LETTERS TO THE EDITORS

The Editors do not hold themselves responsible for opinions expressed by their correspondents. No notice is taken of anonymous communications

Fractional Precipitation of Serum Proteins

IN a recent note¹, we described the purification of renin by means of a fractional precipitation with protein-precipitating agents (sulphosalicylic acid and tungstic acid). In order to test the adaptability of this method, we have investigated the fractional precipitation of blood serum using electrophoresis (Tiselius) for analysing the composition of the products isolated. The most important of the results so far obtained is that it is possible, if we choose the right conditions and suitable precipitating agents, to perform the fractionation with a high degree of selectivity.

(A) To 40 ml. ox serum diluted with 40 ml. phosphate buffer (pH 7.6) was added 1.22 ml. *M* sodium tungstate and then precipitated with *N* hydrochloric acid until it reached pH 6.8. The accompanying electrophoresis curves show a large amount of γ -globulin in the precipitate, considerably less of a component containing α - and β -globulin and only small amounts of albumin. In the filtrate large amounts of albumin, some α -globulin but almost no β - and γ -globulin remained.

(B) To 19 ml. ox serum diluted with 19 ml. phosphate buffer (pH 7.6) was added 10 per cent sulphosalicylic acid until it reached pH 4.0. In the filtrate large amounts of albumin, some γ -globulin but almost no α - and β -globulin remained. The precipitate contained a small amount of albumin, a very large amount of α -globulin, a considerable amount of β -globulin but almost no γ -globulin.

(C) To 20 ml. ox serum diluted with 20 ml. phosphate buffer (pH 7.6) was added 0.7 ml. *M* uranyl nitrate and solid bicarbonate until the precipitate was again dissolved (about pH 8); then *N* hydrochloric acid until it reaches pH 6.3. The compositions of filtrate and precipitate are almost identical and correspond to the composition of the original ox serum.

These three examples show the very conspicuous dissimilarity in the products obtained when different

nature of the agents used for formation of the slightly soluble protein compounds, it is possible to modify this method between much wider limits than the methods usually employed in protein fractionation.

This work was aided by a grant from the Teknisk-kemisk Fond; it is being continued and the results will be published elsewhere.

TAGE ASTRUP

AKSEL BIRCH-ANDERSEN

Biological Institute,
Carlsberg Foundation,
Copenhagen, N.

July 15.

¹ Astrup, T., and Birch-Andersen, A., [*Nature*, **160**, 570 (1947)].² Rinderknecht, H., Noble, R. L., and Williams, P. C., *Biochem. J.*, **33**, 381 (1939).³ Rimington, C., and Rowlands, I. W., *Biochem. J.*, **35**, 736 (1941).

Constitution of Salmine

It has recently been suggested¹ that salmine may be a cyclic peptide because it does not appear to contain any free carboxyl groups when these are estimated by the methods of Fraenkel-Conrat² and Herriot *et al.*³. This suggestion is in direct contrast to the findings obtained in the present work, to be fully reported elsewhere. A complete amino-acid analysis of salmine sulphate suggests a molecular weight of 8,000 and a peptide chain of 58 residues:

Amino-acid	Amino-acid N as % protein	Amino-acid/100 gm. protamine	Min. mol. wt.	No. of residues	Calc. mol. wt.
Arginine	89.0	85.2	204	40	8160
Isoleucine	0.56	1.61	8140	1	8140
Valine	1.20	3.1	3770	2	7540
Proline	2.29	5.8	1980	4	7920
Glycine	1.78	2.94	2550	3	7650
Alanine	0.56	1.10	8100	1	8100
Serine	3.94	9.1	1152	7	8050
	99.33	108.85		58	7930

The protamine sulphate contains 19.85 per cent H_2SO_4 (40 equivalents per molecule), being sufficient to combine with all the arginine residues in the molecule. The amino-acids reported by Block and Bolling⁴

have been confirmed, by isolation as acetyl derivatives; in addition, glycine has been shown to be present in the protamine.

End group analysis by the method of Sanger⁵ (Porter, private communication) has shown that proline occupies the terminal imino position. There is no reason to suppose that the procedure of Sanger has resulted in the opening of a cyclic peptide, since a similar

procedure carried out on gramicidin-*S*⁶ showed that this material was indeed a cyclic peptide.

It seems reasonable to assume that salmine is a normal peptide, and that the methods used by Fraenkel-Conrat and Olcott¹ are not applicable to a protein with such singular properties as salmine.

G. R. TRISTRAM

Biochemical Laboratory,
Cambridge. July 15.

¹ Fraenkel-Conrat and Olcott, *Fed. Proc.*, **6**, 253 (1947).² Fraenkel-Conrat and Olcott, *J. Biol. Chem.*, **161**, 259 (1945).³ Herriot, Anson and Northrop, *J. Gen. Physiol.*, **30**, 185 (1946).⁴ Block and Bolling, *Arch. Biochem.*, **6**, 417 (1945).⁵ Sanger, *Biochem. J.*, **39**, 507 (1945).⁶ Sanger, *Biochem. J.*, **40**, 261 (1946).

FRACTIONAL PRECIPITATION OF OX SERUM WITH (A) TUNGSTIC ACID, (B) SULPHOSALICYLIC ACID, AND (C) URANYL NITRATE. *c* DENOTES THE CONCENTRATION OF THE SOLUTION INVESTIGATED IN RELATION TO THE ORIGINAL SERUM. LEFT-HAND MEMBERS OF PAIRS, FILTRATES; RIGHT-HAND MEMBERS OF PAIRS, PRECIPITATES

conditions and precipitating agents are used. While the uranyl nitrate evidently precipitates the different components to almost the same degree, tungstic acid precipitates chiefly the γ -globulin, and sulphosalicylic acid mainly α - and β -globulin. The only case known to us in which a similar procedure has been used previously is the precipitation of inert proteins from mare serum gonadotrophin by means of sulphosalicylic acid² or metaphosphoric acid³; but here the bulk of proteins is removed and only a very small amount, containing the active substance, remains in the solution.

By changing the conditions of precipitation (pH, salt concentration, dilution, temperature) and the