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Hydrolysis of human dentin collagen

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PROCEEDINGS OF THE 30th MEETING OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY, 16th MARCH, 1960.

AMINO-ANTIPYRINE ESTIMATION FOR THE MEASUREMENT OF TOTAL BODY WATER, *by Ralph E. Bernstein (Electrolyte and Metabolic Research Unit, South African Institute for Medical Research, Johannesburg, South Africa).*

The determination of the "water space" of humans and animals is relevant to investigations on water (and electrolyte) distribution and body composition. The use of the ureas, sulphanilamide or isotopic water to assess the volume of uniform distribution of introduced substances is subject to error or necessitates special equipment, and most methods utilize injected antipyrine or allied non-toxic derivatives. The coupling of 4-amino-antipyrine (or hydrolyzed *N*-acetyl-4-amino-antipyrine) with phenolic substances in the presence of alkaline ferricyanide as an oxidizing agent to form a coloured quinone [Emerson, 1943; Gottlieb and Marsh, 1946] is the basis of the proposed method.

The optimum concentration, temperature, pH, etc., of reagents has been determined; the optical density of the solutions is read immediately at 510 mμ. The reagents required are simple and stable. Phenol and the cresols produce greater colour development than other simple phenols tested, while substituted phenols have little condensing activity. Provided the serum or plasma is clear, protein precipitation is unnecessary since the reagents do not react with the plasma proteins. Results compare well with 4-amino-antipyrine estimations by nitrosation and coupling to form an azo dye [Brodie and Axelrod, 1950]; however, such a method calls for protein precipitation and freshly prepared reagents.

REFERENCES

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HYDROLYSIS OF HUMAN DENTIN COLLAGEN, *by C. C. Solomons (Joint Dental Research Unit of the C.S.I.R. and the University of the Witwatersrand, Johannesburg).*

Peptide fractions of tooth collagen were obtained by heating weighed samples of human dentin (0·2 g.), for various lengths of time in 20 ml. of distilled water in a sealed tube at 117° C. The remaining dentin was washed with a small quantity of cold water, dried at 117° C overnight, and reweighed. From the loss in weight, the percentage of the total protein extracted was calculated. An S-shaped curve was obtained when the percentage of total protein extracted was plotted against time of treatment. Dissolution of protein was complete after 16 hours.

The main products of the water hydrolysis of dentin were peptides, together with minimal amounts of free amino acids and lime salts. Aliquots of the extraction mixture were analysed for their contents of lysine, hydroxylysine and arginine, after acid hydrolysis [Sanger, 1945].

It was observed that the first 52% of the protein to be extracted was poor in lysine, hydroxylysine and arginine when compared with the proportions of these amino acids in the intact protein. Peptides dissolved during the latter half of the extraction procedure were correspondingly rich in these amino acids.

The results suggest that peptides containing large amounts of lysine, hydroxylysine and arginine may be preferentially bound the mineral phase of the dentin.

REFERENCE

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