Relation of Enamel Protein to Dental Caries

I MADE some observations on the nature of enamel protein in 1936 [1]. The possible role of enamel protein in the production of caries is described below.

(*a*) Micro-analysis of enamel protein (Weiler) gave N 12·1 per cent, S 1·2 per cent; other nitrogen determinations (Schoeller) were 9·2 and 11·1 per cent. Levene[2] gives nitrogen determinations for mucoproteins as ranging from 6·7 to 13·3 per cent; nitrogen values for proteins are generally accepted as 12–18 per cent.

(*b*) Sections of developing human enamel stained with thionin provide evidence of limited value of the presence of material resembling mucin. Wassermann[3] has illustrated the secretion of globular material by the enamel-forming cells, the ameloblasts, in guinea pigs.

(*c*) A substrate resembling chondroitin sulphuric acid by qualitative tests has now been prepared from dentine by the use of calcium chloride[4]. Dentine is reduced to powder by the use of a dental engine and a bur, defatted with acetone and ether, and extracted with 10 per cent calcium chloride; the extract is deproteinized with a chloroform – amyl alcohol mixture and centrifuged. Chondroitin sulphuric acid is separated from the supernatant liquid by alcohol and glacial acetic acid, then washed with alcohol. A solution of chondroitin sulphuric acid prepared in this manner reduced Fehling's solution, and was not fermented by yeast. No pentose was detected; naphthoresorcinol gave a brown to purple colour; phloroglucinol a red colour. No free sulphate was detected.

From this substrate, enzymes of a Gram-negative bacillus in pure culture have been found capable of quantitative release of sulphate. The organism used is No. 3247, National Collection of Type Cultures, *Ps. non-liquefaciens*. Neuberg[5] has described three sulphatases in Gram-negative bacilli, of which two are of interest in connexion with the present work: chondrosulphatase, which releases sulphate quantitatively fron chondroitin sulphuric acid, while a similar type of enzyme releases sulphate from the mucoitin sulphuric acid of mucin.

An explanation of caries developing in enamel may be as follows. Enamel protein resembles a mucoprotein in its properties. Gram-negative bacilli, commonly found in caries, can release sulphatase. Enamel is made up of hexagonal prisms each surrounded by a sheath of enamel protein. Enzymatic hydrolysis of these sheaths in caries, with release of sulphuric acid, would result in attack on each prism from the periphery inwards; the relatively insoluble calcium phosphate which forms the inorganic part of the enamel would be slowly changed to the relatively soluble calcium sulphate. It is of interest to notice that Frisbie *et al.*[6] show enamel caries proceeding in just such a manner, each prism decreasing in size from the outer edge inwards.

To summarize: (1) quantitative estimations of nitrogen and of sulphur, together with evidence from the developmental aspect and from histochemical tests, suggest that enamel protein is a mucoprotein; (2) the presence of a chondrosulphatase in certain Gram-negative bacilli is confirmed; (3) the mode of progress of caries in enamel, studied histologically, can be accounted for by the present hypothesis.

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PAUL PINCUS

Department of Physiology,
Middlesex Hospital Medical School,
London, W.1.
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Microflora of the Rumen

QUIN[1], in his study of the rumen micro-organisms occurring in sheep fed exclusively on lucerne hay, noted the appearance of large numbers of ovoid, clear cellular organisms with an average size of 8 $\mu \times 4\,\mu$. He showed that rumen fluid rich in these organisms rapidly fermented glucose with the evolution of gas, and that under these conditions the organism stained mahogany brown on the addition of aqueous iodine solution. He did not state whether these organisms were motile, nor did he record any attempts at isolation by cultural methods. On the basis of their apparent reproduction by binary fission and their fermentative powers, Quin considered that these organisms were a type of false yeast, and accordingly named them '*Schizo-saccharomyces ovis*'.

Baker[2] and Elsden[3] have confirmed these observations, apparently acquiescing in the use of the term '*Schizo-saccharomyces ovis*'.

We have examined repeatedly the rumen contents of sheep, with permanent fistulæ, fed on a diet of meadow hay and mangolds, and have found that ovoid yeast-like forms are the overwhelmingly predominant organisms. In sheep fed on meadow hay only, these ovoid organisms are absent or in small numbers; but within three days of the addition of mangolds to the diet, they appear in great numbers.

In wet preparations under a coverslip, examined under the 1/6th eyepiece of the microscope soon after sampling, the majority of the ovoid forms are seen to be actively motile; but so far, no flagella have been detected either in stained or unstained preparations. Many of the forms appear to have an outer layer resembling an envelope or capsule, around the periphery of which there are a number of small granules, brightly refractile in unstained preparations and staining intensely with Giemsa.

As well as the ovoid forms, there are numerous crescent-shaped forms of approximately the same dimensions; in some individuals one long flagellum, in others two flagella, attached to the centre of the concave side can be detected.

If a relatively pure suspension of these ovoid and crescentic organisms is obtained by fractional centrifugation, mixed with 10 per cent glucose solution and incubated at 37° C., there is a rapid evolution of gas and reduction in *p*H. On microscopic examination, the organisms are found to be non-motile; on mixing with iodine solution, they stain deep mahogany brown, indicating the formation of glycogen.