# Evaluation of Blood Constituents as Diagnostic Markers for Ethmoid Carcinoma in Cattle

By

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THESIS

Submitted in partial fulfilment of the
requirements for the degree

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Centre of Excellence in Pathology
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1993

Dedicated to
my Mother & Father

## DECLARATION

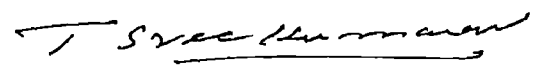
I hereby declare that the thesis entitled **EVALUATION OF BLOOD CONSTITUENTS AS DIAGNOSTIC MARKERS FOR ETHMOID CARCINOMA IN CATTLE** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society

Mannuthy,
3/8/93

S. MANUMOHAN

# CERTIFICATE

Certified that this thesis, entitled **EVALUATION OF BLOOD CONSTITUENTS AS DIAGNOSTIC MARKERS FOR ETHMOID CARCINOMA IN CATTLE** is a record of research work done independently by Sri. S. Manumohan under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.



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# CERTIFICATE

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# Introduction

# INTRODUCTION

The carcinoma of the mucosa of the ethmoid was reported in an endemic form from the Scandinavian countries during the beginning of this century. Subsequently there has been reports of this tumour from many parts of the world and now this tumour has emerged as a specific neoplastic condition in cattle like horn cancer and eye cancer.

In India, since the first record of this tumour by Muthappa (1930) from Tamilnadu the incidence of this tumour has been reported from the other Southern states of India during the last two decades. All these reports clearly indicated that there is a significant increase in the incidence of this tumour during recent years. Although, the incidence was confined to the Southern India for many years of late there has been stray reports of incidence of the tumour from northern states like Haryana, Uttarpradesh, West Bengal and Orissa. This would imply that the tumour incidence is present all over India and it would appear that the geographical barrier has been broken and the tumour has emerged as an important national problem.

The first case in Kerala was recorded in cattle in 1960 by Rajan et al. (1972). Later several cases were recorded in different species of domestic and wild animals. The tumour

affects generally cross-bred cattle in the age group of 5-7 years and particularly in the second semester of pregnancy. The tumour affects the cattle when they are in the prime age of production. Therefore, the economic loss is great and the incidence is on the increase every year. The specific location of the tumour in the ethmoid region and absence of species barrier singles out this tumour as a unique cancer in the field of animal oncology.

The studies made in this department during the last three decades have indicated that cross-bred animals in the age group of six to eight years and in peak production are more susceptible. The increase in incidence of this tumour in high yielding cross-bred cattle during recent years makes this entity an economically important neoplastic condition. The tumour is a deep seated one and the clinical manifestations are exhibited in the advanced stages and therefore, the diagnosis is often made only at the later stage when there is no possibility of attempting any therapeutic measures.

For all neoplasms an effective early diagnosis is the key to successful therapy. As far as this deep seated tumour is concerned, if an early diagnosis could be made there is possibility of undertaking chemotherapy or immunotherapy. For successful immunotherapy which is the

most feasible type of treatment for animal neoplasms the tumour burden should be minimal which depends on the early diagnosis of this tumour.

Blood constituents and circulating immune markers have been used as diagnostic tool in various human and animal neoplasms. No such diagnostic markers have been identified for this tumour. Therefore, an attempt was made to develop tests for early diagnosis by evaluating certain blood constituents and circulating immune markers in the blood of cattle bearing ethmoid carcinoma.

# Review of Literature

# REVIEW OF LITERATURE

## 2.1. Incidence and epidemiology

Ethmoid carcinoma in cattle and horses was reported from Sweden in the beginning of this century (Moussu, 1906, Stenstrom, 1909, 1915, Forsell, 1913, Bergman, 1914, Magnusson, 1916) Horne and Stenerson (1916) reported the incidence of ethmoid carcinoma in cattle from Norway. Jackson (1936) reported the incidence of ethmoid carcinoma in cattle from South Africa. Borkelhammer (1949) described an adenocarcinoma of the nasal epithelium in Shetland sheep dog. Cohrs (1952, 1953) reported the incidence of transmissible adenocarcinoma and infectious adenopapilloma of the olfactory mucosa of sheep. Cotchin (1956) in his review of the neoplasms of domestic animals cited 160 reports of ethmoid cancer in cattle and 24 in horses in Sweden. Bedford (1959) reported the incidence of adenocarcinoma in the maxillary nasal sinus in the dog. Young et al. (1961) reported various neoplasms arising from the olfactory mucous membrane of the sheep. Amaral and Nesti (1963) observed the carcinoma in the ethmoidal sinus of cattle and pigs in Brazil. Nazario et al. (1966) reported carcinoma in the ethmoidal sinus of cattle and pigs. Duncan et al. (1967) reported enzootic nasal adenocarcinoma in sheep. Rubaj and Woloszyn (1967) reported

enzootic adenopapilloma of the nasal cavity in sheep. Becker et al. (1972) observed nasal tumours in cattle. Tokarnia et al. (1972) reported enzootic ethmoid tumour in cattle in Rio-de-Janero, State of Brazil. Inada et al. (1973a) and Inada and Tokarnia (1973b) described ethmoid tumours in pigs and cattle respectively. Bradley and Harvey (1973) made a brief note on the incidence of nasal tumours in dogs. Cho et al. (1974) reported adenocarcinoma in the nasal cavity and brain of a dog. Legendre et al. (1975) reported the incidence of nasal carcinoma in cats. Brownstein et al. (1975) reported nasal carcinoma in a captive eld's deer (Cervus eldi). Mason (1975) recorded a case of spindle cell sarcoma of the equine paranasal sinus and nasal chamber. Stanzi and Hauser (1976) described tumours of the nasal cavity in domestic animals. Madewell et al. (1976) observed 300 cases of primary neoplasms involving the nasal cavity or paranasal sinuses of domestic animals among 12,300 cases studied. The multi species data were compiled from abstracts of medical records maintained by 13 colleges of veterinary medicine in the United States and Canada from 1964-1973. Brown et al. (1977) reported a case of nasal adenocarcinoma in a Taiwan Macaque. Confer and Depaoli (1978) observed sixteen cases of primary neoplasm of the nasal cavity, paranasal sinuses and nasopharynx in the dog. Yonemichi et al. (1978) reported intranasal tumour of the ethmoid

olfactory mucosa in sheep. Njoku et al. (1978) reported the incidence of ovine nasal adenopapilloma from ten sheep . Pospischil et al. (1979,1982) reported five cases of endemic ethmoid carcinoma in cattle and studied their histological and ultrastructural character. Howard et al. (1982) mentioned about the descriptive epidemiology of the carcinoma of the nasal cavity and paranasal sinuses in the dog. Stroud and Amurdson (1983) reported a case of squamous cell carcinoma perforating the hard palate and reaching the nasal cavity in a free ranging white tailed deer (Odocoileus virginianus). Njoku and Chineme (1983) recorded the neoplasms of the nasal cavity of cattle and sheep. Adant et al. (1984) reported a case of congenital ethmoid carcinoma in a foal. Giauffret et al. (1984) reported nasal tumours in caprines. Charray et al. (1985) described an outbreak of adenocarcinoma of the olfactory mucosa in West African Dwarf ewes. Ringe and Rajko (1985) observed growth in the nasal cavity as naturally occurring nasal obstructions in sheep. Steen et al. (1985) reported nasal tumours in a fallow deer (Dama dama L.). The report by Haltgren et al. (1987) described the features of fibrosarcoma in the nasal and maxillary sinuses of two young horses, of which one was congenital. Wendt (1980) described incidence, clinical aspects and diagnosis of ethmoidal tumour in sheep. He studied 11 cases during a period of 10

years. Morrison et al. (1989) reviewed the case records at the Mardoch University Veterinary Hospitals over the period of 1978-1988, and it revealed 37 cases of nasal tumour in dogs.

2.1.1. Incidence and epidemiology in India

In India Muthappa (1930) was the first to record a case of neoplasm in the ethmoid mucosa of a cow. David and Venkataraman (1940) reported the occurrence of malignant growth in the frontal sinus of cattle. Nair and Sastry (1954) in a survey of 2003 neoplasms in domestic animals in Madras State recorded 18 cases of ethmoid cancer in cattle and buffalo. Narayana (1960) reported a case of Carcino Sarcoma of nasal passage and frontal sinus in an eight year old Ongole breeding bull from the state of Andhra Pradesh. Sastry and Rao (1964) recorded a case of adenocarcinoma with fibro-sarcomatous stroma in a bullock. Rajan et al.(1972) reported the incidence and pathology of tumours of paranasal sinuses in domestic animals in Kerala. Damodaran et al. (1974) reported fifty one neoplasms in the ethmoid region of bovines from the state of Tamil Nadu. Balasubramaniyam (1975) studied seventeen cases of sinus tumour in cattle from the state of Karnataka. Prasad and Kohli (1978) recorded a case of nasal osteoma in a bullock. Nayak et al.(1979, 1980) reported tumours of bovine nasal cavity

from Orissa. Jayaraman et al. (1979) stated that in the recent years there has been an increase in the incidence of neoplasm involving ethmoid region in domestic animals. They did not find any breed specificity but neoplasms were common in the age group of 6-9 years. There was preponderance of incidence in the progeny of few sires indicating genetic predisposition. Epidemiological features of the disease indicated an infectious etiology. The tumours of the mucosa of the ethmoid were encountered in Kerala since 1960. One hundred and fifty cases were recorded during 1977-79 period. It consisted of cattle (133), buffalo (7) and goats (10) (Rajan, 1980 and Rajan and Sulochana, 1982). A survey undertaken in Tamil Nadu during a period from 1977-80 revealed the occurrence of the disease more in organised farms where livestock were kept congregated (Viraraghavan et al., 1980). Rajan et al. (1981a) reported ethmoid carcinoma in goats and pigs. Chougnaty and Rao (1982) recorded 60 cases of tumours of the ethmoid mucosa and paranasal sinuses from cattle and buffalo during a period of eight years from 1974 to 1981. Pruthi et al. (1982) recorded a case of fibrosarcoma of nasal region in a bullock. Sreekumaran and Rajan (1983) observed that this tumour has established itself in an endemic form in Kerala. They recorded high incidence in cross-bred Jersey cattle particularly in the age group of 7-10 years. All districts

in the state were equally affected. Kornel et al. (1984) reported the incidence in a herd of purebred Jersey in the age group of 5-11 years. Bovine leukemia virus antibody was detected in 10 out of 21 cases but its relationship to ethmoid neoplasm was not clear. The possibility of a vertical transmission of the neoplasm in the herd was discussed. Rameshmurthi (1984) reported the incidence of ethmoid carcinoma in Jersey cattle in the Red dane project in Karnataka. The animals were in the age group of 4-11 years. Singh and Singh (1984) recorded a case of adenocarcinoma in the nasal cavity of a buffalo. Mahdi (1985) reported a case of squamous cell carcinoma in the nasal cavity of a mare. Muralimanohar et al. (1986) described a case of ethmoid carcinoma in a goat. Swarup et al. (1987) reported two cases of ethmoid carcinoma in adult dairy cow from Uttar Pradesh. Chakrabarthi et al. (1988) described three cases of ethmoid carcinoma in HF cattle from West Bengal. Muralimanohar (1988) made a survey of occurrence of ethmoid neoplasm in domestic animals for a period of 5 years (1983-1987) covering cattle, buffalo, sheep, goat and dog.

## 2.2 Diagnostic procedures

### 2.2.1. Clinical symptoms

Persistent nasal discharge either bilateral or unilateral with or without blood, dyspnoea and unilateral or bilateral exophthalmos were the common clinical symptoms (Moussu, 1906 Stenstrom, 1915, Muthappa, 1930 David and Venkatraman, 1940 Narayana, 1960, Tokarnia et al., 1972 and Jose et al., 1985). Snoring and abdominal type of respiration were found in advanced cases (Rajan et al., 1972 Nair, 1973 Damodaran et al., 1974, Balasubramaniyam, 1975 Njoku et al., 1978 Jayaraman et al., 1979). Nervous symptoms like circling movement, cachexia, perforation of the frontal bone and bulging of the forehead were reported by Nayak et al. (1979), Pospichil et al. (1979), Sreekumaran, (1980), Rajan et al. (1981a) and Pruthi et al. (1982). Swelling of the sub-maxillary lymphnodes was reported by Kornel et al. (1984). Muralimanohar (1988) reported nasal discharge and epistaxis as the clinical symptoms of this condition.

### 2.2.2 Exfoliative cytology

Nair (1973) observed neoplastic cells in the nasal discharge. The neoplastic cells had hyperchromatic nucleus with clumping of chromatin, anisokaryocytosis and

2.2.3 Other methods

Rajan and Vijayan (1981b) evaluated the usefulness of Ehrlich s test as a diagnostic test. The results were encouraging and they suggested the usefulness of this test as one of the battery of tests to be employed for diagnosing the tumour. Vijayan and Rajan (1982b) prepared mucous block from cattle bearing ethmoid carcinoma by collecting mucous using a nasal scoup and by aspiration. Aspiration method gave satisfactory results. An organoid pattern of the tumour tissue was evident and a precise diagnosis of the tumour was possible. Vijayan and Rajan (1982c) evaluated the cerebrospinal fluid of the animal bearing ethmoid carcinoma and recorded slight lymphocytic pleocytosis. Muralimanohar (1988) stated that radiology and ultra sound technique also were useful in the diagnosis. Muralimanohar and Sundararaj (1989) employed mucous block technique to detect ethmoid carcinoma in 14 cases. Thirteen cases showed positive results correlating with the post-mortem finding and suggested that this technique can be effectively employed as a diagnostic tool to detect early cases of ethmoid neoplasm in cattle. Koshy et al. (1989) also suggested the usefulness of radiological techniques in the diagnosis and monitoring of the treatment using chemotherapeutic agents like cyclophosphamide.

Gangadharan (1992) demonstrated Gamma Glutamyl Transpiptidase activity in ethmoid carcinoma tissue sections which was considered as an indirect evidence for the involvement of a gentoxic carcinogen like aflatoxin in the causation of this tumour.

## 2.3 Histopathology

### 2.3.1 Cattle

Adenocarcinoma, squamous cell carcinoma and undifferentiated carcinoma were the common types observed (Stenstrom, 1915, Nair and Sastry, 1954, Rajan et al., 1972 Nair 1973 Damodaran et al., 1974 Balasubramaniyam, 1975 Pospischil et al., 1979 Jayaraman et al., 1979 Sreekumaran, 1980 Rajan, 1980 Rajan and Sulochana, 1982 Choudhary and Rao, 1982 Sreekumaran and Rajan, 1983, Chakrabarthi et al., 1988 and Muralimanohar, 1988). Other types of tumours encountered were osteoma (Moussu, 1906), myxosarcoma (Moussu, 1906 and Nayak et al ,1979), transitional cell carcinoma (Nair, 1973 and Balasubramaniyam 1975), fibroma (Muthappa, 1930), mixed cell sarcoma (David and Venkatraman, 1940), carcinosarcoma (Narayana, 1960 and Sastry and Rao, 1964), fibromyxo-chondro osteoma myxochondro osteosarcoma and fibrosteochondroma (Becker et al., 1972) histiocytic

malignant lymphoma (Madewell et al.,1976, and Choudhary and Rao, 1982), osteoma (Prasad and Kohli, 1978), atypical osteoma (Rumbaugh et al., 1978) and mesenchymal blastoma (Pospischil et al., 1982).

2.3.2 Other species

Young et al. (1961) and Duncan et al. (1967), reported intranasal tumours of epithelial origin in sheep. Cho et al. (1974) reported addenocarcinoma in the nasal cavity of a dog. Adenocarcinoma in the captive Eld's deer was reported by Brownstein et al. (1975). Mason (1975) described a spindle cell sarcoma in the equine paranasal sinus and nasal chamber. Brown et al. (1977) described a papillary adenocarcinoma in a Taiwan macaque monkey. Yonemichi et al. (1978) grouped the intra nasal tumours of sheep as papillary adenoma or adenocarcinoma. Njoku et al. (1978) also reported papillary growths of epithelial cells in the nasal cavity arising from the mucosa of the ethmoid bone in sheep. Confer and Depaoli (1978) reported respiratory epithelial carcinoma, adenocarcinoma, squamous cell carcinoma, chondrosarcoma, and undifferentiated sarcoma in dogs. Rajan (1980) observed adenocarcinoma, papillary adenocarcinoma, and squamous cell carcinoma in goats. Adenocarcinoma was the histological type of ethmoid

carcinoma observed in pigs (Rajan et al., 1981 a). Stroud and Amurdson (1983) reported squamous cell carcinoma in a free ranging white tailed deer, in the nasal cavity. Adant et al. (1984) observed a congenital ethmoid anaplastic carcinoma in a foal. Mahdi (1985) reported a squamous cell carcinoma in the nasal cavity of a mare. Wendt (1989) reported papillary adenoma, fibroadenoma and adenocarcinoma from the nasal cavity of sheep.

## 2.4. Calcium, Phosphorus and Magnesium

Biran et al. (1977) reported ten cases of hypercalcaemia associated with different histological types of ovarian neoplasms. Parathyroid hormone prostaglandins and osteoclast activating factor were the agents mainly involved in the pathogenesis of cancer hypercalcaemia.

According to Barrett et al. (1978) low grade elevation of serum calcium occasionally seen in adult female C3 H/Fg mice may be caused by incipient mammary gland tumours.

Pigadas et al. (1978) reported hypercalcaemia in squamous cell carcinoma of the renal pelvis without bone metastasis and there was a fall in the serum calcium level after the removal of the neoplasm.

Hypercalcaemia was recorded in HSDM murine fibrosarcoma and VX2 carcinoma in rabbits. In these two tumours it was concluded that hypercalcaemia was produced by utilization of prostaglandin E2 as the mediator between neoplasm and bone and analogous or identical mechanisms may operate in a small number of human tumours (Tashjian, 1978).

Regaining normalcy in hypercalcaemia and hypophosphataemia by tumour ablation and recurrence of hypercalcaemia and hypophosphataemia with tumour growth suggested that adenocarcinomas of the apocrine glands of analsac produced a substance that caused hypercalcaemia (Meuten et al., 1981).

Sztern et al. (1981) reported secretion of parathyroid hormone (PTH) like chemicals in breast carcinoma which accounted for hypercalcaemia and hypophosphataemia and treatment with antitumour drugs resulted in reduction of serum calcium level.

Angel et al. (1982) suggested that humoral factor responsible for hypercalcaemia in patients with head and neck cancer was not parathormone Meuten et al. (1982) suggested that mediators of hypercalcaemia associated with adenocarcinoma of apocrine glands of anal sac were other

than parathyroid hormone and these included prostaglandinE2, Vit D sterols and osteoclast activating factor. Ultrastructural studies suggested that the neoplastic cells have well developed synthetic and secretory organelles necessary for production of polypeptide hormones which may be involved in hypercalcaemia.

Weller et al. (1982) observed hypercalcaemia, creatinaemia, azotaemia and hypercalcuria in lymphosarsoma cases. Histopathological studies revealed no changes in the parathyroid and skeletal muscle cells.

Gewirtz et al. (1983) ruled out primary hyperparathyroidism or ectopic parathyroid hormone production as a factor for hypercalcaemia in complicating myelogenous leukaemia. By in vitro studies they observed production of a chemical with potent bone resorbing activity distinct from parathyroid hormone (ipTH) and prostaglandin E2, and the chemical factor resembled chemically to osteoclast activating factor.

Meuten et al. (1983a) observed an increase in the plasma concentration of 13, 14 dihydro-15-keto prostaglandin E2 ($PGE_2M$) in hypercalcaemic dogs with lymphosarcoma. The increased osteoclastic bone resorption in these dogs was not mediated by increased circulating levels of immunoreactive

parathyroid hormone and 1,25-(OH2)D but was dependent upon infiltration of bone marrow by neoplastic cells and presumably, the local production of a bone resorbing stimulating factor.

Meuten et al. (1983b) reported that adenocarcinoma originating from apocrine glands of analsac produced a hypercalcaemic factor other than immunoreactive parathyroid hormone or prostaglandin E2, that increases osteoclastic osteolysis distant from the tumour and results in hypercalcaemia hypercalcuria and hypophosphataemia.

Sica et al. (1983) used a transplantable non-metastasising Leydig cell tumour which occurred spontaneously in aged Fisher rats as a model for tumour hypercalcaemia. This was characterised by hypercalcaemia, hypercalcuria, hypophosphataemia, suppressed parathyroid circulating hormone concentration and production of a bone resorbing factor in vitro by cultured tumour cells.

Norrdin and Powers (1983) concluded that bone remodelling changes do occur in hypercalcaemia associated with lymphosarcoma and analsac apocrine gland adenocarcinoma.

Meuten et al. (1984) reported that excision of adenocarcinoma in dogs associated with hypercalcaemia

originating from the apocrine glands of analsac resulted in normocalcaemia within 24-48 hours and the adenocarcinoma appeared to produce a factor other than iPTH and prostaglandin E2, that increased oesteoclastic osteolysis distant from the tumour and resulted in hypercalcaemia.

Ralston et al. (1984) observed hypercalcaemia in many solid tumours and a humoral mediator similar to the activity of parathyroid hormone was found to play a role in the development of hypercalcaemia by impairing the renal excretion.

According to Dodd et al. (1985) patients with adult T cell leukemia frequently had hypercalcaemia and this was attributed to substances synthesised by the malignant T cells

The review of the pathogenesis of humoral hypercalcaemia produced by neoplasms suggested that the three biological activities associated with tumours that cause humoral hypercalcaemia were adenylate cyclase stimulating activity (parathyroid hormone like activity), in vitro bone resorbing activity and transforming growth factor activity (Rosol and Capen, 1988).

Weir et al (1988) demonstrated in hypercalcaemic dogs with lymphosarcoma an increase in calcium excretion and nephrogenous adenosine monophosphate excretion. They concluded that in some cases, hypercalcaemia in canine lymphosarcoma was mediated by a tumour derived circulating bone resorbing factor which was distinct from parathyroid hormone and adenylate cyclase stimulating activity and was detected only in hypercalcaemic tumour tissue.

Boudaillez et al. (1990) reported that plasma magnesium level remained normal in leukaemia associated hypercalcaemia in a 10 year old boy.

Shek et al. (1990) observed in a hospital population that malignancy associated hypercalcaemia and subsequent studies by them showed that humoral factors were responsible for a relatively high proportion of cases of malignant hypercalcaemia.

Sridhar and Hussain (1990) observed hypercalcaemia refractory to conventional measures of treatment in a case of squamous cell carcinoma of the head and neck.

Wada and Ohtani (1990) reported that no clinical hypercalcaemia was found in those with parathyroid hormone

related protein MRNA positive lung squamous cell carcinomas, breast carcinomas and adenocarcinoma of maxilla.

Buller et al. (1991) reported the first case of a paraneoplastic hypercalcaemia associated with adenosquamous carcinoma of the endometrium and demonstrated atopic parathormone by immunohistochemical method.

The assay of parathyroid hormone related protein (PTHrP) allowed positive identification of patients with PTHrP mediated hypercalcaemia. Therefore, this was considered useful in the clinical investigation of hypercalcaemia patients and furthermore it allowed detection of circulating PTHrP in hypercalcaemic breast cancer patients with bone metastasis. This indicated a significant role for PTHrP in the disease and it was found to be of value as diagnostic tool (Grill et al., 1991).

Miyake et al. (1991) observed in tumour xenografts in nudemice with hypercalcaemia that parathyroid hormone related protein alone released from tumour cells could induce hypercalcaemia.

Mosekilde et al. (1991) described that tumour extracts from patients with humoral hypercalcaemia often contained PTH like bioactivity.

Ratcliffe et al. (1991) proved direct evidence for a humoral role of tumour derived PTHrP in solid tumours and haematological malignancies and showed that PTHrP assays can be used appropriately to investigate hypercalcaemia of malignancy.

A two and one half fold increase of plasma calcium concentration was found in human squamous cell carcinoma of the maxilla transplanted in athymic nude mice and the level dropped on surgical excision of the tumour (Yoneda et al., 1991).

Bundred et al. (1992) implicated parathyroid hormone related protein as the humoral factor responsible for hypercalcaemia associated with breast cancer and hypercalcaemia was suggested as a useful indicator to predict bone metastasis.

## 2.5. Lipid Bound Sialic Acid

Studies on the gangliosides [lipid bound sialic acid (LSA)] of plasma membrane from normal rat liver and Morris hepatoma cases revealed that the LSA originated from the tumour cell surface (Dristrain et al., 1975, 1977).

Kloppel et al. (1978) reported that the level of LSA in the serum was elevated in tumour bearing dogs indicating that this could also be employed as a diagnostic tool.

Skipski et al. (1978) used serum level of LSA as a potential diagnostic tool for cancer detection.

Katopodis et al. (1980) observed that the difference in total lipid bound sialic acid levels in blood plasma of cancer patients and assymptomatic healthy individuals was highly significant and this procedure provided precise data in a short time.

According to Dristrain et al. (1981 & 1983), plasma lipid bound sialic acid was found to be a useful biochemical marker in patients with leukemias, lymphomas, Hodgkins disease, melanoma, cancer of prostate, bladder, breast, lung colon and ovary.

Kloppel et al (1981) stated that the level of LSA in the serum was elevated in tumour bearing horses which decreased markedly and returned to normal after the surgical removal of the growth and chemotherapy indicating that this could also be utilized as a diagnostic tool.

Shanmugam and Nagarajan (1986) observed a consistent rise in serum LSA in cervical cancer patients.

Muralimanohar (1988) recorded increased levels of LSA in white cattle as well as in buffaloes bearing carcinoma of ethmoid mucosa

Baxi et al (1991) stated that evaluation of lipid bound sialic acid levels in the serum could be used in the early detection and staging of the disease in precancerous conditions of the oral cavity.

Chondros et al. (1991) demonstrated a correlation between serum lipid bound sialic acid and cytology of the body fluid They stated that this could be used as an early tumour marker to detect the extent of metastasis and to monitor therapy

Kakari et al (1991) stated that lipid bound sialic acid levels in the serum was a highly sensitive marker in lung cancer but their specificity was low

Patel et al (1991) observed that lipid bound sialic acid levels in the serum could be used as a sensitive tumour marker in leukemia and could also be used for differentiating anaemia from leukemia

## 2.6. Total Sialic Acid

The study of Kalant *et al.* (1964) indicated that diaminoazobenzene induced rat hepatoma cells possessed more surface sialic acid than normal.

Singh *et al.* (1967) reported elevated serum sialic acid levels in patients bearing malignant growth.

According to Warren *et al.* (1972) one of the most consistent feature associated with tumourogenesis was the altered expression of cell surface glycoproteins and glycolipids to which sialic acid residues were attached.

Van Beck *et al.* (1973) reported higher amount of sialic acid in the transformed cells *in vitro* than the normal cell culture.

Rapin and Burger (1974) noticed alteration of glycolipids, glycoproteins and glycosyl transferase in tumour tissues. They also observed that tumour cells possessed an increased amount of sialic acid on the cell surface. During tumourogenesis sialic acid associated with glycoproteins and glycolipids were shed into the circulation from the cell surface of tumour cell which caused increased serum sialic acid levels in tumour bearing animals and man.

Khadapkar et al. (1975) reported that the increased serum sialic acid level was a general feature of proliferative cell growth and not unique to tumourogenesis alone. Elevated serum sialic acid levels were reported in rats with mammary tumours (Bernacki and Kim, 1977).

Bhide et al. (1977) observed that the level of sialic acid content per million cells in malignant human foetal muscle cells grown in vitro was more than that of normal skeletal muscle cells.

Lipton et al. (1978) stated that patients with malignant disease of the lung, gastrointestinal tract gyaenocological cancers, lymphomas and malignant melanoma had elevated serum sialic acid levels.

Lincheyskaya et al. (1978) demonstrated rise in sialic acid content of the gastric mucosa in cancer of the stomach.

Silver et al. (1978) recorded significantly elevated serum sialic acid concentrations in malignant melanoma patients. According to them the levels tended to be greater in those with large tumour burden. According to Waalkes et al. (1978) serum sialic acid levels were above the normal range in majority of metastatic breast cancer patients and can be used to monitor the disease.

Precise determination of serum sialic acid level in the serum provides information about the stage of malignancy and helps in monitoring of the clinical course and evaluation of treatment (Weiss et al., 1978).

A significant correlation was found between serum sialic acid level in ovarian tumours and tumour burden. Serial estimation of the levels suggested that this may be clinically valuable in guiding patient management (Silver et al., 1980).

Shamberger (1984) stated that the diagnostic usefulness of sialic acid was superior to carcinoembryonic antigen and other tumour antigens associated with a limited spectrum of tumours.

Uehan et al. (1984) stated that majority of gastrointestinal cancer patients showed abnormally high serum sialic acid levels. It increased with progress of malignancy. This was found to be of value as a marker for the disease.

Motoi et al. (1985) observed that elevated serum sialic acid levels were noticed in hepatic abscess in cattle and estimation of sialic acid level in the serum as a tumour marker was limited.

Plasma sialic acid levels were elevated in patients with cancer of the lung, ovary, cervix, pancreas, prostate, thyroid, uterus, oesophagus and endometrium. There was a correlation between the level and progression and regression of disease (Diwedi et al., 1987).

Kinoshita et al. (1987) observed that serum concentration of sialic acid was significantly higher in cows suffering from bovine leukosis than the normal healthy controls.

Muralimanohar (1988) reported increased levels of total serum sialic acid in white cattle as well as in buffaloes bearing ethmoid neoplasms

Serum sialic acid levels did not vary significantly between healthy cattle and cattle which were haematologically and serologically positive for bovine leukosis virus. In contrast, human cancer patients had significantly higher serum sialic acid concentrations than healthy subjects. Foetal and newborn calves had higher levels of sialic acid than adults (Sydow et al., 1988). According to Scherblom et al. (1988) the sialic acid content of new born calf was approximately three fold higher than that of mature animals.

Estimation of total sialic acid level in the serum can be used in the early detection and staging of the oral precancerous and cancerous conditions (Baxi et al., 1991).

Bhatavdekar et al. (1991) showed a correlation between serum protein bound sialic acid level and progression of the disease and response to therapy in patients with advanced head and neck cancer.

Chondros et al. (1991) demonstrated a correlation between total sialic acid level in the serum and cytology of the body fluid. They stated that this was an early tumour marker and was useful to detect the extent of metastasis and to monitor the therapy.

According to Kakari et al. (1991) total sialic acid level in the serum was a highly sensitive marker in lung cancer but its specificity was low.

Patel et al. (1991) observed that total sialic acid level in the serum was a sensitive tumour marker in leukemia and was useful for differentiating anaemia from leukemia.

Reuter et al. (1992) observed that free sialic acid levels and sialidase activity in the oral secretions can be

considered as markers for carcinomas of the upper digestive tract.

## 2.7. Immunological studies/Tumour immune markers

Tumour specific antigens were demonstrated in human colon adenocarcinoma by agar gel immunodiffusion test (Gold and Fredman, 1965).

Most of the neoplastic cell types were immunogenic for the host, in which they arise due to their acquisition of certain antigens called transplantation specific cellular antigens and in individuals with metastasis it might bring about inhibition of serum mediated immunological reaction of the patient to his tumour (Currie and Bashams, 1972).

Mavlight et al. (1973) prepared soluble antigens from solid tumour in man using 3M KCl and found to be useful in studying cell-mediated immunity.

Human thymus leukemia associated antigen (H Thy-h) was detected by immunodiffusion in significant quantities in normal thymocytes and also in lymphocytes from patients with E-rosette positive acute lymphoblastic leukemia It was found to a lesser extent in cells from patients with E rosette negative lymphoblastic leukemia and acute myeloblastic leukemia (Boris et al., 1973).

Dutta (1977) demonstrated the presence of horn cancer antigen by immunodiffusion with antiserum raised in rabbit against crude horn cancer antigen.

Antisera raised in rabbits against NaCl extract of squamous cell carcinoma of the cervix showed precipitin bands in double gel diffusion to normal and malignant cervical tissues. The antisera adsorbed with normal cervical extracts revealed precipitin reactivity against a variety of other cancers including those of the stomach colon, lung and endometrium. The antigens which showed identity with BOFA (Beta oncofetal antigen) were identified from cervical squamous cell carcinoma. This was present in a number of adult normal and neoplastic human tissues (Goldenberg et al., 1977).

Howell and Goldrosen (1977) reported that 4 M KCl extract preparations of colon carcinoma (MCA-3S) and melanoma tumour (B16) in mice showed tumour specific antigenic activity associated with a cell surface glycoprotein. This was not similar to murine histocompatibility antigens and the antigen preparation elicited leucocyte adherence inhibition.

Colorectal tumour of different grades exhibited significant difference when tested for leukocyte adherence

inhibition by using 3 M KCl extracts of the tumour tissue and decreased non adherence index was noticed with extensive tumour burden with the same antigen (Ichiki et al., 1977).

Imamura et al. (1977) produced antibodies in rabbits against extracts of human ovarian cystadenocarcinoma and studied the reactivity of sera of cancer patients by double diffusion in agar. It was found that in normal healthy individual sera, antigens were not detected and in patients with ovarian tumours antigens were present in the serum.

Keyahou et al. (1977) demonstrated antibodies against organ specific as well as tumour associated antigens by immunodiffusion using antihuman glioblastoma serum prepared against saline extracts of the tumour.

Mc Intire et al. (1977) identified a human lung tumour associated antigen in the serum of patients with lung cancer.

Periman (1977) revealed considerable variation in response to skin tests in cancer patients. Among different lots of membrane antigens identically prepared by 3M KCl extraction from the same lymphoid cell line in the same laboratory, there was considerable variation in response to these antigens.

Serum from hypernephroma patients were assayed in micro complement fixation for the presence of tumour associated antibody using membrane rich fractions of hypernephroma cells obtained by hypotonic cell lysis as the source of antigen and it was found that complement fixing antibody reacted against hypernephroma cell extracts. The antibody was found only in the patients who had a small tumour burden and as the disease progressed the antibody titer came down to zero. These antibodies were not found in normal healthy controls (Raming et al., 1977).

Rangel et al. (1977) demonstrated melanoma associated antigen and antibody by ultra complement fixation using melanoma extracts as antigen and rabbit antimelanoma serum as antibody respectively.

Reisfeld et al. (1977) successfully employed melanoma associated antigen prepared by 3M KCl extract of cultured melanoma cells to detect antibody to melanoma cells in the serum from melanoma patients.

Extracts of pooled induced pancreatic neoplasms in Syrian golden hamsters showed apparently identical precipitin bands which indicated the presence of circulating tumour specific antigen (Runge et al., 1977).

Winters et al. (1977) reported that fifty per cent of dogs with lymphosarcoma had serum antibodies to tumour associated antigen while twelve per cent of normal dogs and dogs with other type of tumours had serum antibodies to the same tumour associated antigen.

*Emmrich* et al. (1978) compared the macrophage electrophoretic mobility test with leukocyte migration inhibition test for the detection of tumour associated antigens using agarose with allogenic KCl extracts of tumours and normal tissue to find out positive reactions.

Faulconer et al. (1978) prepared soluble extracts from fresh renal carcinoma tissue by extraction with 3M KCl and measured the antigenecity of extract using leukocyte migration inhibition. Sixty three per cent gave positive reaction.

An organ specific tumour membrane associated antigen was partially purified and identified from a spontaneously metastasising rat mammary carcinoma. Antiserum produced in the rabbit against this antigen was used to detect the tumour antigen in the serum of tumour bearing animals. (Ghosh et al., 1978)

Maxim et al. (1978) prepared antisera to cervical carcinoma antigen from tumour biopsies by low frequency membrane sonication. This did not react with normal serum or normal tissue pools of cervix. The antisera produced specific fluorescence of squamous cells only with cervical carcinoma scrapings.

Hellstrom and Brown (1979) reported that tumours induced by irradiation, chemicals or viral carcinogens contained tumour associated antigens.

A heteroantiserum raised in rabbits against KCl extracts of both normal lung and bronchogenic squamous cell carcinoma was used to monitor the tumour associated component of the extract. A protein isolated from extract which did not appear to be present at detectable levels in an equivalent fraction of normal lung extract reacted with the heteroantiserum and appeared to be present in all extracts of squamous cell carcinoma (Kelly and Bevy, 1979).

Kuchroo et al. (1979) described the preparation of tumour antigen from horn cancer tissue. The mass was homogenised, sonicated and centrifuged. The extract revealed antigenic similarity between squamous cell carcinoma of horn and bovine foetal tissue.

Crude 3 M KCl extracts of the chemically induced3 H/HeJ fibrasarcoma, MCA-F possessed two immunobiologically active components upon fractionation by preparative isoelectric focussing (Pellis et al., 1979).

Indirect immunofluorescence method was successfully employed in the diagnosis of ethmoidal neoplasms. The study revealed presence of circulating antibodies against tumour antigens (Sundararaj, 1979, Masillamony et al., 1980).

Jayaraman et al. (1980) performed serological tests to detect bovine sinus neoplasms by employing homogenised tumour tissue suspension as antigen for gel diffusion and hypersensitivity reactions without success

Sulochana et al. (1980) investigated the etiological factors associated with tumours of the ethmoid mucosa of domestic animals. Haemagglutinating agents were isolated from 17 processed samples by inoculation of samples into the CAM of chicken embryos. Haemagglutination of all the seven isolates were inhibited by New Castle disease antiserum (ND). Serum neutralization tests done in chicken embryos with ND antiserum showed that they were not ND virus although they possessed some antigenic relationship with it as evidenced from HI tests. Delibrate exposure of

unvaccinated, week old, chicks to tumour bearing animals showed that these animals do shed the virus through expired air and chicks can pick up infection without showing any clinical symptoms.

Sulochana and Rajan (1981) made a study on the usefulness of agar gel diffusion and passive haemagglutination tests for the diagnosis of endemic ethmoid carcinoma in cattle by employing 3 M KCl extracts of pooled tumour tissue collected from tumour animals, but did not succeed in their attempt.

Alturu et al. (1982a) studied the reactivity of serum collected from six cattle with plaque and malignant stages of ocular squamous cell carcinoma Indirect immuno flourescence detected antibodies produced against tumour cells. There was no surface reactivity when these sera were tested against normal epithelial cells. Sera adsorbed with autologous and allogeneic cells showed no reactivity to surface antigens or allogenic cells. They suggested that a shared antigen existed among the cells from plaque and malignant lesions.

Serum samples from cattle in various stages of ocular squamous cell carcinoma were collected and tested for reactivity with surface antigens from cultured autologous

allogenic cells The serum possessed high titres of antibodies to autologous cells. But the reactivity of allogeneic serum to cultured cells varied. Furthermore surface reactivity was not observed in these serum samples in tests for reactivity with normal epithelial cells. Absorption of serum with precanerous cells eliminated the reactivity of serum from carcinoma bearing animals towards cultured tumour cells. From these observations it was concluded that there was a shared antigen among the cells from precarcinoma and carcinoma lesions (Alturu et al., 1982b).

Kuchroo et al. (1982) demonstrated tumour associated antigens in bovine horn cancer by immunodiffusion in agar gel and also by immunoflourescence. Antiserum raised in rabbits against sonicated pooled horn cancer tissue extracts reacted only with horn cancer extracts on agar gel diffusion. Preparations of normal antigen and extract from other tumours did not show any reactivity against antiserum by this test.

Kuchroo and Spradbrow (1985) noticed that majority of the twenty one squamous cell carcinoma (OSCC) serum samples reacted with autologous and allogenic OSCC cells and did not show any significant reactivity with normal skin cells.

These serum samples when further tested on seven different allogenic or xenogenic tumour cell types (other than OSCC) showed significant reactivity only with cultured equine sarcoid and cutaneous papilloma cells. Most of the control serum samples did not show any significant reactivity against OSCC cells except serum from one cow bearing ocular papilloma and two healthy normals that were in contact with OSCC bearing animals. This study suggested that OSCC cells possessed tumour associated antigens that are common to a number of tumours.

Ristau and Klima (1985) produced hyperimmune serum in rabbits from plasma membrane preparations of tumour lymph nodes from three leukosis cows. The serum that had been adsorbed three times with a plasma membrane preparation from healthy cattle remained positive in ELISA to autologous plasma membrane preparations but did not with preparations from healthy animals. Three hyperimmune sera reacted positively to a variable proportion (12 23 & 45 per cent) of 15 pooled plasma membrane preparations from leukocytic lymph nodes.

Filchev et al. (1987) made experimental studies with hepatoma cell extracts and demonstrated that the proteins separated from hepatoma tissue by polyacrylamide gel electrophoresis contained tumour associated antigens.

Pillai (1987) used different stabilising agents like pyruvic aldehedye, gluteraldehyde and formaldehyde and found that gluteraldehyde is the best aldehyde for stabilising sheep red blood cells. Such stabilized and antigen *sensitized* sheep red blood cells could be stored for six months without any change in quality.

Muralimanohar (1988) made an attempt in ethmoid carcinoma bearing cattle to identify antibodies to tumour specific antigens. No antibodies could be demonstrated by agar gel immunodiffusion test, but specific antibodies could be demonstrated using flourescent antibody technique.

Antisera produced to 3 M KCl extracts of malignant ascites cells obtained from a patient with colon cancer reacted in double immuno diffusion with 10/10 malignant ascites effusion and 0/3 ascites effusion (Sarauis et al., 1989).

# Materials and Methods

# MATERIALS AND METHODS

## Design of the experiment

Twenty-nine tumour bearing animals in various stages of ethmoid carcinoma, procured from different parts of Kerala and twelve clinically healthy animals maintained at the University Livestock Farm, Mannuthy (controls) were bled aseptically and the blood was collected in a clean sterile vial. After clotting the serum was separated and half of the serum stored at -50°C and the remaining half was inactivated at 56°C for 30 minutes and stored at -50°C.

## TECHNIQUES

### 3.1 Calcium estimation

Serum calcium level of 29 tumour bearing animals and 12 clinically healthy control animals were estimated by employing the method described by Henry and Dryer (1963) in Chemitrix spectrophotometer.

### 3.2 Phosphorus estimation

The method of Amador and Urban (1977) was employed for the determination of serum phosphorus level in 29 tumour bearing animals and 12 control animals using Chemitrix spectrophotometer.

### 3.3. Magnesium estimation

Serum Magnesium was estimated by the method of Fernandez and Kahn (1971) in Perkin-Elmer 2380 atomic absorption spectrophotometer.

### 3.4. Lipid bound sialic acid estimation

The serum lipid bound sialic acid was determined as per the method described by Katopodis and Stock (1980).

### 3.5. Total sialic acid estimation

The serum total sialic acid was estimated by the method described by Horgan (1981).

### 3.6. Antigen preparation

The antigen was extracted from freshly collected pooled tumour tissues by the method followed by Mavlight et al. (1973) with slight modification. The freshly collected pooled tumour tissues were minced and the red blood cells were removed by washing with 0.8 per cent Ammonium chloride in phosphate buffered saline (pH 7.2). The mixture was placed in a beaker and rocked overnight at 4°C The contents were then centrifuged in a refrigerated centrifuge at 1900 rpm for 60 min. The slightly viscous supernatent was transferred to a dialysis tube and dialysed against deionised water for one hour. Afterwards it was dialysed

against phosphate buffered saline for 24 hours, with change of dialysing solution every eight hours. The dialysed extract was again centrifuged at 4°C at 19000 rpm for 30 min. The supernatent was concentrated by freeze drying. The concentration of protein was estimated by Biuret's method and concentration of protein was adjusted to 4.5 g/100 ml. The tumour antigen was distributed in 1 ml aliquots and were stored at -50°C until further use.

### 3.7. Antisera preparation

Antisera to the prepared tumour antigen was raised in rabbits. Initially 1 ml of antigen mixed with an equal quantity of Freund's complete adjuvant was administered subcutaneously and 2 ml of antigen without adjuvant was given intravenously. Subsequently they received four injections each of 2 ml antigen alone at three days interval beginning from the third day after the first injection. Ten days after the last injection, the antibody response was tested by agar gel precipitation test against homologous antigen. When a satisfactory response was obtained the rabbits were bled by cardiac puncture and sera were separated. The separated sera was inactivated at 56°C for 30 min and stored at -20°C in 1 ml aliquots for later use.

One ml of the antisera was mixed with an equal quantity of normal clinically healthy bovine serum, inactivated at

56°C for 30 min and stored in 2 ml aliquot at -20°C for later use.

### 3.8. Agar gel precipitation test

The procedure followed for Agar gel precipitation test was the double diffusion method of Ouchterlony (1958). One per cent agarose in normal saline solution was used for the preparation of slides. The test was done on 3x1 slide, each slide having a set of seven wells one central and six peripheral. Tumour antigen was taken in central well, inactivated test sera and antisera raised in rabbits in peripheral wells. After adding antigen and sera, the slides were kept in a humid chamber and incubated at 37°C. The formation of any precipitation lines was examined at every 4 hours under darkground illumination. After 72 hours the slides were stained with Coomassie blue. The pretest inoculation serum collected from rabbits was also tested against tumour antigen as above.

### 3.9. Passsive haemagglutination

#### 3.9.1. Preparation of stabilized sheep red blood cells (SRBC)

The procedure described by Jagannath *et al.* (1984) was followed in this study. SRBC collected from two sheep in Alsevers solution from the slaughter house were kept at 4°C

for 24 hours. SRBC were washed three times in sterile PBS (pH 7.2) and a 50 per cent cell suspension was preparation in PBS.

The gluteraldehyde SRBC mixture was prepared as follows.

1. Neutral gluteraldehyde - 1.5 ml
2. Normal saline solution - 5.0 ml
3. Phosphate buffer (pHs) - 1.0 ml
4. Sheep red blood cells - 1.0 ml

The reaction mixture was stored at 4°C for 24 hours with occasional stirring. The gluteraldehyde SRBC mixture, stored at 4°C for 24 hours was washed with PBS five minutes and filtered through gauze cloth and stored as 10 per cent suspension at 4°C

### 3.9.2. Sensitization of stabilized sheep red blood cells with tumour antigen.

For sensitization of SRBC, the method cited by Jagnnath et al. (1984) was followed. A suspension of SRBC stabilized with gluteraldehyde was washed thrice in PBS and centrifuged at 100 g for 10 minutes. The cell deposits were further diluted with 2 volumes of acidic PBS (pH 6.4) and

gently mixed to provide a uniform suspension of cells. Three millilitre aliquots of the stabilized SRBC prepared were dropwise mixed with 25, 50, 75, 100, 150 and 200 ul of tumour antigen. SRBC antigen mixture was incubated for 6 hours at 37°C and washed in PBS (pH 7.2). After centrifugation at 800 g for 5 min, the cell deposits were resuspended in 1 per cent Bovine serum albumin (BSA) in PBS (pH 7.2) to provide 1 per cent suspension and stored at 4°C till further use.

### 3.9.3. Determination of optimum concentration of Tumour Antigen by Passive Haemagglutination for sensitization of SRBC Checker Board Titration

Rabbit antiserum against the antigen was twofold serially diluted to provide dilution of 1 2 to 1 4096 with 1 per cent mixture in microtiter plates of BSA in PBS (pH 7.2).

Fifty microlitre of different concentration of antigen stabilized SRBC was added to each serum dilution. The mixture was gently agitated and incubated at room temperature for 1 to 1½ hours before reading. The lowest concentration of the antigen that gave a maximum haemagglutination titre with the highest serum dilution was taken as the optimum concentration of antigen for bulk sensitization of SRBC. Since 75 ul of antigen treated stabilized SRBC and 100 ul of antigen treated SRBC gave

similar titre of 1 256, 100 ul of antigen was used for bulk sensitization of stabilized SRBC. Proper controls were also put up along with all the test samples.

3.9.4. Protocol for passive haemagglutination test

A two fold serial dilution of suspected inactivated serum samples and inactivated normal control serum samples were prepared in 1 percentage BSA in PBS (pH 7.2) to provide 1 2 through 1 4096 dilutions in a microtitre plate. Fifty microlitre of antigen sensitized SRBC was added to all the dilutions of sera and mixed thoroughly.

Readings were taken after 1 to 1½ hours of incubation at room temperature. The reciprocal of highest dilution of the serum at which complete haemagglutination occured was taken as the titre of the serum. Proper controls were also put up along with all the test samples.

3.10. Isolation of haemagglutinating agents

At the time of slaughter, tumour tissues were collected from 7 animals under sterile conditions in Hanks Buffered Salt solution (HBSS) containing 1000 IU penicillin and 50 mg of streptomycin/ml. The tissues were then tricturated with the help of a Tenbrock tissue grinder with sterile sand to

prepare a 10 per cent suspension. The suspension was allowed to stand at 4°C for one hour and 0.1 ml of centrifuged clear supernatent solution was inoculated at the rate of 0.1 ml into the chorioallantoic membrane (CAM) of 10-11 day old embryonated eggs. Embryos that died within 24 hours were discarded. The inoculated embryos alive after 24 hours were candled at 8 hours interval. The embryos alive after 5 days of inoculation were chilled at 4°C for 4-6 hours before harvesting. While harvesting, the embryos and CAM were examined for the presence of any lesions. The allantoic and amniotic fluid were subjected to haemagglutination test employing chicken red blood cells. Three blind passages were done using pooled suspensions prepared from the chicken embryo CAM and allantoic fluid, before discarding any sample as negative for haemagglutinating agent.

# Results

## RESULTS

The serum calcium, phosphorus, magnesium, lipid bound sialic acid and total sialic acid levels of 29 tumour bearing animals and 12 control animals are tabulated in table 1.

### 4.1. Serum calcium

The mean serum calcium level in tumour bearing animals was found to be 11.78±0.23 mg% and in control animals it was 10.23±0.10 mg% (Fig. 1). There was increase in mean serum calcium level in tumour bearing animals when compared to normal healthy animals and the increase was statistically significant.

### 4.2. Serum phosphorus

The mean serum phosphorus level in tumour bearing animals was 6.11±0.10 mg% and in control animals it was 6.66±0.09 mg% (Fig.1). The mean serum phosphorus level showed reduction in tumour bearing animals when compared with that of control animals and the reduction was statistically significant.

### 4.3. Serum magnesium

The mean serum magnesium level in tumour bearing animals was 2.35±0.05 mg% and that in control animals it was

2.27±0.07 mg% (Fig.1). The difference in mean serum magnesium level between tumour bearing animals and control animals was not statistically significant.

The serum total sialic acid and serum lipid bound sialic acid level in tumour bearing and control animals are tabulated in table 2.

### 4.4. Serum lipid bound sialic acid

The mean serum lipid bound sialic acid level estimated in 29 tumour bearing animals was 30.83±0.36 ng/dl and that in control animals was 11.67±0.43 ng/dl (Fig. 2). There was increase in mean serum lipid bound sialic acid in tumour bearing animals when compared with that of normal animals and the increase was statistically significant.

### 4.5. Serum total sialic acid

The mean serum total sialic acid level in tumour bearing animals was found to be 95.21 ± 0.78 ng/dl and that in control animals it was 60.67±0.87 ng/dl (Fig.2). The rise in mean serum total sialic acid level in tumour bearing animals when compared with control animals was statistically significant.


## 4.6. Agar gel precipitation test

Distinct precipitin band was observed when the tumour antigen prepared was tested against the antiserum raised against the tumour antigen in rabbit. One distinct precipitin band only was obtained against the tumour antigen (Fig.3).

Pretest inoculation serum did not reveal the presence of any precipitin lines.

All the 29 serum samples collected from the tumour bearing animals showed two distinct precipitin lines. One band was thick and was more closer towards the serum well whereas the sharp band was observed closer towards the antigen well. This second precipitin line was similar to the precipitin band obtained in the case of hyperimmune serum and the antigen (Fig. 3) This indicated that tumour antigen contained soluble antigen and serum of the tumour bearing animals possessed soluble antibody against the tumour antigen.

The serum samples obtained from 12 control animals gave a distinct thick precipitin band whereas majority of the control sera also showed the second sharp band observed closer towards the antigen well that was obsered in tumour bearing animals. In the case of tumour samples as well as

the antiserum raised against the tumour antigen (Fig.3), control sera showed the second precipitin line also.

### 4.7. Passive haemagglutination test (PHA)

Gluteraldehyde stabilised SRBC were found to be suitable for sensitization with tumour antigen. The stabilized and tumour antigen sensitized SRBC could be stored under refrigeration conditions (±4°C) for six months without loss of quality

The optimum concentration of tumour antigen for sensitization of gluteraldehyde SRBC was 75 ul/3 ml of packed volume of SRBC. The passive haemagglutination titre was 1/256 using hyperimmune sera raised against tumour antigen in rabbits (Fig. 4). The PHA titre was the same while using higher concentration of antigens (100 ul, 150 ul and 200 ul) for sensitization. The antigen concentration less than 75 ul/3ml of SRBC was found to be not optimal because the lower concentrations of antigens (10 ul, 25 ul and 50 ul) showed a reduction in PHA titre.

The PHA titre values of 29 serum samples collected from the tumour bearing animals are presented in table 3. The highest titre value obtained was 1 256 and the lowest titre obtained was 1 8.

The titre values of serum samples collected from 12 control animals are presented in table 3. The highest titre obtained was 1 128 and lowest was 1 32. The titre values of two control animals and five tumour animals are shown in Fig 5.

## 4.8. Isolation of haemagglutinating agents

None of the tumour samples processed and inoculated into embryonated eggs revealed the presence of haemagglutinating agents. No haemagglutinating agents could be obtained even after three blind passages of the same samples. None of the embryos showed any lesions indicating the involvement of an infectious agent. Since no haemagglutinating agents could be isolated in the present study haemagglutination inhibition test could not be performed.

Table 1. Serum calcium phosphorus and magnesium in tumour bearing and control animals

| | Sl. No. | Animal No. | Calcium (mg %) | Phosphorus (mg %) | Magnesium (mg %) |
|---|---|---|---|---|---|
| | 1 | T1 | 11.1 | 6.1 | 2.3 |
| | 2 | T2 | 12.2 | 7.2 | 3.0 |
| | 3 | T3 | 11.9 | 5.8 | 2.1 |
| | 4 | T4 | 13.2 | 5.6 | 3.0 |
| | 5 | T5 | 10.9 | 7.6 | 2.6 |
| | 6 | T6 | 12.6 | 6.2 | 2.1 |
| | 7 | T7 | 12.8 | 6.1 | 1.9 |
| | 8 | T8 | 11.2 | 7.1 | 2.2 |
| | 9 | T9 | 12.1 | 6.2 | 2.4 |
| | 10 | T10 | 10.8 | 7.3 | 2.6 |
| | 11 | T11 | 9.9 | 6.1 | 2.3 |
| | 12 | T12 | 13.1 | 5.5 | 2.4 |
| | 13 | T13 | 12.9 | 5.6 | 2.1 |
| Tumour | 14 | T14 | 12.2 | 6.0 | 2.8 |
| animals | 15 | T15 | 13.2 | 5.8 | 2.7 |
| | 16 | T16 | 12.1 | 6.3 | 2.2 |
| | 17 | T17 | 12.3 | 6.0 | 2.3 |
| | 18 | T18 | 9.8 | 5.6 | 2.5 |
| | 19 | T19 | 13.2 | 5.8 | 1.9 |
| | 20 | T20 | 12.5 | 6.0 | 2.0 |
| | 21 | T21 | 11.9 | 6.2 | 2.1 |
| | 22 | T22 | 12.2 | 5.4 | 2.4 |
| | 23 | T23 | 10.8 | 5.7 | 2.5 |
| | 24 | T24 | 12.1 | 6.1 | 2.4 |
| | 25 | T25 | 10.7 | 6.4 | 2.3 |
| | 26 | T26 | 9.2 | 5.3 | 2.8 |
| | 27 | T27 | 8.9 | 6.1 | 1.9 |
| | 28 | T28 | 12.5 | 6.2 | 2.1 |
| | 29 | T29 | 13.2 | 5.9 | 2.3 |
| Mean | | | 11.78±0.22 | 6.11±0.10 | 2.35±0.05 |
| S.D. | | | 1.22 | 0.56 | 0.31 |
| | 1 | C1 | 10.2 | 7.1 | 2.1 |
| | 2 | C2 | 10 1 | 6.3 | 2.6 |
| | 3 | C3 | 10.5 | 6.9 | 1.9 |
| | 4 | C4 | 10.1 | 6.5 | 2.3 |
| | 5 | C5 | 9.8 | 6.8 | 2.7 |
| Control | 6 | C6 | 10.4 | 6.5 | 2.1 |
| animals | 7 | C7 | 10.1 | 6.1 | 2.4 |
| | 8 | C8 | 9.9 | 6.4 | 2.1 |
| | 9 | C9 | 11.2 | 6.9 | 2.5 |
| | 10 | C10 | 10.2 | 6.4 | 2.1 |
| | 11 | C11 | 10.2 | 6.9 | 2.0 |
| | 12 | C12 | 10.1 | 7.1 | 2.4 |
| Mean | | | 10.23±0.10 | 6.66±0.09 | 2.27±0.07 |
| S.D. | | | 0.36 | 0.33 | 0.25 |

Table 2. Serum lipid bound sialic acid and total sialic acid in tumour bearing and control animals

| | Sl. No | Animal No. | Lipid Bound sialic acid (ng/dl) | Total sialic acid (ng/dl) |
|---|---|---|---|---|
| Tumour animals | 1 | T1 | 29 | 92 |
| | 2 | T2 | 31 | 88 |
| | 3 | T3 | 30 | 90 |
| | 4 | T4 | 33 | 94 |
| | 5 | T5 | 30 | 91 |
| | 6 | T6 | 28 | 89 |
| | 7 | T7 | 32 | 90 |
| | 8 | T8 | 31 | 92 |
| | 9 | T9 | 34 | 98 |
| | 10 | T10 | 30 | 100 |
| | 11 | T11 | 28 | 92 |
| | 12 | T12 | 33 | 98 |
| | 13 | T13 | 28 | 101 |
| | 14 | T14 | 32 | 102 |
| | 15 | T15 | 29 | 99 |
| | 16 | T16 | 34 | 98 |
| | 17 | T17 | 27 | 101 |
| | 18 | T18 | 32 | 96 |
| | 19 | T19 | 31 | 95 |
| | 20 | T20 | 32 | 100 |
| | 21 | T21 | 29 | 99 |
| | 22 | T22 | 33 | 98 |
| | 23 | T23 | 30 | 95 |
| | 24 | T24 | 31 | 92 |
| | 25 | T25 | 29 | 90 |
| | 26 | T26 | 32 | 98 |
| | 27 | T27 | 33 | 91 |
| | 28 | T28 | 30 | 97 |
| | 29 | T29 | 33 | 95 |
| Mean | | | 30.83±0.36 | 92.21±0.78 |
| S.D. | | | 1.95 | 4.14 |
| Control animals | 1 | C1 | 11 | 61 |
| | 2 | C2 | 10 | 60 |
| | 3 | C3 | 12 | 62 |
| | 4 | C4 | 13 | 63 |
| | 5 | C5 | 10 | 58 |
| | 6 | C6 | 11 | 60 |
| | 7 | C7 | 13 | 59 |
| | 8 | C8 | 15 | 65 |
| | 9 | C9 | 12 | 61 |
| | 10 | C10 | 11 | 56 |
| | 11 | C11 | 10 | 57 |
| | 12 | C12 | 12 | 66 |
| Mean | | | 11.67±0.43 | 60.67±0.87 |
| S.D. | | | 1.50 | 3.03 |

Table 3. Passive Haemagglutination titre values in serum of tumour bearing and control animals.

| Sl. No. | Animal No. | Titre value in tumour animal serum | Animal No. | Titre value in control animal serum |
|---|---|---|---|---|
| 1 | T1 | 64 | C1 | 32 |
| 2 | T2 | 32 | C2 | 64 |
| 3 | T3 | 16 | C3 | 128 |
| 4 | T4 | 16 | C4 | 64 |
| 5 | T5 | 16 | C5 | 64 |
| 6 | T6 | 64 | C6 | 32 |
| 7 | T7 | 32 | C7 | 32 |
| 8 | T8 | 16 | C8 | 64 |
| 9 | T9 | 16 | C9 | 128 |
| 10 | T10 | 32 | C10 | 64 |
| 11 | T11 | 64 | C11 | 64 |
| 12 | T12 | 8 | C12 | 32 |
| 13 | T13 | 32 | | |
| 14 | T14 | 8 | | |
| 15 | T15 | 128 | | |
| 16 | T16 | 16 | | |
| 17 | T17 | 32 | | |
| 18 | T18 | 16 | | |
| 19 | T19 | 32 | | |
| 20 | T20 | 16 | | |
| 21 | T21 | 32 | | |
| 22 | T22 | 256 | | |
| 23 | T23 | 32 | | |
| 24 | T24 | 128 | | |
| 25 | T25 | 16 | | |
| 26 | T26 | 16 | | |
| 27 | T27 | 64 | | |
| 28 | T28 | 16 | | |
| 29 | T29 | 8 | | |

* Titre values are expressed as reciprocal.

FIG. 1 MEAN SERUM VALUES OF CALCIUM, PHOSPHORUS AND MAGNESIUM

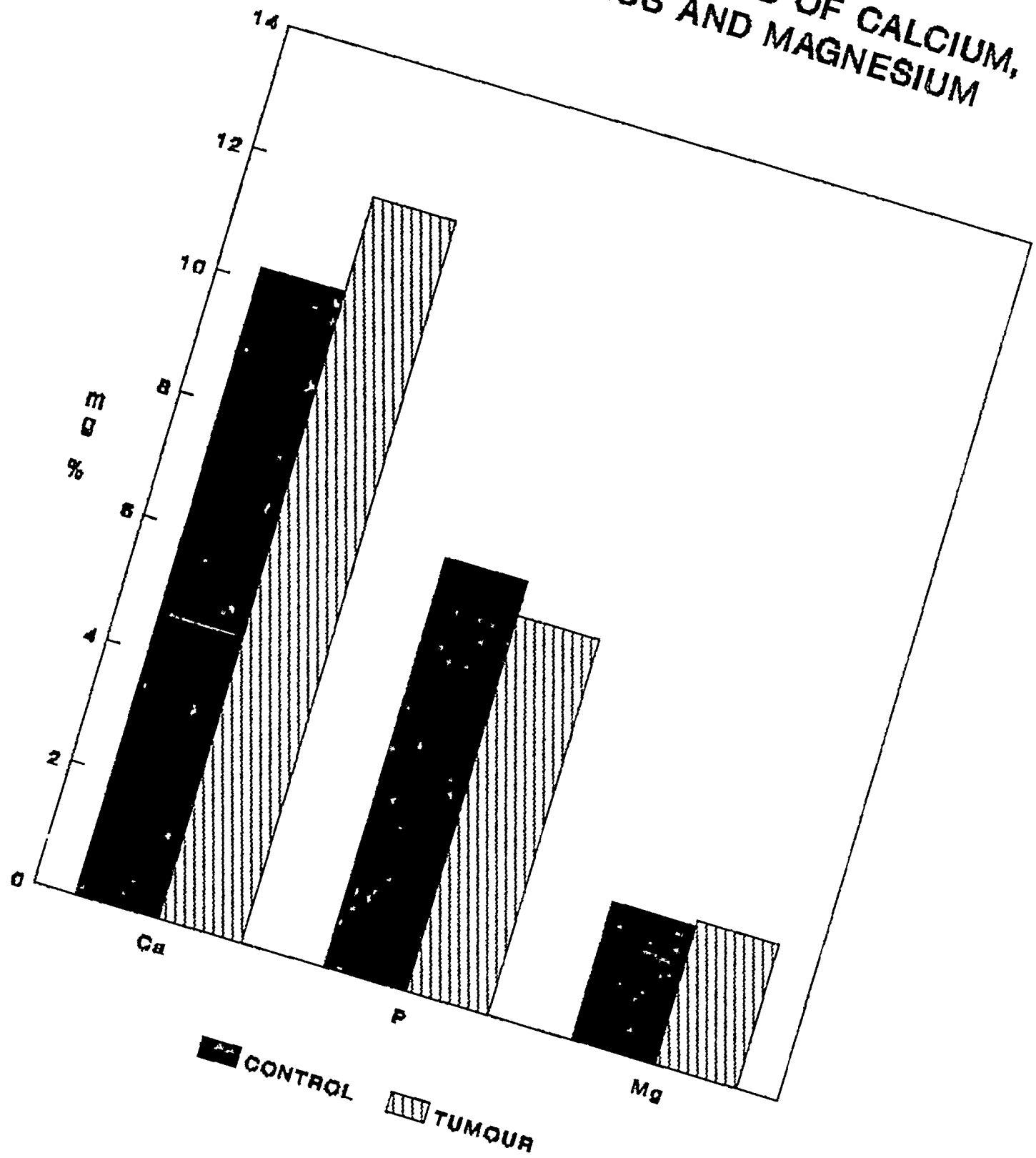


FIG. 2 MEAN SERUM VALUES OF SIALIC ACID AND LIPID BOUND SIALIC ACID

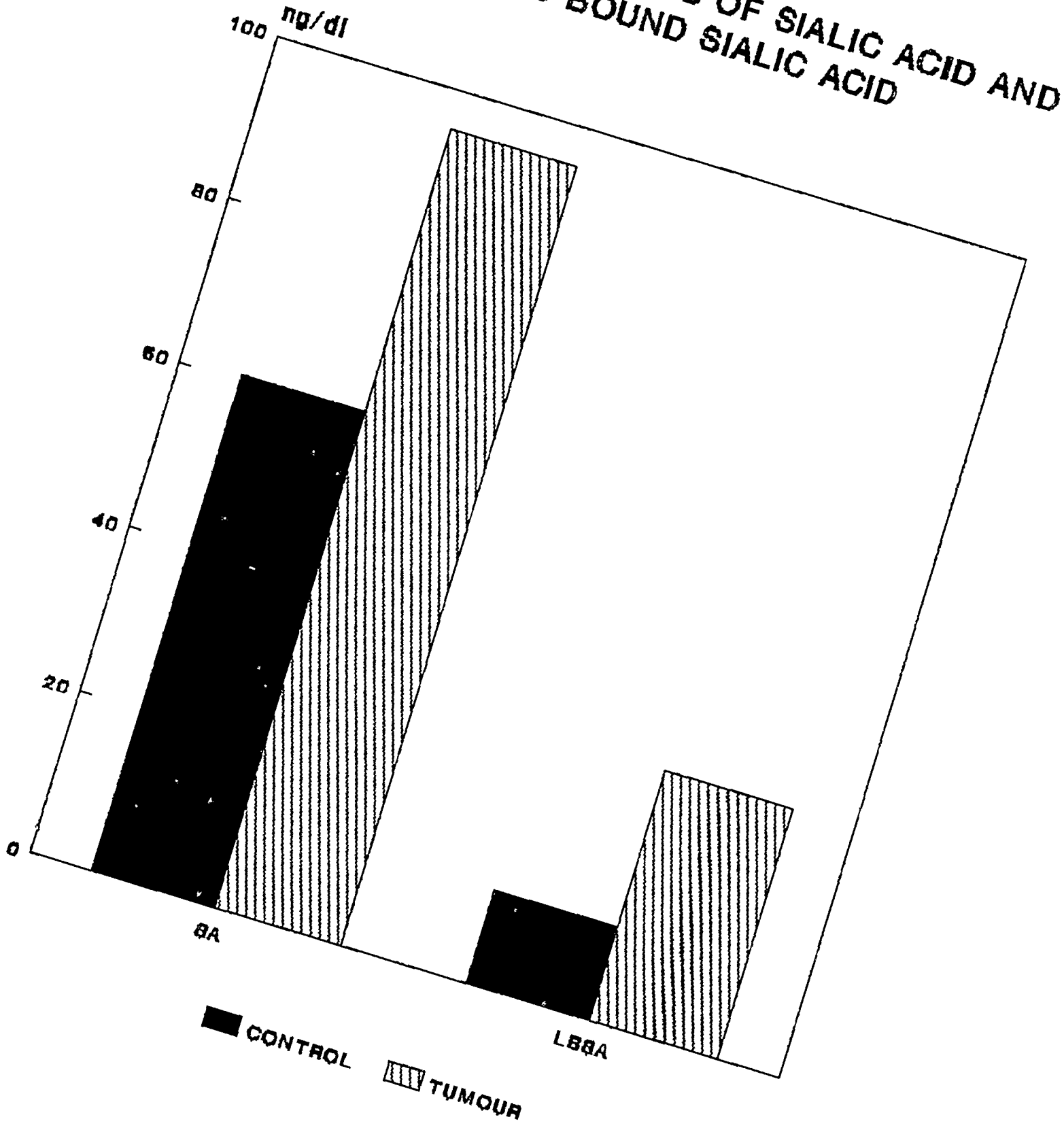


Fig 3. Precipitation in agar gel

Antigen and antibody diffusing from their respective well, precipitate in a region where optimal proportion are achieved

In this Ag - Tumour antigen

As - Antiserum raised in rabbit against the tumour antigen

1 - $T_{21}$ Tumour animal serum

2 - $C_{\_}$ Control animal serum

3 - $T_{20}$ Tumour animal serum

4 $T_{29}$ Tumour animal serum

5 - $C_4$ Control animal serum

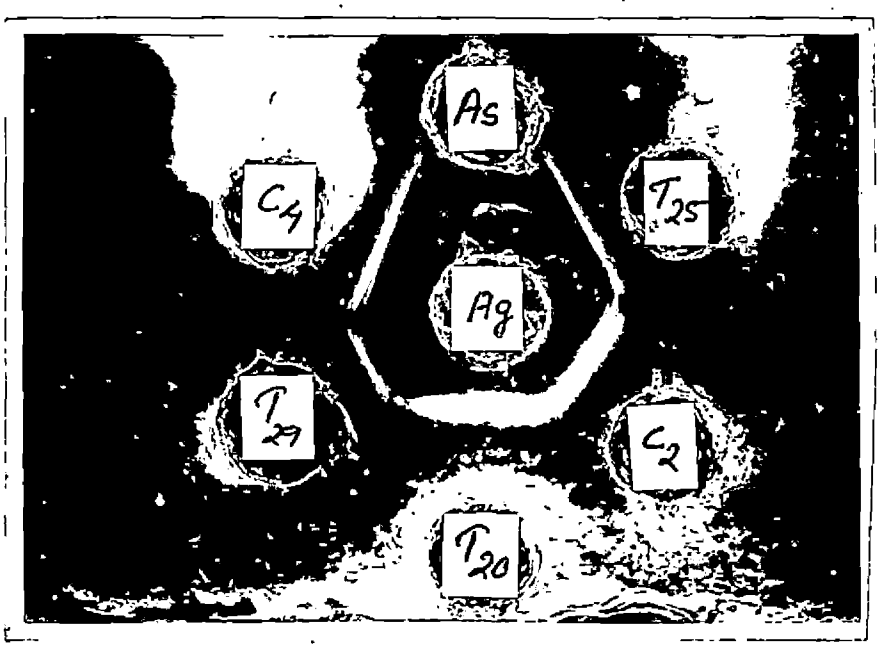

$A_5$
$C_4$
$T_{25}$
$A_g$
$T_{24}$
$C_2$
$T_{20}$

Fig.4. Microtitre plate showing the assay of tumour antigen concentration to find out the optimum titre for sensitization of formalinized sheep RBI

Optimum concentration was found to be 75 ul/5ml of formol stabilized sheep RBC became at high concentration also same titre was observed.

Fig. 5. Microtitre plate showing the results of passive haemagglutination titre in normal animal serum and tumour animal serum.

A. Control - $C_3$ - 1:128

B. Control - $C_1$ - 1:32

C. Tumour - $T_6$ - 1:64

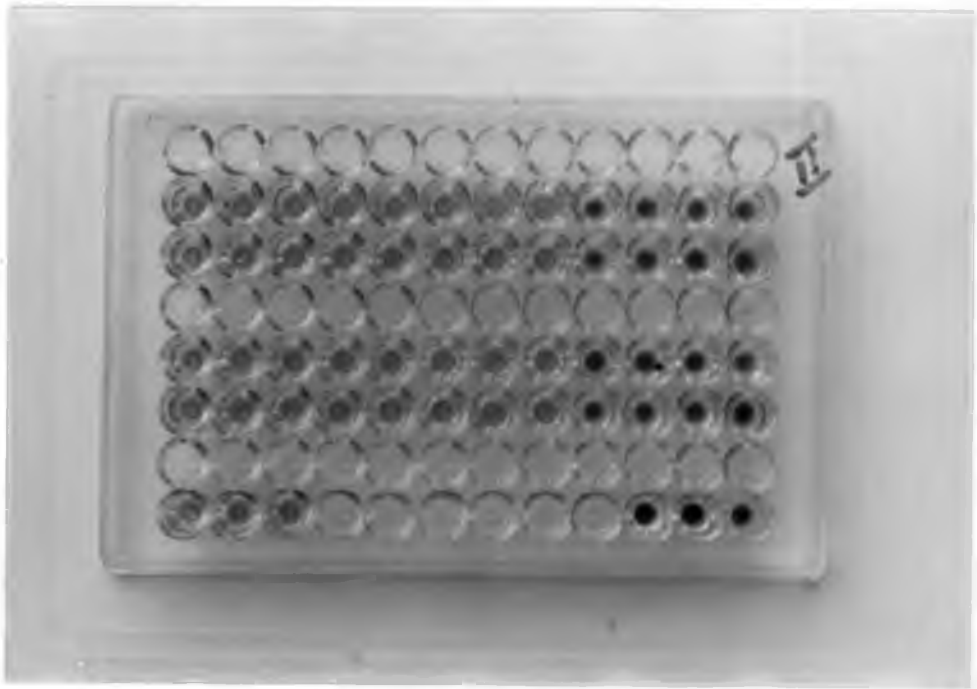
D. Tumour - $T_{15}$ - 1:128

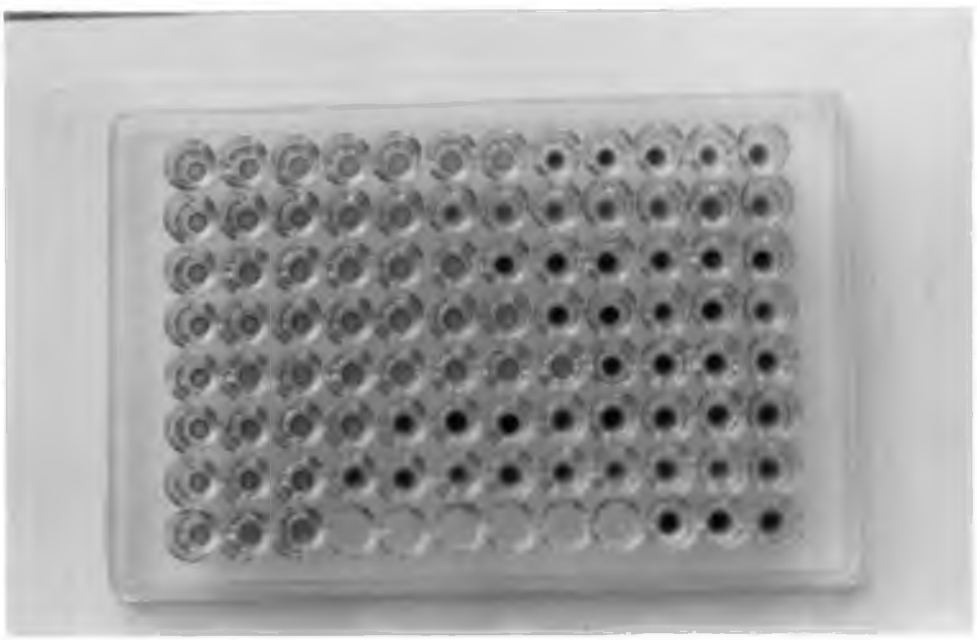
E. Tumour - $T_{22}$ - 1:256

F. Tumour - $T_8$ - 1:16

G. Tumour - $T_{14}$ - 1:8

H. Positive control * * * * * * Negative control





# Discussion

# DISCUSSION

Ethmoid carcinoma in domestic animals is an important neoplastic condition in Kerala. The etiological aspect of this tumour is not fully understood, and an early serologic diagnostic marker has not been developed. In the present study attempts were made to evaluate the serum level of various blood constituents with the objective of identifying useful diagnostic markers. For the present study blood was collected from 29 tumour bearing animals and 12 clinically healthy animals for evaluating various blood constituents as markers for early diagnosis. Tumour tissue was also collected from seven tumour bearing animals for production of tumour antigen and for isolation of haemagglutinating agents.

During the present study, it was found that the serum calcium level of tumour bearing animals was significantly higher when compared to that of the control animals. Hypercalcaemia associated with different varieties of neoplasms has been reported both in animals and human neoplasms. Hypercalcaemia associated with neoplasms was observed in mammary gland tumours in mice (Barrett et al., 1978), HSDM Murine fibrosarcoma and VX2 carcinoma of the apocrine glands of anal sac in dogs (Meuten et al., 1983), lymphsarcoma in dogs (Meuten et al., 1983 Weir et al., 1988), transplantable non metastasising Leydig cell tumour

in rats (Sica et al., 1983), lymphosarcoma and analsac apocrine gland adenocarcinoma in dogs (Norrdin and Powers, 1983) in tumour xenografts in nudemice (Miyake et al., 1991) in neoplasms of the ovary in women (Biran et al., 1977), Squamous cell carcinoma of the renal pelvis (Pigadas et al., 1978) breast carcinoma in women (Sztern et al., 1981, Grill et al., 1991 Bundred et al., 1992) head and neck tumour in human beings (Angel et al., 1982, Sridhar and Hussain, 1990), lymphosarcoma in human beings (Weller et al., 1982), complicating myelogenous leukemia in human beings (Gewirtz et al., 1983), adult T cell lymphoma in human (Dodd et al., 1983), adenosquamous carcinoma of the endometrium and in haematological malignancies in human beings (Ratcliffe et al., 1991).

It was reported that in cancer hypercalcaemia associated with carcinoma of the renal pelvis there was a fall in calcium level after surgical removal of the tumour (Pigadas et al , 1978). Reduction in calcium level was also been reported after treatment with antitumour drugs in breast carcinoma (Sztern et al., 1981), other tumour abalation in adenocarcinoma of apocrine glands of the analsac (Meuten et al., 1981, Meuten et al., 1984) and after surgical excision of human squamous cell carcinoma of the maxilla transplanted in athymic nude mice (Yoneda et al., 1991).

The observation of hypercalcaemia in ethmoid tumour bearing animals during the present study is also pointer that would support the observation of hypercalcaemia in neoplasia.

The Parathyroid hormone, prostaglandins and osteoclast activating factor (Biran et al., 1977) parathyroid hormone like chemicals secreted by the carcinoma (Sztern et al., 1981) prostaglandin E2 which acts as the mediator between neoplasm and bone (Tashjian, 1978), prostaglandin E2 Vitasterols, Osteoclast activity factors (Angel et al., 1982), a chemical and potent bone resorbing activity distinct from parathyroid hormone and prostaglandin E2 and chemically similar to osteoclast activating factor (Gewirtz et al., 1983) 13, 14, dihydro-15-keto prostaglandin E2 and local production of a bone resorption stimulating factor (Meuten et al., 1983), a bone resorbing factor (Sica et al., 1983), a hypercalcaemic factor produced by tumour other than immunoreactive parathyroid hormone or prostaglandin E2 that increases osteoclastic osteolysis distant from tumour (Meuten et al., 1983, Meuten et al., 1984) a humoral mediator similar to the activity of parathyroid (Ralston and Fogelman, 1984), substances synthesised by malignant cells due to activity of osteoclasts (Dodd et al. 1985), tumour derived circulating bone resorbing factor (Weir et al., 1988), atopic parathormone (Buller et al., 1991),

parathyroid hormone like protein (Grill *et al.*, 1991), parathyroid hormone related protein released from tumour cells (Miyake *et al.*, 1991), Mosekilde *et al.*, 1991 Ratcliffe *et al.*, 1991 Bundred *et al.*, 1992) were the agents mainly reported to be involved in the pathogenesis of cancer hypercalcaemia. The three biological activities that cause humoral hypercalcaemia were adenylate cyclase stimulating activity, *in vitro* bone resorbing activity and transforming growth factor activity (Rosol and Capen, 1988). Hypercalcaemia refractory to conventional measures of treatment was observed in a case of squamous cell carcinoma of the head and neck (Sridhar and Hussain, 1990). During the present study, no attempts were made to find out the cause of cancer hypercalcaemia and any one or more than one of the factors mentioned above may be involved in the pathogenesis of ethmoid cancer associated hypercalcaemia. In ethmoid carcinoma tumour invasion and destruction of the bone is a feature and the destructive rarefying lesion of the bone may induce hypercalcaemia. Although there was consistent hypercalcaemia this cannot be used as a diagnostic marker, since this may be seen in many other disease conditions

During the present study it was observed that the mean serum level of phosphorus was found to be significantly low in tumour bearing animals when compared with the mean

serum level in control animals. Similar findings of hypophosphataemia was observed in breast cancer in women (Sztern et al., 1981) and adenocarcinoma of the apocrine glands of analsac in dogs (Meuten et al., 1981). The factors that were indicated in the pathogenesis of hypophosphataemia in cancer were parathormone like chemicals in breast carcinoma (Sztern et al., 1981) and a substance produced by the tumour of the apocrine glands in dogs (Meuten et al., 1981). It was also observed by Meuten et al. (1981) that phosphorus level came back to normal by tumour abalation and hypophosphataemia recurred with tumour growth in apocrine adenocarcinoma of the anal gland. The agents involved in the pathogenesis of hypophosphataemia were not investigated during the present study and the agents as parathormone like chemicals or substances produced by the tumour may be involved in the pathogenesis of hypophosphataemia and this needs further investigation.

During the present study it was found that the serum megnesium level of the tumour bearing animals did not show any significant difference when compared to that of control animals The literature on magnesium level in tumour bearing neoplastic conditions are very few Boudaillez (1990) reported that magnesium level did not show any difference in a hypercalcaemic, leukemic 10 year old boy and this finding

supports the results of the present work and therefore serum magnesium level in tumour bearing animals cannot be used as an early diagnostic marker.

Significant increase with level of serum lipid bound sialic acid was found in ethmoid tumour bearing animals when compared with control animals during the present investigation. Increased lipid bound sialic acid was recorded in tumour bearing dogs (Kloppel et al., 1978). Tumour bearing horses in which the level decreased markedly and returned to normal following surgical removal of the growth and chemotherapy, indicated that it can be used as diagnostic tool (Kloppel and Richardson 1981). In cervical cancer patients, (Shanmugam and Nagarajan, 1986) and in white cattle as well as buffaloes bearing carcinoma of ethmoid mucosa (Muralimanohar, 1988). Increase in serum lipid sialic acid was reported.

Level of lipid bound sialic acid can be used as a potential diagnostic tool (Skipski et al., 1978 Katopodis et al., 1980), in patients with leukemias, lymphomas Hodgkins disease, melanoma, cancer of prostate, bladder, breast, lung, colon and ovary (Dristrain et al., 1981 and 1983). This was suggested as a diagnostic tool in the early detection and staging of the disease in precancerous

conditions of the oral cavity (Baxi et al., 1991) and in detecting the extent of metastasis and to monitor therapy in cancer (Chondros et al., 1991). This can considered as a highly sensitive marker in lung cancer (Kakri et al. 1991) and as a sensitive tumour marker in leukemia and also for differentiating anaemia from leukemias (Patel et al , 1991). Dristrain et al. (1975, 1977) stated that lipid bound sialic acid originated from the tumour cell surface in Morris hepatoma cases. The findings of the present study demonstrated an increase in lipid bound sialic acid level in the serum of tumour bearing animals. This observation is in agreement with the earlier reports in various neoplastic condition. Therefore, the serum level of lipid bound sialic acid can be used as a sensitive diagnostic marker in carcinoma of ethmoid mucosa.

Increased level of total serum sialic acid was reported in human patients bearing malignant growth (Singh et al., 1967), in rats with mammary tumours in malignant diseases of the lung, gastrointestinal tract, gyanaecological cancers, lymphomas and malignant melanoma (Lipton et al., 1978) in malignant melonoma in human beings (Silver et al., 1978), in metastatic breast cancer human patients (Waalkes et al., 1978) in gastrointestinal cancer of the human beings (Uehan et al., 1984), in human beings with cancer of the lung,

ovary, cervix, pancreas, prostate thyroid, uterus, eosophagus and endometrium in cows suffering from bovine leukosis (Kinoshita et al., 1987), in white cattle as well as in buffaloes bearing ethmoid neoplasms (Muralimanohar, 1988) in precancerous and cancerous stages of oral cancer so that it can be used in the early detection and staging of the disease (Baxi et al., 1991) in lung cancer (Kakari et al., 1991) and in leukemia where the rise in serum level can be used for differntiating anaemia from leukemia (Patel et al., 1991). There was a correlation between the levels during progression and regression of the tumour (Diwedi et al., 1987).

Increased sialic acid level was recorded in diaminobenzene induced rat hepatoma cell surface (Kalant et al., 1964) transformed cells in vitro (Van Beck et al., 1973) on tumour cell surfaces (Rapin and Burger, 1974), in malignant human foetal muscle cells grown in vitro (Bhide et al., 1977), in gastric mucosa during cancer of the stomach in human beings (Linchyskaya et al., 1978) and in oral secretions in carcinomas of the upper digestive tract, which can be used as a marker for carcinomas of the upper digestive tract (Reuter et al., 1992).

There exists a significant correlation between serum sialic acid level in ovarian tumours and tumour burden and

serial estimation helps in guiding patient management (Silver et al., 1980). It was also observed that elevated serum sialic acid levels were noticed in hepatic abscess in cattle (Motoi et al., 1985) and also in foetal and new born calves in which there was a three fold increase in serum sialic acid level when compared to mature animals (Sydow et al., 1988 Scherblom et al., 1988).

During tumourogenesis there is altered expression of cell surface glycoproteins and glycolipid to which sialic acid residues are attached (Warren et al., 1972). During tumourogenesis sialic acid associated with glycoproteins and glycolipids are shed into the circulation from the cell surface of tumour cell and this was considered as the reason for increase in the total serum sialic acid levels in tumour bearing animals and man (Rapin and Borger, 1974).

In this investigation it was found that the serum total sialic acid level was significantly high in ethmoid tumour bearing animals. The studies made earlier in man and animals regarding the usefulness of serum sialic acid level as a tumour marker also point out that serum total sialic acid level in carcinoma of the ethmoid mucosa can be used as one of the tumour markers in the diagnosis of this tumour.

Pretest inoculation serum collected from the rabbit could not give any precipitin lines when precipitation test was carried out against the tumour antigen whereas the antiserum raised in rabbit against the tumour antigen gave a sharp precipitin line. This indicated that the tumour antigen contained soluble antigen and antiserum raised in the rabbit against the tumour antigen had soluble antibody.

The presence of soluble antigen in the rat mammary carcinoma and the presence of soluble antibody in hyperimmune sera produced against this antigen was reported by Ghosh et al. (1978). They used the immunodiffusion test for detecting the presence of antigen. Studies by Dutta (1977) also demonstrated the presence of horn cancer antigen by immunodiffusion test with antigen raised in rabbit against crude horn cancer antigen.

Antisera raised in the rabbit against the sodium chloride extract of squamous cell carcinoma of the cervix (Goldenberg, 1977) showed precipitin bands in double diffusion test. The study by Kuchroo et al. (1982) demonstrated tumour associated antigen in bovine horn cancer by immunodiffusion test.

From the studies undertaken it was evident that 3 M KCl extract of tumour antigen obtained from the ethmoid tumour bearing animals and the antisera raised in rabbit against this antigen could produce visible precipitin line by agar gel precipitation test.

When instead of the hyperimmune serum raised in the rabbit, the serum from the tumour bearing animals was used for immunodiffusion test, two distinct precipitin lines was obtained, of which one was a thick band closer towards the serum well which was absent in the case of hyperimmune serum raised in the rabbit. The second strip band was similating the band obtained while using hyperimmune serum raised in the rabbit. The observations indicated that the serum of the tumour bearing animals had two types of soluble antibodies. All the 12 control animal sera also gave the thick precipitin lines indicating the possibility that this band may be the antibodies against bovine serum. Eventhough in this study care was taken to remove the blood from the tumour tissue before antigen preparation, complete removal might not have been effected by the methodology adopted. The presence of the thick band while testing rabbit antiserum and the presence of the same band while using control animal serum and tumour animal serum indicated that this precipitin line was not having any significance in the diagnosis of ethmoid tumour.

The second precipitin line which is sharp and close towards the antigen well in the case of tumour bearing animal serum appears to have significance because the same precipitin line was produced by the hyperimmune serum produced in the rabbit. But majority of the control animals also gave this precipitin line even though all the control test serum could not gave this precipitin line.

Since both bands were given by the sera collected from all the tumour bearing animals and majority of the control animals the agar gel precipitation test cannot be recommended as a diagostic tool or an immune marker for ethmoid carcinoma. The results obtained in this studies are in conformity with Jayarman et al. (1980). They did not consider gel diffusion test as a tool for the diagnosis of bovine sinus neoplasms. Sulochana and Rajan (1981) in their studies also could not find the usefulness of agar gel diffusion for the diagnosis of ethmoid carcinoma in cattle. Muralimanohar (1988) also could not find any use for the agar gel diffusion test for the diagnosis of ethmoid carcinoma in cattle.

Gluteraldehyde stabilized sheep red blood cells (SRBC) was found suitable for sensitisation with tumour antigen because such stabilised and treated cells could be stored more than 6 months without any reduction in titre. The

result of this study is in conformity with the studies reported by Pillai (1987) where they have used different stabilizing agents like pyruvic aldehyde, gluteraldehyde and formal dehyde. He found that gluteraldehyde is the best aldehyde for stabilization. Such stabilized and antigen sensitized SRBC could be stored for 6 months without any change in quality, eventhough different concentration of antigens was used for sensitisation.

The optimum concentration of tumour antigen for sensitisation of SRBC was 75 ul/3 ml of packed SRBC. The maximum PHA titre obtained was 1.256 using hyperimmune sera raised in rabbit against tumour antigen.

The PHA titre obtained with the hyperimmune serum raised in rabbit against tumour antigen was 1 256. The PHA titre values of 29 tumour bearing animals ranged from 1 8 to 1 256. The hignest titre was 1 256, the same titre was obtained with the hyperimmune serum raised in rabbit against the tumour antigen. The highest PHA titres of test control animals, was 1 128 and the lowest titre was 1 32. Since in the control animals the titre values showed only the difference of one well the comparison of the control and tumour bearing animal titre values indicated that a base

line cut off value cannot be obtained to differentiate the tumour infected and control animals

So the PHA test could not be taken as a diagnostic tool or an immune marker test for carcinoma of the ethmoid mucosa. The same was the report of Sulochana and Rajan (1981) on the applicability of this test for diagnosis where they used tannic acid coated cells In this study the sonicated antigen was absorbed to the gluteraldehyde stabilised cells. In the present study none of the tumour samples revealed the presence of any haemagglutinating agents even though the earlier studies (Sulochana,1980) indicated the possible involvement of seven haemagglutinating agents This study was also aimed to carry out the haemagglutination inhibition studies Since no haemagglutinating agent was isolated haemagglutination inhibition test was not perfomed.

# Summary

## SUMMARY

1. During the period of the study, 29 ethmoid tumour bearing animals were procured from different parts of the state for investigation. Serum was collected from 29 tumour bearing animals and also from 12 clinically healthy animals maintained at the University Livestock Farm (controls) for the study.

2. Serum calcium, phosphorus and magnesium levels of 29 tumour bearing animals and 12 control animals were estimated. Average serum calcium, phosphorus and magnesium levels in the control animals was 10.23±0.10 mg per cent, 6.66±0.09 mg per cent and 2.27±0.07 mg per cent respectively. The average serum calcium, phosphorus and magnesium levels in tumour bearing animals was 11.78±0.23 mg per cent, 6.11±0.10 mg per cent and 2.35±0.05 mg per cent respectively. There was statistically significant difference between serum calcium and serum phosphorus levels of tumour bearing animals when compared with that of the control animals. The difference in the serum magnesium level between tumour bearing animals and control animals was not statistically significant.

3. The serum total sialic acid and serum lipid bound sialic acid levels of tumour bearing animals and control animals were estimated. There was increase in

both serum total sialic acid and serum lipid bound sialic acid in the tumour bearing animals when compared with that of the control animals and the difference was statistically significant. This could be used as one of the non specific clinical markers for diagnosis.

4. Tumour antigen was extracted from pooled tumour tissues using 3MKCl and antisera to the prepared tumour antigen was raised in rabbits

5. Agar gel precipitation test was conducted using the prepared tumour antigen antisera prepared against tumour antigen suspected serum samples of tumour bearing animals and serum samples of control animals. The serum from tumour bearing animals gave two distinct precipitin bands of which one was a thick band closer towards the serum well and a second strip band closer to antigen well similating the band obtained while using antisera raised against tumour antigen in rabbits. Since majority of the control animals also gave the two precipitin lines it was concluded that the agar gel precipitation cannot be used as a diagnostic tool.

6. The passive haemagglutination test (PHA) was conducted using the serum of tumour bearing animals and control animals. The PHA titre values of tumour bearing animals and control animals showed only a difference of one

well and this indicated that a base line cut off value cannot be obtained to differentiate the tumour infected and control animals.

7. At the time of slaughter, tumour tissues were collected from seven animals and the pooled tumour tissue was employed to make a suspension to inoculate embryonated eggs. The allantoic and amniotic fluids were subjected to haemagglutination test employing chicken red blood cells, but none of the tumour samples revealed the presence of any haemagglutinating agents and so haemagglutination inhibition test could not be carried out.

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# Evaluation of Blood Constituents as Diagnostic Markers for Ethmoid Carcinoma in Cattle

By

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Abstract of a Thesis

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## ABSTRACT

An investigation was carried out to evaluate the usefulness of blood constituents as tumour markers for carcinoma of ethmoid mucosa in cattle. During the period of two years, twenty nine tumour bearing cattle in various stages of ethmoid carcinoma procured from different parts of Kerala and twelve clinically healthy animals maintained at the University Livestock Farm Mannuthy (controls) were bled and serum samples were collected.

Serum calcium serum phosphorus and serum magnesium levels of tumour animals were compared with that of the control animals. Tumour animals had a mean serum calcium level of 11.78±0.23 mg per cent, mean serum phosphorus level of 6.11±0.10 mg per cent and mean serum magnesium level of 2.35±0.05 mg per cent. In control animals the mean serum calcium phosphorus and magnesium levels were 10.23±0.10 mg per cent 6.66±0.09 mg per cent and 2.27±0.07 mg per cent respectively. There was increase in the serum calcium level and reduction in serum phosphorus level in tumour bearing animals was statistically significant. The serum magnesium level did not reveal any statistical significance.

The mean serum total sialic acid and mean serum lipid bound sialic acid levels in tumour bearing animals was 95.21±0.78 ng/dl and 30.83±0.36 ng/dl respectively and in

control animals it was 60.67±0.87 ng/dl and 11.67±0.43 ng/dl respectively. Both serum total sialic acid and serum lipid bound sialic acid level in tumour bearing animals was high when compared with that of control animals and the increase was statistically significant.

Serum from twenty-nine tumour bearing animals and serum control animals were tested against the prepared tumour antigen using agar gel precipitation test for detecting tumour antibodies. All the twenty nine serum samples from tumour animals showed two distinct precipitin bands, one band close to the serum well and another sharp band closer towards the antigen well. Majority of the control animal serum samples also showed both the precipitin bands although some of the control samples did not show the second sharp band nearer to the antigen well.

Passive haemagglutination test was conducted using tumour antigen sensitized gluteraldehyde stabilized sheep red blood cells tumour animal serum control animal serum and proper controls. The highest titre value obtained for serum of tumour animals was 1 256 and the lowest was 1 8. In control animals it was 1 128 and 1 32 respectively for highest and lowest titre values. Since in the control animals the titre value showed only the difference of one well the comparison of the control and tumour animals titre

values indicated that a base line cut off value cannot be obtained to differentiate the tumour infected and control animals. None of the tumour samples processed and inoculated into embryonated eggs revealed the presence of any haemagglutinating agents.