Nitrogen in Human Dental Enamel

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Dental enamel is the structure which is first exposed to the attack of dental caries, and the success or failure of this attack depends on many factors, among them the chemical nature and structure of the enamel. Some authors (Pincus, 1939; Gottlieb, 1947) go so far as to postulate that the caries process is initially a proteolytic one. In this connexion the nitrogenous material of enamel is of interest.

The object of the present experiments was to estimate the nitrogen content of enamel on samples of material which were representative of the main enamel mass and uncontaminated by dentine. Enamel is firmly attached to the underlying dentine which contains about 3·5% nitrogen compared with about 0·15% in enamel. On these figures, even a 2% dentine impurity would increase the enamel nitrogen by about 60%.

METHOD

The following methods are available for the preparation of enamel samples: (i) The enamel may be ground off with various cutting instruments. With this method, apart from the difficulty in judging when the dentine is about to be exposed, there must always be a variable amount of enamel attached to underlying dentine which is not taken for analysis. (ii) The dentine may be removed from the inside with burs, leaving a hollow enamel cap. (iii) The whole calcified crown of the tooth may be removed and powdered, and the dentine and enamel separated by a flotation method relying on the difference of density between the two materials. This method was used by Manley & Hodge (1939), who claimed for it an enamel purity of 99·4%, but a loss of enamel of 0·3%. Since this fraction is necessarily slightly less dense than the remainder, it is probable that it contains proportionally more organic material, so that the nitrogen loss may be considerably more than 9·3% of the total.

Method (ii) was considered the most reliable. The teeth used in the first part of the investigation were sound premolars which had been extracted for orthodontic purposes from patients not more than 16 yr. old. The teeth were collected in 70% ethanol and thoroughly scraped and brushed on a lathe with pumice powder before use. The crowns were then removed and dentine burnished out leaving a hollow enamel cap.

With round burs of graded size, dentine was removed rapidly and completely. The matt appearance of dentine, compared with the shiny undersurface of the enamel coupled with the change in burring sound and resistance, made the arrival at the amelo-dentine junction easily detectable. There was small risk of removing enamel with these burs as very light pressure was used.

For each batch the enamel from 6 to 10 crowns was crushed in a diamond mortar to a powder which would pass a 80-mesh sieve. The purity of the powder was tested by the method described by Manley & Hodge (1939), relying on the different refractive indices of enamel and dentine. With this method it was possible to recognize under the microscope particles of dentine in a field of enamel particles. Tests were made on every batch and never more than three dentine particles per thousand enamel particles were found. The powder was then divided into three samples of between 500 and 700 mg., dried to constant weight at 105–110° and each sample was transferred to a 100 ml. Kjeldahl digestion flask containing 7 ml. of HCl (1 vol. conc. HCl plus 4 vol. water) to dissolve the calcified material. Digestion with concentrated H₂SO₄, using the catalyst of Chibnall, Rees & Williams (1943), was carried out for 11 hr. The NH₃ was steam-distilled into 1% (w/v) boric acid and titrated with 0·01N-HCl, using the methyl red-methylene blue indicator mixture of Pierre, Tully & Ashburn (quoted by Britton, 1942).

It was thought that the presence of CaSO₄ in the digest might interfere with the recovery of the NH₃. To test this point estimations were carried out on egg albumin solutions in the absence and presence of CaSO₄. The results given below showed that there was no interference. (Sample 1: without CaSO₄, 0·119 mg./l.; with CaSO₄, 0·118 mg./l. Sample 2: without CaSO₄, 0·122 mg./l.; with CaSO₄, 0·122 mg. of N/l.)

RESULTS

Table 1 shows the nitrogen content of ten batches of enamel. In all cases except two the values given are the means of estimations on three samples of a batch of enamel obtained by pooling 6–10 crowns. Wide variation is shown about the mean of 0·071 g./100 g.

Table 1. Nitrogen content of dental enamel taken from (a) sound young premolars and (b) sound teeth from patients in higher age groups

<table>
<thead>
<tr>
<th>Orthodontic extractions (under 17 yr. of age)</th>
<th>Caries-resistant older age groups</th>
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<tbody>
<tr>
<td>0·110</td>
<td>0·071</td>
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<tr>
<td>0·052</td>
<td>0·086</td>
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<td>0·078</td>
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<td>0·097</td>
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<td>0·077</td>
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<td>0·059</td>
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Mean ± s.d. 0·071 ± 0·015 0·083 ± 0·021
enamel, and this is expressed by the standard deviation of $\pm 0.015$ g./100 g.

A second series of estimations was conducted on teeth which had resisted caries. The teeth used were sound premolars extracted because of paradontal disease or to clear an otherwise empty mouth, and came from the higher age groups. The values, again the means of three samples, are also shown in Table 1. With a mean of $0.083$ g./100 g., enamel, and standard deviation of $0.021$ g./100 g. there is no statistically significant difference between the nitrogen content of this enamel and that of the first series.

**DISCUSSION**

The values for enamel nitrogen found in the experiments reported here are lower than most reported to date. Bowes & Murray (1935) found $0.156$ % in enamel separated by grinding it from the surface of the dentine. Deakins & Volker (1941) used the flotation method for separating enamel and dentine. From their figures expressed as protein with a nitrogen content assumed to be $16$ % the range of nitrogen values was found to be $0.078-0.31$ %. It is considered that the low results given in the present paper were obtained by reducing the dentine impurity to a minimum. A simple comparison between the enamel-nitrogen content of the two groups of teeth gives little information. In this series at least two variants may operate. First, there is the possibility that age changes may influence nitrogen content. Secondly, on the basis of a proteolytic theory of dental caries there is a possible relationship between nitrogen content and caries susceptibility. Although the second series of estimations were conducted on teeth of proven caries resistance, the orthodontic premolars were of unknown susceptibility to caries, some would have survived, others would have succumbed. Further work is necessary to test the effect of these and other variants on enamel-nitrogen values.

**SUMMARY**

Nitrogen estimations carried out on the carefully separated enamel of sound premolar teeth give a mean value of $0.071 \pm 0.015$ % for the age group up to 16 years and $0.083 \pm 0.021$ % for older age groups. These values are lower than those previously reported.

My thanks are due to Dr Agnes Shore, Prof. W. R. Spurrell and Prof. M. A. Rushton for much helpful advice and criticism.

**REFERENCES**


**Interrelationship of Certain Vitamins of the B Group in Aneurin, Riboflavin and Biotin Deficiencies**

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A close interrelationship of vitamins of the B group may be expected, since they are fundamental to all forms of life, and are the controlling factors in the chain of oxidative removal of certain metabolites. Definite interrelationships among these vitamins have been established by investigations on human subjects and on animals. The treatment of multiple deficiencies in man with aneurin alone results in the development of pellagrous skin changes which disappear on administration of either nicotinic acid or yeast (Lehmann & Nielsen, 1939, Salvesen, 1940; Braendstrup, 1940). Sydenstriker (1941) pointed out that, in deficiencies of the individual vitamins of the B group, treatment with one vitamin may be rapidly followed by the development of severe signs of deficiencies of others. Klopp, Abels & Rhoads (1943) found no clinical signs of riboflavin deficiency in subjects on normal diets receiving large doses of aneurin, but found an increased urinary excretion of riboflavin. Increased excretion of riboflavin in the urine in chronic and acute aneurin deficiencies was observed by Sure (1944). Unna & Clark (1942) were