The Swelling of Collagen in Alkaline Solutions

1. SWELLING IN SOLUTIONS OF SODIUM HYDROXIDE

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The swelling of collagen and other fibrous proteins in acid and alkaline solutions is governed by the osmotic pressure differences arising between the protein phase and the external solution as a result of the formation of protein salts—Donnan membrane effect—(Procter, 1914; Bolam, 1932; Donnan, 1933), and by the cohesion of the protein (Procter, 1914; Jordan Lloyd, 1920, 1938), i.e. the forces opposing swelling, such as interweaving of the fibres and intermolecular forces. In order to interpret the effect of pH changes on swelling it is, therefore, necessary to consider the influence of the acid or alkali both on the Donnan equilibria and on the cohesion of the protein.

The swelling of collagen and gelatin in alkaline solutions has received much less attention than that in acid solutions. A number of swelling curves of gelatin in alkaline solutions have been reported (Jordan Lloyd, 1920, 1930; Jordan Lloyd & Pleass, 1927, 1928; Loeb, 1921*a*, *b*, *c*, *d*; Kunitz, 1923–4; Pleass, 1930), but in general these do not extend above pH 12.0 and make no allowance for solution of the gelatin. A few alkaline swelling curves of collagen have also been reported (Kaye & Jordan Lloyd, 1924*a*, *b*; Jordan Lloyd, Marriott & Pleass, 1933; Highberger, 1936), but in all these there are insufficient points to determine the exact shape of the curves above pH 11.0. They show, however, no definite decrease in swelling at high pH values corresponding to that occurring in acid solutions below pH 2.0. With keratin (Jordan Lloyd & Marriott, 1934) and silk (Jordan Lloyd & Marriott, 1933; Coleman & Howitt, 1947) there is also an increase rather than a decrease in swelling at high pH values. With silk this increased swelling is accompanied by splitting up of the fibres, and is attributed to a weakening of the cohesive forces of the fibre.

The swelling of gelatin in alkaline solutions up to pH 10 is reduced by the addition of sodium chloride or other appropriate salts, in the same manner as swelling in acid solutions, but at higher pH values the curves have an upward trend, and the suppression of swelling is less marked (Jordan Lloyd & Pleass, 1927, 1928). There is also some evidence that the nature of the anion has some influence on swelling; e.g. sodium nitrate depresses swelling to a lesser extent than sodium chloride at the same molar concentration (Jordan Lloyd & Pleass, 1927, 1928). The few

experiments which have been carried out indicate that salts do not suppress swelling of collagen in alkaline solutions to the same extent as swelling in acid solutions, nor to the same extent as swelling of gelatin in alkaline solutions (Kaye & Jordan Lloyd, 1924*b*).

Thus, it appears that the swelling of collagen in alkaline solutions is more complicated than in acid solutions, and that loss of cohesion of the collagen plays a more important part. The swelling of collagen in solutions of different bases, with and without the addition of salts, is, therefore, being investigated. The present paper deals with swelling in solutions of sodium hydroxide with and without the addition of sodium chloride.

EXPERIMENTAL

Raw material. Oxhide and alkali-treated sheepskin collagen D and E were prepared as described by Bowes & Kenten (1948*a*, *b*). The alkali-treated sheepskin was preferred for the present work, since, owing to its looser structure, it is able to take up more water and hence shows greater variations in swelling with changes in external conditions.

Swelling curves. Air-dry collagen (0.5 g.) in the form of pieces about 0.5 cm. square was placed in 100 ml. of the appropriate solution for 3 days at 20°. The pH of the solution was then determined and the pieces lightly blotted and weighed.

Preliminary experiments showed that, provided the pH was determined quickly, it was unnecessary to take any special precautions to avoid contamination with CO₂. The Cambridge glass electrode standardized with phthalate buffer (pH 4.0) and borate buffer (pH 9.22) was used for pH values up to 9.5 and the special Beckman electrode, standardized with Na₂CO₃-sodium borate buffer (pH 10.0) for pH values above 9.5. An electrode assembly mounted in a thermostat maintained at 20° (Coates, 1945) was used for the high pH values.

When the effect of time was being investigated, the pieces were removed, weighed, and returned to the solution again. During these experiments the pH fell slightly, probably owing to hydrolysis of the collagen and absorption of CO₂. This fall was small and insufficient to affect the conclusions drawn from the results.

For the swelling curve of gelatin, 0.05 g. of leaf gelatin, in pieces of 1 sq.cm. area were placed in 100 ml. of NaOH solutions of varying pH values. The solutions were previously cooled to 0° and kept at this temperature for 3 days. This temperature was chosen in preference to 20° in

order to minimize solution of the gelatin. The pieces of gelatin were removed and lightly wiped with blotting paper. The solutions were warmed to 20° and the pH values determined. The N contents of the majority of the solutions were determined by a micro-Kjeldahl method (Markham, 1942). Assuming that the N was all derived from solution of the gelatin (an assumption which will not be greatly in error except where low concentrations of N are involved), and taking the N content of the gelatin as 18%, the amount of gelatin which had dissolved was calculated. Allowance was made for dissolved gelatin in calculating the percentage water uptake of the gelatin.

RESULTS

The effect of time on swelling. The effect of time on the swelling of oxhide collagen in solutions of sodium and calcium hydroxides is shown in Fig. 1. The pH values of the solutions at the end of 3 and 18 days are recorded on the graph. The curves are similar in shape to those obtained by Jordan Lloyd (1920) with gelatin, and by Balfe, Beakbane & Wallis (1945) for collagen in acid solutions. There was a rapid uptake of water from all the solutions during

Fig. 1. The effect of time on the swelling of collagen in alkaline solutions.

	pH at 3 days	pH at 18 days		pH at 3 days	pH at 18 days
×, NaOH	11.34	10.80	△, NaOH	13.65	—
⊙, NaOH	12.84	12.83	+, NaOH + 0.1% Na ₂ S	12.72	12.67
▽, NaOH	13.05	13.02	⊕, Saturated Ca(OH) ₂	12.66	12.66
□, NaOH	13.32	13.21	⊗, Saturated Ca(OH) ₂ + 0.1% Na ₂ S	12.72	12.69

In all experiments swelling was taken as the amount of solution held expressed as a percentage of the weight of the moisture- and ash-free collagen or gelatin.

The pH values quoted refer to the pH at 20°. In alkaline solutions the pH decreases as the temperature increases, whereas the pOH, which is the important factor governing the swelling, remains unaltered. For this reason the solutions used in the determination of the swelling curve of gelatin were warmed to 20° before the pH was determined.

the first 2 days, followed, with one exception, by a further increase which was not complete at the end of 72 days. This further increase in water uptake became more pronounced as the pH increased. At pH 13.32 the curve rose quite sharply and after 21 days the collagen began to disintegrate. At 13.65, the highest pH value examined, the water uptake increased steadily and at the end of the fourth day the collagen was almost completely dissolved.

At the end of 3 days the swelling in excess of that occurring in the isoelectric range was half as great with calcium as with sodium hydroxide at the same pH, but as time elapsed swelling increased more rapidly in calcium than in sodium hydroxide solutions, so that at the end of 70 days there was little difference in the swelling of the two samples.

11.5 and 13.0 and then increased again very rapidly with further rise in pH. There is no evidence that the guanidino groups in collagen begin to titrate below pH 13.3 (Bowes & Kenten, 1948*a*), or that there is any large increase in the number of titratable groups during 3 days' exposure to pH values of this order (Bowes & Kenten, 1948*b*). Further, the amide N

Fig. 2. Swelling of collagen D in hydrochloric acid/sodium chloride and sodium hydroxide/sodium chloride systems at 20°; $\odot$, no salt; $\triangle$, 0.05M-NaCl; +, 0.1M-NaCl; $\times$, 0.2M-NaCl; $\square$, 0.5M-NaCl; ∇ , 2M-NaCl.

The swelling of collagen in sodium hydroxide and sodium hydroxide/sodium chloride solutions. The swelling of alkali-treated sheepskin collagen in solutions of sodium hydroxide and hydrochloric acid over the pH range 0.5–13.5 is shown in Figs. 2 and 3. The curve for hydrochloric acid is included for purposes of comparison; it has the usual shape of such curves and calls for no special comment.

Swelling increased fairly sharply with rise in pH up to about 11.5, increased less rapidly between pH

of sheepskin E was only reduced from 0.39 to 0.22 mmol./g. on treatment in a sodium hydroxide solution at pH 13.4 for 3 days. It is unlikely, therefore, that this last rapid increase in swelling is due to increased formation of protein salt and hence to increase in the forces drawing the water into the collagen (swelling pressure). Thus, swelling in alkaline solutions differs markedly from that in acid solutions in showing no decrease at high concentrations as predicted by the Donnan theory.

In acid solutions swelling decreased progressively as the concentration of sodium chloride was increased, and at concentrations of 0.5M was almost completely suppressed (Fig. 2). In solutions of sodium hydroxide the effect of salt was much less marked; although there was an appreciable decrease in swelling in the presence of 0.05M-sodium chloride, further increase in the salt concentration had only a comparatively small effect and at pH values above 13.0 the effect was hardly perceptible (Figs. 2 and 3).

collagen, breakdown of structural factors is also involved. The existing swelling curves of gelatin do not offer conclusive evidence on this point, and the swelling curve of gelatin was accordingly determined at 0°, this temperature being chosen in preference to 20° in order to minimize solution of the gelatin. Corrections were made for the gelatin dissolved (p. 2).

The swelling of gelatin (Fig. 4) increased with pH up to 12, decreased between pH 12.0 and 12.8, and

Fig. 3. Swelling of collagen E in sodium hydroxide/sodium chloride systems at 20°; $\circ$, no salt; $\times$, 0.2M-NaCl; $\square$, 0.4M-NaCl.

The swelling of gelatin in sodium hydroxide solutions at 0°. The increased swelling of collagen at high pH values may be due to breakdown of reticular tissue and elastin, to the removal of other structural factors restricting swelling, or to loosening of intermolecular forces such as salt links and hydrogen bonds. If the swelling curves of gelatin and collagen at high alkalinities are similar in shape, then it is probable that breakdown of intermolecular forces is the main cause of the decrease in cohesion, whereas if the curves differ in shape it suggests that, with

then increased again rapidly with further rise in pH, until at pH values above 13.5 the gelatin disintegrated. There was a definite minimum at pH 12.7–12.8; hence it would appear that swelling takes place in accordance with the Donnan theory up to about pH 12.8 and only at higher pH values does some other factor come into play. This additional factor presumably involves the breakdown of intermolecular linkages, which leads to loss of cohesion and eventually to solution of the gelatin.

Consideration of the swelling curves in relation to the Donnan theory of membrane equilibria

From observation of the swelling curves alone it is difficult to estimate the extent to which water uptake diverges from that predicted by the Donnan theory, and hence to deduce the part played by decrease in cohesion in determining swelling. Swelling of fibrous proteins in acid and alkaline solutions is considered to be the result of the excess osmotic pressure (swelling pressure) in the protein phase

thus a measure of the forces restricting swelling, i.e. the cohesion of the protein. Cohesion here includes any force restricting swelling, such as interweaving of the fibres, network formation of reticular tissue or elastin and intermolecular forces such as salt links and hydrogen bonds. On the basis of the Donnan theory

$$x^2 = y(y + z),$$

and

$$e' = 2y + z - 2x,$$

where x and y are the molar concentrations of hydrogen or hydroxyl ions in the external and protein

Fig. 4. Swelling of gelatin in solutions of sodium hydroxide at 0°.

arising from the difference in concentration of the diffusible ions in the protein and the external phase, equilibrium being reached when the osmotic pressure is balanced by the cohesive forces of the protein (Procter & Wilson, 1916). With gelatin, Procter & Burton (1916) have shown that $e' = CV$, where e' is the difference in concentration of diffusible ions, V is the increase in volume of a given amount of gelatin, and C is a constant, which they term the 'bulk modulus' of the protein. For any given value of e' the increase in volume is dependent on C which is

phases respectively, and z is the equivalent concentration of the protein anion or cation, whence

$$e' = -2x + \sqrt{(4x^2 + z^2)}.$$

Hence, e' and C may be calculated from a knowledge of the external pH, the acid or alkali bound by the protein and the increase in volume. With a fibrous protein, such as collagen, such calculations cannot be precise. Difficulty arises in allotting a value to z , since the volume available for solution in the internal phase is in doubt, and the increase in volume V is

difficult to determine accurately. In making the following calculations it has been assumed that all the water held by the collagen is available for solution and as an approximation the water uptake, in excess of that occurring in the isoelectric range, in ml./g., was taken as V .

On this basis e' and C were calculated for the following samples of collagen: (1) oxhide, using the

closely woven than those of oxhide, thus offering less resistance to swelling, and the large difference in the value of C for the two types of skin indicates that the interweaving of the fibres must play a large part in determining cohesion. The corresponding value of C for gelatin in hydrochloric acid solutions is 0.00021 (Procter & Burton, 1916), i.e. 100 times smaller than the value for alkali-treated sheepskin.

Table 1. *Typical values of C , Procter's 'bulk modulus'*

($C = e'/V$, where V is the increase in volume of the protein in ml./g. and e' is the difference in concentration between the protein and the external phase in mol./l.)

	Sodium chloride concentration (M)					
	0.0	0.05	0.10	0.2	0.4	0.5
Oxhide:						
Acid solutions	0.127	—	—	—	—	—
Alkaline solutions, pH 10–12.5	0.075	—	—	—	—	—
Alkaline solutions, pH 13.5	0.008	—	—	—	—	—
Alkali-treated oxhide:						
Acid solutions	0.111	—	—	0.122	—	Large
Alkali-treated sheepskin D:						
Acid solutions	0.019	0.024	—	0.026	—	Large
Alkaline solutions, pH 10–12.5	0.013	0.016	0.013	—	—	0.003
Alkaline solutions, pH 13.5	0.001	0.005	0.002	—	—	<0.001
Alkali-treated sheepskin E:						
Acid solutions	0.021	—	—	—	—	—
Alkaline solutions, pH 10–12.5	0.020	—	—	0.016	0.010	—
Alkaline solutions, pH 13.5	0.002	—	—	<0.001	<0.001	—

swelling and titration curves obtained by Bowes & Kenten (1948*a*, *b*); (2) alkali-treated oxhide, using the swelling curves obtained by Balfe & Fowler (1948) and the titration curves of Retterova (1948); (3) alkali-treated sheepskin, using the present curves and the titration curves of Bowes & Kenten (1948*b*).

These calculations show that e' decreases as the pH increases above 12.0, e.g. with sheepskin collagen the value of e' decreases from 0.056 at pH 12.0 to 0.005 at pH 13.5. It follows, therefore, that the increased water uptake at high pH values cannot be due to an increase in swelling pressure, but must be attributed to a decrease in cohesion.

Typical values of C under various conditions are given in Table 1. For any one sample the values of C are approximately constant between pH 1 and 3 and between 10.5 and 11.0, the values being slightly lower in alkaline solutions than in the corresponding acid solutions. Above pH 12.5 the values of C decrease sharply. C is not affected by additions of sodium chloride up to 0.1 M, but at higher concentrations it begins to decrease and with 0.5 M-sodium chloride it is reduced to about one-quarter of the value found in the absence of salt. The value of C for alkali-treated collagen is slightly less than that of untreated collagen, showing that such treatment reduces cohesion. The values for the alkali-treated sheepskin collagens are only about one-fifth of those of the alkali-treated oxhide collagen under corresponding conditions. The fibres of sheepskin are much less

DISCUSSION

This investigation shows that decreases in cohesion must play a much more important part in determining the swelling of fibrous proteins in alkaline solutions than in acid solutions. This can be qualitatively deduced from the shape of the curves and is borne out by mathematical analysis of the results on the basis of Procter's theory.

On the assumption that the value of C is an approximate measure of cohesion, it may be concluded that the cohesion of collagen in alkaline solutions is less than in acid solutions at corresponding pH values. The effect is small at pH values up to 12.5, but becomes increasingly marked at higher pH values, the value of C decreasing nearly tenfold between pH 12.5 and 13.5. This decrease in cohesion should eventually lead to solution of collagen and this in practice does occur, if the pH and the time of treatment is sufficiently long (p. 2). In this connexion it may be noted that collagen may be left in a suspension of calcium hydroxide (pH 12.6 at 20°) for long periods without obvious damage, but on subsequent warming the collagen is much more readily converted to gelatin.

The presence of sodium chloride at concentrations of 0.2 M or greater also appears to decrease cohesion, with 0.5 M-sodium chloride the effect being equivalent to a 25% decrease in C . Salts are known to affect the swelling of proteins in the isoelectric

range, but in general the effect appears to be smaller than at higher pH values, and takes place at higher concentrations of salt (cf. Page & Page, 1927). Kunitz (1929-30) finds that the elasticity of gelatin in the isoelectric range is only appreciably affected when the salt concentration exceeds 0.8 M.

The reason why salts almost completely suppress the swelling of gelatin in alkaline solutions, while only having a comparatively small effect on the swelling of collagen, is related to the much larger uptake of water by gelatin. Because of this the concentration of diffusible ions in the gelatin phase is comparatively low, and hence the difference in concentration between the internal and external phases is reduced to negligible proportions by quite low concentrations of salt which have no effect on cohesion.

The first rapid swelling observed when collagen is placed in alkaline solutions is probably due to osmotic effects. The further slow increase taking place over long periods of time may be due to combination of base with additional groups liberated as a result of hydrolysis of peptide and amide bonds and the consequent formation of an increased amount of protein salt; or more probably, in view of the limited chemical changes of this kind which take place (Bowes & Kenten, 1948*b*), to a decrease in the cohesion of the collagen. Since the further increase in swelling is more marked with calcium than with sodium hydroxide at the same pH value, the calcium ion appears to be more effective in decreasing cohesion than the sodium ion.

The decrease in cohesion observed in alkaline solutions is apparently not primarily associated with breakdown of the main polypeptide chains, since apart from some loss of amide nitrogen and hydrolysis of a very small number of peptide links there is little chemical change in the collagen (Bowes & Kenten, 1948*b*). Further, since a prolonged alkaline soak is an important pretreatment in one method of preparation of gelatin, the collagen presumably eventually goes into solution in the form of large polypeptides and not as peptides. Comparison of the swelling curves of collagen and gelatin suggests that the increased swelling of both proteins at high pH values is primarily due to the same cause, probably

breaking of intermolecular links such as hydrogen bonds. However, the existence of a minimum in the pH range 12.0-12.8 with gelatin, but not with collagen, suggests that with collagen breakdown of structural features restricting swelling, such as reticular tissue, elastin, etc., may also be involved.

It is uncertain how far the effects on cohesion observed in alkaline solutions are permanent and influence the subsequent behaviour of the collagen. Some effect certainly remains, since the increase in swelling in the acid or isoelectric range following alkaline treatment is greater than can be accounted for, on the basis of an increase in the number of reactive groups (Bowes & Kenten, 1948*b*). Provided the decrease in cohesion is not too great and the polypeptide chains are not forced too far apart, it would appear reasonable to assume that the process can largely be reversed, the intermolecular links between the chains reforming as water is withdrawn.

SUMMARY

1. The swelling of collagen in sodium hydroxide solution increases progressively with increase in pH, and shows no decrease at high pH values corresponding to that occurring in acid solutions below pH 2.0.

2. The addition of small amounts of sodium chloride (0.05 M) decreases swelling at pH values up to 12.5, but at higher pH values has little effect. Higher concentrations of salt cause little further decrease in swelling.

3. Consideration of the curves in relation to the Donnan theory indicates that decrease in cohesion plays an important part in determining swelling in alkaline solutions. There is some decrease in cohesion at all pH values above 11.0, but more especially above 13.

4. Sodium chloride tends to decrease cohesion.

5. Decrease in cohesion is in part due to breakdown of structural features, but in the main to breaking of intermolecular links.

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REFERENCES

- Balfe, M. P., Beakbane, H. R. & Wallis, R. G. (1945). Unpublished work in the British Leather Manufacturers' Research Association Laboratories.
- Balfe, M. P. & Fowler, D. (1948). See *Progress in Leather Science* 1920-45, p. 588. London: British Leather Manufacturers' Research Association.
- Bolam, T. R. (1932). *The Donnan Equilibria*. London: G. Bell and Sons.
- Bowes, J. H. & Kenten, R. H. (1948*a*). *Biochem. J.* **43**, 358.
- Bowes, J. H. & Kenten, R. H. (1948*b*). *Biochem. J.* **43**, 365.
- Coates, G. E. (1945). *J. chem. Soc.* 489.
- Coleman, D. & Howitt, F. O. (1947). *Proc. Roy. Soc. A*, **190**, 145.
- Donnan, J. (1933). *J. int. Soc. Leath. Chem.* **17**, 36.
- Highberger, J. H. (1936). *J. Amer. Leath. Chem. Ass.* **31**, 345.
- Kaye, M. & Jordan Lloyd, D. (1924*a*). *Biochem. J.* **18**, 1043.
- Kaye, M. & Jordan Lloyd, D. (1924*b*). *Proc. Roy. Soc. B*, **96**, 293.
- Kunitz, M. (1923-4). *J. gen. Physiol.* **6**, 547.
- Kunitz, M. (1929-30). *J. gen. Physiol.* **13**, 565.
- Lloyd, D. Jordan (1920). *J. int. Soc. Leath. Chem.* **14**, 163.

- Lloyd, D. Jordan (1930). *Biochem. J.* **24**, 1460.
 Lloyd, D. Jordan (1938). *J. phys. Chem.* **42**, 7.
 Lloyd, D. Jordan & Marriott, R. H. (1933). *Trans. Faraday Soc.* **29**, 1228.
 Lloyd, D. Jordan & Marriott, R. H. (1934). *Trans. Faraday Soc.* **30**, 944.
 Lloyd, D. Jordan, Marriott, R. H. & Pleass, W. B. (1933). *Trans. Faraday Soc.* **29**, 554.
 Lloyd, D. Jordan & Pleass, W. B. (1927). *Biochem. J.* **21**, 1352.
 Lloyd, D. Jordan & Pleass, W. B. (1928). *Biochem. J.* **22**, 1007.
 Loeb, J. (1921*a*). *J. gen. Physiol.* **2**, 85.
 Loeb, J. (1921*b*). *J. gen. Physiol.* **2**, 547.
 Loeb, J. (1921*c*). *J. gen. Physiol.* **2**, 667.
 Loeb, J. (1921*d*). *J. gen. Physiol.* **2**, 691.
 Markham, R. (1942). *Biochem. J.* **36**, 790.
 Page, A. W. & Page, R. O. (1927). *Industr. Engng Chem.* **19**, 1264.
 Pleass, W. B. (1930). *Biochem. J.* **24**, 1472.
 Procter, H. R. (1914). *J. chem. Soc.* **105**, 313.
 Procter, H. R. & Burton, D. (1916). *J. Soc. chem. Ind., Lond.*, **35**, 404.
 Procter, H. R. & Wilson, J. A. (1916). *J. chem. Soc.* **109**, 307.
 Retterova, E. (1948). See *Progress in Leather Science* 1920-45, p. 87. London: British Leather Manufacturers' Research Association.

A Study of the Peptides of Cystine in Partial Hydrolysates of Wool

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Although much work has been carried out on the disulphide group of wool, no information has been obtained as to which particular amino-acid residues are linked with cystine in the wool polypeptide chain. Such information seems specially desirable because wool and the related keratins are characterized by a high cystine content. Both amino and both carboxyl groups of cystine are probably involved in peptide linkages (Geiger, Harris, Mizell & Patterson, 1941; Lindley & Phillips, 1945), and Phillips (1946), on the basis of differences of reactivity of the combined cystine of wool towards sodium bisulphite and other mild reagents, has suggested that the more reactive fraction is associated with a polar environment. Consden, Gordon & Martin (1949) succeeded in identifying a number of lower peptides of glutamic and aspartic acids in partial hydrolysates of wool, and we were encouraged to apply similar methods to identify the peptides of cystine. Although Consden *et al.* (1949) found that some peptides of cystine with dicarboxylic amino-acids were present in a 3-day partial hydrolysate of wool, much more of the cystine of such hydrolysates occurred in the neutral ionophoretic fraction. The problem was, therefore, to separate the cystine peptides from this complex mixture, before attempting to employ paper chromatography. This has been achieved by oxidation which easily converts the disulphide groups into sulphonic acids. The cysteic acid and cysteic acid compounds thus produced could then be separated

from the non-acidic constituents. The latter would of course include any peptide which contained an equal number of cysteic acid and basic residues. Oxidation has additional advantages: first, acidic substances are the easiest to investigate (Consden *et al.* 1949); secondly, conversion to sulphonic acids avoids working with cystine compounds, which in general do not behave satisfactorily on paper chromatograms.

The object of the present investigation was to demonstrate the presence of as many peptides of cystine as possible (in the derived form of peptides of cysteic acid). This has proved much simpler than the investigation of the peptides of aspartic and glutamic acids, since, among other reasons, only one acidic amino-acid is here involved. The results obtained have already been given in a preliminary report (Consden & Gordon, 1948*a*).

The stages of the analysis are shown in Fig. 1. Acidic peptides and amino-acids in partial hydrolysates of wool were first removed chromatographically on an anion-exchange resin and the non-acidic fraction was then oxidized with bromine water. The cysteic acid and acidic cysteic acid peptides were then separated from non-acidic substances by repeating chromatographic separation on the anion-exchange resin. This was followed by ionophoresis, which separated the acidic peptides into groups with similar electrical mobilities. Each group was then examined by chromatography on paper and the sub-fractions thus obtained were hydrolysed and the constituent amino-acids identified. Further, those amino groups not involved in peptide linkage were destroyed by deamination and the amino-acids re-

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