

The Extraction of the Protein Components of Human Hair

I. Aqueous Extraction as a Function of pH and Temperature after Reductive or Oxidative Treatments

JAMES P. DANEHY, University of Notre Dame

Introduction

Keratin fibers, such as wool and human hair, are structures insoluble without previous chemical modification. They have a remarkably, perhaps uniquely, high protein content. By reason of their morphological differentiation one would expect these fibers to contain more than one protein component. Qualitative and semiquantitative support for this view were furnished by Geiger's data on the amino acid contents of cortex and cuticle fractions of wool which had been separated by mechanical means (1). Later studies on the physical properties of partial solutions of wool substance have cast some light on the range of molecular weights and shapes of degradation products of wool (2, 3). A chemical study of a hydrolysate of wool which had been treated with fluorodinitrobenzene has furnished a quantitative estimation of end-groups and a calculation of average chain weight, and has led to the proposal of a molecular model, as yet highly hypothetical (4). More recently an Australian group has described some of the properties of protein fractions obtained by the extraction of wool at 50° C. with alkaline thioglycolate solutions (5-14).

Yet much work remains to be done before the individual protein components of any keratin fiber can be characterized and their morphological distribution ascertained. In particular, more attention must be paid than has been in the previously reported studies to an essential requirement: to obtain solutions which are as representative as possible of the total protein substance, under conditions in which degradation of the linear units (peptide hydrolysis) will be kept to a minimum. The presently reported experimental study of human hair has been directed especially to this question.

While at present we are unable to specify all of the forces responsible for the cohesion of the fiber and for its resistance to solution, there is no doubt but that the disulfide linkages of cystinyl residues are the most important. An essential prerequisite for any procedure for dissolving intact peptide chains of hair is the cleavage, by oxidative or reductive methods, of as many of these disulfide bonds as are responsible for inter-chain, rather than intra-chain, linkages. The solubility of such chemically modified fibers can then be studied as a function of those factors (pH, ionic strength, specific solvent, temperature) which are known to govern the behavior of potentially soluble proteins.

The Extractability of Reduced Hair as a Function of pH, with or without Protein Swelling Agents

Experimental Procedures: 1) Bundles of hair fibers (about 4 in. in length; 0.5-1.0 g.) were treated over-night at room temperature in test tubes with 25 ml. 0.47 *M* sodium thioglycolate (TG) adjusted to different pH values. The TG solutions were decanted and discarded, the hair briefly rinsed with water, and 20 ml. of saturated aqueous urea or of 90% formic acid was added. After two days the solutions were decanted, the hair samples washed repeatedly with water, de-watered with acetone, air-dried and weighed (Table 1).

TABLE 1
Extraction With Aqueous Urea or Formic Acid After
Neutral Reduction*

Sample	Treatment Initial**	Final	Original wt., g.	Wt. after extraction, g.	Percentage extraction
1	pH 4.68	Sat'd aq. urea	0.68	0.66	3
2	pH 7.19	"	0.76	0.63	17
3	pH 8.10	"	0.53	0.43	19
4	pH 4.68	90% formic acid	0.50	0.50	0
5	pH 8.42	"	0.79	0.78	2

* All operations at room temperature: 26-28°C.

** Over-night reduction with 0.47*M* sodium thioglycolate adjusted to pH value given.

2) Bundles of hair fibers were treated at room temperature with 25 ml. portions of 0.47 *M* TG adjusted to increasingly alkaline pH values. After 48 hours the extracts were decanted, combined, and dialyzed. The swollen fibers were further extracted with 25 ml. water for 24 hours followed by two aqueous extractions for shorter periods. Protein was precipitated from both the dialyzed urea solution and the combined aqueous extracts by acidification with acetic acid to pH 5.5-6.0. The washed hair samples were dewatered with acetone, air-dried and weighed (Table 2).

TABLE 2
Extraction at 27-28°C. With 0.47*M* Sodium Thioglycolate for 48
Hours as a Function of pH

Sample	pH of solution Initial	Final	Original wt., g.	Wt. after extraction, g.	Percentage extraction
1	9.08	8.81	0.58	0.58	0
2	10.02	9.87	1.01	0.53	47.5
3	11.02	10.84	0.76	0.21	72.4

3) Bundles of hair fibers were treated at room temperature with 25 ml. portions of 0.47 *M* TG, half-saturated with urea, adjusted to increasingly alkaline pH values. After 72 hours the solutions were decanted, combined and dialyzed. The swollen fibers were further extracted with water, dewatered with acetone, air-dried and weighed (Table 3). Protein was precipitated from both the dialyzed urea-TG solution and the combined aqueous extract by acidification with acetic acid to pH 5.6.

TABLE 3

Extraction at 27-8° C. With 0.47*M* Sodium Thioglycolate, Half-Saturated With Urea, for 72 Hours as a Function of pH

Sample	pH of solution		Original wt., g.	Wt. after extraction, g.	Percentage extraction
	Initial	Final			
1	8.95	8.82	0.62	0.54	12.9
2	9.52	9.44	0.52	0.29	44.2
3	10.00	9.94	0.81	0.48*	40.7
4	10.62	10.51	1.16	0.39	66.4

Discussion: The first experiments were prompted by the quickly dissipated hope that, following extensive reduction in the neutral range, the hair substance could be dissolved with the aid of powerful protein swelling agents such as aqueous urea or aqueous formic acid, thus avoiding exposure of the fibers to high pH values. Consideration of the data in Table I reveals that extraction of fibers with saturated aqueous urea followed by water, after reduction with TG over the pH range of 5 to 8, results in the solution of only 3 to 19% of the hair substance. Extraction with 90% formic acid followed by water, after reduction with TG at pH 4.68 or 8.42, is almost completely ineffective in dissolving protein.

Systematic comparison of the effectiveness as extracting agents of TG, and of TG plus urea, at increasing pH values shows that urea does seem to have some effect on lowering the pH value for incipient solubilization (Tables 2 and 3). But at pH 10 and above urea seems to offer no advantage.

Influence of Lithium Bromide on Extraction with Aqueous, Alkaline Thioglycolate

Experimental Procedures: 1) From a stock solution, 0.94 *M* TG at pH 9.11, 20 ml. aliquots were added to weighed amounts of lithium bromide which had been added to a 50 ml. graduated cylinder. Water was added to give 40 ml. of solution, the pH value determined, and the entire solution poured into a large test tube to which a weighed amount of hair had already been added. The tubes were held 48 hours at room temperature (23-25° C.) with occasional shaking. While the strongest lithium bromide solution seemed the least active in swelling the fibers during the first few hours there was no doubt but that it caused by far the greatest swelling after 48 hours. The extracting solutions were then decanted and replaced with water. Replacement of water was carried out twice daily for several days. Neither the original extracts, nor the subsequent aqueous extracts, were further investigated when it was seen that virtually no protein had been extracted from the hair. The hair samples were finally dehydrated with acetone, air-dried and weighed (Table 4).

2) Procedures were the same as in the preceding series except that the 0.94 *M* TG stock solution had a pH of 9.80. In addition, a control

* Residue whitened when treated with acetone, indicating insufficient rinsing with water; percentage extraction figure therefore low.

TABLE 4

Extraction at 23-25° C. With 0.47*M* Sodium Thioglycolate Containing Lithium Bromide

<i>Sample</i>	<i>LiBr, M</i>	<i>pH</i>	<i>Original wt., g.</i>	<i>Wt. after extraction, g.</i>	<i>Percentage extraction</i>
1	0.879	8.95	1.005	1.003	0.2
2	1.758	8.80	1.085	1.078	0.1
3	3.516	8.51	1.072	1.059	0.1
4	7.032	8.05	1.136	1.080	4.5

was included: *i.e.*, one hair sample was extracted with diluted stock solutions containing no lithium bromide. Also, since appreciable protein was extracted, the first 500 ml. of combined water rinsing from all five hair samples (not including the original TG - LiBr extract) was analyzed and found to contain 123.4 mg. nitrogen; after acidification of this extract with acetic acid and settling of the precipitated protein the supernatant solution contained 55.4 mg. nitrogen (Table 5).

TABLE 5

Extraction at 23-25° C. With 0.47*M* Sodium Thioglycolate Containing Lithium Bromide at a Higher pH Level

<i>Sample</i>	<i>LiBr, M</i>	<i>pH</i>		<i>Original wt., g.</i>	<i>Wt. after extraction, g.</i>	<i>Percentage extraction</i>
		<i>Initial</i>	<i>Final</i>			
1	0.879	9.85	9.71	1.0039	0.4586	54.2
2	1.758	9.70	9.50	1.0010	0.5358	46.4
3	3.516	9.44	9.24	1.0000	0.6028	39.7
4	7.032	8.82	8.70	1.0006	0.7125	28.7
5	0	10.09	9.85	1.0000	0.4307	56.9

3) The procedure for the preparation of the reagent was modified so that the solutions, varying in lithium bromide concentration, would have constant pH values while in contact with the hair. As anticipated, however, this caused the subsequent water rinsings to be carried out over a wide pH range. The stock solution, 0.94 *M* TG, was at pH 8.0. A 20 ml. aliquot was added to the weighed amount of lithium bromide, strong sodium hydroxide solution added to the pH desired, and the solution then brought to 40 ml. with water. The extraction was then carried out exactly as in the two preceding series (Table 6).

TABLE 6

Attempts to Extract Hair With Thioglycolate-Lithium Bromide Solutions at Constant pH Values

<i>Sample</i>	<i>LiBr, M</i>	<i>pH</i>		<i>First rinsing</i>	<i>Wt. after extraction, g.</i>	<i>Percentage extraction</i>
		<i>Initial</i>	<i>Final</i>			
1	0	9.49	9.43	9.64	0.7254	27.5
2	0.879	9.49	9.50	9.81	0.5423	45.8
3	1.758	9.50	9.50	9.91	0.5583	44.2
4	3.516	9.47	9.61	10.18	0.4370	56.3
5	7.032	9.27	9.61	11.39	0.2514	74.9

All original weights were 1.0000 g.

4) As a pretreatment, the hair was subjected to a supercontraction procedure, the details of which were supplied by C. C. Jensen (15). Hair (about 6 g.) was soaked over-night in 100 ml. sodium phosphate buffer at pH 7.30, rinsed with distilled water, and air-dried. The hair was then added to 100 ml. of aqueous solution containing 57.5 g. lithium bromide which had been preheated to 95° C. and the mixture was held at 95° C. for 30 minutes. During this time the hair became so gelatinous that it would have been very easy to disintegrate mechanically. After the mixture had cooled the solution was discarded and the hair washed repeatedly with distilled water (several days) until the decanted water no longer clouded on addition of silver nitrate solution. The hair was then air-dried. Despite the drastic swelling it had undergone, neither in visible appearance nor in handle did it seem to differ from hair of the same lot which had not been so treated. Whether or not the fibers shortened was not measured. Five samples of this pretreated hair were then extracted with 40 ml. of 0.47 *M* TG which had been adjusted to different pH values. The extraction procedure was the same as in the preceding series (Table 7). The combined extracts and water washings contained 176.6 mg. nitrogen before precipitation and 98.0 mg. nitrogen after precipitation. The precipitate was washed with water and lyophilized (0.86 g.).

Discussion: The clearly intended purpose of the first two series of experiments was to determine the influence of increasing concentrations of lithium bromide on the extraction of protein from hair fibers reduced with TG at pH 9 and 10 (Tables 4 and 5). In both series, however, the range of concentrations of lithium bromide employed caused a gradual decrease in pH values amounting to one full unit. A third series, in which the initial pH values of the extracting solutions were kept closer by adding increasing amounts of sodium hydroxide, did not solve the problem since on subsequent extraction with water the pH values rose very steeply (Table 6). Despite the unsatisfactory control of the interdependent variables it can be concluded that lithium bromide is not a particularly useful adjunct for such extractions. The total amount of protein extracted, considering the highest pH value attained in each case on subsequent extraction with water, is comparable to what would have been attained if no lithium bromide had been used. It should be noted that only about 45 to 55% of the nitrogen extracted by the TG - LiBr solutions is precipitable by adjusting the pH values of the extracts to the point of maximum precipitation (about pH 5.5).

TABLE 7
Extraction of Supercontracted Hair With 0.47*M* Sodium
Thioglycolate as a Function of pH

Sample	pH		Original wt., g.	Weight after extraction, g.	Percentage extraction
	Initial	Final			
1	8.59	8.44	1.0023	0.9990	0.2
2	9.03	8.98	1.0002	0.9046	9.5
3	9.50	9.45	1.0003	0.6028	39.7
4	10.00	9.97	1.0007	0.4777	52.2
5	10.52	10.42	1.0010	0.3593	64.1

The results of the fourth series of experiments, in which hair was submitted to a rather drastic supercontraction procedure before extracting with aqueous TG at five different pH values, show that this physically modified hair has substantially the same extractability as untreated hair (Table 7).

Extractability of Hair With Alkaline Buffers as a Function of Extent of Reduction of the Hair

The established fact, that the amount of protein extracted from hair by alkaline TG solutions increases with the pH of the solution used, suggested the possibility of fractionation of hair proteins by reduction with a neutral solution of TG followed by extraction of the reduced hair with a series of buffers of increasing alkalinity. Experimentally, however, it was found that such a procedure resulted in negligible extraction of protein when the hair had been reduced at pH 6.68. This finding prompted an investigation of the actual extent of reduction of hair by TG over the pH range of 2 to 8.5 and the subsequent extractability of these reduced hair samples by phosphate buffer at pH 11.0.

Procedure: Approximately one gram samples of hair were weighed accurately into 125 ml. Erlenmeyer flasks and smaller samples (unweighed, for blocking and cystine analysis immediately after removal of TG) were placed in test tubes. Solutions, each 0.47 *M* in TG and adjusted to different pH values, were added to the hair samples: 75 ml. to the weighed samples and 25 ml. to the unweighed samples. After 48 hours the solutions were decanted from the flasks, their pH values determined, and the solutions discarded. Each hair sample was rinsed for a few seconds with 20 ml. water, the latter discarded, and 50 ml. of phosphate buffer at pH 11.02 added. Over a period of 48 hours each sample was extracted successively with three 50 ml. portions of phosphate buffer (pH 11.02) and three 50 ml. portions of water. The hair samples were then repeatedly rinsed with water until the pH values of the rinsings were below 10, and the still rather swollen hair samples were then lyophilized, moisture equilibrated with air, and weighed. Two such series were run at different times. In the first series the smaller samples, in the test tubes, were rinsed with water after 48 hours in the TG solutions, then 25 ml. aqueous ethylene oxide was added (30 g. ethylene oxide plus 100 g. water) in order to block the free sulfhydryl groups in the reduced hair, the sample drained after 24 hours, rinsed several times with water, and finally air-dried. These samples were then hydrolyzed in sealed tubes with 6 *N* HCl at 118-120° C. for six hours, and the cystine contents of the hydrolyzates were determined by the method of Shinohara (16). The extent of reduction was estimated from the differences in cystine content between the original hair lot and the reduced and blocked samples. In the second series the procedure was the same except that the blocking solution was a mixture of 20 ml. aqueous ethylene oxide and 10 ml. phosphate buffer at pH 6.98. Only in the second series are the cystine analyses considered reliable. (In the absence of strong buffering in the neutral range reaction between

ethylene oxide and sulfhydryl groups causes a sharp increase in pH which disintegrates the reduced hair samples (17)). (Table 8)

TABLE 8

Extraction of Hair at pH 11.02 After Reduction With 0.47M Sodium Thioglycolate at Various pH Values

Sample	pH of reduction		Reduction level, %	Percentage extraction
	Initial	Final		
1	1.96	2.20	54.8	13.5
2	2.89	2.95		11.8
3	2.90	2.88	61.7	11.7
4	5.02	4.88	71.5	8.19
5	5.41	5.18		8.57
6	6.94	5.75		8.18
7	8.45	8.34		7.57
8	8.48	8.21	83.6	5.86
9	...*			0.00

Discussion: The data presented in Table 8 show clearly that the extent of alkaline extraction is not a function of the degree of reduction over the range of 55 to 84% reduction. While the differences in amount extracted by alkali are small they are consistent: more protein is extracted at lower degrees of reduction. The minimum amount extracted (5.9%) was from the sample with the maximum degree of reduction (84.6%). Why, then, does extraction with alkaline TG increase steeply as the pH is increased over the next 1.5 pH units? At pH 10 is reduction substantially complete? Is complete reduction a prerequisite for high degrees of extraction? These questions are not easily answered since the difficulties of determining the degree of reduction in a sample, a large part of which is streaming into solution, are formidable. Also, what is the significance of the increasing alkaline extraction of samples of decreasing degrees of reduction? In view of these results it cannot be assumed that the mechanism of the solution of hair protein in alkaline TG is simply reduction followed by alkaline solution of the reduced components.

Influence of Temperature on the Extraction of Hair With Alkaline Thioglycolate and on the Nature of the Extracted Fractions

Since the extraction of 50 to 75% of the hair substance requires TG solutions at pH 10 to 11.5 the question is raised as to whether or not some alkaline hydrolysis of peptides may take place under these conditions. Dialysis of extracts and degree of precipitability of protein from extracts have been used as rough screening methods for detection of low molecular weight fragments. Since some of the latter may be natural components of the fibers the mere presence of dialyzable nitrogen or of non-precipitable nitrogen would not demonstrate peptide hydrolysis, but differences in these values as a function of temperature would be significant.

Procedure: Duplicate one gram samples of hair were weighed into 125 ml. Erlenmeyer flasks. To each flask was added 50 ml. of a 0.47 M

* Not reduced, but extracted with the phosphate buffer at pH 11.02.

TG solution at pH 9.29, 10.00, or 10.92. After 48 hours at 26-28° C. the solutions were decanted into volumetric flasks and 25 ml. water was added to each hair sample. At approximately ten hour intervals four aqueous extracts were decanted from each hair sample into the appropriate volumetric flask containing the original TG extract. Characteristically, the pH values of successive aqueous extracts increase considerably before they decline. For the samples, in the order of increasing pH value of the original TG solution, the pH values of successive aqueous extracts were: 9.53, 9.93, 10.52, 10.98; 10.38, 10.81, 11.34, 11.38; 11.61, 11.77, 11.70, 11.27. Each collective solution was then diluted volumetrically for subsequent sampling. One hundred ml. of each solution, in a dialysis tube, was placed in a one liter cylinder containing 900 ml. water. After sampling the remainder of each solution for total nitrogen determination (micro-Kjeldahl procedure) each solution was carefully acidified by drop-wise addition of glacial acetic acid.

- A. pH 9.59; still clear at pH 8.7; opalescent at pH 7.1; flocculated at pH 5.95.
- B. pH 10.75; only faint opalescence at pH 6.6; increased opalescence but no precipitate at pH 5.82; flocculated at pH 5.55.
- C. pH 11.80; opalescence did not develop until pH 4.90; flocculated at pH 4.72.

After each precipitate had settled the supernatants were again sampled for total nitrogen. After 99 hours of dialysis duplicate aliquots of dialysand were taken for nitrogen analysis. Finally, the hair residues were repeatedly washed with water and lyophilized before weight loss determination. A second series of experiments was conducted in exactly the same manner except that all operations were carried out in a refrigerator at 1-2° C. (Table 9).

Discussion: In all of the earlier experiments the values for percentage weight loss were taken to be equivalent to the percentage extraction of protein. It is worth noting that the values for percentage extraction (weight loss) given in Table 9 agree well with the values for percentage of total nitrogen extracted.

While less nitrogen is extracted at 1-2° C. than at 27-28° C. several observations point to the conclusion that degradation (peptide hydrolysis) is appreciably greater in the latter case. The percentage of nitrogen extracted which is precipitable is consistently higher at 1-2° than at 27-28°; conversely, the amounts of nitrogen dialyzable in the 1-2° extracts are consistently lower than the amounts of nitrogen dialyzable in the 27-28° extracts. At the higher temperature while the percentage of nitrogen extracted which is precipitable first increases, then decreases, the absolute difference between nitrogen extracted and nitrogen precipitated increases sharply (20.5, 25.4, 67.1 mg.). At 1-2°, however, the absolute difference between the nitrogen extracted and the nitrogen precipitated is almost constant (6.5, 6.1, 6.8 mg.) while the percentage of the nitrogen extracted which is precipitable rises from a minimum of 61% (for only 11% extraction) to a maximum of 91% (for 48% extraction). These facts suggest either a certain amount of naturally occurring polypeptides of low molecular weight, or amide nitrogen readily susceptible to alkaline hydrolysis. In neither series, however, could all

TABLE 9
Extraction With 0.47*M* Sodium Thioglycolate at 27-28° C.

Sample	Initial pH of extraction	Percentage extraction	Nitrogen extracted mg.	%*	Nitrogen pptd. by pH adjustment mg.	% of N extd.	Nitrogen dialyzed mg. % of N extd.
1	9.29	20.5	27.5	18.0	7.0	25.5	11.8
2	10.00	63.9	96.5	63.1	71.1	73.6	42.9
3	10.92	76.6	112.7	73.9	45.6	40.5	14.0
							13.7
4	9.22	8.27	16.5	10.7	10.0	60.6	15.2
5	9.92	31.9	50.5	33.0	44.4	87.9	2.50
6	10.86	51.6	73.3	47.8	66.5	90.7	5.24
							2.75
							3.75

* Hair contained 16.8% N, dry basis, and 9.7% moisture; one g. hair, as is, contained 153 mg. N.

of the non-precipitable nitrogen be assigned to ammonia arising from hydrolysis of side-chain amide groups, for all of this ammonia would dialyze readily. The results of this study certainly indicate that all extractions should be carried out at temperatures as low as possible.

Aqueous Extraction as a Function of pH of Hair Oxidized With Peracetic Acid

Procedure: Five hair samples were treated with 50 ml. portions of 2.4% peracetic acid in stoppered 125 ml. Erlenmeyer flasks at 27-28° C. for 21 hours. The five solutions were decanted, together with a small water rinse in each case, into the same 500 ml. volumetric flask. Each hair sample was then watered to about 50 ml. and adjusted to a selected pH value with 0.15 N NaOH. The pH values given in Table 10 are approxi-

TABLE 10

Extraction of Hair, Oxidized With Peracetic Acid, With Water as a Function of pH

Sample	pH of Solution		Original wt., g.	Wt. after extraction, g.	Percentage extraction	Nitrogen in extract after pH adjustment, mg.*
	Initial	Final				
1	7.0	5.52	1.08	0.40	62.9	56.5
2	8.0	6.58	1.04	0.19	81.7	99.8
3	9.1	6.92	1.05	0.18	82.9	109.3
4	10.0	7.88	1.07	0.13	87.8	110.8
5	11.0	8.68	1.05	0.08	92.3	116.6

mate since the interaction of the aqueous alkali with the fibers from which excess peracetic acid (or acetic acid) was diffusing continuously for some time tended to keep the pH values going down slowly. After 20 hours an unsuccessful attempt was made to separate the greatly swollen, but still distinctly fibrous, residues by centrifugation. Accordingly, the separation was made by gravity filtration through a glass wool plug and the residues were similarly washed, though not very efficiently, with water. Each residue was lyophilized. Each alkaline solution and its corresponding water washings were collected in a volumetric flask, aliquoted for nitrogen determination, and the five extracts combined. Upon acidification with acetic acid no permanent turbidity appeared until the pH reached 4.5; between pH 4.1 and 4.0 the protein precipitated in flocculent form. This precipitate, after washing with water by settling and decantation, was redissolved to give a perfectly clear solution by raising the pH value to 5.1.

Discussion: The oxidation of keratin fibers with peracetic acid was first investigated by Alexander (18) who claimed that "... in the reaction with wool only the oxidation of cystine is significant." This statement is based, however, on the findings of Toennies (19) that the

* The five, combined peracetic acid solutions, decanted from the fibers before pH adjustment of the latter, contained 49.0 mg. nitrogen; each sample, therefore, lost 9.8 mg. nitrogen during the peracetic acid treatment.

only natural amino acids which react with performic acid are cystine, methionine and tryptophan. More recently Earland and Knight (20) have recognized that peracetic acid "... is not, in fact, completely specific for oxidizing the cystine residues in keratin." In this connection it should be noted that in the present investigation 7.1% of the nitrogen of the hair showed up in the peracetic acid solution before any upward pH adjustment was made. This indicates either a degradation product resulting from non-specific oxidation, or a very soluble modified protein component. The question of specificity to one side, the data in Table 10 certainly show that protein is extracted in large quantities and at relatively low pH values from hair which has been treated with peracetic acid. There are at least two possible reasons for this increase in extractability, either or both of which may be operative. First, more of the cystine is oxidized (about 90%) by peracetic acid than is reduced by thioglycolate (maximum measured value of 84%). Second, the conversion of the cystinyl disulfide links to sulfonic acid groups greatly increases the acidity of the protein; this is clearly shown experimentally by the lower pH value to which solutions must be adjusted in order to precipitate the modified protein.

Literature Cited

1. GEIGER, WALTON B. 1944. J. Research Natl. Bureau Standards **32**:127.
2. MERCER, E. H. and OLOFSSON, BERTIL. 1951. J. Polymer Sci. **6**:671.
3. WARD, W. H. 1952. Textile Research J. **22**:405.
4. MIDDLEBROOK, W. R. 1951. Biochim. Biophys. Acta **7**:547.
5. GILLESPIE, J. M. and LENNOX, F. G. 1953. Biochim. Biophys. Acta **12**:481.
6. FRASER, R. D. B. 1953. Biochim. Biophys. Acta **12**:482.
7. FRASER, R. D. B. and ROGERS, G. E. 1953. Biochim. Biophys. Acta. **12**:484.
8. GILLESPIE, J. M. and LENNOX, F. G. 1955. Australian J. Biol. Sci. **8**:97.
9. HARRAP, B. S. 1955. Australian J. Biol. Sci. **8**:122.
10. GILLESPIE, J. M. and LENNOX, F. G. 1955. Australian J. Biol. Sci. **8**:378.
11. GILLESPIE, J. M. and LENNOX, F. G. 1957. Australian J. Biol. Sci. **10**:95.
12. GILLESPIE, J. M. 1957. Australian J. Biol. Sci. **10**:105.
13. GILLESPIE, J. M. Proc. International Wool Textile Research Conf., Melbourne **1955B**:35.
14. GILLESPIE, J. M. 1958. Biochim Biophys. Acta **27**:225.
15. JENSEN, C. C. Private communication.
16. SHINOHARA, K. J. 1935. Biol. Chem. **112**:683.
17. DANEHY, J. P. J. Am. Chem. Soc.: in press.
18. ALEXANDER, P., HUDSON, R. F. and FOX, M. 1950. Biochem. J. **47**:27.
19. TOENNIES, G. and HOMMILLER, R. P. 1942. J. Am. Chem. Soc. **64**:3054.
20. EARLAND, C. and KNIGHT, C. S. 1956. Biochim. Biophys. Acta **22**:405.