physectomized rats than by ribosomes from normal rat liver.

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Bacterial Degradation of Glycoprotein Sugars in Human Saliva

Previous reports[1,2] have indicated that sialic acid in human, wax-stimulated saliva is rapidly released and metabolized by the oral flora. Heat (100°, 5 min), or antibiotic, prevents both these reactions from taking place. Further investigations have now shown that the other sugars associated with the salivary glycoproteins (3 hexoses, 2 hexosamines and fucose) are all spontaneously lost from saliva by bacterial action, and this loss may also be prevented by either heat or antibiotics. Wax-stimulated saliva contains, with the exception of fucose, all these sugars in two distinct forms: those in the salivary glycoproteins and those in the many and varied bacteria (10^8/ml.) also present in the saliva. Many of the bacteria in the saliva contain rhamnose, a 6-deoxyhexose of the same group of sugars as fucose, but it is possible to distinguish between these two sugars by either paper or thin-layer chromatography[3]. By this method it was shown that the 6-deoxyhexoses in freshly collected, wax-stimulated saliva consisted of rhamnose and fucose. It was also shown that, on incubation, the saliva rapidly lost all its fucose and sialic acid, while the rhamnose remained. The hexoses and hexosamines, however, occurred in the salivary glycoproteins and the bacteria, and it was not possible to distinguish between these sources by direct analysis or by chromatography. On incubating wax-stimulated saliva, 50–70 per cent of the hexoses and hexosamines were lost, but it was not possible in this instance to distinguish whether the remaining sugar was derived from the salivary glycoproteins or from the bacteria. If the saliva was collected directly from the salivary ducts (parotid, submaxillary and sublingual) and pooled, there were now present so few bacteria that all the sugars from the glycoproteins remained intact on incubation and the saliva remained clear and viscous. The addition of bacteria, in the form of dental plaque or salivary sediment, to the viscous duct saliva caused a rapid loss of sugars, a drop in viscosity and the formation of a precipitate. After incubation no fucose or sialic acid was present and the amount of residual sugars was approximately equal to that from the added bacteria. In none of the experiments described here has it been possible to detect any but traces of free sugars in the saliva when bacteria have been present, and these sugars were also rapidly metabolized when added in a free state to the saliva. It is thus apparent that the carbohydrate components of the glycoproteins in saliva are labile in the oral environment and are rapidly released and metabolized by bacterial action. The loss of these sugars, particularly sialic acid, provides an explanation for the observed drop in viscosity and formation of a precipitate that occurs spontaneously in wax-stimulated saliva[1,2]. Previous suggestions that the drop in viscosity is due to a mucinolytic depolymerization of the salivary glycoproteins[4] are valid so far as the viscosity-drop is concerned, but this mechanism should produce a product of increased solubility, and, in fact, a heavy precipitate always appears concomitantly with the drop in viscosity. It is tempting to postulate that this is the mechanism whereby the material from the saliva forms a matrix holding together the many bacteria adjacent to the teeth, which is commonly referred to as dental plaque. Support for this is found in the observation that dental plaque is practically devoid of both sialic acid and fucose (<0·002 per cent), the two essential sugar constituents of salivary glycoproteins that can be identified as belonging exclusively to the glycoproteins and not to the oral bacteria.

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High Content of Hyaluronic Acid in Normal Human Heart Valves

During the course of investigations of the effect of age on acid mucopolysaccharide content of the human heart valves, we found hyaluronic acid to be a major component. In all it represented about 60 per cent of the acid mucopolysaccharide present in the tissue. This observation is in striking contrast with reports from other laboratories[1,2] which have shown hyaluronic acid to be a minor component of both bovine and pig heart valves. This communication reports the distribution of the acid mucopolysaccharide fractions of the normal human heart valves.

For total acid mucopolysaccharide determination, individual heart valves (approximately 50 mg) were dried, de-fatted and subjected to papain digestion and the protein separated by precipitation with trichloroacetic acid. The acid mucopolysaccharide is precipitated by cetylpyridinium chloride, dissolved in sodium chloride and reprecipitated with ethanol. For fractionation, extracted acid mucopolysaccharide of about ten valves of the same age-group was pooled. The acid mucopolysaccharide was fractionated to hyaluronic acid, chondroitin sulphuric acids and heparin according to Schiller *et al.*[3]. Applying this method, hyaluronic acid, chondroitin sulphates, and heparin were obtained at 0·4 M, 1·2 M, 2·1 M sodium chloride solution, respectively. Chondroitin sulphate *B* was further separated from chondroitin sulphate *A* or *C* by hyaluronidase (testicular) digestion. The total acid mucopolysaccharide is expressed as uronic acid and determined by the carbazole reaction[4]. The results of such a fractionation procedure are shown in Table 1. The total acid mucopolysaccharide present in the mitral heart valve is 0·93 mg uronic acid/100 mg dry weight and is significantly higher than in the tricuspid valve ($P < 0·01$ analyses were carried out on fourteen individual valves). In both valves there is no change in the distribution of various fractions of acid mucopolysaccharide and it can be seen that hyaluronic acid represents 58 per cent

Table 1. Total Acid Mucopolysaccharide (AMPS) Content and Individual AMPS Fractions from Human Heart Valves

Heart valves*	Total AMPS†	Fraction (per cent) HA	CSA—*A*/*C*	CSA—*B*	Heparin
Mitral	0·93	58·8	27·6	13·6	Trace
Tricuspid	0·74	57·7	28·0	14·3	Trace

HA, hyaluronic acid; CSA—*A*/*C*; chondroitin sulphate *A* or *C*; CSA—*B* chondroitin sulphate *B*.
* These heart valves were obtained from subjects of 20–30 years of age.
† Mg of uronic acid/100 mg dry de-fatted valve.