TOTAL SULFUR, CYSTINE, AND METHIONINE CONTENT OF BLOOD GLOBINS OF FIVE MAMMALIAN SPECIES*

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The interest of this laboratory in the composition of the erythrocyte has been centered upon the stromal portion of the cell. However, the hemoglobin, which makes up by far the largest portion of the cell solid, is of great importance in determining the composition of the whole erythrocyte unit. Amino acid analyses of the protein moiety of the stromal materials obtained from five mammalian species failed to show the existence of any significant species variations in composition (1). Identity of the hemoglobins of mammalian bloods has been proved with reference to their heme groups, their molecular weights, and their basic amino acid composition (2). Nevertheless, hemoglobins are known to differ among the various mammalian species because they give specific immune reactions and they also differ in their crystal form. Inasmuch as the heme groups are identical, differences among the hemoglobins must find their basis largely in differences in the amino acid composition of the globins.

It has long been known that the sulfur contents of hemoglobins of different species vary. Therefore one or both of the sulfur-containing amino acids must exist in different quantities in the various globins. Cystine analyses of three hemoglobins have been carried out by Vickery and White (3). If one subtracts the cystine sulfur from the total sulfur of these three hemoglobins, a constant residual sulfur is not obtained, indicating that not only cystine but also methionine may exist in different quantities in

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the different globins. Therefore the globins of five mammalian species have been studied with respect to the distribution of their total sulfur between cystine and methionine.

EXPERIMENTAL

Erythrocytes of horse, hog, beef, sheep, and human bloods were freed of serum proteins and leucocytes. The cells were hemolyzed and the insoluble posthemolytic residue removed by high speed centrifugation. The resulting hemoglobin solutions were saturated with carbon monoxide and the globins precipitated according to the method of Anson and Mirsky (4), by the addition of acetone containing oxalic acid.

The dried globins containing oxalic acid were then analyzed for total nitrogen by the macro-Kjeldahl method; for oxalic acid and for total sulfur gravimetrically after combustion in the oxygen bomb. The proteins were also analyzed for methionine by the volatile iodide method of Baernstein (5) after a preliminary removal of oxalic acid as its calcium salt from hydrochloric acid-digested globin samples. Analyses for cystine content were carried out by the micro cysteine-cuprous mercaptide method of Graff, Maculla, and Graff (6) and checked by the polarographic method (7). The analytical data were all recalculated to an oxalic acid-free basis to obtain the composition of the free globin.

DISCUSSION

The results of the total nitrogen, total sulfur, and cystine and methionine analyses are shown in Table I. The nitrogen content of the preparations varied from 15.73 to 16.58 per cent. The total sulfur values varied widely, ranging from 0.37 to 0.60 per cent. Variations were found in the cystine contents of some of the globins, although three of the five contained nearly identical quantities of this amino acid. The variations in cystine content of the globins are stepwise in character. The beef globin contained the least cystine, or 0.38 per cent. The sheep, hog, and horse globins contained approximately twice this quantity of cystine, while human globin contained 1.21 per cent cystine, or approximately 3 times as much cystine as did the beef globin.

To obtain a check upon the validity of the cystine values obtained by the Graff, Maculla, and Graff method, the analyses of the

globins[1] were repeated by a modification (7) of the polarographic method of Brdicka. In the Graff method the cystine is estimated from the nitrogen content of the sulfhydryl compounds precipitated by cuprous oxide, while the polarographic method is based on the interpretation of current voltage curves obtained with the hydrolysates in the presence of cobaltous chloride and ammonia in a cell in which the cathode consists of mercury falling in small drops from a glass tube with a very narrow capillary (8). Good checks between the cystine values yielded by the two methods were found. It is significant in establishing the values for cystine content of these proteins that the physical method and purely chemical method yield results in close agreement.

TABLE I

Composition (Per Cent) of Globins

	N	Total S	Cystine	Methionine	Cystine S	Methionine S	Cystine + methionine S	Per cent total S determined
Beef	15.73	0.43	0.38	1.26	0.10	0.27	0.37	87
Sheep	16.11	0.56	0.81	1.22	0.22	0.26	0.48	85
Horse	16.40	0.40	0.85	0.75	0.23	0.16	0.39	96
Hog	16.50	0.37	0.79	0.75	0.21	0.16	0.37	100
Human	16.58	0.60	1.21	1.23	0.33	0.26	0.59	97

Horse, sheep, and dog hemoglobins have been analyzed for cystine by Vickery and White (3). The cystine content of 0.60 per cent for their preparation of sheep hemoglobin is in fair agreement with the one reported in the present paper. The cystine content of horse hemoglobin of 0.4 per cent obtained by Vickery and White is approximately half the value found in the present experiment and about equal to the cystine content found in beef globin. These discrepancies in cystine content are less surprising in view of the fact that wide individual variations in the sulfur content of globins from the same species have been observed by several investigators (9, 10). The dog hemoglobin, reported by Vickery and White to contain 1.16 per cent cystine, checks the value of 1.21 per cent found for human globin in this study.

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With reference to methionine the five mammalian globin preparations fall into two groups. Horse and hog globins were found to contain 0.75 per cent methionine, while beef, sheep, and human globins contained 1.24 (average). Together, the cystine and methionine sulfur determined from the results of the amino acid analyses accounted for from 85 to 100 per cent of the total sulfur of the preparations, as shown in Table I.

Using the molecular weight of globin and its per cent composition with respect to cystine and methionine, we have calculated the molecular distribution of cystine and methionine in the globins. Adair (11) has determined the molecular weight of hemoglobin as

TABLE II

*Molecular Composition of Globins with Respect to Cystine and Methionine**

	Weight per gm. mole globin		Moles per mole globin	
	Cystine	Methionine	Cystine	Methionine
	gm.	*gm.*		
Beef	247	811	1.0	5.4
Sheep	520	786	2.2	5.3
Horse	546	483	2.3	3.2
Hog	510	483	2.1	3.2
Human	730	792	3.2	5.3

* Assuming the molecular weight of globin to be 64,400 (11).

66,800. By subtracting 2400 (which is the approximate weight of four heme groups), one obtains 64,400 as the molecular weight of the globin part of the molecule. In Table II the moles of cystine (mol. wt. 240) and of methionine (mol. wt. 149) contained in 1 molecule of globin (mol. wt. 64,400) are shown. It will be seen that the beef globin contained 1 molecule of cystine per molecule of globin, or the least amount of cystine possible in a molecule of that weight. (If, however, the amino acid exists as cysteine in the native protein, a protein of molecular weight 64,400 conceivably could contain half as much cysteine as did our sample of beef globin.) The sheep, hog, and horse globins contained 2 molecules of cystine, and human globin contained 3 molecules of cystine. With reference to methionine the globins fall into two groups. The horse and hog globins contained 3 molecules of methionine, while the beef, sheep, and human contained approxi-

mately 5 molecules. The molecular ratios do not calculate to whole numbers when the physically determined molecular weight of 64,400 is used. A value of 60,000 for the molecular weight of globin would yield molecular values within ±0.1 of a molecule for all the preparations.

The results clearly demonstrate that the globin preparations obtained from five mammalian species differ from each other in composition with respect to the sulfur-containing amino acids. The differences in the content of certain amino acids such as cystine and methionine in the different globins may account for the differences in behavior of hemoglobins of different species or possibly of individuals of the same species. This is in agreement with the statement of Block (12) concerning various hemoglobins, "Thus of the constituents analyzed, only sulfur and cysteine vary and these in molecular proportions. This variation may account not only for differences in crystalline form but also for differences in rate of denaturation, change in molecular weight in urea solution, etc."

SUMMARY

The cystine, methionine, and total sulfur contents of globins from the bloods of five mammalian species were determined and found to vary. These results suggest that the specificity of hemoglobins depends upon differences in their amino acid composition.

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