BRIEF COMMUNICATION

SOME STUDIES ON THE TRICHLOROACETIC ACID-PRECIPITATED PROTEINS OF SEMINAL PLASMA IN THE BULL, BUFFALO AND GOAT

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Summary. Precipitation of bull, buffalo and goat seminal plasma with trichloroacetic acid did not render the seminal plasma proteins insoluble. On the basis of solubility and dialysibility, the acid-precipitated seminal plasma proteins from bull and buffalo were resolved into three fractions which were quantitatively estimated. Some of the properties of the largest of these fractions from bull, were studied. There was a slight change in the relative proportion of these fractions on storage of semen at room temperature.

INTRODUCTION

During the course of earlier work (Bhargava, Bishop & Work, 1959) on protein synthesis in mammalian semen, a part of the trichloroacetic acid-precipitated seminal plasma protein was observed to be soluble in water and/or dilute salt solution, suggesting that it may not have been denatured. Precipitation of blood plasma albumin with trichloroacetic acid (TCA) has been shown to have no effect on its physical, chemical and biological properties (Schwert, 1957). These observations prompted a further investigation of the TCA-precipitated seminal plasma proteins; preliminary findings are reported here.

METHODS

Seminal plasma was separated by centrifugation from a known amount of suitably diluted bull, buffalo or goat semen, stored in the cold for less than 3 hr after collection. The diluted seminal plasma was treated with an equal volume of 10 % TCA at room temperature for 5 min. The TCA precipitate was washed twice each with 5 % TCA and 5 % sodium chloride solution (NaCl) and then extracted exhaustively with water; the NaCl washes contained less than 2 % of the protein in the TCA precipitate. Almost all the precipitate (which had 13 to 14 % nitrogen, and a typical protein spectrum in the ultraviolet) dissolved in water; the insoluble residue accounted for less than 3 % of the dry weight of seminal plasma for all the animals studied, and did not contain any protein or lipids, which provides further evidence (Mann, 1954) in favour of the

TABLE I

Animal	No. experiments	Dry wt of seminal plasma (mg)	Water-extractable protein in NaCl-washed rca ppt. of seminal plasma* (% §)	Fraction A* (% ¶)	Fraction B† (% ¶)	Fraction C‡ (% ¶)
Bull (Kerry)	5	97.8 ± 6.7	47.8 ± 4.4	13.4	61.4	25.3
Bull (Deoni)	4	95.8 ± 7.3	59.9 ± 5.7	11.8 ± 1.6	54.6 ± 3.5	33.6 ± 1.8
Buffalo	4	52.4 ± 3.9	52.2 ± 1.9	12.2 ± 1.7	61.1 ± 2.0	26.7 ± 1.8
Goat	2	137.5	55.0	—	—	—

All the data given are for 1 ml of semen. For details of Fractions A, B and C, see text. The values given are the mean, and are followed by the standard deviation. Where no standard deviation is given, the estimations were done only in two experiments.

* Estimated spectrophotometrically (an optical density of 1.25 at 280 mμ in a 1-cm cell was taken to correspond to 1 mg protein, in typical experiments, Fraction A, Fraction B, and the total extractable protein in the NaCl-washed rca precipitate, gave the same absorption at 280 mμ, 1 mg/ml giving an optical density of approximately 1.25 in each case).

† Estimated by determination of dry weight.

‡ Estimated by difference (total rca-precipitable protein minus the sum of Fractions A and B).

§ Percentage of the dry weight of seminal plasma.

¶ Percentage of the water-extractable protein in NaCl-washed rca ppt. of seminal plasma.

absence of free, acid-insoluble lipids (or lipoprotein) in mammalian seminal plasma. The dry weight of seminal plasma and the protein content of the aqueous extract of the TCA precipitate of seminal plasma, are given in Table 1 for a series of experiments on a Kerry bull, a Deoni bull, a Murrah buffalo and a Jamnapari goat. The TCA-precipitated sperm proteins were completely insoluble in water and/or dilute salt solutions.

RESULTS

Dialysis against water for 72 hr of the aqueous solution (obtained as above) of the washed TCA-precipitated seminal plasma proteins yielded two non-dialysable fractions, A and B, and one dialysable fraction, C. Of the former, one (Fraction A) was soluble in both water and dilute salt solutions, while the other (Fraction B) was soluble only in dilute salt solutions; Fraction B was initially extracted in water owing to the presence of salt left over in the TCA precipitate after the NaCl washing, and was precipitated on dialysis. The relative proportion of Fractions A, B and C from two bulls and a buffalo are given in Table 1. No significant loss of TCA-precipitable nitrogen on dialysis of bull seminal plasma against a phosphate buffer was observed earlier (Larsen & Salisbury, 1953); the origin of Fraction C, is, therefore, not clear. Storage of buffalo semen for 3 hr at room temperature (30° C) caused no reduction in the total TCA-precipitable protein but resulted in a 65 to 80 % decrease in the non-dialysable fraction, A. Most of the protein lost from Fraction A was recovered in the other non-dialysable fraction, B, and there was only a 10 to 15 % increase of protein in Fraction C. Fraction C is, therefore, unlikely to be derived as a result of proteolysis (Mann, 1954) of Fractions A and/or B during the storage of semen before treatment with TCA.

Following Sanger's fluorodinitrobenzene method, the average molecular weight of the polypeptide chains of Fraction B from the Kerry bull was found to be 2150, 2410 and 2560 in three experiments, suggesting that this fraction consists of small polypeptide chains; arginine, glycine, serine and alanine were detected as the N-terminal amino acids of Fraction B, indicating a heterogeneity in its polypeptide chains. Preliminary experiments on the amino acid composition of Fraction B from Kerry bull indicated the presence of a high concentration of the sulphur-containing amino acids (methionine and cysteine, approximately 12 and 6 g, respectively, per 100 g protein). It is possible that each component of Fraction B consists of several peptide chains joined together by the abundant cysteine residues.

This investigation also indicates that proteins of seminal plasma are qualitatively different from those of blood plasma. The latter (including globulins) are completely soluble in water after precipitation with TCA from whole plasma and the removal of TCA by dialysis (Abraham & Bhargava, 1963).

Although precipitation with TCA does not render any of the seminal plasma proteins studied insoluble in water and/or dilute salt solutions, it may affect the structure, the biological properties and the other physical properties of the proteins. Further work on the characterization of the TCA-precipitated proteins, and on their relation to the native proteins of mammalian seminal plasma, is in progress.

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