TABLE II

CONCENTRATION OF GAG FRACTIONS EXPRESSED AS μg URONIC ACID PER mg DNA

Tissue	DNA (Mg/g dry. defatted tissue)	GAG concentration					
		HA	HS	Ch-4-S	Ch-6-S	DS	H
Normal	1268 ± 48.4	75 ± 4.2	95 ± 8.6	108 ± 4.8	93 ± 3.6	132 ± 3.2	54 ± 0.86
Oral Cancer	1893 ± 106.1	104 ± 5.1	83 ± 6.9	106 ± 5.2	156 ± 4.9	241 ± 6.8	56 ± 1.45
P Value	< 0.01	< 0.01	N.S.	N.S.	< 0.01	< 0.01	N.S.

Values given are the average of 6 different experients ± SEM.
N.S. = Not Significant.

(b) Concentration of GAGs expressed in terms of gram, dry, defatted tissue weight:

The GAG content of tumour tissue as compared to normals, expressed as μ g uronic acid/g of dry, defatted tissue are given in Table I.

There is a significant increase in the amount of HA and the total sulphated GAG content of tumour tissues. Of the sulphated GAGs, all the fractions showed a significantly higher concentration in tumour tissue. Both normal and tumour tissues showed the same pattern of GAGs, DS showing the maximum amount, and H the least.

c) Concentration of GAG expressed in terms of mg DNA:

Tumour tissues are known to have increased cell mass as compared to normal counterparts. Although the function of GAG in cell recognition and adhersion are extracellular, their synthesis depends on the total cell number. In view of these reasons, the absolute amount of GAG fractions expressed in terms of DNA content was determined (Table II). As expected, the DNA content per gram of dry, defatted tissue was higher for the tumour tissue. Ch-6-S, DS and HA show increased levels in tumour tissue whereas all the other GAG fractions did not show any significant difference from the normal tissue. The increased amounts of HS, Ch-4-S and H in tumours observed when expressed in terms of dry tissue disappeared when the data was expressed in terms of DNA content.

DISCUSSION

The idea that the external membrane has an important role in growth control in normal animal cells and that partial or complete loss of control in transformed cells can reside in membrane changes has been supported by mu new evidence in recent years. Numerous chang in the cell surface carbohydrates (Satoh *et* 1973) membrane glycolipids (Steiner a Melnick, 1974) and cell surface glycosamin glycans (Robin *et al*, 1975; Kraemer and Tobe 1972) have been reported as a result of tran formation.

Oral cancer tissues show about two-fo increase in Ch-6-S and DS content. The increa in the amount of DS is of particular intere since it is observed that DS containing prote glycans interact more extensively to collage than all other GAGs (Obrink, 1973). It wou seem that the primary function of DS is stim lation of collagen production and binding collagen fibres (Kennedy, 1979). The increase collagenous, fibrillar structure of oral tumo tissues could be due to their increased D content.

Oral cancer tissues are known to be differentiated squamous cell carcinoma. Class cally, they form epithelial pearls with abundar keratinization. Macroscopically, the tumour are will be hard and composed of increased amoun of fibrous elements. The genesis of this fibroti reaction is not fully explained. It is possible th the increased amount of DS is partly respcnsibl for this reaction by stimulating the synthesis c collagen.

The present data is in agreement with th reported increase of HA in tumour cells, but doe not confirm reports that sulphated GAGs ar generally decreased in transformed cell line (see introduction). This apparent discrepanc can be explained by the fact that all the report cited above have been on cells in culture. In th present study, since tumour tissue itself is usec all the accumulated extracellular GAG of th tissue mass has also to be accounted for. More

er, the present data is in agreement with the servation of Dietrich *et al* (1979) that sulphated AG is increased in tumour tissues.

A mechanism has been proposed (Dietrich *al*, (1979) for the possible mode of action of phated GAGs in stimulation of cell division. cording to this, the inhibition of cell growth brought about by the contact of HS molecules adjacent cells. In the case of the tumour cells, increased amount of Ch-6-S and DS on the l surface, prevents the normal contact of HS lecules of adjacent cells. This would result a lack of cell-to-cell contact inhibition and is uncontrolled growth of cells leading to malignancy.

Recent reports indicate the involvement GAGs in nuclear function and protein bios thesis (Arnold *et al*, 1972; Cook and Aika 1973; Saiga and Kinoshita, 1976; Kraemer a Coffey, 1970). Do membrane changes, especia cell surface GAG changes, lead to subseque nuclear alterations and fiinally uncontrolled c division and tumour growth? This question mains unanswered for the present, due to la of sufficient data on changes of tumour c surface GAGs, and so it is too early to draw a definite conclusion on the role of GAG on tra formation of normal cell to tumour cell.

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Glycosaminoglycan Composition of Human Oral Cancer Tissue

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SUMMARY

Changes in the Glycosaminoglycan (GAG) composition of human oral cancer tissue as compared to normal tissue was studied using cellulose column chromatography to separate the different GAG fractions. The results indicated an absolute increase in the amounts of hyaluronic acid (HA) chondroitin-6-Sulphate (Ch-6-S) and dermatan sulphate (DS) fractions of the oral cancer tissue. The importance of these findings on the role of GAG as cellular growth regulators and in cell recognition and adhesion are discussed. A possible mechanism by which GAG may affect the transformation of a normal cell to a tumour cell is also discussed.

INTRODUCTION

MAMMALIAN cells contain a variety of glycosaminoglycans (GAGs) associated ith their surfaces (Lindahl and Hook, 1978). ıe physiological role of these cell surface ılysaccharides is not understood; among the verse functions suggested, regulation of cell owth (Ohnishi et al, 1975) and mediation of cell cell communication (Robin et al, 1975) have en proposed. Many of the cell surface prorties have been found to be modified in tumour transformed cells, although few of these seem be universal for the neoplastic state (Nicolson, 976). Virus-transformed cell lines have been own to have enhanced rate of synthesis of cell rface hyaluronic acid compared to untransrmed parental cell lines (Makita and Shimojo, 73; Satoh et al, 1973). Other reports show a nerally lower rate of synthesis of sulphated AG by transformed cells (Satoh et al, 1973; ito et al, 1975; Robin et al, 1975; Kraemer and ıbey, 1973). However, this may be an over nplification due to the large number of fferent classes of glycosaminoglycan at the cell rface and the fact that few systems have en fully investigated (Nigam and Cantero, 72).

Since the incidence of oral cancer is rather gh in India, and no detailed investigation has far been done in this particular kind of ncer, this study was carried out to find the anges in GAG content of oral cancer tissue.

MATERIALS AND METHODS

Oral cancer tissues were obtained fre from the operation room in sterile conditic The normal tissue surrounding the tumour ma was used as normal counterparts for comparisc Histological examination of the tissue sampl

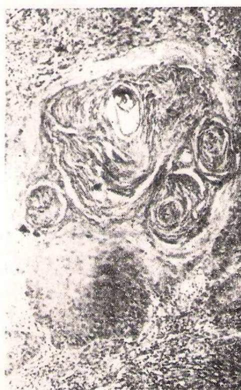
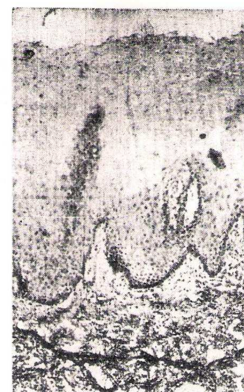


Fig. 1 Microphotograph of a well-differentiated squa ous cell carcinoma of the oral cavity, showi groups of large squamous cells with keratinization the centre to form the pearls that are characteris of this tumour. (X 150)



. 2 Microphotograph of the normal mucosal layer of oral cavity, surrounding the tumour mass showing no characteristics of malignancy. (X 150)

ıs carried out to assertain the normalcy of the ntrol tissues used. Sections from frankly ılignant areas showed anaplastic cells with ithelial pearls (Fig. I), whereas control normal sue did not show any characteristic malignant anges (Fig. II). Tissues were washed peatedly in sterile saline before defatting.

fatting of Tissues :

The minced tissues were defatted twice with ıanol; diethyl ether (3 : 1v/v) at 60°C. for o hrs. followed by chloroform : methanol : 1v/v) for one hr. The defatted tissues were ied in a vacuum desiccator till constant ight.

Estimation of GAG :

The dry, defatted tissue was subjected digestion with papain according to the procedı of Laurent (1960) and the digest was pas through a column of cellulose (Merck, microc stelline) previously washed with 1 per c cetyl pyridenium chloride (CPC). The differe GAG fractions were eluted according to the p cedure of Svejcar and Robertson (1967). T individual GAG fractions were quantitated the modified carbazole reaction of Bitter a Muir (1962). Complete resolution of the G fractions is not achieved by any of the meth available at present. In this procedure al heparan sulphate (HS), chondroitin-4-sulph (Ch-4-S), chondroitin-6-sulphate (Ch-6-S) a dermatan sulphate (DS) fractions were fou to contain traces of Ch-4-S, Ch-6-S, DS a heparin (H) respectively when analysed by t enzymatic method of Murata et al (197 Hyaluronic acid (HA) and heparin (H) we mostly uncontaminated as seen by cellul acetate electrophoresis. Since a large number samples had to be analysed, the procedure w restricted to the chromatography of GAG-C complex over cellulose.

DNA was estimated by the diphenyl ami method (Schneider, 1957).

RESULTS

(a) Recovery of GAG from the Cellul Column :

Normal and tumour tissue GAGs sh comparable recoveries from the cellulose colur (Normal —92 ± 2.8%; Oral Cancer —83 3.9%). About 10-15 per cent of the total G applied on the column remain unrecover during fractionation.

TABLE I

CONCENTRATION OF GLYCOSAMINOGLYCAN FRACTIONS EXPRESSED AS μg URONIC ACI PER GRAM DRY DEFATTED TISSUE

sue	GAG concentration					
	HA	HS	Ch-4-S	Ch-6-S	DS	H
rmal	89 ± 3.8	128 ± 4.5	145 ± 7.8	119 ± 4.9	186 ± 6.1	69 ± 3.2
al Cancer	206 ± 13.6	142 ± 5.9	186 ± 16.4	296 ± 13.1	466 ± 15.1	106 ± 8.4
Value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

ılues given are the average of 6 different experiments ± SEM.