Availability to Dinitrofluorobenzene of some Amino Groups in Human Dentin and Ox Hide Collagen

Human dentin (containing 80 per cent by weight of mineral material), ox hide collagen, and samples of dentin which were partially demineralized at 4° C. by soaking in either 1 *N* hydrochloric acid or 1 *N* trichloracetic acid, or 15 per cent versene (*p*H 7·3), were assayed for α- and ε-amino groups using Sanger's dinitrofluorobenzene method[1].

0·2 gm. of tissue was added to 0·5 gm. dinitrofluorobenzene (excess) in 66 per cent ethanol saturated with sodium bicarbonate and shaken for 36 hr. at room temperature. The dinitrophenyl protein thus formed was washed with water and ethanol, and hydrolysed for 16 hr. with 6 *N* hydrochloric acid in a sealed tube at 105° C. After extraction of the hydrolysate with peroxide-free ether, separation of the water-soluble ε-N dinitrophenyl lysine and ε-N dinitrophenyl hydroxylysine was achieved by means of paper-chromatography, using butanol/acetic acid/water (4 : 1 : 5) as solvent. The yellow spots were cut out, eluted into 5 ml. of 1 *N* hydrochloric acid, and their optical densities at 360μ recorded on the Beckman spectrophotometer.

Distinct differences between the availability of amino groups of dentin and those of similarly treated hide collagen were observed :

(1) Only 50–60 per cent of the total lysyl and hydroxylysyl ε-amino groups of hide collagen were available to the dinitrofluorobenzene[2], whereas in human dentin the availability of these amino groups increased linearly from 6 per cent in untreated dentin to more than 90 per cent in the fully demineralized dentin[3].

(ii) A linear increase (from zero to 10 micromoles/gm. protein) in the concentration of N-terminal amino groups during demineralization of dentin was observed. In accordance with other workers[2], no N-terminal amino groups were detected in hide collagen.

(iii) The molar ratio (*R*) of ε-N dinitrophenyl lysine to ε-N dinitrophenyl hydroxylysine, obtained from the dinitrophenylation of hide collagen, was almost theoretical (3·9) [4]; but in dentin collagen this ratio increased in a linear manner from 0·8 to 2·9 (theoretical maximum 3·7) [5], when plotted against the percentage dissolution of mineral material (Fig. 1). This implies that, especially in the early stages of the decalcification process, the availability to dinitrofluorobenzene of hydroxylysyl ε-amino groups exceeds that of the lysyl ε-amino groups, in spite of the much higher concentration of lysine residues. The same results were obtained using each of the above-mentioned demineralizing solutions.

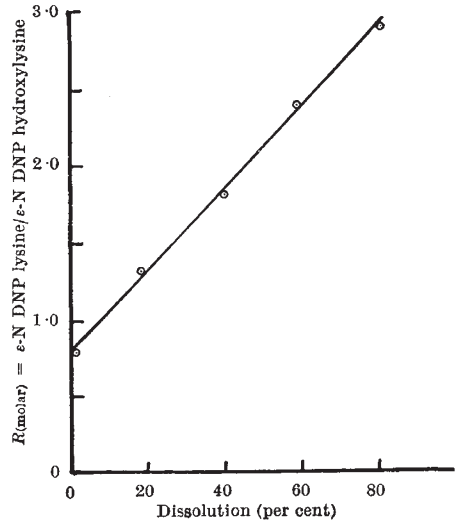

Fig. 1. Relative availability, to dinitrofluorobenzene, of lysyl and hydroxylysyl ε-amino groups of dentin collagen, during mineralization

Other workers[6] have suggested that polar side-chains of amino-acid residues in proteins and peptides may act as binding sites for ions; and since the anionic binding power of the ε-amino groups of hydroxylysine is weaker than that of lysine[7], bound anions are expected to be liberated at a greater rate from hydroxylysyl than from lysyl amino groups, during demineralization.

It is therefore suggested that the ε-amino groups of lysine and hydroxylysine may play a part in the combination of mineral material with the protein matrix of human dentin.

C. C. Solomons
J. T. Irving

Joint Dental Research Unit of the
Council for Scientific and Industrial Research
and the University of the Witwatersrand,
Johannesburg.

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Correlation between Deoxyribonucleic Acid Content and Volume of Individual Nuclei in Different Tissues of the Rat

It has been shown that in the liver[1], and in several other organs[2], of the rat the nuclear classes determined according to the deoxyribonucleic acid content correspond to the nuclear volume classes. An objective analysis was performed in order to see if in the individual nuclei of a given organ a correlation exists between deoxyribonucleic acid content and volume. The results of measurements obtained with E. Pisi[2] were used in this study. The relative deoxyribonucleic acid content of the individual nuclei was obtained by means of the histophotometrical method of L. Lison[3]. The great majority of the nuclei being practically spherical in shape, the nuclear volume was calculated by a simple geometrical formula from the projection surface.

Fig. 1 summarizes the results obtained for the thyroid gland for two rats (105 and 106). The regression lines and the correlation coefficients *r* (0·816 and 0·536) show that, if the degree of correlation can be varying in the same organ from one individual to another, in each case a real linear correlation exists between the deoxyribonucleic acid content of the different nuclei and their volume.

Similar results were obtained for other organs, which like the thyroid gland contain practically only one nuclear class (kidney, endocrine pancreas). In organs with different nuclear classes (liver, exocrine pancreas) conditions seem to be more complicated; they will be the subject of a further study.