THE BASIC AMINO ACIDS OF SERUM PROTEINS

III. A CHEMICAL RELATIONSHIP BETWEEN SERUM PROTEINS OF VARIOUS ORIGINS

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The importance of a classification of proteins based on amino acid composition has been previously pointed out (1, 2). A measure of success has been achieved only with the keratinoid tissues and the protamines (3). The protamines, moreover, seem to form a special class of proteins. This leaves the keratins as the only true proteins which have been classified on a purely chemical basis. Block and Vickery (2) showed that keratins of widely differing origins yielded on acid hydrolysis amounts of histidine, lysine, and arginine in the molecular ratios of approximately 1:4:12 (2, 4). In every case the material analyzed comprised the entire protein portion of the tissue. In other words, the keratinoid tissues contained histidine, lysine, and arginine in a remarkably constant proportion although the actual percentage of these bases present in the tissue varied as much as 1000 per cent.

On the other hand, it has been indicated that the albumins and globulins obtained from cattle serum by precipitation with various neutral salts are not of constant basic amino acid composition but are simply artificial products produced by the reagents employed in their preparation. However, in every case, the more soluble protein fractions (albumins) obtained by this technique yielded more lysine than the less soluble fractions (globulins). These studies indicated that if the albumin-globulin determinations as carried out in the clinic were real estimations of the amounts of albumin and globulin present in a serum, then two sera with

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dissimilar albumin-globulin contents should yield unlike amounts of lysine. We have found that this is not the case.

Our previous investigations indicated that a better characterization of serum protein could be effected by a study of the whole protein rather than by attempting to isolate any "pure" preparation even though it might be crystalline.

**EXPERIMENTAL**

*Preparation of Serum Protein*—The freshly drawn blood was centrifuged and a small aliquot of serum was removed for the estimation of total protein, albumin, and globulin by the phosphate method of Howe (5). The remainder of the serum was poured into 10 volumes of ice-cold acetone, allowed to stand at 4° for 5 hours, and the precipitate was centrifuged off. The protein was washed successively with hot acetone, benzene, alcohol, and ether. It was then dried at 110° for 24 hours. The ash content was not determined because of the small amounts of material at our disposal.

*Preparation of Urine Protein*—The urine was heated to boiling and dilute acetic acid was carefully added until no further precipitate occurred. The protein was washed with water, organic solvents, and dried at 110°.

*Determination of Basic Amino Acids*—Histidine, arginine, and lysine were isolated as the diflavianate, flavianate, and picrate respectively in the manner previously described (4).

**Results**

The analytical data are summarized in Table I. When these results are considered in the light of our previous investigations on cattle serum (1), we may draw the following conclusions.

1. Although the amount of total protein in various samples of blood serum may vary 100 per cent, its basic amino acid composition is constant.

2. In spite of the fact that our previous investigations have shown that the more soluble (albumin) fractions of the serum protein always contain the higher amounts of lysine, nevertheless even with 10-fold variations in the amount of albumin, the basic amino acid yield of the total serum protein remains the same.

3. The urinary protein from a patient with nephrosis closely
resembles the serum protein (6, 7) and is not "nearly all albumin" as has been stated in a recent text (8).

4. The basic amino acids yielded by acid hydrolysis of three kinds of mammalian serum proteins are constant both in absolute and relative amounts. Thus we have another instance of a tissue protein which, like the keratins, may be classified according to

| TABLE I |
|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
|                  | Amount of serum protein | Albumin | Globulin | Ratio albumin to globulin | Amount of protein hydrolyzed | Basic amino acids | Molecular ratio arginine to lysine |
| Source of protein | per cent              | per cent | per cent | g/ml. | per cent | g/ml. | per cent | per cent | per cent | per cent | per cent |
| Human serum, normal | 6.99               | 4.24 | 2.75 | 1.51:1 | 2.67 | 14.7 | 1.0 | 4.3 | 6.2 | 10:17 |
| Human serum, female | 7.09               | 4.41 | 2.68 | 1.58:1 | 2.67 | 14.7 | 1.0 | 4.5 | 6.9 | 10:18 |
| Human serum, nephrosis, female | 3.35 | 0.44 | 2.91 | 0.15:1 | 1.54 | 14.5 | 0.9 | 4.8 | 6.8 | 10:17 |
| Human urine, nephrosis, male | 3.00 | 15.2 | 6.9 | 10:18 |
| Dog 1, serum, normal | 7.80               | 4.07 | 3.73 | 1.10:1 | 2.10 | 14.5 | 0.9 | 4.7 | 7.2 | 10:18 |
| Dog 2, serum, normal | 6.88               | 4.29 | 2.59 | 1.65:1 | 1.24 | 14.7 | 0.7 | 4.9 | 7.3 | 10:18 |
| Dog 3, serum, normal | 6.60               | 3.31 | 3.29 | 1.00:1 | 1.16 | 14.6 | 0.8 | 4.9 | 7.0 | 10:17 |
| Cow serum, normal    | 7.80               | 9.20 | 14.2 | 1.2 | 4.8 | 6.9 | 10:17 |

the molecular ratios of the basic amino acids it yields on acid hydrolysis.

These observations should throw doubt on the idea that living serum contains two proteins or two groups of proteins which are usually classified as albumins and globulins. They support our previous suggestion (1) that the proteins obtained from blood serum by the usual physicochemical methods are not of a constant amino acid composition but are artificial products produced by
the reagents employed in their preparation (cf. (9)). Our studies on the serum proteins emphasize the idea of recognizing that products isolated from living tissue even by the mildest chemical procedures are not necessarily present as such in that tissue during life.

BIBLIOGRAPHY

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