, **15**, 75.
 Dole, M. (1941). *The Glass Electrode*. New York: John Wiley and Sons, Inc.
 Fruton, J. S. & Lavin, G. I. (1939). *J. biol. Chem.* **130**, 375.
 Gale, E. F. (1945). *Biochem. J.* **39**, 46.
 Gilbert, G. A. & Rideal, E. R. (1944). *Proc. Roy. Soc. A*, **182**, 335.

- Hac, L. R. & Snell, E. E. (1945). *J. biol. Chem.* **159**, 291.
- Hac, L. R., Snell, E. E. & Williams, R. J. (1945). *J. biol. Chem.* **159**, 273.
- Hier, S. W., Graham, C. E., Friedes, R. & Klein, D. (1945). *J. biol. Chem.* **161**, 705.
- Highberger, J. H. & Stecker, H. C. (1941). *J. Amer. Leath. Chem. Ass.* **36**, 368.
- Horn, M. J., Jones, D. B. & Blum, A. E. (1946). *J. biol. Chem.* **166**, 313.
- Kanagy, J. R. & Harris, M. (1935). *Bur. Stand. J. Res., Wash.*, **14**, 563.
- Kritzinger, C. C. (1948). In the Press.
- Lewes, J. C. & Olcott, H. S. (1945). *J. biol. Chem.* **157**, 265.
- Lichtenstein, I. (1940). *Biochem. Z.* **303**, 20.
- Lloyd, D. Jordan (1920). *J. Soc. Leath. Tr. Chem.* **4**, 163.
- Lloyd, D. Jordan & Bidder, P. B. (1934). *Trans. Faraday Soc.* **29**, 148.
- Lugg, W. J. H. (1938). *Biochem. J.* **32**, 2125.
- Lyman, C. M., Moseky, O., Butler, B., Wood, S. & Hale, F. (1946). *J. biol. Chem.* **166**, 161.
- Macpherson, H. T. (1946). *Biochem. J.* **40**, 470.
- Markham, R. (1942). *Biochem. J.* **36**, 790.
- Neuberger, A. (1943). *Biochem. J.* **37**, 302.
- Rees, M. W. (1946). *Biochem. J.* **40**, 362.
- Rutherford, H., Harris, M. & Smith, A. L. (1937). *Bur. Stand. J. Res., Wash.*, **19**, 467.
- Schneider, F. (1940). *Collegium, Darmstadt*, p. 97.
- Speakman, J. B. & Hirst, N. C. (1933). *Trans. Faraday Soc.* **29**, 148.
- Steinhardt, J. (1939). *J. biol. Chem.* **129**, 135.
- Steinhardt, J. & Harris, M. (1940). *Bur. Stand. J. Res., Wash.*, **24**, 335.
- Stokes, J. L. & Gunness, M. (1945). *J. biol. Chem.* **157**, 651.
- Theis, E. R. & Jacoby, T. F. (1940). *J. Amer. Leath. Chem. Ass.* **35**, 257.
- Theis, E. R. & Jacoby, T. F. (1941a). *J. Amer. Leath. Chem. Ass.* **36**, 260.
- Theis, E. R. & Jacoby, T. F. (1941b). *J. Amer. Leath. Chem. Ass.* **36**, 375.
- Theis, E. R. & Jacoby, T. F. (1941c). *J. Amer. Leath. Chem. Ass.* **36**, 545.
- Theis, E. R. & Jacoby, T. F. (1942a). *J. Amer. Leath. Chem. Ass.* **37**, 611.
- Theis, E. R. & Jacoby, T. F. (1942b). *J. biol. Chem.* **146**, 163.
- Theis, E. R. & Jacoby, T. F. (1943a). *J. biol. Chem.* **148**, 105.
- Theis, E. R. & Jacoby, T. F. (1943b). *J. biol. Chem.* **148**, 603.
- Tristram, G. R. (1946). *Biochem. J.* **40**, 721.
- Van Slyke, D. D., Hiller, A., Macfadyen, J. A., Hastings, A. B. & Klemperer, F. W. (1940). *J. biol. Chem.* **133**, 287.

The Effect of Alkalis on Collagen

BY JOANE H. BOWES AND R. H. KENTEN

The British Leather Manufacturers' Research Association, London, S.E. 1

(Received 17 February 1948)

An essential process in the conversion of hides and skins to most types of leather is the removal of the hair or wool by treatment in suspensions of lime and sodium sulphide, usually in the cold, for periods varying from a few hours to several weeks.

Although several workers have investigated the effect of mild alkaline treatment on collagen (Marriott, 1928, 1931; Küntzel & Phillips, 1933; Kubelka & Knöedel, 1938; Highberger & Stecker, 1941; Ames, 1944), each has dealt only with one or two of the possible changes which may occur, and in only one instance (Marriott, 1931) has a collagen been used which has had no previous alkaline or enzymic treatment.

The present investigation has been carried out, therefore, on a collagen which has received no previous chemical treatment likely to cause chemical modification (see Bowes & Kenten, 1948), with the aim of obtaining more precise information regarding possible changes occurring during mild alkaline treatment.

EXPERIMENTAL

Alkali treatment of collagen. Collagen prepared as previously described (Bowes & Kenten, 1948) was cut into cubes about 1 cm. square, and 100 g. of this material, together with 310 ml. of water, 1.55 g. of $\text{Ca}(\text{OH})_2$, and 90 ml. of N-NaOH were incubated in a sealed flask at 20° . A preliminary experiment showed that these quantities gave an initial OH^- concentration of 135 m-equiv./l. (pH 13.3 at 20°), and a final value of 68 m-equiv./l. (pH 13.0 at 20°). After 14 days the flask was cautiously opened to avoid loss of NH_3 , sufficient HCl added to bring the contents to pH 4.5–5.0, the flask resealed and left at 20° for 3 days. Two batches of collagen (I and II) were treated in this way.

The collagen was filtered off, washed in running water for several hours, treated repeatedly with acetic acid pH 3 containing 1.5% NaCl , exhaustively washed with water at pH 8, finally with water at pH 6, then dehydrated with acetone. The pH of the last wash water was 5.5. (A little toluene was added to all wash waters to inhibit the growth of micro-organisms.)