that two protons are bound in the expansion,[23] *i.e.*

$$N_\alpha + 2H^+ \rightleftharpoons N_\beta \cdot 2H^+$$

Gibbs[17] found the slope of his plot of log rate *versus* pH to be two in this pH range and postulated that loss of two protons from the low pH form must precede denaturation.

As attractive as this mechanism is, it leaves unanswered several important questions, notably the apparent increase in rate below pH 2, seen both in our data and in that of Levy and Warner[16] and of Gibbs.[17] Further study of the process by combined use of precipitation, viscosity and optical rotation should lead to clarification of such points.

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(23) This is readily demonstrated by plotting the logarithm of the apparent equilibrium constant *versus* pH, the experimental slope being −1.8. We are here comparing results on bovine plasma albumin with results on the human protein. The data of Levy and Warner[16] on the bovine protein show a pH dependence in better accord with the binding of three hydrogen ions.

It should be pointed out that this substantial agreement of the pH dependence on kinetics and equilibrium could be fortuitous. Strictly speaking, it only shows that the transition complex in the irreversible process has a charge similar to or identical with that of the unexpanded form. On the other hand, the fact that the plasma albumins are rather unusual in showing the low pH expansion, and are also unusual in that they denature less rapidly in acid solution than at the isoelectric point, seems significant.

[CONTRIBUTION NO. 1973 FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY]

The Amino Acid Composition of Certain Morphologically Distinct Parts of White Turkey Feathers, and of Goose Feather Barbs and Goose Down

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Chromatographic analyses have established that differences exist in the amino acid composition of morphologically distinct parts of white turkey feathers. Furthermore, it is probable that different types of feathers from a given species of bird are identical in amino acid composition but that feathers from different species of birds are dissimilar. The high content of proline in feather proteins makes it unlikely that the polypeptide chains of these proteins assume simple pleated sheet or helical configurations.

Feather rachis keratin has long been known to produce a complicated and well-defined X-ray diffraction pattern[1] and hence to possess a highly oriented structure. Rudall[2] has found that essentially the same X-ray pattern which is given by the rachis is also shown by the barbules, barbs, calamus and medulla of the feather (Fig. 1 of the present paper may be consulted for a definition of the various feather parts). However, if one examines the individual feather parts under a polarizing microscope, the rachis appears to be highly oriented but the other parts are much less so or virtually unoriented. Furthermore, although these parts of a feather arise from the *stratum intermedium*, one need not conclude that they must be identical in composition. Thus, the work of Lillie[3] and collaborators proves that the various portions of the papilla from which the *stratum intermedium* arises are highly specific in their ability to produce the individual parts of a feather: the barbs cannot be considered to be branches of the rachis nor the calamus an elongation of it. This information, therefore, lends credence to the possibility which arises on mere visual inspection that, perhaps, the morphologically distinct parts of a feather may not be identical in composition.

As part of an investigation of the structure of feather keratin, we have made an exact quantitative determination of the amino acid content of four distinct parts of a feather in order to determine whether or not the composition is uniform. White turkey feathers have been used for analysis because the absence of color simplified the determinations and because the size of the feathers facilitated their sectioning. In addition, less exact analyses have been made of goose feather barbs and of goose down in order to ascertain (1) whether species differences exist and (2) whether various types of feathers from the same kind of bird are identical in composition.

(1) W. T. Astbury and T. C. Marwick, *Nature*, **130**, 309 (1932).

(2) K. M. Rudall, *Biochim. Biophys. Acta*, **1**, 549 (1947).

(3) F. R. Lillie, *Biol. Rev.*, **17**, 247 (1942). This review of Lillie as well as the papers of R. M. Strong (*Bull. Mus. Comp. Zool. Harvard*, **40**, 147 (1902)) and H. R. Davies (*Morph. Jahrb.*, **15**, 560 (1889)) may be consulted for a description of entire developmental history of a feather

Experimental

Materials.—Figure 1 presents a drawing of a typical white turkey feather and a definition of terms. In this figure, the feather is viewed from the *dorsal* side, that is, the side which the feather presents to the outside world; the opposite side toward the bird itself is termed the *ventral* side. The portion of the feather from R to A is above the skin level of the bird and that from C to A is below. The *rachis* bears the *barbs* which in themselves are complex structures and support *barbules*. The distal barbules of each barb possess hooklets which interlock with the proximal barbules of the adjoining distal barb. This interlocking of the barbules maintains the form of the feather. If the hooklets are absent, the feather is fluffy as is often the case near the junction of rachis and calamus. Throughout its length, the rachis is filled with a pithy cellular substance of low apparent density which is termed the *medulla*. The barbs also contain medullary material which does not join that of the rachis. The interior dorsal side of the rachis contains grooves which extend from R approximately to A; like the rest of the interior, these grooves are packed tightly with the medulla. The rachis is thickest on the dorsal side and very thin at the junction with the barbs and at the bottom of the ventral groove. The *calamus* is essentially cylindrical but slightly thickened on the dorsal side. The interior of the calamus contains pulp caps, the origin of

which is described in detail by Lillie[3]; the outside is covered with a sheath which is the last vestige of the *stratum corneum*.

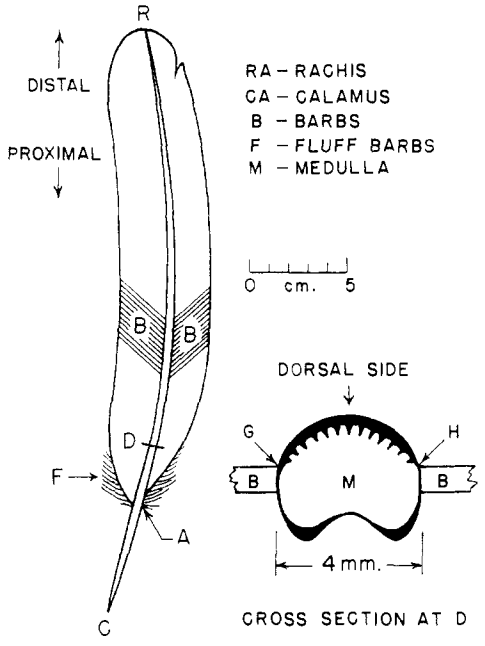

Fig. 1.—Anatomy of white turkey feather; view from dorsal side.

The white turkey feathers, obtained in April, 1954, were mature feathers from immature birds. The feathers which were used for analysis may have been obtained from more than one bird. They were superficially degreased by soaking in benzene for 1/2 hour and were then dried in air. The dissection of the feathers was most conveniently done by means of a jeweler's saw and a surgical scalpel.

The *barbs* were cut off as close to the rachis as possible and the fluffy portion was discarded. The barbs (225 mg.) from one feather were sufficient for this work.

The *calamus* was cut off 1.5 cm. below the barbs and the very thin, waxy sheath was removed by scraping with a fingernail. It was then split dorsiventrally and the pulp caps were removed and discarded. The pulp caps were easily removed except in the lower 7 mm. of the calamus so this portion was cut off and discarded. Two feathers were required to give an adequate sample of calamus.

The *rachis* was sampled from the dorsal side (between points G and H of Fig. 1) between 12 and 24 cm. from the distal end. The medulla was scraped from between the grooves with the point of a surgical scalpel and, likewise, the outside surface was scraped off because the orientation in the thin outer layer is said to be different[4] from that of the main body of the rachis. Two feathers were used in order to obtain an adequate sample.

The *medulla* was cut and scraped from the inside of the rachis. The medulla from three feathers was required.

The *goose feather barbs* were cut from the rachis with scissors, and the fluffy portion was discarded.

Goose down was used whole. The goose feathers and goose down were white; they were taken from European geese and were not degreased.

All samples except the goose down were cut into small pieces and thoroughly mixed. Table I shows the nitrogen, sulfur, moisture and ash contents of these samples.

Acidic Hydrolyses.—Samples of 30 or 50 mg. of feather parts were hydrolyzed in 10 ml. of refluxing doubly distilled 6 *N* hydrochloric acid for periods of 24 or 73 hr. The 24-hr. hydrolysates were all light yellow or brown in color and the intensity of color was not greatly different after 73 hr. of hydrolysis.

At the end of the hydrolysis, the hydrochloric acid was evaporated *in vacuo* at 50–60°, a few ml. of water was added and evaporated, and the residue was transferred with 0.1 *N* hydrochloric acid to a volumetric flask. A portion was used immediately and the remainder was stored at 0–5°.

(4) W. T. Astbury and F. O. Bell, *Tabulae Biologicae*, **17**, 90 (1939).

TABLE I

PERCENTAGE OF NITROGEN, SULFUR, MOISTURE AND ASH IN FEATHER PARTS[a]

Feather part	Nitrogen[b] Kjeldahl	Dumas	Sulfur[b]	Moisture[c]	Ash
Turkey barbs	16.42	16.48	2.48, 2.54	7.76, 7.69	1.34, 1.39
Turkey calamus	17.34	17.44	2.68, 2.59	8.04, 8.15	1.88, 1.79
Turkey medulla	16.98	17.06	2.47, 2.53	9.08, 8.99	3.48, 3.44
Turkey rachis	16.73	16.87	2.57, 2.54	9.66, 9.57	0.97, 0.90
Goose barbs	17.40	17.48	3.32, 3.28	7.78, 7.69	2.71, 2.74
Goose down	16.44	16.50	3.33, 3.38	5.83, 5.91	5.83, 5.75

[a] Microanalyses by Dr. Adalbert Elek. [b] In moisture- and ash-free protein. [c] At 100–105°.

Oxidation of the Proteins and Subsequent Acidic Hydrolysis.—The oxidation of the proteins with performic acid and their subsequent acidic hydrolysis was required for the determination of cystine as cysteic acid according to the method of Schram, Moore and Bigwood.[5] Thirty-mg. samples of protein were oxidized for 16 hr. exactly as these authors[5] describe and were then hydrolyzed for 20 hr. in 10 ml. of double distilled 6 *N* hydrochloric acid. After removal of the hydrochloric acid *in vacuo*, water was added and evaporated, and the residue was transferred to a volumetric flask with water. These hydrolysates were yellow in color and contained dark brown suspended matter. A portion was used immediately and the remainder was stored at 0–5°.

Chromatographic Methods.—The chromatographic determination of the amino acid composition of the feather parts was made by means of the starch[6] and ion-exchange[7] methods of Moore and Stein. Their procedures were used with relatively minor modification.

The 100-cm. columns[7] of Dowex 50-X 8 were used for the determination of aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine and valine. Cystine may also be determined but the quantity cannot be considered a reliable indicator of the actual amount in the protein because of losses during hydrolysis. Portions of hydrolysate corresponding to 2.5 to 3 mg. of protein were used and were placed on the column in 2 or 3 ml. of *p*H 2 buffer. These chromatograms were run at 37–38° with *p*H 3.42 buffer until the emergence of valine and were then reconditioned for further use with sodium hydroxide followed by buffer. They were not used for the determination of amino acids which emerge after valine. The detergent, BRIJ 35, and thiodiglycol were not added to the buffer.

Histidine, lysine, ammonia and arginine were estimated by means of 15-cm. columns of Dowex 50-X 8 with no modifications of procedure. The sample of hydrolysate corresponded to 6 to 7 mg. of protein and was placed on the column in 2 or 3 ml. of *p*H 4.25 buffer.

Starch chromatograms[6] developed with *n*-butanol–benzyl alcohol–water were used for the determination of phenylalanine, leucine, isoleucine, methionine, tyrosine and valine. These chromatograms were maintained at 15° in order to achieve a satisfactory separation of tyrosine and valine.[8] The sample was equivalent to 2.5 to 3 mg. of protein and was made up for chromatography in the usual manner.[6]

Cystine was determined as cysteic acid by the method of Schram, Moore and Bigwood.[5] Chromatography on Dowex 2-X 10 was done as they describe except that BRIJ 35 was omitted from the developer. The yield in a control oxidation of cystine as a check on the procedure averaged 93% as compared to 90% obtained by the above authors. Consequently, our values for cystine have been corrected by assuming a 93% yield of cysteic acid from cystine in the protein. The sample consisted of about 3 mg. of oxidized and hydrolyzed protein and was applied to the column in 1 ml. of aqueous solution.

Analysis of Effluent Fractions.—The quantity of amino acid in each effluent fraction was estimated by means of the

(5) E. Schram, S. Moore and E. J. Bigwood, *Biochem. J.*, **57**, 33 (1954).

(6) W. H. Stein and S. Moore, *J. Biol. Chem.*, **176**, 337 (1948).

(7) S. Moore and W. H. Stein, *ibid.*, **192**, 663 (1951).

(8) W. A. Schroeder, L. M. Kay and J. C. Wells, *ibid.*, **187**, 221 (1950).

photometric ninhydrin method of Moore and Stein.[7,9] Previous experience with this procedure[8] had shown that the color yields reported by Moore and Stein[7,9] were applicable without modification; one exception was cysteic acid which gave a color yield of 0.95 under our conditions instead of 1.01.[5] As recommended by Moore and Stein,[9] the color yield of ammonia was checked with the same batch of reagent which was used whenever a zone of ammonia was encountered on a chromatogram. As a further check on the reliability of our use of these methods, mixtures of known quantities of amino acids were chromatographed and the recoveries were calculated. The results, in general, were satisfactory and the average recovery was 99%. Histidine, however, showed consistent recoveries of 90%; check of the color yield showed satisfactory agreement with that of Moore and Stein.

Identification.—The identification of the amino acids in these hydrolysates rests upon the chromatographic behavior of the zones as compared to that of known amino acids except in the case of proline where the reddish-brown color with ninhydrin is an obvious aid in identification. Although it is possible that such uncommon substances as citrulline, aminoadipic acid and α-amino-*n*-butyric acid could interfere with certain portions of the chromatogram on the 100-cm. columns of Dowex 50,[7] it is more probable that the identification of zones both on the 100-cm. columns and on the starch columns is correct. However, on the 15-cm. columns of Dowex 50, lysine might be confused with ornithine and, likewise, histidine with hydroxylysine (ref. 7, p. 676). It is assumed that lysine and histidine are present in the proteins but, because of the small amounts, no attempt at certain identification has been made.

Results

Table II presents the results of the amino acid analyses of the white turkey feather parts, and of goose feather barbs and goose down.

Each of the turkey feather parts has been subjected to one 24-hr. hydrolysis and one 73-hr. hydrolysis. In all of these hydrolysates, a single determination of each amino acid has been made. The individual amounts so obtained are listed under the times of hydrolysis. The final values for the most part are the averages of the individual results from the two times of hydrolysis. However, for reasons which will be discussed below, the final values in certain instances are not averages. Thus, the data from the 24-hr. hydrolysate only are used for the methionine and proline of all parts and for threonine in calamus and medulla. The final value for valine is the average of the quantities found in the 73-hr. hydrolysate by both starch and ion exchange chromatography. An extrapolation of the 24- and 73-hr. results to zero time gave the final values for serine and ammonia.

Only a single 24-hr. hydrolysis of goose feather barbs and goose down was made. Because of difficulties during the running of the 100-cm. columns, the determinations were duplicated. Where the duplicate values are acceptable, the final value is an average but, in all other instances, it is the result of a single hydrolysate.

Despite the fact that our recoveries with known histidine are low, the data for histidine in Table II have not been corrected because the amount of this amino acid in the feather parts is very minor and the determinations themselves, thus, are subject to a more than normal error. No correction for loss of glutamic acid has been made.[7]

Although the number of significant figures which have been retained in the data of Table II is greater than is justified, they have been kept because rounding off would in some instances increase the apparent similarity or dissimilarity of the results.

Discussion

Chromatographic Aspects of the Results.—No difficulty was experienced in determining the contents of alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, proline, tyrosine, valine and ammonia. These amino acids, in general, produced well-formed and well-separated zones.

Cystine itself in hydrolysates of unoxidized protein formed a well-separated but wide zone between alanine and valine. Cystine, when determined directly in this way, amounted to 80 to 90% of the quantity which was found by oxidation to cysteic acid. The separation of cysteic acid from other constituents of the oxidized and hydrolyzed protein was excellent on the columns of Dowex 2.

The identification of the methionine is definitely suspect. The material which emerged at the position of known methionine has been calculated as methionine. However, this zone was minute and ill-defined and the optical densities in the ninhydrin procedure did not rise more than a few hundredths above the blank.

Approximately 90% of the sulfur is accounted for in terms of cystine and methionine. Inability to account for 10% of the sulfur may lie in several sources of error. If cystine itself is oxidized to cysteic acid and determined chromatographically, the recovery is 93% under our conditions. When the cystine content of a protein is calculated, it is corrected to take into account this incomplete recovery. However, if the correction factor is different for a protein than for cystine itself, the determination of cystine and the sulfur balance will be in error. The estimation of methionine in these proteins may be very inaccurate and this fact would also be reflected in the sulfur balance. Finally, no attempt has been made to determine whether inorganic sulfur is present in these materials. In view of the high ash content of certain parts, it may well be there.

Zones of known phenylalanine and of phenylalanine from the hydrolysates had distorted leading edges which were not improved by increasing the quantity of 8-hydroxyquinoline in the pretreatment of the columns.[6] This distortion apparently resulted from some abnormal behavior of the starch because well-formed zones appeared when another portion of the same sample of starch was used in some of the final chromatograms.

The separation of threonine and serine on the 100-cm. columns was not entirely satisfactory. Usually, the corrected optical density of the valley between threonine and serine was not less than one-half of the optical density at the peak of threonine. As a result, the quantity of threonine has been calculated by the method for badly overlapping zones (ref. 6, p. 351). When thus calculated, the results are approximately 10% higher than when the usual procedure for slightly overlapping zones is used.[10]

(9) S. Moore and W. H. Stein, *J. Biol. Chem.*, **176**, 367 (1948).

(10) S. Moore and W. H. Stein, *ibid.*, **178**, 53 (1949); see p. 64.

TABLE II

THE AMINO ACID COMPOSITION OF WHITE TURKEY FEATHER PARTS, AND OF GOOSE FEATHER BARBS AND GOOSE DOWN

(Values are in terms of g. of amino acid per 100 g. of moisture- and ash-free material.)

Amino acid	Turkey barbs: Time of hydrolysis 24 hr.	Turkey barbs: Time of hydrolysis 72 hr.	Turkey barbs: Final value[a]	Turkey calamus: Time of hydrolysis 24 hr.	Turkey calamus: Time of hydrolysis 72 hr.	Turkey calamus: Final value[a]	Turkey medulla: Time of hydrolysis 24 hr.	Turkey medulla: Time of hydrolysis 72 hr.	Turkey medulla: Final value[a]	Turkey rachis: Time of hydrolysis 24 hr.	Turkey rachis: Time of hydrolysis 72 hr.	Turkey rachis: Final value[a]	Goose barbs: 24-hr. hydrolysis	Goose barbs: Final value[a]	Goose down: 24-hr. hydrolysis	Goose down: Final value[a]	Literature[d]
Alanine	4.01	3.98	4.01	7.38	6.86	7.12	5.94	5.83	5.89	7.85	7.47	7.66	4.11, 4.08	4.10	4.01, 3.91	3.96	1.8[e]-G
Arginine	6.48	6.40	6.44	6.79	6.58	6.69	6.49	6.61	6.57	6.25	6.10	6.18	6.04	6.04	6.52	6.52	7.5[j]-C; 5.0[h]-G; 6.0[g]-H
Aspartic acid	6.21	6.89	6.55	7.17	7.01	7.09	7.00	7.02	7.01	7.38	7.43	7.41	(6.40),[c] 7.47	7.47	7.31, 7.20	7.26	7.0[j]-C; 1.1[e]-G; 7.5[f]-H; 6.57[m]-S
Cystine[b]	8.63	8.72	8.68	8.33	8.24	8.29	8.11	8.06	8.10	8.69	8.27	8.48	10.84, 10.66	10.75	11.37, 11.25	11.31	8.2,[j] 7.2,[k] 7.6[l]-C; 9.2,[k] 8.8 and 12.2[n]-D; 6.4,[h] 9.1 and 10.7[n]-G; 6.8[g]-H; 7.1–8.9[n]-T
Glutamic acid	9.15	9.00	9.08	8.92	8.55	8.74	8.35	8.85	8.60	9.21	8.47	8.84	9.03, 8.94	8.99	8.96, 9.15	9.06	9.7,[j] 12.3[l]-C; 2.3[e]-C; 9.4[f]-H; 9.72[m]-S
Glycine	7.27	7.22	7.25	9.72	9.47	9.60	8.78	9.01	8.90	10.26	10.01	10.14	8.38, 8.37	8.38	7.19, 7.33	7.26	2.6[e]-G; 9.5[g]-H
Histidine	0.38	0.40	0.39	0.54	0.64	0.59	0.78	0.78	0.78	0.27	0.40	0.34	0.44	0.44	0.33	0.33	0.4[j]-C; 0.35[h]-G; 0.3[g]-H
Isoleucine	5.05	4.91	4.98	3.86	4.01	3.94	3.77	4.15	3.96	3.92	3.88	3.90	4.58	4.58	4.76	4.76	6.0[j]-C
Leucine	7.24	7.27	7.26	8.70	8.99	8.85	8.13	8.00	8.07	9.32	9.41	9.37	7.68	7.68	7.74	7.74	8.0[j]-C; 8.0[e]-G
Lysine	1.16	1.29	1.23	0.87	1.08	0.98	1.31	1.33	1.32	0.87	0.89	0.88	1.30	1.30	1.41	1.41	1.3[j]-C; 1.04[h]-G; 1.6[g]-H
Methionine (?)	0.36	0.20	0.36	0.34	...	0.34	0.44	0.30	0.44	0.39	...	0.39	0.25	0.25	0.32	0.32	0.5[j]-C
Phenylalanine	4.90	5.01	4.96	5.44	6.07	5.76	5.52	5.65	5.59	5.97	5.53	5.75	4.04	4.04	3.80	3.80	5.2[j]-C; 5.3[g]-H
Proline	10.50	11.32	10.50	10.98	10.48	10.98	10.87	10.22	10.87	10.97	9.70	10.97	(10.69),[c] 10.05	10.05	9.81, (11.49)[c]	9.81	8.8[j]-C; 3.5[e]-G
Serine	12.25	10.92	12.90	13.43	10.00	15.09	11.53	9.79	12.37	12.90	10.44	14.09	12.69, 12.37	12.53	12.69, 12.06	12.38	0.4[e]-G; 10.2[f]-H
Threonine	4.70	4.65	4.68	4.73	3.77	4.73	4.35	3.78	4.35	4.61	4.41	4.51	5.36, 4.52	4.94	5.90, 4.94	5.42	4.4[j]-C; 4.6[f]-H
Tyrosine	2.46	2.18	2.32	3.78	4.15	3.97	3.63	3.98	3.81	3.10	2.71	2.91	4.46	4.46	3.69	3.69	2.2[j]-C; 3.6[e]-G; 2.2[g]-H; 3.2[i]-?
Valine																	
Ion exchange	7.68	8.82	}	7.49	8.32	}	6.95	8.60	}	7.29	8.51	}	(6.54),[c] 7.36	}	7.92, 7.76	}	8.3[j]-C; 0.5[e]-G
Starch	8.24	8.38	} 8.60	7.82	8.54	} 8.43	7.40	8.58	} 8.59	8.12	8.79	} 8.65	7.31	} 7.34	8.00	} 7.89	
Ammonia	1.96	2.19	1.85	1.65	2.10	1.43	1.60	2.11	1.35	1.71	2.17	1.49	1.91	1.91	1.89	1.89	
Total			102.0			112.6			106.6			112.0		105.3		104.8	
% of wt. accounted for			86.9			95.5			90.5			94.8		89.6		89.3	
% of N accounted for			91.1			93.5			90.2			96.0		88.1		93.1	
% of S accounted for			95.6			86.4			90.0			91.4		88.5		92.0	

[a] The considerations which lead to these final values are presented in the text under "Results." [b] Duplicate determinations of cysteic acid from one oxidation and 20-hr. hydrolysis. Calculated as cystine and corrected as described in the text. [c] Values in parentheses were stricken from further consideration, mainly because of chromatographic difficulties. [d] Type of feather—C, chicken; D, duck; G, goose; H, hen; S, sea gull; T, turkey. [e] E. Abderhalden and E. R. LeCount, *Z. physiol. Chem.*, **46**, 40 (1905). [f] R. J. Block and D. Bolling, "The Amino Acid Composition of Proteins and Foods," 2nd Ed., Charles C Thomas, Springfield, Ill. [g] R. J. Block, D. Bolling, F. C. Brand and A. Schein, *J. Biol. Chem.*, **128**, 181 (1939). [h] R. J. Block and H. B. Vickery, *ibid.*, **93**, 113 (1931). [i] O. Fürth and A. Fischer, *Biochem. Z.*, **154**, 1 (1924). [j] C. E. Graham, H. K. Waitkoff and S. W. Hier, *J. Biol. Chem.*, **177**, 529 (1949). [k] C. B. Jones and D. K. Mecham, *Arch. Biochem.*, **3**, 193 (1943). [l] H. S. Olcott, *J. Biol. Chem.*, **153**, 71 (1944). [m] J. B. Speakman and F. Townend, *Nature*, **139**, 41 (1937). [n] R. H. Wilson and H. B. Lewis, *J. Biol. Chem.*, **73**, 543 (1927).

It is of interest to note that on our sample of resin, proline forms a well-separated peak almost midway between glutamic acid and glycine but tending slightly toward glycine. This behavior should be contrasted with that observed by Moore and Stein,[7] Smith and Stockell,[11] and Simmonds[12] who found that proline and glutamic acid formed closely adjacent or overlapping zones.

Effect of Time of Hydrolysis.—The analyses of insulin by Harfenist[13] and of carboxypeptidase[11] and papain[14] by Smith and co-workers have emphasized the inaccuracies which may enter into a determination of amino acid composition unless more than one time of hydrolysis is used. In the present investigation, therefore, the turkey feather parts were hydrolyzed for two different periods, namely, 24 and 73 hr. When the results of the amino acid analyses on these two hydrolysates are compared, the agreement of results is excellent. The two values from a given feather part usually differ by less than 6% in the cases of alanine, arginine, aspartic acid, glutamic acid, glycine, isoleucine, leucine, phenylalanine and tyrosine. Even when the absolute amount is small, as, for example, histidine and lysine, few determinations differ by more than 10%. Among these eleven amino acids, the higher result is observed as frequently from a 24-hr. as from a 73-hr. hydrolysate, that is, the distribution is random. The conclusion may be reached that extended hydrolysis is without influence on these amino acids and also that the precision of the analytical method is very satisfactory.

Extended hydrolysis definitely affects the amounts of serine, valine and ammonia and perhaps also the amount of proline. These results are not unexpected because of the known stability of peptide bonds involving the carboxyl group of valine,[15,16] because of the destruction of serine during hydrolysis,[17] and because of the increase of ammonia on continued hydrolysis. One cannot unequivocally conclude that destruction of proline occurs although in three of the four feather parts the quantity is less in the 73-hr. hydrolysate; for this reason, the results from the 24-hr. hydrolysates have been taken as the final values in Table II.

Smith and co-workers[11,14] have observed the destruction of serine, threonine, aspartic acid and lysine on extended hydrolysis in both carboxypeptidase and papain and also of glutamic acid in papain. Hirs, Stein and Moore[18] detected the destruction of aspartic acid, glutamic acid, serine, threonine, proline, tyrosine and arginine on long hydrolysis of ribonuclease. The present results show destruction of serine and probably proline but there is no evidence for destruction of the other amino acids listed by the above authors; threonine may have been destroyed in the present work but, because of the poor separations from serine, the results lack the necessary accuracy for definite decision. It is apparent that the composition of the protein itself will greatly influence the destruction of the component amino acids and that some decomposition of serine and threonine may almost certainly be expected.

In Table II, the final values of serine and ammonia in the turkey feather parts were obtained by straight line extrapolation to zero time. Because only one determination was made in each hydrolysate, such an extrapolation is unquestionably subject to considerable error but the extrapolated result is no doubt more nearly correct than either of the individual ones. The data on valine from the 73-hr. hydrolysates should give a satisfactorily accurate measure of this amino acid because the increase over the amounts in the 24-hr. hydrolysates was relatively slight. It should be noted that under our conditions the hydrolysis of these proteins was essentially complete in 24 hr.

General Composition of the Proteins.—The most abundant amino acids in feather proteins are serine, glycine and proline; the first two each constitute about 15% of the residues and the proline about 10%. Alanine, valine and leucine each compose about 8% of the residues and isoleucine about 4%. Approximately equal residues of aspartic acid and glutamic acid are present and they total about 12%. The ammonia content would suggest that in the barbs most of the acidic amino acid residues are in the form of glutamine and asparagine while in the other parts only about 75% are in this form. Of the basic amino acids, arginine alone is present in appreciable amount. The residues of basic amino acids total only about half the number of the residues of the acidic amino acids.

Table II lists the literature values for the amino acid content of various types of feathers. The data of the present work generally agree with the results obtained by relatively modern methods of analysis.

Comparison of the Amino Acid Composition of the White Turkey Feather Parts.—The primary objective of the present investigation has been to determine whether or not the various parts of a feather differ in amino acid composition. However, before worthwhile consideration of similarities or dissimilarities can be made, it is necessary to decide (1) the extent by which two results must differ in order to be significantly different, and (2) the manner in which the results should be compared in order to eliminate the effect of extraneous influences.

The determination of the amino acid composition of known mixtures[6,7] and of proteins[11,13,14,18] by the chromatographic methods employed in the present work would indicate that a single estimation of a given amino acid may be inaccurate to the extent of ±3 to 5%. Thus, if a single value of a given amino acid in two materials is compared, the quantities could be identical and yet appear to differ by 6 to 10% or they could differ by 6 to 10% and appear to be identical. When duplicate determinations of a given amino acid fall well within the

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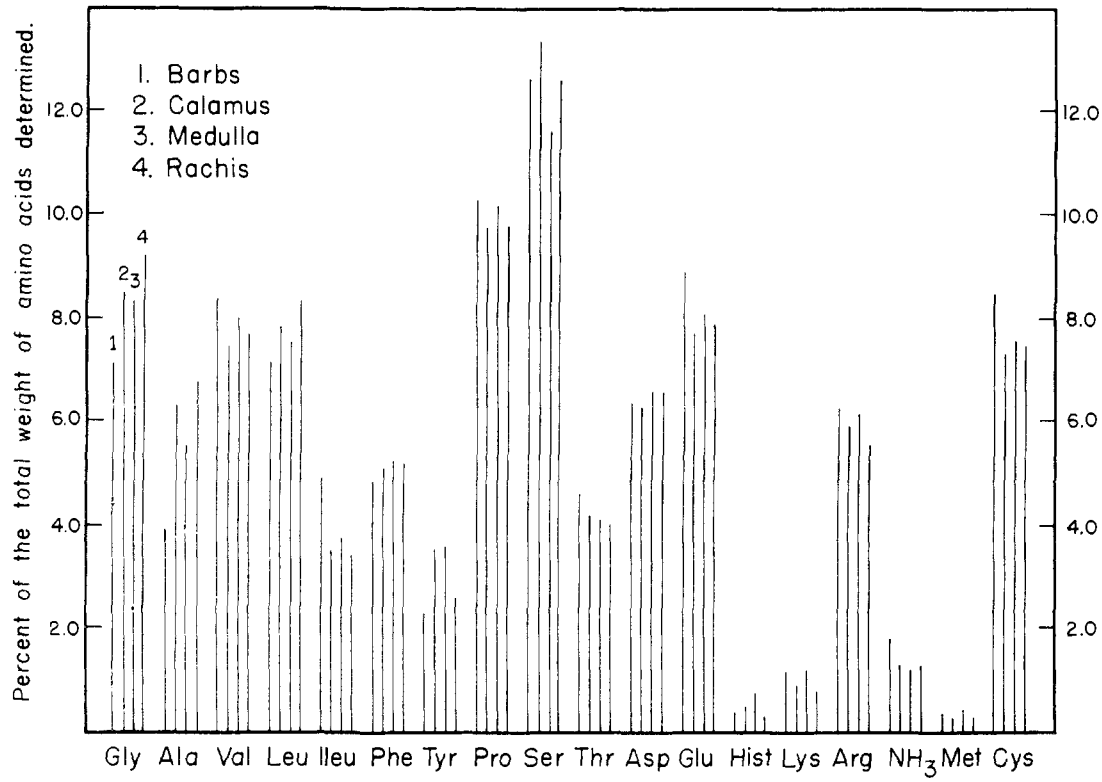

Fig. 2.—Comparison of the amino acid composition of white turkey feather parts.

range of ±3 to 5%, the average of the two must give a rather accurate picture of the actual content. The accuracy of the average value is even better if single determinations from two hydrolysates agree well. As mentioned above, the agreement of our single determinations in two hydrolysates of the same material is excellent and, with few exceptions, is well within the limits of ±3 to 5%. Accordingly, it might be suggested that the results are accurate ±2 or 3%. However, the replication of determinations is far too small to allow one to assume such accuracy and we have concluded that the contents of a given amino acid in two parts of the feather must differ by at least 10% before the difference can be considered significant.

In Table II, the data are presented in the commonly used system of g. of amino acid per 100 g. of protein. From these data, the percentages of weight, nitrogen, and sulfur accounted for have been calculated. It is apparent that the accounting is more complete in the calamus and rachis than in the barbs and medulla. It is reasonable to conclude that these differences arise largely because of the presence of greater amounts of cell debris in the barbs and medulla: thus, if the various parts are examined microscopically, it is difficult to discern any cell outlines in the calamus and rachis but they are very apparent in the medulla and to some extent also in the barbs which in themselves possess a medulla. Apparently, then, the barbs and medulla contain greater amounts of non-proteinaceous material. If, therefore, we compare the amino acid compositions on the basis of g. of amino acid per 100 g. of material, the basis of comparison will be false because varying percentages of the total will have been accounted for in the different parts. Furthermore, comparison of such derived data as g. of residue per 100 g. of protein, g. of nitrogen per 100 g. of protein, or residues of amino acid per 10^5 g. of protein will likewise be unsatisfactory. On the other hand, comparison is satisfactory on the basis of percentage of the total weight of amino acids determined. Thus, if we compared the amino acid composition of a pure protein with that of the protein component of a 1:1 mixture of this same protein and carbohydrate, it is apparent that, on the basis of g. of amino acid per 100 g. of material, the protein component of the mixture would appear to have a different composition from that of the pure protein. However, on the basis of the percentage of the total weight of amino acids determined, the identity of the pure protein with the protein of the mixture would be evident. Accordingly, the data on the turkey feather parts have been recalculated on the basis of percentage of the total weight of amino acids determined and the results have been plotted in Fig. 2.

Examination of Fig. 2 shows that each amino acid is present to some extent in each part, that the composition of the parts is very similar, but that some differences greater than 10% occur. On closer study, it is apparent that the calamus and rachis are identical in composition within the limits mentioned above, with the exception of the tyrosine content and possibly the histidine content. Furthermore, the medulla resembles the calamus and rachis very closely but the barbs are appreciably dissimilar. In Table III the final conclusions are presented.

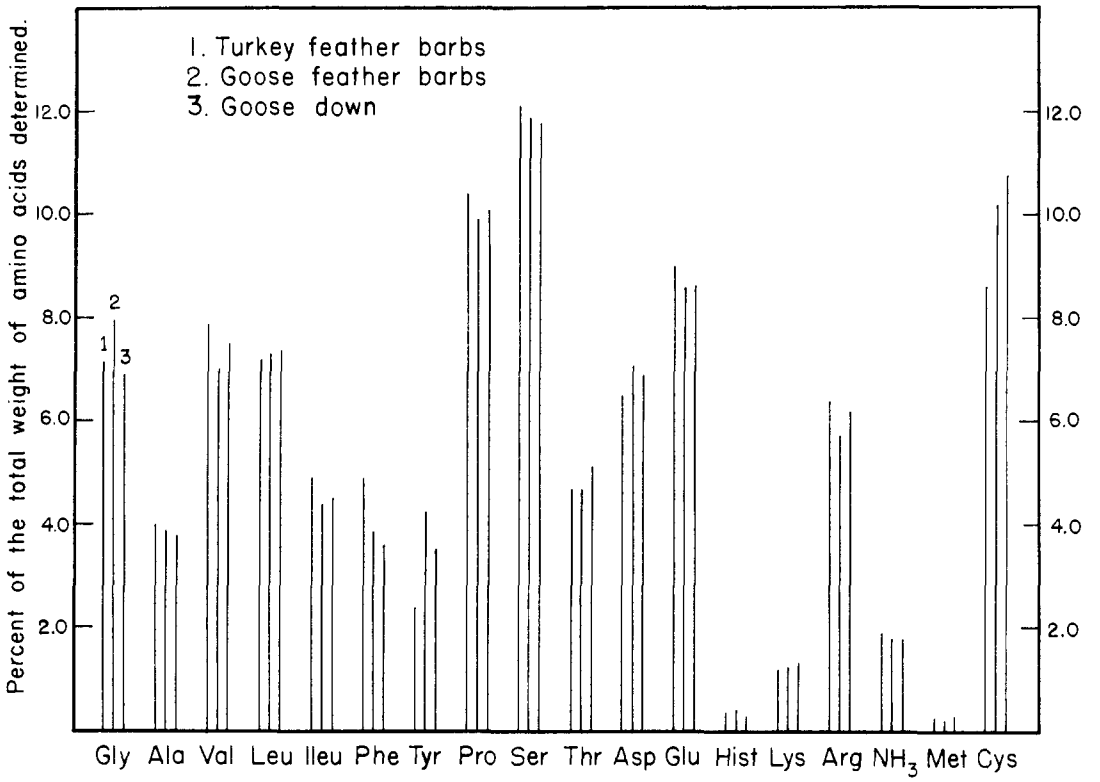

Fig. 3.—Comparison of the amino acid composition of turkey feather barbs, goose feather barbs and goose down.

TABLE III

CONCLUSIONS ABOUT THE AMINO ACID COMPOSITION OF WHITE TURKEY FEATHER PARTS

Identical	Different
Aspartic acid	Alanine—B[a] < M < C = R
Phenylalanine	Ammonia—B > C = M = R
Proline	Cystine—B > C = M = R
.....	Glutamic acid—B > C = M = R
Arginine	Glycine—B < C = M = R
Methionone	Isoleucine—B > C = M = R
Serine	Tyrosine—B = R < C = M
Threonine	.....
Valine	Histidine—B = R ≤ C < M
	Leucine—B < C ≈ M ≈ R
	Lysine—B = M > C = R

[a] B = barbs; C = calamus; M = medulla; R = rachis.

For some amino acids, the conclusion can be made that the quantities are identical or that they differ by at least 10%; these are listed above the dotted lines under the headings of Table III. Those amino acids which are listed below the dotted lines have been placed in the indicated categories but their placement is less certain for various reasons. Thus, arginine, leucine and valine are borderline cases which are most reasonably placed as shown. Methionine probably is present in the same amount in all parts but because of the small amount and the poor analyses the agreement may be fortuitous. The extrapolation which is required to arrive at the final values for serine tends to introduce a larger than normal error; hence, it is reasonable to conclude that the quantities are identical. The determination of threonine is rather unsatisfactory and the agreement may be fortuitous. The excellent agreement of the duplicate values of histidine and lysine in the various parts would lead one to conclude that the quantities differ but this conclusion must be treated with some reserve because of the small absolute amounts of these amino acids.

Comparison of the Amino Acid Composition of Turkey Feather Barbs, Goose Feather Barbs and Goose Down.—A secondary objective of the present work was to determine whether or not there were species variations in feather composition and whether or not there were variations in different types of feather from the same species. In order to ascertain the effects of these variations, goose feather barbs and goose down were chosen for comparison with turkey feather barbs. Barbs and down were selected because of morphological similarities and also because the barbs are easily separated from the remainder of the feather.

The results are recorded in Table II. Only one hydrolysis of the goose barbs and goose down has been made and, in general, only a single determination of most of the amino acids. As a result, the comparison must be somewhat less exact than in the case of the turkey feather parts. However, it will again be assumed that a difference of at least 10% is necessary to be significant. Comparison on the basis of per cent. of the total weight of amino acids determined is shown in Fig. 3. Since only a 24-hr. hydrolysis of the goose barbs and goose down was made, the comparison in Fig. 3 is made with the results of the 24-hr. hydrolysate of turkey barbs.

Inspection of Fig. 3 reveals only two possibilities (namely, glycine and tyrosine) in which the goose

barbs and down may differ. However, both instances are borderline and because duplicate hydrolyses of goose barbs and down were not made, one cannot conclude with confidence that differences exist: probably they do not. Thus, in all likelihood, appreciable dissimilarities in the amino acid content of the two types of goose feathers are negligible.

On the other hand, it would appear that turkey barbs differ from goose barbs and down in content of cystine, glycine, isoleucine, phenylalanine, proline, tyrosine and valine. However, the differences in glycine, isoleucine, proline and valine are borderline and probably are not significant. It seems quite definite, however, that turkey feather barbs contain less cystine and tyrosine and more phenylalanine than goose feather barbs or goose down. The sulfur contents (Table I) substantiate the conclusion about differences in cystine content. Thus, species variations are indicated.

Conclusions

The results of the present investigation show that the morphologically distinct parts of a white turkey feather differ in amino acid composition. The barbs are most distinctly different from the other parts and the contents of alanine, glycine, isoleucine and tyrosine are most manifestly dissimilar. Although one cannot assume *a priori* that feathers of other birds will show similar variations from part to part, it is apparent that in studies of so-called "feather keratin" definite portions rather than the whole feather should be investigated if the results are to be meaningful.

Although the data are less extensive, it may tentatively be concluded that different types of feathers from a given species of bird probably do not vary greatly in amino acid content but, on the other hand, some variation in amino acid composition of feathers from different species seems to be probable. This conclusion is also suggested by the work of Wilson and Lewis[19] on the cystine content of feathers from several types of birds.

The general amino acid composition of the feather proteins is such that about 10% of the residues are proline and another 40% are composed of amino acids with small side chains, that is, glycine, alanine and serine. Serine and threonine with their polar side chains make up 20% of the residues. If any attempt is to be made to interpret the complex X-ray diffraction pattern of the rachis in terms of the structure of the protein, the general amino acid composition must be kept in mind. For example, it is of interest to note that a proline residue will profoundly influence the configuration of a polypeptide chain and that the feather proteins contain one residue of proline in ten. Therefore, if the proline residues are at all randomly distributed in the feather keratin, it is highly unlikely that the polypeptide chains could assume any of the pleated sheet or simple helical configurations,[20,21] which have thus far been suggested.

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The Amino Acid Composition of *Bombyx mori* Silk Fibroin and of Tussah Silk Fibroin

BY W. A. SCHROEDER AND LOIS M. KAY WITH THE TECHNICAL ASSISTANCE OF BARRY LEWIS AND NANCY MUNGER

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Chromatographic determinations of the amino acid content of *Bombyx mori* silk fibroin and of Tussah silk fibroin (*Antheraea pernyi*) have revealed marked differences in composition. Although glycine, alanine, serine and tyrosine account for about 90% of the residues in both fibroins, the major difference lies in the almost exact reversal of the amounts of glycine and alanine in one fibroin as compared to the other. Some of the minor constituents also are present in very different amounts.

The composition and structure of the silk fibroin of *Bombyx mori* has been the subject of study and speculation over a period of many years. One evidence of this interest lies in the many fragmentary determinations of amino acid composition which have been described in the literature. It is, therefore, the more surprising to find that apparently the only complete analysis of a single sample of silk fibroin in one laboratory was made by Tristram who cites his own unpublished results in two review articles.[1] Bergmann and collaborators[2-4] determined certain amino acids in a sample of silk fibroin and portions of this sample were apparently used by many other workers who report that they obtained silk fibroin from Bergmann and co-workers: thus, a complete analysis of this sample seems to have been made.

The common cultivated silkworm, *Bombyx mori*, feeds on mulberry leaves and produces a silk which after degumming is almost white. Tussah silk, however, is produced by wild silk-

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