quares of the precipitation ring diameter of ifferent dilutions of standard sera of known nmunoglobulin concentration against the im- unoglobulin content of each dilution. The oncentration of Igs in the samples were calcu- ted from the standard graphs.

(3) The presence of amylase in the tissue xtracts were looked for by the method of omogyi (1960).

Protein content of the tissue extract was etermined by Lowry's method (Lowry *et al*, 951).

Goat anti-serum to human IgG, IgA and gM were purchased from Kallestad, Chaska, Mn. SA.

RESULT

(1) **α-amylase activity of the tissue extract:** aliva contains high amounts of IgA. Although ie tissues used were washed several times, to ile out any possibility of contamination from aliva, α-amylase activity was measured in ie tissue extracts. No measurable amount of nzyme activity was detected in any of the ssue extracts indicating the absence of saliva ontamination in the extracts.

(2) **Detection and quantitation of IgG, IgA nd IgM:** The saline and Kcl extracts of normal nd tumour samples were run in the respective nti-immunoglobulin containing plates. Results re shown in Table I. Only the saline extract om oral tumour tissue showed the presence of gG and IgA. IgM was absent in all the mples.

TABLE I

ETECTION OF IMMUNOGLOBULINS IN SALINE XTRACT AND Kcl EXTRACT OF ORAL TUMOUR AND NORMAL TISSUES

xtract	Protein content mg/ml.	IgG	IgA	IgM
ALINE				
'ormal	9.0	—	—	—
'umour	12.8	+	+	—
Kcl				
'ormal	3.8	—	—	—
'umour	4.7	—	—	—

Results are from 6 different experiments.

The IgG and IgA content of saline extract of tumour tissues were quantitated. Results are shown in Table II. The immunoglobulin content is expressed as percentage of the total protein content of the tissue extract.

DISCUSSION

Initial experiments in our laboratory (Abdul Khader *et al*, 1981) indicate the presence of a tumour-associated antigen in oral cancer tissue. Antibodies to tumour-associated antigens might reasonably be expected to attach to tumour cells **in vivo** and be detectable on such cells. Therefore, the present observation that immunoglobulins are present only in the tumour extract further supports the presence of tumour-associated antigens.

TABLE II

QUANTITATION OF IMMUNOGLOBULINS IN SALINE EXTRACT OF TUMOUR TISSUE

Immunoglobulin	Protein content (mg/ml) quantitated from single radial immunodiffusion	Expressed as percentage of total protein
IgG	1.5	0.6
IgA	0.5	0.2

Total protein content in the concentrated salin extract was 250 mg/ml.

Values are the average of 6 different experiment

A common procedure, 3 Molar Kcl extrac tions, was used to extract membrane integra proteins or proteins which are bound tightly t membrane surface into soluble form. Absenc of Ig in this extract, but its presence in the salin extract suggests that the Igs are present eithe on the soluble cytoplasmic component of the cel extract, or they are present on the surface of th tumour cells loosely attached to other cell mem brane proteins. However, it remains unclea whether the Ig are produced locally in the cel or they are coated from the outside.

Another interesting observation in patient with oral cancer is the presence of large amount of antibodies against Herpes Simplex Virus i their sera (Kumari *et al*, 1980). A characteristi of many cells transformed by DNA viruses i the presence of virus specific antigens on th tumour surface (Rapp and Duff, 1973). If viru infection is the initial step in the pathogenesi

ncer, one might expect to find viral n the tumour cell surface, and anti- odies might be present on the tumour ce. Therefore, the possibility exists nmunoglobulins present in the tumour ght be directed against the virus specific hich are expressed on the tumour

igh several investigators (Dorval *et al*, 1973) have reported the presence of und immunoglobulins, their biological e remain obscure. It has been sug- gested that these probably form the factors protecting the tumour from im gical attack by the host. Beneficial ef tumour-associated antibodies have als reported in experimental animals (Ra 1976; Haskill *et al*, 1977). Hence it appe tumour-bound antibodies belong to 2 detrimental or beneficial to the host's to growing tumours. Isolation of these noglobulins and a study of their functio prove a good correlation of the progno clinical staging of the disease.

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Detection and Quantitation of Immunoglobulins Associated with Tumours of the Human Oral Cavity

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SUMMARY

The presence of immunoglobulin G (IgG) and immunoglobulin A (IgA) were detected in extracts of tumours of the oral cavity by single radial immuno-diffusion on agar plates containing the respective anti-immunoglobulin sera. The amount of IgG and IgA were quantitated by comparison with standard serum of known immunoglobulin content. Results obtained indicate that probably the immunoglobulins are present on the tumour cell-surface, losely attached to the membrane integral proteins, or they are produced locally inside the tumour cells.

INTRODUCTION

IF human tumours bear surface antigens which differ from those of normal cells they should evoke detectable immune responses which could induce regression or retard metastasis. Detection and quantification of anti-tumor antibodies may be of value in diagnosis, clinical staging and monitoring the development of metastases. Antibodies may be sought free in plasma or attached to their natural substrate—the tumour cells. It is probable that some of the tumour-associated antibodies are anti-tumour antibodies although non-specific attachment of globulins to cells can also occur.

The subject of antibody association with tumours is a controversial one; some authors have found no tumour cell associated immunoglobulin (Ig) (Kopf *et al*, 1966; Nairn *et al*, 1971) while others report a relatively high incidence of tumour cells coated with Ig (Gutterman *et al*, 973; Izsak *et al*, 1974; Vanky *et al*, 1976). The presence of IgG and IgA have been reported in tumour extracts (Antony and Parsons, 1965; Witz and Ran, 1970) by low pH elution (Vanky *et al*, 1976). In view of the controversial nature of the problem, and its diagnostic value, the present investigation was carried out to look for the possible presence and quantitation of Ig in extracts of tumours of the human oral cavity.

MATERIALS AND METHODS

(1) **Preparation of tumour tissue extracts** Tumour tissue samples were obtained eith from the operation theatre or from biops material. The cancerous nature of the tissu was ascertained by histopathological examin tions. Normal oral mucosal tissues were obtai ed from fresh autopsy samples or pinch biops specimens. The tissues were washed six tim with sterile saline and twice with phospha buffered saline (PBS). The tissues were hom genised in PBS (1:4 w/v) and centrifuged 20,000 g for 30 minutes at 4°C. The supernate (saline extract) was used for the study. Th precipitate was resuspended in 3 M Kcl and ke overnight at 4°C. The supernatent after centr fugation at 20,000 g for 30 minutes, is dialyse against three changes of normal saline for 2 hrs. The dialysed extract is again centrifuge to obtain the clear supernatent (Kcl extract The extracts were concentrated by lyophilisatio for quantitation of immunoglobulin.

(2) **Radial Immunodiffusion:** The saline an Kcl extracts were used for radial immunodiffu sion in plates containing antisera of IgA, IgG and IgM. The mmunoglobulins were quantitate by single radial immunodiffusion according t the procedure of Mancini *et al* (1965). Linea standard graphs were obtained by plotting th