triethanolamine and bismuth triethanolamine are reported. These compounds represent new types of complex bismuth salts, and their pharmaceutical action is being investigated.

CORNELL UNIVERSITY
ITHACA, N. Y.

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[CONTRIBUTION FROM THE BIOCHEMICAL LABORATORY, STATE UNIVERSITY OF IOWA]

Studies in the Composition of Human Hair

BY RICHARD C. CLAY, KENNETH COOK AND JOSEPH I. ROUTH

It seems well established that there are differences in the chemical composition of various keratins. Eukeratins, *e. g.*, wool and hair, are distinguished from pseudokeratins in that their basic amino acids (histidine, lysine, and arginine) are found in a relatively constant molecular ratio of 1:4:12.[1] On the other hand, the proportions of their non-basic amino acids show wide variations, particularly with respect to cystine, a high content of which is characteristic of keratins. Whether secondary factors, such as age and sex, give rise to further variations is less definitely established.

As early as 1806[2] an attempt was made to correlate the pigmentation of human hair with variations in its sulfur content. Rutherford and Hawk[3] studied the relation of age, sex, pigmentation, and race to the chemical composition of hair. Conflicting results have since been reported by many investigators. For example, from the analyses of twenty-three samples of hair from children (thirteen years and under) and six adults, Wilson and Lewis[4] could deduce no consistent relation between the cystine content and color, age, or sex, although there was an "apparent tendency" for the values to be larger in adult hair. With reference to the many previous studies, the general statement seems valid that actual relations were not definitely established because of the small number of samples, the relatively wide dispersion of the data, and the small obtained differences. At times deviations from the mean were almost as great within the groups as between them.

This paper represents an extended investigation of the cystine, cysteine,[5] nitrogen, and sulfur content of human hair.

(1) R. J. Block, *J. Biol. Chem.*, **121**, 761 (1937).
(2) L. N. Vauquelin, *Ann. chim. phys.*, **58**, 41 (1806).
(3) T. A. Rutherford and P. B. Hawk, *J. Biol. Chem.*, **3**, 459 (1907).
(4) R. H. Wilson and H. B. Lewis, *ibid.*, **73**, 543 (1927).
(5) Some doubt exists that cysteine is a constituent of the protein molecule. However, work now in progress in this Laboratory indicates that cysteine is a primary hydrolysis product of keratins. The values reported here are to be regarded as minimal.

Experimental

The hair was cleaned and defatted by washing in benzene, dried in a vacuum oven for three to five hours at 65–70°, and stored in a vacuum desiccator over phosphorus pentoxide until analyzed. Total nitrogen was determined by the macro-Kjeldahl method. Total sulfur was determined gravimetrically by the Benedict–Denis method after preliminary oxidation with concentrated nitric acid, as suggested by Wilson and Lewis.[4]

Cystine was determined by the Sullivan[6] and Shinohara[7] methods, and cysteine by the methods of Shinohara[8] and Lavine.[9] The hair was hyrolyzed with 20% hydrochloric acid for ten hours in an electric oven at 124–127°. The hydrolysates were decolorized with kaolin.

Discussion

The results of analyses of 120 samples of human hair are summarized in the table; they are averages of determinations on at least two portions of each hair sample. Uniform methods of analysis were used throughout and, insofar as was possible, the variables were limited to age, sex, and pigmentation. The range of values for each of the constituents is given in the table. There was considerable variation within the groups, this being particularly true of the cystine and cysteine values.

The presence of cysteine as shown by the Shinohara method is not in agreement with the observations of Wilson and Lewis,[4] who did not obtain a reduction of the uric acid reagent without the addition of sodium sulfite. Perhaps the explanation of this discrepancy lies in variations in procedure. Their more thorough extraction (total of ninety-six hours) and drying (several days at 100°) may have destroyed the small amount of cysteine present. Further, in agreement with the observations of previous workers,[10,11] we have found that the cysteine content of the hydroly-

(6) W. C. Hess and M. X. Sullivan, *J. Biol. Chem.*, **117**, 423 (1937).
(7) K. Shinohara, *ibid.*, **112**, 683 (1936).
(8) K. Shinohara, *ibid.*, **109**, 665 (1935).
(9) T. F. Lavine, *ibid.*, **109**, 141 (1935).
(10) Y. Okuda, *J. Dept. Agr. Kyushu Imp. Univ.*, **1**, 4 (1925).
(11) B. Kassel and E. Brand, *J. Biol. Chem.*, **125**, 435 (1938).

TABLE I

RELATION OF AGE, SEX AND PIGMENTATION TO CHEMICAL COMPOSITION OF HUMAN HAIR

Samples		1–14 years Light	1–14 years Dark	15–29 years Light	15–29 years Dark	30 years and over Light	30 years and over Dark	Range of values
	Male	12	8	12	11	6	9	
	Female	11	9	12	7	8	15	
				Cystine, %				
Shinohara	Male	16.2 ±0.3[a]	17.1 ±0.3	17.0 ±0.3	17.6 ±0.2	16.5 ±0.3	16.8 ±0.2	14.0–18.2
	Female	15.2 ± .4	16.3 ± .3	15.3 ± .3	15.7 ± .4	15.5 ± .7	15.5 ± .1	12.6–18.4
Sullivan	Male	14.6 ± .3	15.2 ± .5	14.5 ± .4	15.2 ± .6	14.9 ± .4	14.5 ± .4	11.2–16.3
	Female	12.8 ± .6	13.4 ± .3	13.1 ± .4	14.3 ± .5	13.9 ± .5	13.8 ± .3	9.4–16.5
				Cysteine %				
Shinohara	Male	0.49 ±0.05	0.57 ±0.02	0.51 ±0.05	0.48 ±0.02	0.61 ±0.05	0.57 ±0.03	0.21–0.80
	Female	.51 ± .04	.51 ± .05	.44 ± .02	.42 ± .04	.47 ± .05	.47 ± .03	.23– .84
Lavine	Male	.57 ± .03	.59 ± .05	.59 ± .03	.58 ± .03	.67 ± .04	.62 ± .03	.27– .82
	Female	.53 ± .04	.54 ± .04	.47 ± .03	.47 ± .04	.48 ± .04	.49 ± .04	.23– .72
Nitrogen	Male	16.07 ±0.16	15.40 ±0.18	15.61 ±0.15	15.48 ±0.10	15.26 ±0.13	15.41 ±0.18	14.81–16.83
	Female	15.42 ± .12	15.51 ± .10	15.29 ± .11	14.96 ± .12	15.63 ± .21	15.33 ± .08	14.68–16.46
Sulfur	Male	5.34 ± .03	5.38 ± .09	5.30 ± .07	5.43 ± .06	5.10 ± .17	5.25 ± .05	4.54–5.67
	Female	5.30 ± .06	5.40 ± .08	5.27 ± .11	5.13 ± .06	5.20 ± .12	5.23 ± .05	4.76–5.78

[a] Standard error of the mean.

sates decreases on standing. From their paper it cannot be determined whether this was a factor in their negative findings.

There are few data on the cysteine content of human hair available for comparison. Using the iodate titration method Okuda[12] found 0.44%, and Tadokoro and Ugami[13] 0.72–1.90% cysteine in Japanese hair. As determined by both the Shinohara and Lavine methods, the cysteine values reported in the present paper showed an approximate range of from 0.2 to 0.8%.

Of the many possible inter-relationships among the variables under consideration, a few of the more significant ones perhaps should be mentioned. There was no consistent relation between the composition of hair and age. In part this may have been due to the relatively small number of samples in each sub-group. A comparison of the average values in the table showed that there was more cystine and cysteine in male hair than in the hair of females. Dark hair contained more cystine than did light hair; the cysteine values were the same. A partial analysis of the data indicated that these differences were statistically significant (S. R. greater than 3.0).[14]

Six samples of red hair (ages 20 to 34) and two of female albino hair (ages 18 and 30) showed no striking differences from hair of other colors except that the cystine of the albino hair (13.99 and 12.42%) was lower than the average for light hair (15.29%).

Summary

The cystine, cysteine, nitrogen, and sulfur content of 120 samples of human hair was determined. The relation of age, sex and pigmentation to the composition of hair was studied. No consistent relation between age and composition was found. There was more cystine and cysteine in male hair than in female hair. Dark hair contained more cystine than did light hair. These differences were statistically significant. No significant variations in the nitrogen and sulfur content were observed.

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(12) Y. Okuda, *Proc. Imp. Acad. Jap.*, **5**, 246 (1929).

(13) T. Tadokoro and H. Ugami, *J. Biochem. (Japan)*, **12**, 187 (1930).

(14) S. R. (significance ratio) = obtained difference/standard error of difference.