HOMOLOGOUS AND HETEROLOGOUS TRANSPLANTATION OF BOVINE ETHMOID CARCINOMA CELLS

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled **"Homologous and Heterologous Transplantation of Bovine Ethmoid Carcinoma Cells"** 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.

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CERTIFICATE

Certified that the thesis entitled **"Homologous and Heterologous Transplantation of Bovine Ethmoid Carcinoma Cells"** is a record of research work done independently by **Shri. Ajith Jacob George** 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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Introduction

INTRODUCTION

Carcinoma of the mucosa of the ethmoturbinate which was first reported in cattle by Moussu in 1906 from Sweden has emerged as an important neoplastic condition at present. The tumour was reported for the first time from India by Muttappa in 1930 from Madras State. Since then the prevalence of this tumour was reported from many parts of India including the northern parts of the country like Uttar Pradesh. The high incidence of this tumours in a specific location without any species barrier in domestic animals captured the interest of Veterinary Oncologists.

Among cattle, this tumour is mostly seen in cross-bred high yielders in the advanced stage of pregnancy and perforce leads to severe economic loss. Since its first reports from Kerala in 1960 it has established in an endemic form in the state.

During the last twelve years investigations were undertaken in a systematic manner at the Centre of Excellence in Pathology. Incidence, symptomatology, diagnostic criteria, pathological features and immunological background of the tumour bearing animal were studied in detail. Cyclophosphamide was found to be of value for therapy in the early stages of the tumour growth. Evidence was also obtained on the possible role of a virus in the etiology of tumour.

Tumour transplantation studies have great importance in experimental cancer research. A transplanted tumour could be used for studying the basic difference between normal and malignant tissue and also for obtaining more informations regarding cell kinetics of the neoplastic cells. It offers an excellent model system for evaluating immunoprophylactic measures, chemosensitivity and tumour metastasis.[9] The major use of transplanted tumour is to prescreen chemotherapeutic agent that acts against the proliferating tumour, in vivo. Studies in the tumour-host relationship in experimental animal would provide important clues which aid in the clinical management of tumour in the primary host. Conditional and unconditional host systems have been extensively employed to achieve this objective and the results have been variable with different types of tumour.

So far, a systematic effort has not been made to transplant the tumour of the ethmoturbinate mucosa of cattle in homologous and heterologous host system. The limited studies carried out by scientists at different places have not been very satisfactory. Therefore, the present study was undertaken to assess the transplantability of the tumour of the ethmoturbinate mucosa of cattle to homologous and heterologous species of animals.

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 and 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 dog.

Young et al. (1961) reported various neoplasms arising from the olfactory mucosal 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 bovines and swines.

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. Bradley and Harvey (1973) made a brief note on the incidence of nasal tumours in dogs. Inada et al. (1973) and Inada and Tokarnia (1973) described ethmoid tumours in pigs and cattle respectively. Cho et al. (1974) reported adenocarcinoma in the nasal cavity and brain of a dog. Brownstein et al. (1975) reported nasal carcinoma in a captive eld's deer (Cervus eldi). Legendre et al. (1975) reported the incidence of nasal tumours in cats. Mason (1975) recorded a case of spindle cell sarcoma of the equine paranasal sinus and nasal chamber. Madewell et al. (1976) observed 300 cases of primary neoplasm 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. Stanzi and Hauser (1976) described tumours of the nasal cavity in domestic animals. Brown et al. (1977) reported a case of nasal adenocarcinoma in a Taiwan Macaque. Confer and Depaoli (1978) observed sixteen cases of primary neoplasms of

the nasal cavity, paranasal sinuses and nasopharynx in the dog. Njoku et al. (1978) reported the incidence of ovine nasal adenopappiloma from ten sheep. Yonemichi et al. (1978) reported intranasal tumours of the ethmoid olfactory mucosa in 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 sinus in the dog. Njoku and Chineme (1983) reported the neoplasms of the nasal cavity of cattle and sheep. Stroud and Amundson (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). 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 Drawf ewes. Ringe and Rajko (1985) observed growth in the nasal cavity as naturally occurring nasal obstruction in sheep. Steen et al. (1985) reported nasal tumours in a fallow deer (Dama dama L.). A 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, and a case of spindle cell sarcoma in a young horse. Morrison et al. (1989) reviewed the case records at Mardoch University Veterinary Hospital over the period of 1978-1988, and it revealed 37 cases of nasal tumour in dogs. Wendt (1989) described incidence, clinical aspects and diagnosis of ethmoidal tumours in sheep. He studied 11 cases during a period of 10 years.

2.1.1 Incidence and epidemiology in India

In India Muttappa (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 a eight year old Ongole breeding bull from the State of Andra Pradesh. Sastry and Rao (1964) recorded a case of tumour consisting of adenocarcinoma with a fibro-sarcomatous stroma in a bullock. Rajan et al. (1972) reported fifty one neoplasms in the ethmoid region of cattle from the state of Kerala. In their study they reported that the tumour was encountered in Kerala since 1960.

Balasubrahmanian (1975) studied seventeen cases of sinus tumour in bovine from the State of Karnataka. Prasad and Kohli (1978) recorded a case of nasal osteoma in a bullock. 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 a few sires indicating genetic predisposition. Epidemiological features of the disease indicated an infectious etiology. Nayak et al. (1979, 1980) reported tumours of the bovine nasal cavity from Orissa.

One hundred and fifty cases were recorded during 1977-1979 period. It consisted of cattle (133), buffalo (7) and goats (10) (Rajan, 1980 and Rajan and Sulochana, 1982). Rajan et al. (1980, 1981) reported ethmoid carcinoma in goats and pigs. 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). Chaudhary 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 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. 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 cows from Uttar Pradesh Chakrabarthi et al. (1988) described three cases of ethmoid carcinoma in HF cattle from West Bengal. Muralimanohar (1988) made a survey on the occurrence of ethmoid neoplasm in domestic animals for a period of five years (1983-87) covering cattle, buffalo, sheep, goat and dog.

According to the records maintained in the Centre of Excellence in Pathology, Kerala Agricultural University 220 positive cases of ethmoid tumour were recorded out of the 320

suspected field cases, during 1992-94. These included cases from different parts of the state.

2.2 Tumour transplantation

Duncan et al. (1967) failed to produce enzootic nasal adenocarcinoma of sheep and lambs by intranasal inoculation of macerated tumour material.

Rajan et al. (1972) attempted experimental transmission of tumours of bovine paranasal sinuses in heifers by intraperitoneal and intranasal route. Nair (1973) failed to produce the tumour by inoculation of tumour pieces into the frontal sinus of calves and also by intraperitoneal inoculation of a fine suspension of tumour cells in normal saline. He also attempted to transplant the tumour by intraperitoneal inoculation in guinea pigs and white mice.

Hill and Stanley (1975) transplanted B16 melanoma cells into nude mice. Stanbridge et al. (1975) studied about the growth of human and animal malignant cell populations in immunosuppressed mice.

Dutta (1977) observed regression of horn cancer tissue transplanted in the anterior chamber of eyes of guinea pigs within two to three weeks. However, the transplants grew in immunosuppressed guinea pigs. A successful autotransplantation

on the pinna of the ear of 11 cases of horn cancer was also attempted. Hoffmann et al. (1977) successfully transplanted fragments of bovine squamous cell carcinoma tissue and single cell suspension in Tricine buffered media 199 supplemented with 15 per cent foetal calf serum, obtained by trypsinisation of the tumour tissue, into nude mice. Irvin et al. (1977) successfully transplanted bovine lymphosarcoma cells into athymic nude mice.

Wynn-Williams and McCulloch (1977), Giovanella et al. (1978) studied the growth of human cancers and other transplants in nude mice.

Kuchroo et al. (1978) attempted to transplant horn cancer cells into the cheek pouch of day old hamster, and a small soft growth was seen after three weeks post-inoculation, but regressed slowly and completely after two months.

Jayaraman et al. (1979) attempted transplantation of sinus neoplasms subcutaneously into irradiated and non-irradiated mice, rabbits and rats. They also tried intranasal transplantation in rats and calves. Attempts to cultivate the tumour cells in vivo in medium Na 199 or BHSS with foetal calf serum were also made without success.

Chachinian et al. (1980) transplanted fragments of human malignant mesothelioma subcutaneously and intra-

peritoneally in nude mice. Huang et al. (1980) successfully transplanted and characterised NPC/HK1 cell line from a differentiated squamous carcinoma of the nasopharynx in nude mice.

Matsubara et al. (1980) transplanted Ehrlich ascites tumour in DDI mice by intraperitoneal inoculation of the cells at the rate of 10^5 cells per inoculum. Stocco and Hutson (1980) transplanted Novihoff-hepatoma cells into female sprague-Dauley rats by intraperitoneal inoculation. Stragand et al. (1980) transplanted human colonic adenocarcinoma into athymic mice by intraperitoneal, intravenous and subcutaneous routes.

Several attempts were made for transplantation of the ethmoturbinate neoplasms of the domestic animals into experimental animals with or without immunosuppression without success (Sulochana, 1980 and Pospischil et al. 1982).

Tanooka et al. (1980) transplanted methylcholantrine induced tumours subcutaneously into mice using solid pieces of the tumour. Ehrlich tumour cells were inoculated subcutaneously at the rate of 2×10^6 cells per inoculum into mice (Yamanaka et al. 1980).

Immunosuppression with drugs, antilymphocytic serum or whole body irradiation have been used to increase the

acceptance of leukemia and lymphosarcoma (Watanabe et al., 1980). It was revealed that number of cells collected was higher in subcutaneously transplanted tumours than intraperitoneal or intravenous inoculation.

Tumerogenicity of human hepatoma cell line was assessed by subcutaneous inoculation of the tumour cells in nude mice (Shouval et al. 1981).

Al-Yaman and Willenborg (1983) successfully transplanted ovine squamous cell carcinoma in nude mice and observed similar growth pattern as in the original host. Temporal morphologic changes in human colorectal carcinoma following xenografting in nude mice was studied by Barkla and Tutton (1983). Tumerogenicity of an established epithelial cell line (CNE-2) obtained from a nasopharyngeal carcinoma patient with poorly differentiated squamous cell carcinoma was assessed by Gu et al. (1983). Neely et al. (1983) hetero-transplanted 85 pediatric tumours into nude mice.

Al-Yaman and Willenborg (1984) implanted ovine squamous cell carcinoma from various sites into nude mice. An association was found between the site of origin of the tumour in sheep and its acceptance as a xenograft, most successful growth was obtained from tumours of the skin of nose. Zimber et al. (1984) transplanted experimentally induced sheep lung

adenomatosis subcutaneously into nude mice. Small freely movable cysts lined by proliferating epithelium which were similar to its original were observed at the site of inoculation.

Baille-Johnson et al. (1985) and Engelholm et al. (1986) studied the tumerogenicity of human lung cancer. Human endometrium transplanted into nude mice retained the light microscopic and ultrastructural appearance. The chromosomal and biochemical properties were also preserved. This confirmed that human tissue transplanted into nude mice retained human characteristics (Bergqvist et al. 1985). They also concluded that the take rate of human tumour was above 50 per cent, normal tissue lower and those from endocrine target tissue still lower. Human hepatocellular carcinoma was transplanted subcutaneously into nude mice after culturing the cell in vivo (Laohathai and Pravathi, 1985). They observed that the growth rate of cultures seeded from tissues that were initially longer than 2 mm and then minced was considerably slower than smaller macrofragments. The tumour grew within ten days after inoculation in mice. They were solid and well encapsulated.

Yeger et al. (1985) transplanted Wilm's tumour into nude mice. They inoculated small tissue fragments and single cell suspension subcutaneously. One million cells were

inoculated intraperitoneally also. Of the 18 hetero-transplants made, he succeeded in 10 (56 per cent). They concluded that in tumour transplantation the route of administration influenced the degree of acceptance and the latent period.

Ebbers et al. (1986) suggested that nasopharyngeal carcinomas have difficulty in surviving in tissue culture system and even the transplantation of solid tumour mass transplanted into nude mouse was also not easy.

Human colorectal adenocarcinomas were transplanted by subcutaneous inoculation of trypsinised, cell culture maintained tumour cells in female BALB/C nude mice by Kirkland and Bailey (1986). Histologically the tumour was similar. There was a central area of necrosis. No metastasis was observed. Successful transplantation of rat pituitary tumours MtTSA5 subcutaneously into Wistar rats was achieved by Mori et al. (1986). The tumour grew in 8-10 weeks.

Garvin et al. (1987) successfully transplanted the blastemal component of Wilm's tumour subcutaneously into nude mice. They used minced tumour fragments for inoculation. Six weeks after inoculation multilobulated tumour growth was observed.

Cell line LIM 2210 was propagated as subcutaneous xenografts in the flanks of nude mice directly from small pieces of tissues obtained from a resected specimen of a poorly differentiated human colon carcinoma by Barkla et al. (1988). Small cell carcinoma was grown as monolayer in RPMI-1640 with 10 per cent neonatal calf serum and antibiotics, until the required cell number was obtained (Bergh, 1988). Cells were inoculated at the rate of $3\text{-}10 \times 10^7$ cells subcutaneously into mice and was observed once a week until they were approximately 0.5-2 cm in diameter.

Busson et al. (1988) transplanted EBV containing nasopharyngeal carcinoma subcutaneously into the back of previously irradiated Swiss nude mice. They used tumour fragments and single cell suspension for inoculation. Out of the sixty heterotransplants only one resulted in successful grafting. They observed that five million viable cells were sufficient to generate a 1 cm^3 tumour after five weeks of growth. He reported that only malignant epithelial cells retain the ability to proliferate in nude mice thus allowing the tumour cells to separate from non malignant and infiltrating cells. They found that heterotransplantation with metastatic tissue was more successful than with primary tumour.

Corrier and Norman (1988) found that T-2 mycotoxin can increase the susceptibility of mice to tumour transplants. Fredrickson et al. (1988) successfully transplanted heavy cell suspension of pancreatic tumour intraperitonially into weanlings or subcutaneously into nu/nu adults. The tumour grew with 56 days. Friedman et al. (1988) successfully transplanted human medulloblastoma cell line subcutaneously and intraceribrally into BALB/c mice. The tumours developed in all the animals in four weeks.

Tumerogenicity of GGI cell line obtained from human nasopharyngeal carcinoma was assessed by Chang et al. (1989) by inoculating $1x10^7$ cells in PBS subcutaneously into nu/nu mice. Tumours of 1 cm^3 developed at the site of inoculation within four weeks. Hashimura et al. (1989) successfully transplanted renal cell carcinoma inoculated at the rate of $1x10^7$ cell in 0.2 ml PBS subcutaneously in nude mice. Huang et al. (1989) successfully transplanted undifferentiated nasopharyngeal carcinoma cell lines subcutaneously in nude mice. The xenograft was free of lymphoid cells and remained undifferentiated upto thirty passage. Tumerogenicity of human colorectal carcinoma was assessed by implanting $3x10^6$ dissociated viable cells into the middle of the quadriceps femoris of nude mice (Jessup et al., 1989). The tumour

appeared in three weeks and was histologically similar to the original tumour.

Lin et al. (1990) transplanted nasopharyngeal carcinoma cells. subcutaneously into the back of BALB/c nude mice, The tumour grew upto 2-3 months, when it was 2.2 cm in diameter. Ultrastructurally the cells were seen as dark and light cells.

Price et al. (1990) suggested that many human tumours proliferate when injected subcutaneously into nude mice but metastasis from the skin was rare. However, implants from orthotopic sites metastasised readily than ectopic sites. They inoculated human breast carcinoma cells subcutaneously and into the mammary fat pad. At high doses of 10^6 and $5x10^6$ tumour developed in all mice in both the sites at similar intervals. However, at lower dose of 10^5 cells tumour grew in 2 of 5 mice. The tropic effect was specific for breast carcinoma as melanoma, renal cell carcinoma and colon carcinoma did not make any difference in growth. Histologically, they observed poor vascularity and more extensive necrosis in the subcutaneous tumour and a reduced fibrous capsule around the mammary fat pad tumours. They suggested that trophic effect was, specific tumour-host cell interaction and allowed growth of tumour at a lower inoculum than in subcutis. Yao et al. (1990) successfully transplanted

NHE-1 and HONE-1 cells obtained from Epsteen-Barr virus infected nasopharyngeal carcinoma, subcutaneously into nude mice. The growth of HNE-1 was faster.

Bucana et al. (1992) successfully transplanted various human and murine tumours subcutaneously into nude mice. Line et al. (1993) successfully transplanted subcutaneously nasopharyngeal carcinoma cells into nude mice.

Chaudhary (1994) attempted transplantation of tumour of the ethmoturbinate mucosa of cattle which were propagated in vitro, in mice subcutaneously at the rate of $1x10^6$ cells per inoculum, which were immunosuppressed by giving cyclosporine A 15 mg/kg orally. But the animals did not reveal any growth.

2.2 Cyclophosphamide

Berenbaum (1964) observed prolongation of homograft survival in mice with single dose of cyclophospamide. Fox (1964) observed suppression of tissue immunity by cyclophosphamide treatment.

Brody et al. (1965) studied the influence of cyclophosphamide on ear-skin homograft rejection in rabbits receiving the drug at the rate of 25 mg/kg/day. They observed soft purulent swelling at the site of transplantation and

histologically congestion of blood vessels, thrombi, perivascular cuffing and infiltration and destruction of epidermal appendages by mononuclear cells, in untreated animals. In drug treated animals rejection was slower and infiltration of mononuclears were very low.

Studies on the recovery of lymphocyte transformation in response to PHA and PWM conducted by Stockman (1973) indicated a severe effect on B cells than on T cells. PHA responsiveness was shown to recover within three days after 400 mg/kg. The blastogenic response to PWM did not recover until 10-14 days. Histologically, red pulp and B cell areas were severely depleted.

Yumashwa et al. (1974) observed depression of haemopoietic elements and of the immunological function of spleen three to four days after the administration of cyclophosphamide in mice. There was hyper-regeneration of myeloid and erythroid elements and stem cells were noted whereas the lymphoid cells and plasmocyte series of cells remained depressed.

Mansour and Nelson (1977) observed depression in the response of rats peripheral blood lymphocytes to PHA after cyclophosphamide treatment. Steinbach (1977) observed

depression of immunological response by parental administration of cyclophosphamide.

Studies on six bone marrow biopsy samples, one before and five spread over 22 days after I/V injection of cyclophosphamide in yearling sheep conducted by Hofirek and Drabek (1980) showed depression of haemopoiesis 2 to 4 days after the injection.

Corrier et al. (1981) observed recrudescence of clinical anaplasma infection in two Anaplasma carrier spleenectomized calves following injection of cyclophosphamide. They observed a depression of humoral mediated immunity.

Rccardi et al. (1981) observed that in mice treated with cyclophosphamide the clearance of IUdR-labelled YAC-1 tumour cells given I.V. was reduced and was most marked by the fourth day after administration of the drug. Similar depression of splenic NK activity was also seen with strong depression on day four.

Wander and Hilgard (1981) observed depression of GVH reaction following cyclophosphamide administration.

Heilmann et al. (1982) in their experiment using 26 calves aged 4-98 days observed that when calves were given

10-180 mg/kg cyclophosphamide intravenously at the dose of 50 mg/kg it caused pneumonia or enteritis with death in 1-2 weeks. Thirty mg/kg caused thymic atrophy and changes in other lymphatic organs. Impaired granulopoiesis and damage to megakaryocytes in bone marrow was dose dependent.

Therapy with cyclophosphamide was associated with diminished lympho-proliferation, reduced antibody levels and prolonged survival of MRL/I mice (Smith et al. 1984). At 100 mg/kg per week there was arrested lymphoid hyperplasia and decreased severity of renal diseases.

Six week old B10 hp/cpb nude mice were inoculated with fragments of ovarian tumours subcutaneously by Nauta et al. (1986). Cyclophosphamide was given at single dose of 100 mg/kg intraperitoneally 24 h before tumour implantation. They observed increase serial transplantability in treated mice. It did not cause toxicity in mice or inhibition of tumour transplantability. The latency period and the doubling time of the tumour were not affected and there was decreased natural killer cell activity in treated mice.

2.3 Hydrocortisone

Fauci (1976) and Roth and Kaeberle (1982) studied the effect of glucocorticoids on the immune system. They reported decreased serum antibody concentration in glucocorticoid

treated animals by suppressing antibody production or by increasing the antibody catabolism. This depended on the type of glucocorticoid administered, the dosage and the duration of treatment. In cattle, it had been shown to suppress lymphocyte blastogenesis in response to PHA. In calves, increased plasma cortisol had a profound effect on circulating T-lymphocytes than on B lymphocytes. It inhibited T-cell growth factor production by mononuclear cells.

Berkelhammer and Hook (1980) transplanted Sinclan swine melanoma in Hamster cheek pouch by administering 3 mg of cortisone acetate subcutaneously immediately after inoculation. Thereafter cortisone was administered to each hamster twice weekly by subcutaneous injection of 2.5 mg of the drug.

Koptopoulos et al. (1992) in their experiment using six goats observed depression of lymphoid organs and lymphopenia when glucocorticoid was administered alone. When cyclophosphamide was also administered there was severe leukopenia.

2.4 Cyclosporine A

White et al. (1979) observed that cyclosporine A inhibited proliferation of procine T lymphocytes, lymphocytes which were not proliferating and leukocyte migration.

Borel and Merzados (1980) showed that CS-A acts on immunocompetent T cells in the very early stage of lymphocyte stimulation and this effect was reversible. They observed that the sensitisation phase remains inhibited as long as this drug was given.

Hellmann and Goldman (1980) observed that CS-A inhibited the incorporation of H-thymidine into lymphocytes responding to mitogen or allogenic cells but had little effect on unstimulated lymphocytes. Significant suppression of granulopoiesis did not occur.

Gunn et al. (1981) showed that CY-A has a preferential effect on T cell-mediated immunity. They suggested that CY-A does not affect the generation of T_S but inhibited the generation of T_C cells by Con. A.

Galfand et al. (1987) observed that cyclosporine A depressed the IL-2 induced proliferation of IL-2 receptor expressing cells. It also inhibited cytosolic free Ca^{2+} and also resulted in membrane depolarization of T lymphocytes.

Materials and Methods

MATERIALS AND METHODS

In the present study efforts were made to transplant tumour of the ethmoturbinate mucosa of cattle into homologous and heterologous animals. In order to prevent tissue rejection the animals were treated with immunosuppressant drugs. The tumour was transplanted as fragments, single cell suspension and cells from tissue culture.

3.1 Source of animals

3.1.1 Donor animals

Cattle bearing tumour of the ethmoturbinate mucosa were brought to the Centre of Excellence in Pathology from different parts of Kerala.

3.1.2 Recipient animals

3.1.2.1 Homologous animals

Eighteen neonatal calves which were not fed with colostrum were procured from the University Livestock Farm.

3.1.2.2 Heterologous animals

One hundred and twenty weaned white rats (Wistar), white mice (Swiss albino) each and eighteen weaned NewZealand

white rabbits were procured from the small animal breeding station attached to the College of Veterinary and Animal Sciences, Mannuthy.

3.2 Immunosuppression

3.2.1 Homologous species

3.2.1.1 Cyclophosphamide

Cyclophosphamide (Cycloxan-Bio Pharmaceuticals) was administered to six calves intravenously at the rate of 35 mg/kg body weight four days prior to transplantation.

3.2.1.2 Hydrocortisone

Hydrocortisone sodium succinate (Neon) was administered to six calves subcutaneously at the rate of three mg/kg body weight one day prior to transplantation and was repeated for three days.

3.2.2 Heterologous species

3.2.2.1 Rats

3.2.2.1.1 Cyclophosphamide

Cyclophosphamide was administered to twenty rats intraperitoneally at the rate of 200 mg/kg body weight four days prior to transplantation.

3.2.2.1.2 Hydrocortisone

Hydrocortisone sodium succinate was administered to twenty rats at the rate of 2 mg per animal subcutaneously a day prior to transplantation.

3.2.2.1.3 Cyclosporine A

Cyclosporine A (Sandimun - Sandoz) was administered orally to forty rats daily for fourteen days at the rate of 15 mg/kg body weight beginning from twelve hours prior to transplantation.

3.2.2.2 Mice

3.2.2.2.1 Cyclophosphamide

Cyclophosphamide was administered to twenty mice intraperitoneally at the rate of 200 mg/kg body weight four days prior to the transplantation.

3.2.2.2.2 Hydrocortisone

Hydrocortisone sodium succinate was administered to twenty mice subcutaneously at the rate of 1 mg per animal one day prior to transplantation.

3.2.2.2.3 Cyclosporine A

Cyclosporin A was administered orally to forty mice at the rate of 15 mg/kg body weight for fourteen days starting twenty four hours prior to transplantation.

3.2.2.3 Rabbits

3.2.2.3.1 Cyclophosphamide

Cyclophosphamide was administered to six rabbits intraperitoneally at the rate of 25 mg/kg body weight four days prior to transplantation.

3.2.2.3.2 Hydrocortisone

Hydrocortisone sodium succinate was administered to six rabbits subcutaneously at the rate of 3 mg per animal one day prior to transplantation.

3.2.2.3.3 Cyclosporine A

Cyclosporine A was administered to six rabbits orally for seven days at the rate of 30 mg/kg body weight starting twenty four hours prior to transplantation.

3.3 Experimental groups

Rats and mice were divided into four main groups with 12 subgroups (Table 1). Group A, B and C were given

cyclophosphamide, hydrocortisone and cyclosporine A respectively. Group D was maintained without immuno-suppression and was kept as control group. Animals belong to subgroups 1 and 2 of all the groups were given trypsinised single cell suspension subcutaneously and intraperitoneally respectively. Animals of subgroups 3 and 4 of groups C and D were inoculated subcutaneously with tumour tissue fragments and cells from tissue culture respectively.

Rabbits were divided into four groups. Group A, B and C were given cyclophosphamide, hydrocortisone and cyclosporine A respectively. Group D was maintained without immuno-suppression and was kept as control. In all the groups trypsinised single cell suspension of the tumour was given subcutaneously.

Calves were grouped into three. Groups A and B were given cyclophosphamide and hydrocortisone respectively. Group D was maintained without immunosuppression and was kept as control to all the groups trypsinised single cell suspension of the tumour was given subcutaneously.

Ten mice were inoculated intraperitoneally with Ehrlich's ascites tumour cells at the rate of 1×10^6 cells per inoculum and were maintained as control.

Table 1. Experimental groups

Species	A		B		C				D				Total
	1	2	1	2	1	2	3	4	1	2	3	4	
Rats	10	10	10	10	10	10	10	10	10	10	10	10	120
Mice	10	10	10	10	10	10	10	10	10	10	10	10	120
Rabbits	6		6		6				6				24
Calves	6		6						6				18

A - Cyclophosphamide
B - Hydrocortisone
C - Cyclosporine A
D - Control

1. Trypsinised single cell suspension
2. Typsinised single cell suspension intraperitoneally
3. Tumour tissue fragments subcutaneously
4. Cells from tissue culture subcutaneously

3.4 Media

The following media were used for the study.

3.4.1 Maintenance media

Hank's balanced salt solution (HI-MEDIA)

The content of one vial (9.55 g) was dissolved in one litre of deionized double distilled water and was filtered through 0.2 micron membrane filter (Saratorius) under positive pressure. Antibiotics were added.

3.4.2 Culture media

3.4.2.1 TC 199 (HI-MEDIA)

The content of one vial (10.9 g) was dissolved in one litre of deionized double distilled water and was filtered through 0.2 micronmembrane filter under positive pressure. It was supplemented with 10 per cent foetal calf serum (CSIR, New Delhi).

3.4.2.2 Tissue culture media

RPMI 1640 and HAM F 12 nutrient mixture - 1:1
Foetal calf serum - 10 per cent
Insulin - 20 ug/ml
Penicillin G - 100 IU/ml
Streptomycin - 50 ug/ml
Nystatin - 100 ug/ml

3.5 Antibiotics

Penicillin G	- 200 IU/ml
Streptomycin	- 150 ug/ml
Gentamycin	- 50 ug/ml
Nystatin	- 100 IU/ml

3.6 Trypsin

3.6.1 Trypsin 0.25 per cent

0.25 per cent trypsin (1:250 Difco) in phosphate buffered saline (Ca and Mg free) (Hi-Media) was prepared and was sterilized by filtering through 0.2 micronmembrane filter.

3.6.2 Trypsin for tissue culture (TVG)

Trypsin	- 250 mg
Glucose	- 500 mg
EDTA	- 125 mg
PBS	- 100 ml

3.7 Collection of tumour tissue

Tumour bearing cows were euthanised by exsanguination after stunning with captive bolt pistol. After decapitation the head was bisected with an electric saw. Fresh soft healthy tumour tissue was dissected out from the deeper portion avoiding the necrotic area, under sterile condition.

The tumour tissue was collected in Hank's balanced salt solution with antibiotics. A part of the tumour tissue was taken in 10% formalin for histopthology.

3.8 Preparation of the tumour tissue

The tumour tissue was washed using phosphate buffered saline containing antibiotics, several times to remove the debris. It was transferred into a Petri dish containing PBS. The superficial fascia was removed from the tumour mass. The tissue was then cut into small cubes of 1 mm size and washed several times with PBS.

3.9 Tissue dissociation

A few cubes of the tumour tissue was transferred into a beaker containing 100 ml of 0.25 per cent trypsin solution in PBS. The beaker was placed on a magnetic stirrer and stirred for 10 minutes using a magnetic stirring paddle. The supernatent was decanned and replaced with fresh 100 ml of 0.25 per cent trypsin. It was stirred using magnetic stirrer for another 10 minutes. The serum (3 ml) was added to the suspension to neutralize the trypsin. The suspension was sieved through a double layered sterile muslin cloth into a sterile flask. The suspension was transferred to a centrifuge tube and was centrifuged at 1000 rpm for 5 minutes. The supernatent was poured off and the cell pellet was suspended

in media with antibiotic. The viable cell concentration was adjusted to 1 x 10^6 viable cells per 0.25 ml.

3.10 Tumour tissue culture

3.10.1 Explant culture

The tumour tissue collected in HBSS was cut into small pieces and was washed with PBS containing antibiotics. These were again cut into small cubes with a sterile scalpel blade and was washed with PBS containing antibiotics. Five to eight such pieces were kept in a petri dish and 4 ml of tissue culture media was poured in it. The Petri dish was kept in a carbon dioxide incubator (Cintex) at 37°C and 5 per cent CO_2. After 24 h the media was changed after removing the floating tissue cubes. The media was changed every 4th day.

3.10.2 Single cell suspension

Single cell suspension prepared by tissue dissociation was seeded into tissue culture bottles with 5 ml of tissue culture media. The bottles were kept partially open with a rubber stopper and were kept in a carbon dioxide incubator at 37°C and 5 per cent CO_2. The media was changed after 24 h and later on every fourth day.

3.11 Transplantation

3.11.1 Tumour pieces

The tissue pieces which were cut into small cubes (1 mm^3) and were suspended in tissue culture media and were injected subcutaneously in the flank region.

3.11.2 Single cell suspension

Single cell suspension obtained by trypsinization and tissue culture were injected separately into experimental animals, by the subcutaneous and intraperitoneal route at the rate of $1x10^6$ viable cells per inculum.

3.12 Observation for growth

The thickness of the skin was measured after inoculation and repeated weekly in case of heterologous hosts and monthly in case of homologous hosts. The site of inoculation was observed visually and by palpation daily from the third day onwards after inoculation. The homologous hosts were observed for four months and the heterologous hosts for a month. All animals were sacrificed after the observation period. The site of inoculation, spleen, lungs, heart, liver and kidney were taken for histopathology. Peritoneal fluid was also taken from animals which were given cells intraperitoneally.

3.13 Total leucocyte count

Blood was collected in EDTA at the time of sacrifice for finding out the total leucocyte count.

3.14 Differential count

Blood for differential leucocyte count was taken at the time of sacrifice.

3.15 Determintion of T-lymphocytes in the peripheral blood smear (ANAE staining technique)

Peripheral blood smears obtained at the time of sacrifice were immediately fixed in the fixative which contained six parts of acetone and four parts of 0.038 M sodium citrate (pH 5.4). The smears were kept in the fixative for 30 seconds, rinsed in distilled water and dried.

For staining the smears, a reaction mixture was prepared as follows.

In 40 ml of 0.067 M phosphate buffer (pH 5.0), 2.4 ml of hexazotised pararosanaline and 10mg of alphanaphthylacetate (Loba, India) dissolved in 0.4 ml acetone was added, and the final pH of the reaction mixture was adjusted to 5.8 with 2N sodium hydroxide.

The hexazotised parasosaline was prepared in the following manner.

Equal volumes of two solutions:

1. Freshly prepared 4 per cent sodium nitrite in distilled water.

2. One gram of pararosanaline hydrochloride (Sigma) dissolved in 20 ml of distilled water and 5 ml of 12N hydrochloride acid.

These two solutions were combined. The hexazotised pararosanaline which formed was shaken and then allowed to stand for one minute before adding it to the reaction mixture.

The slides were incubated in the reaction mixture for 18-21 h at room temperature then rinsed thoroughly with distilled water and then counterstained with 1 per cent toludine blue for 45-60 minutes. The slides were then rinsed thoroughly with distilled water, dehydrated in ascending grades of ethyl alcohol, cleared in xylol and mounted in DPX and examined under oil emission objective. Those lymphocytes with localised orange and nodular reaction product in the cytoplasm were considered as positive cells (T lymphocytes). The number of positive cells in every hundred cells were counted and recorded.

Results

RESULTS

Tumour tissue for the transplantation study was obtained from seven clinically affected cows.

4.1 Gross pathology of the tumour

In all the instances the tumour was found to occupy the posterior third of the nasal passage, causing partial to complete obstruction (Fig.1). In one case the tumour extended anteriorly into the frontal sinus, laterally into the maxillary sinus and ventrally into the spheno-palatine sinus. In another case the tumour extended posteriorly into the pharyngeal passage. Growth of the tumour into the cranial cavity was noticed in one case without any meningeal adhesion. Horn core was free of neoplastic invasion in all instances. Involvement of the retrobulbar region was a constant finding. A thick capsule covering the tumour mass was observed in one case. The tumour mass was pale yellow to cream in colour and was either fleshy or soft in consistency. Haemorrhagic, necrotic and suppurative foci were frequently observed in those parts of the tumour which were directly exposed to the atmosphere. There was partial to complete destruction of the turbinates. Hyperemia and oedema of the nasal mucosa were constantly observed.

Regional lymphnodes or other organs were free of any gross lesions.

4.2 Histopathology of the tumour

The tumours encountered were carcinomas of different degree of malignancy. They were diagnosed as adenocarcinoma (3), papillary adenocarcinoma (2), undifferentiated carcinoma (1) and squamous cell carcinoma (1).

4.2.1 Adenocarcinoma

Adenocarcinomas were characterised by non-ciliated columnar cells. Numerous acini of varying size and shape were observed (Fig.2). The acini were lined by layers of proliferating neoplastic epithelial cells of columnar or cuboidal type. A few of the acini were filled with desqumated epithelial cells. The nucleus of the cells were vesicular, hyperchromatic and basally placed. Most of them contained more than one nucleolus. Many of the cells revealed mitotic figures. In some areas sheets of isolated clumps of neoplastic cells without tendency to form acini were observed. Those cells were round or polygonal in shape with hyperchromatic nucleus.

A few degenerative foci with moderate infiltration of lymphocytes and macrophages were observed.

4.2.2 Papillary adenocarcinoma

Tumours characterised by papillary processes lined by multilayered tall non-ciliated columnar cells were classified as papillary adenocarcinoma. These neoplastic cells revealed basally placed hyperchromatic vesicular nucleus with one or more nucleoli (Fig.3). Varying degree of mitotic figures were observed in the tissue.

4.2.3 Squamous cell carcinoma

Microscopical features of non-keratinising squamous cell carcinoma were observed. The neoplastic cells were large with centrally placed nucleus. Mitotic division was evident in large number of cells (Fig.4). Imperfectly developed epithelial pearls were seen scattered throughout the parenchyma. In certain areas the neoplastic cells were distributed in irregular masses in narrow and/or wide branching anastomosing columns spreading through the loose fibrous stroma.

Moderate stromal reaction was observed. Focal areas of congestion and haemorrhage were noticed. Focal infiltration of lymphocytes and macrophages were observed in degenerated areas.

4.2.4 Undifferentiated carcinoma

The neoplastic cells were oval, round without any differentiation into either columnar or squamous type. These cells were arranged in groups separated by thin stromal tissue. The cells had scanty eosinophilic cytoplasm with hyperchromatic nucleus (Fig.5). Different stages of mitosis were seen in a number of neoplastic cells.

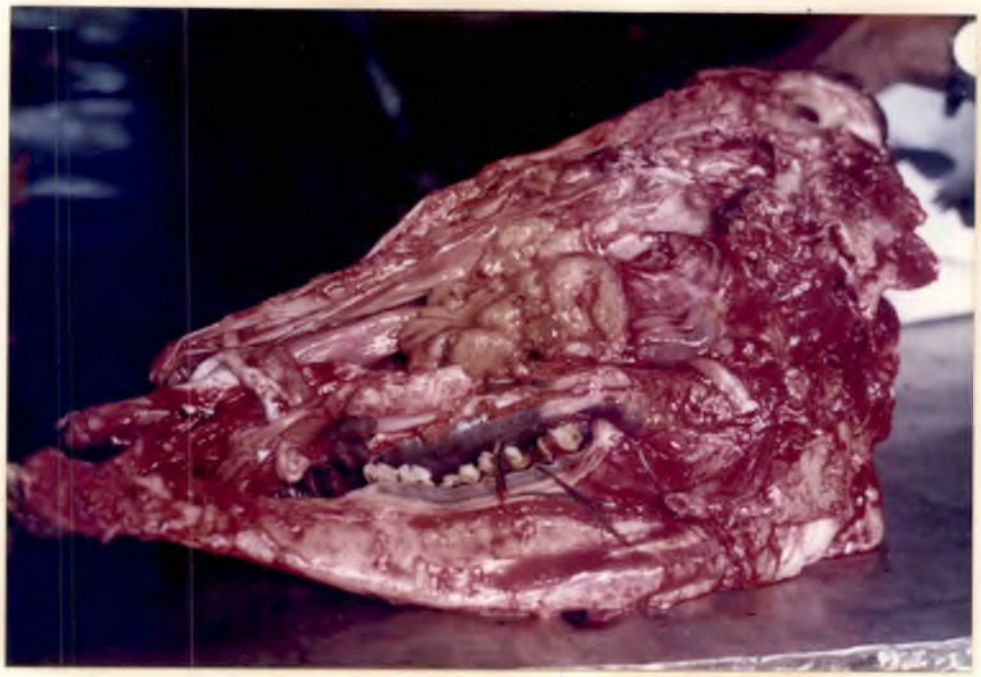
Stromal content and infiltration of lymphocytes and macrophages were minimal.

4.3 Tumour tissue culture

Cells were found adhered to the glass surface of the tissue culture bottles and petri dishes which were seeded with trypsinised single cell suspension and tumour tissue pieces respectively. The tumours were of adenocarcinoma, papillary adenocarcinoma and squamous cell carcinoma type. The cell layer became confluent within 12 days (Fig.6 and 7). Cell growth was faster in bottles than those from explant culture. The monolayer was dissociated using trypsin versene and was seeded in two bottles each. The smears prepared from the primary culture and stained by Papanicolaou's method revealed large cells in various stages of division with well defined nucleus and distinct cytoplasm (Fig.8).

Fig.1 Ethmoid tumour occupying the posterior third of nasal passage

Fig.2 Adenocarcinoma - acini lined by columnar cells - H&E x 450



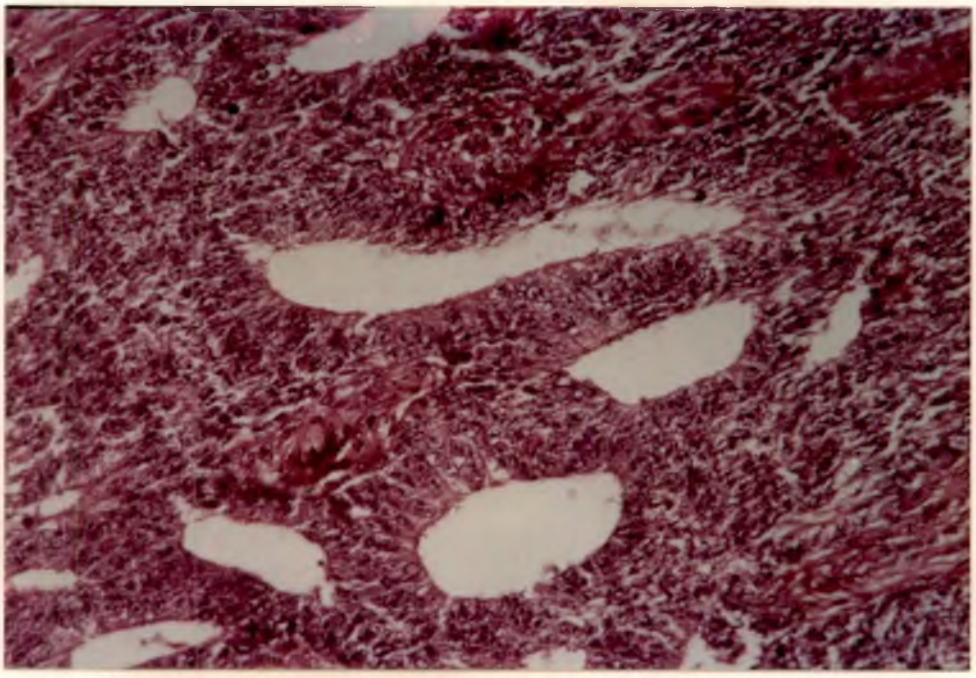
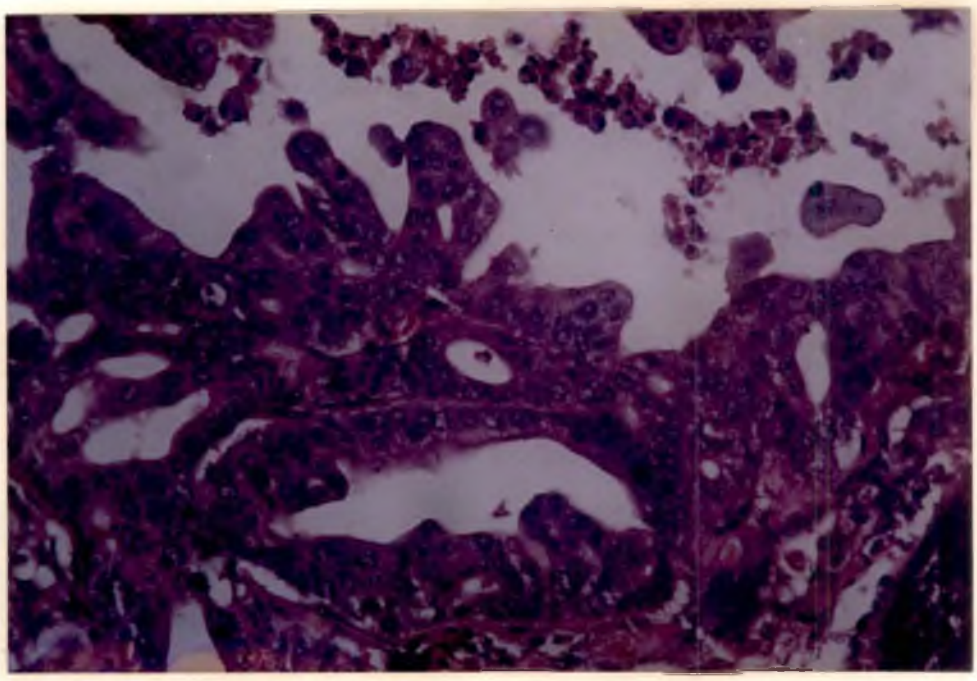


Fig.3 Papillary adenocarcinoma - acini with papillary projection - H&E x 450

Fig.4 Squamous cell carcinoma - neoplastic squamous cells - H&E x 450



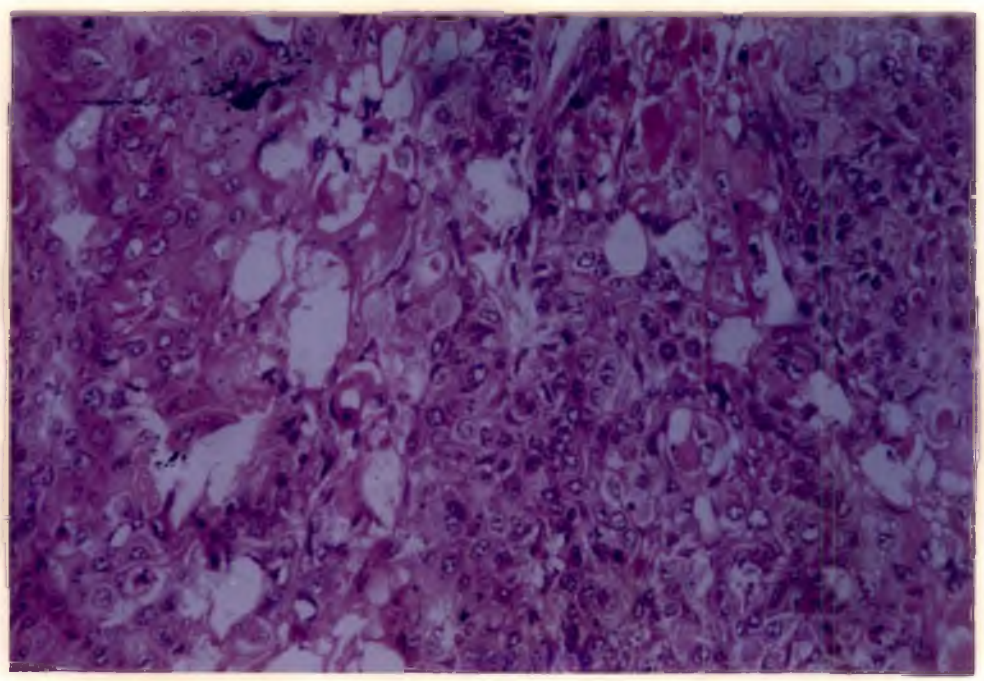
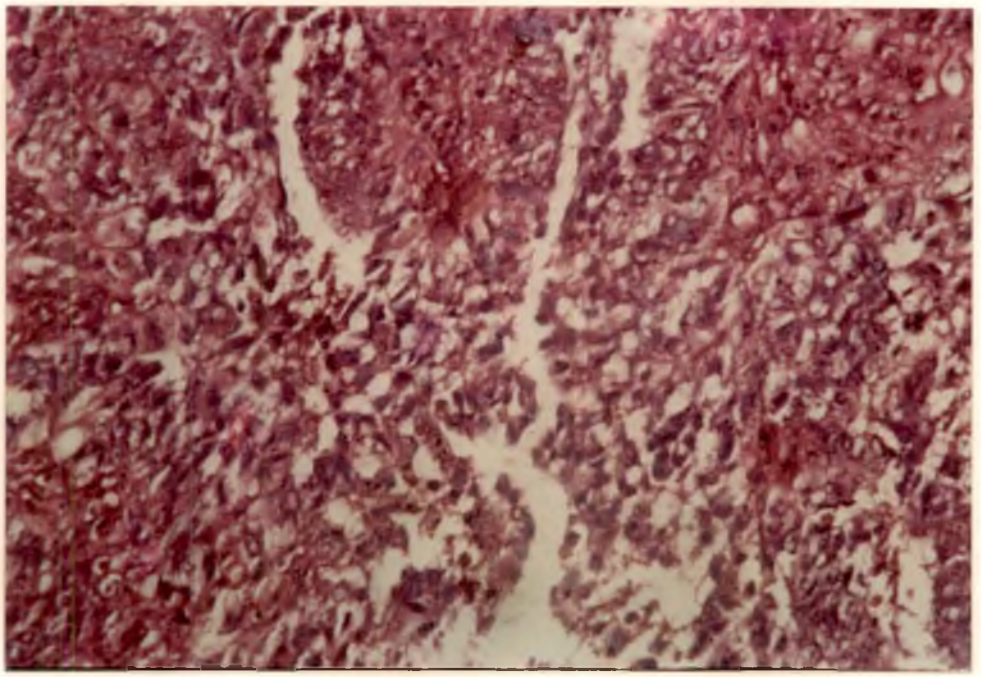


Fig.5 Undifferentiated carcinoma - cells with scanty cytoplasm and hyperchromatic nucleus - H&E x 450

Fig.6 Neoplastic cells adhering to the glass surface - x 100



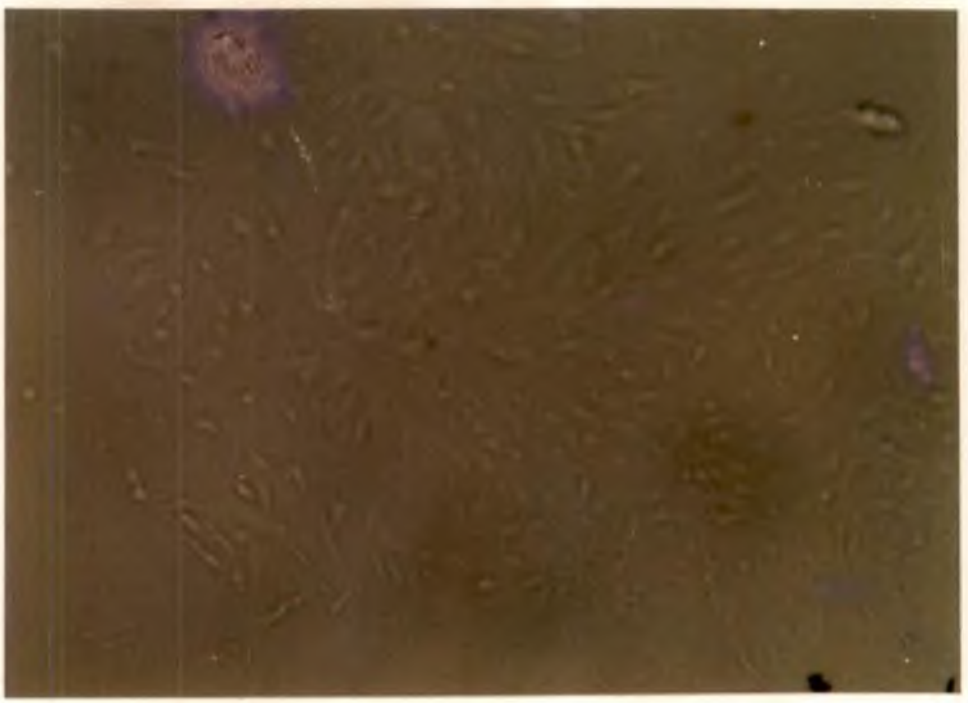
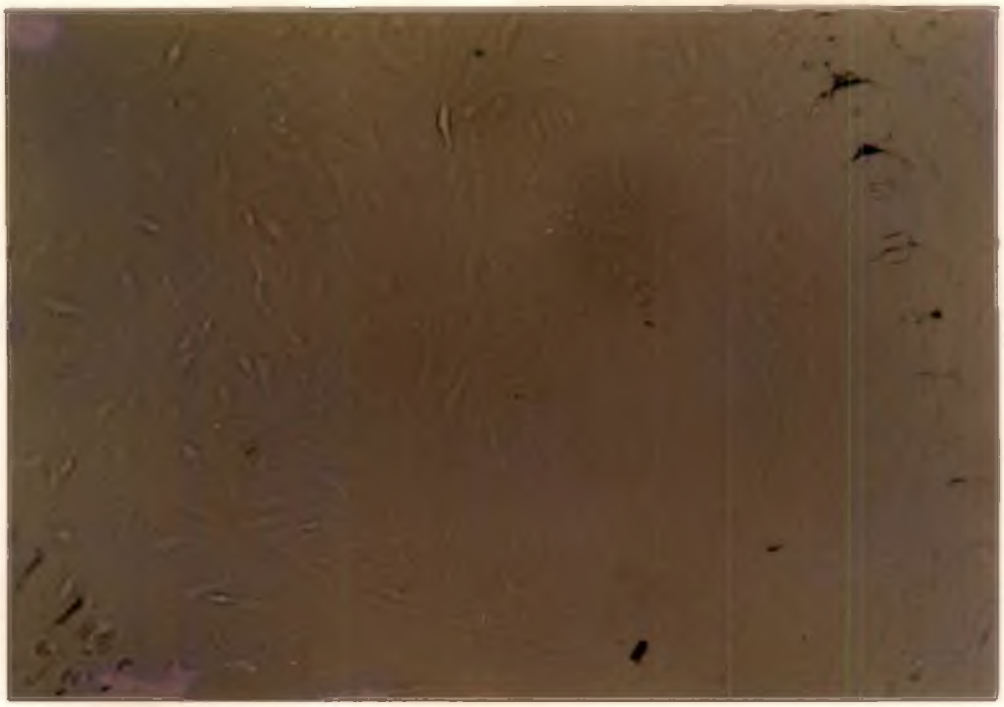
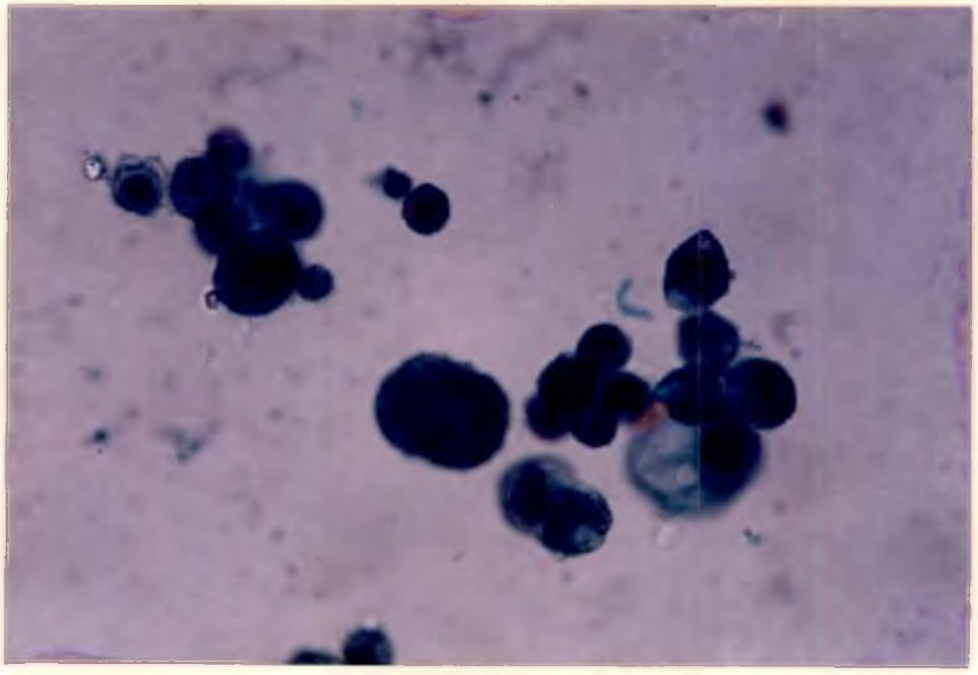


Fig.7 Neoplastic cells adhering to the glass surface showing epithelial morphology - x 100

Fig.8 Papanicolaou's staining - cells with greenish cytoplasm and pink staining nucleus x 1000





After the next monolayer formation the cells were harvested by scrapping with a piolet. The viable cell count was adjusted to 1×10^6 cells per 0.25 ml.

4.4 Gross pathology of the inoculation site

4.4.1 Inoculation with tumour fragments

In all the ten rats and mice immunosuppressed using cyclosporine A and subcutaneously implanted with tissue fragments obtained from adenocarcinoma, papillary adenocarcinoma and squamous cell carcinoma, revealed small palpable swelling at the site of inoculation for a week. The swelling gradually decreased in size and finally disappeared after the second week, as shown in Fig.9 and Tables 2 and 3. Other detectable gross lesions indicating inflammatory reactions were absent during the period of observation.

Control animals revealed local oedema at the inoculation site twenty-four hours after the transplantation and attained maximum by the first week. It gradually decreased in size and completely disappeared after the 2nd week as shown in Tables 2 and 3 and Fig.9.

4.4.2 Inoculation with trypsinised single cell suspension

In all homologous (Table 5) and heterologous transplantations (Tables 2, 3, and 4) the animals inoculated

subcutaneously, the initial swelling completely disappeared within twenty-four hours. There was no evidence of tumour growth in animals immunosuppressed with cyclophosphamide, hydrocortisone and cyclosporine A, except in mice immunosuppressed with cyclosporine A. The immunosuppressed mice which were administered adenocarcinoma cells, a small fluctuating firm nodule was observed after 2 week. The nodule increased in size progressively and by the end of the fourth week it was easily palpable (Fig.10).

In all immunosuppressed heterologous animals inoculated intraperitoneally with the tumour cells, no gross lesions were seen. Microscopical examination of the fluid from the abdominal cavity at the termination of the period of observation did not reveal any neoplastic cell.

In homologous and heterologous animals belonging to the control group evidence of tumour growth was not observed. A swelling which was observed at the site of inoculation in the first week, regressed in size gradually and disappeared within two weeks.

4.4.3 Inoculation of cells from tissue culture

There was no evidence of tumour growth or inflammation in any of the immunosuppressed animals.

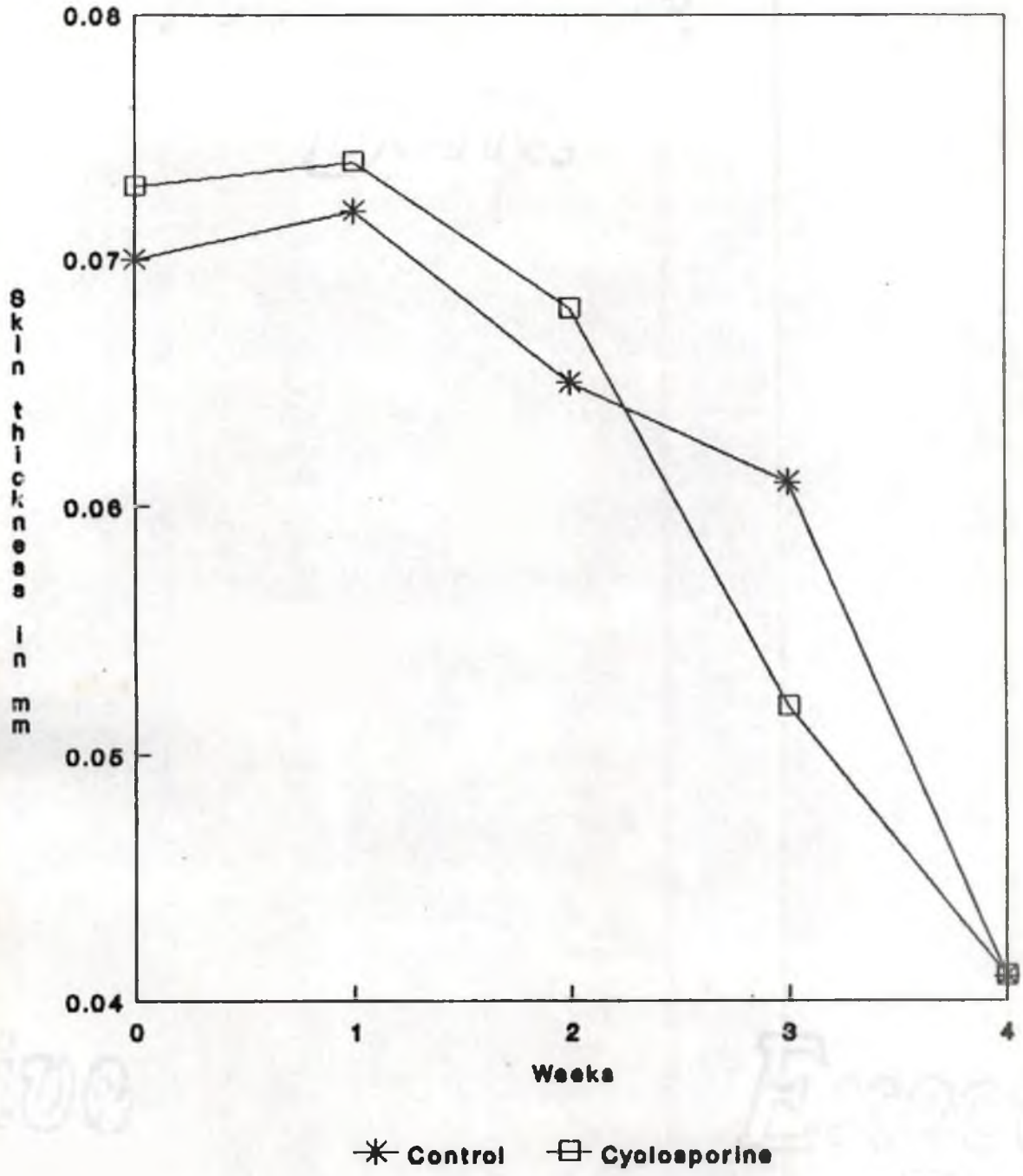


FIG.9 SKIN THICKNESS OF MICE TREATED WITH CYCLOSPORINE: IMPLANTED WITH TUMOUR TISSUE FRAGMENTS (mm)

Table 2. Skin thickness of mice (mm)

Period (weeks)	Cyclophosphamide	Hydrocortisone	Cyclosporine A			Control		
	S/C	S/C cell suspension	S/C	Tumour fragments	Tissue culture	S/C cell suspension	Tumour fragments	Tissue culture
0	0.055± 0.002	0.059± 0.009	0.040± 0.006	0.073± 0.006	0.048± 0.003	0.058± 0.019	0.070± 0.007	0.051± 0.007
1	0.044± 0.003	0.047± 0.004	0.043± 0.006	0.074± 0.004	0.039± 0.004	0.059± 0.005	0.072± 0.001	0.051± 0.005
2	0.040± 0.002	0.043± 0.003	0.052± 0.004	0.068± 0.004	0.045± 0.004	0.062± 0.053	0.065± 0.004	0.045± 0.004
3	0.040± 0.002	0.045± 0.003	0.066± 0.005	0.052± 0.004	0.042± 0.004	0.065± 0.005	0.061± 0.005	0.045± 0.004
4	0.042± 0.051	0.045± 0.002	0.074± 0.004	0.041± 0.004	0.042± 0.005	0.067± 0.084	0.041± 0.005	0.046± 0.002

Table 3. Skin thickness of rats (mm)

Period (weeks)	Cyclophosphamide	Hydrocortisone	Cyclosporine A			Control		
	S/C	S/C cell suspension	S/C	Tumour fragments	Tissue culture	S/C cell suspension	Tumour fragments	Tissue culture
0	0.135± 0.002	0.135± 0.064	0.140± 0.006	0.152± 0.006	0.134± 0.009	0.139± 0.008	0.150± 0.006	0.132± 0.006
1	0.126± 0.006	0.127± 0.006	0.136± 0.007	0.149± 0.006	0.127± 0.006	0.142± 0.008	0.153± 0.004	0.133± 0.008
2	0.128± 0.006	0.127± 0.005	0.133± 0.005	0.140± 0.038	0.125± 0.005	0.137± 0.006	0.142± 0.005	0.124± 0.005
3	0.130± 0.005	0.131± 0.005	0.132± 0.005	0.141± 0.005	0.128± 0.004	0.133± 0.013	0.143± 0.004	0.127± 0.004
4	0.131± 0.003	0.132± 0.004	0.132± 0.150	0.142± 0.005	0.131± 0.004	0.131± 0.020	0.144± 0.004	0.128± 0.004

Table 4. Skin thickness of rabbits (mm)

Period (weeks)	Cyclophosphamide	Hydrocortisone	Cyclosporine A	Control
0	0.167 ± 0.006	0.170 ± 0.004	0.166 ± 0.004	0.157 ± 0.005
1	0.150 ± 0.004	0.157 ± 0.003	0.151 ± 0.003	0.159 ± 0.002
2	0.151 ± 0.004	0.159 ± 0.003	0.153 ± 0.003	0.145 ± 0.004
3	0.151 ± 0.004	0.159 ± 0.003	0.153 ± 0.004	0.146 ± 0.004
4	0.152 ± 0.005	0.161 ± 0.004	0.154 ± 0.003	0.148 ± 0.005

Table 5. Skin thickness of calves (mm)

Period (months)	Cyclophosphamide	Hydrocortisone	Control
0	0.400 ± 0.006	0.410 ± 0.010	0.419 ± 0.006
1	0.312 ± 0.004	0.313 ± 0.004	0.410 ± 0.075
2	0.322 ± 0.008	0.320 ± 0.004	0.368 ± 0.093
3	0.352 ± 0.007	0.331 ± 0.003	0.417 ± 0.005
4	0.388 ± 0.007	0.392 ± 0.003	0.420 ± 0.006

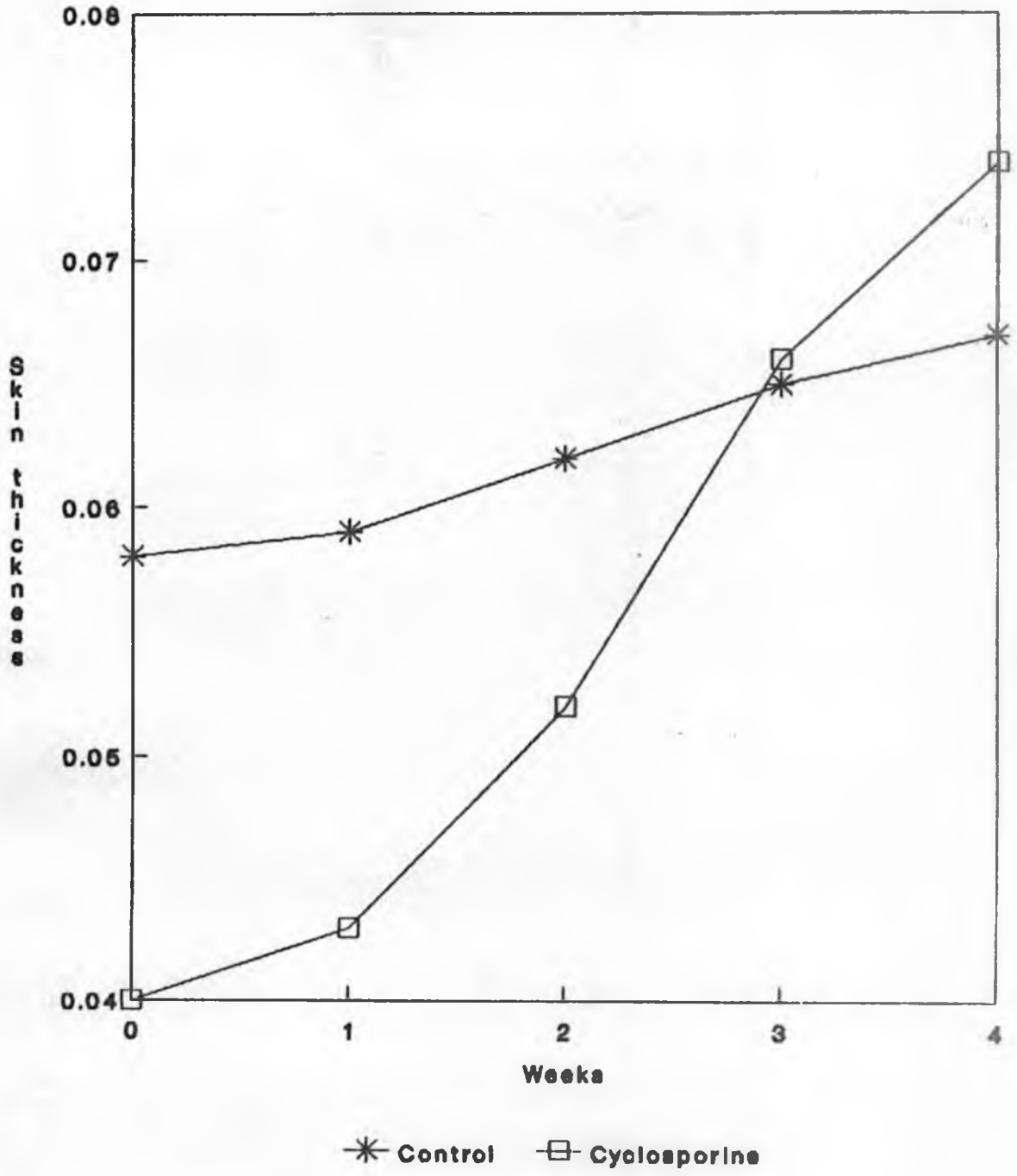


FIG.10 SKIN THICKNESS OF CYCLOSPORINE TREATED MICE WITH TRYPSINISED CELL SUSPENSION (mm)

In the animals of the control group a swelling observed at the site during the first week disappeared completely after two weeks.

In control mice inoculated with Ehrlich's ascites tumour cells, ascites was seen with 10-15 days and was observed through out the observation period. At the end of the observation period the ascites fluid was collected and Ehrlich's ascites cells were seen microscopically (Fig.11).

4.5 Histopathology of the inoculation site

In both homologous and heterologous immunosuppressed animals inoculated with the tumour, no tumour growth was evident. In eight mice immunosuppressed with cyclosporine A and inoculated with trypsinised single cell suspension of adenocarcinoma, neoplastic foci were identified microscopically.

A round or oval zone of proliferating tumour cells was seen embedded in the subcutaneous fat of eight mice (Fig.12). In all the cases the proliferating tumour mass was encapsulated by a very thin layer of fibrous tissue and were always in close proximity to the blood vessels (Fig.13). All the tumours were well vascularised from the murine epidermis with few blood vessels within the proliferating tumour mass

(Fig.14 and 15). The tumour growth was well differentiated with diffuse acini formation (Fig.16). The acini were lined by a single layer of columnar cells with a basally placed hyperchromatic vesicular nucleus. The cells in the area without acini were polygonal in shape with hyperchromatic vesicular nucleus. Pleomorphisim was also observed (Fig.17). The cells were in varying degrees of mitosis. Binucleated cells were also observed (Fig.18).

The underlying musculature was free of invasion by the neoplastic cells.

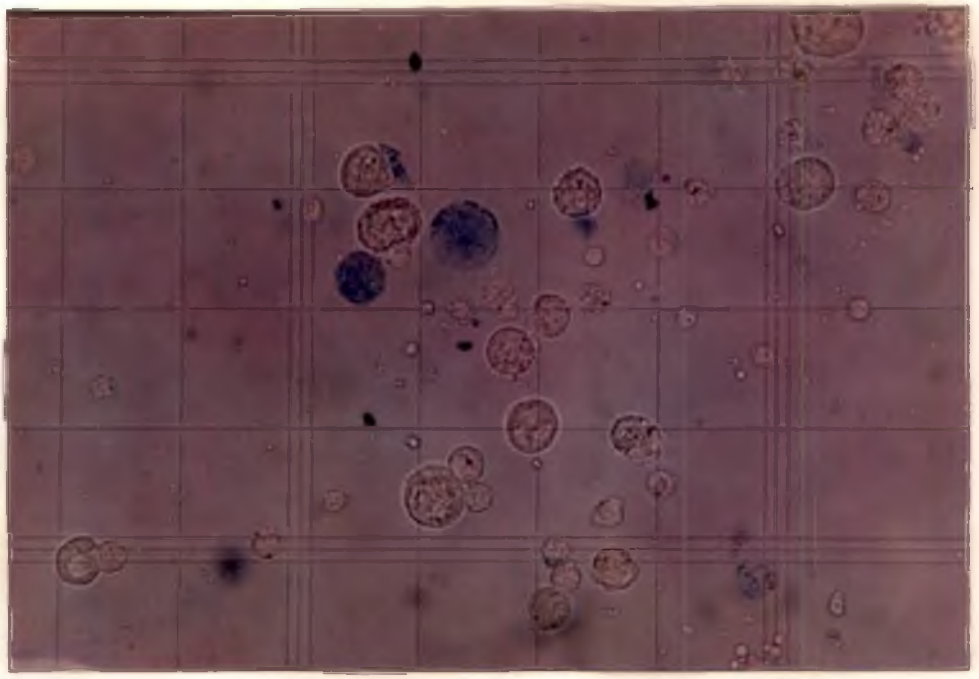
In all the control animals, there was congestion of subcutaneous blood vessels and infiltration with a few lymphocytes and macrophages in the inoculation site. Neoplastic cells were not detected at the site of inoculation.

4.6 Other histopathological observations

Microscopically the periarteriolar lymphoid sheath of the spleen of all homologous and heterologous immunosuppressed hosts contained only scanty lymphocytes when compared to that of the control. Red pulp was abundant than the white pulp. Germinal centers were not seen in immunosuppressed animals.

Fig.11 Ehrilich's ascites cells-dead cells stained blue - Trypan blue staining x 450

Fig.12 Tumour xenograft in mice - proliferating tumour cells embedded in the subcutaneous fat H&E x 60



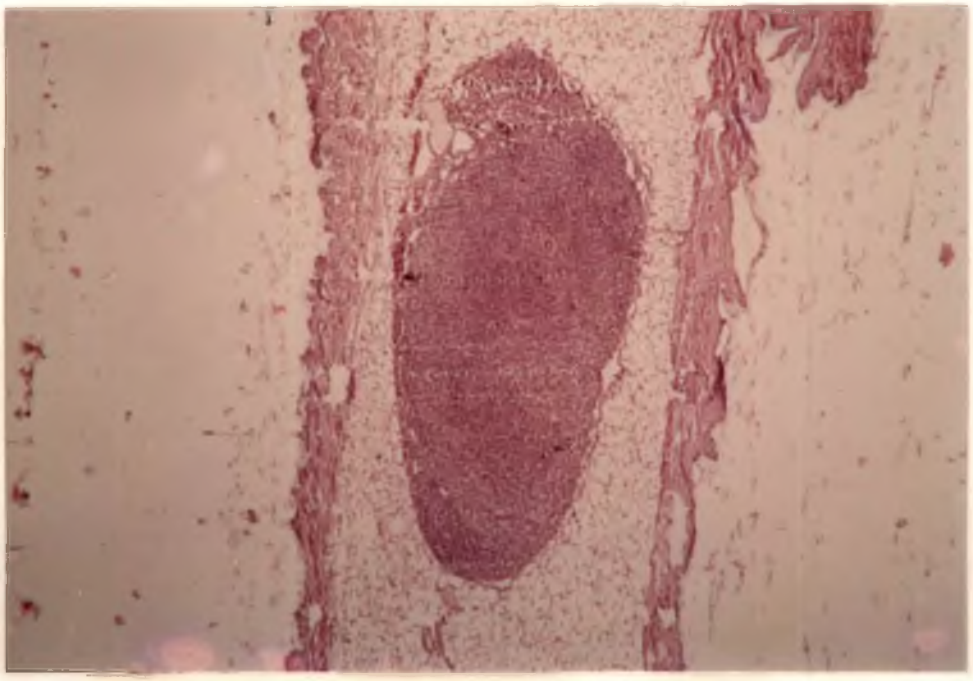
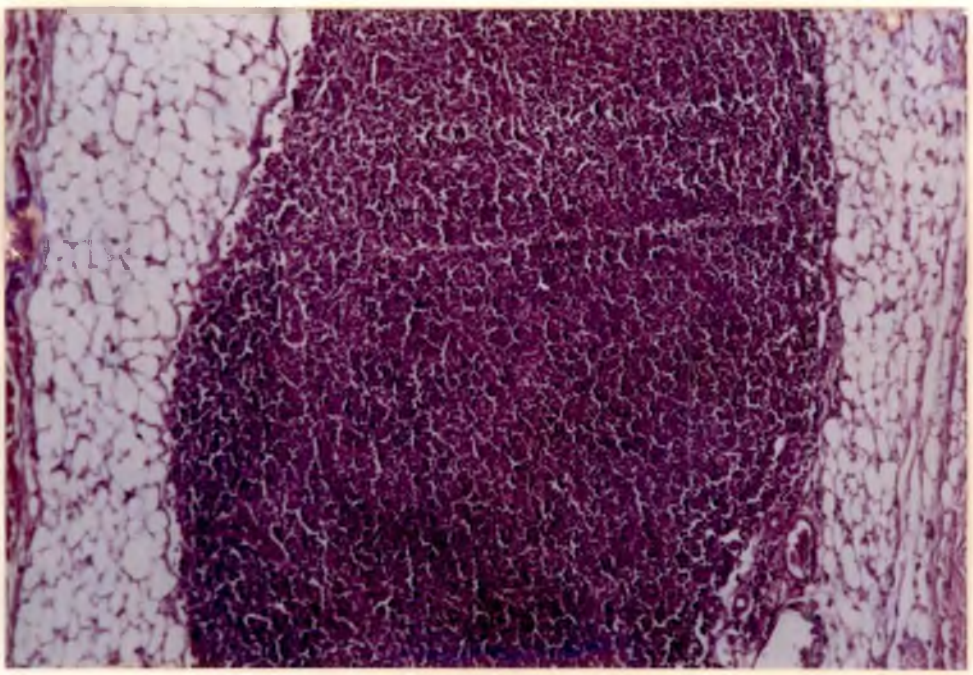


Fig.13 Tumour xenograft in mice - blood vesels near the graft and very thin fibrous tissue covering - H&E x 100

Fig.14 Tumour xenograft in mice - blood vessels near the graft - H&E x 450



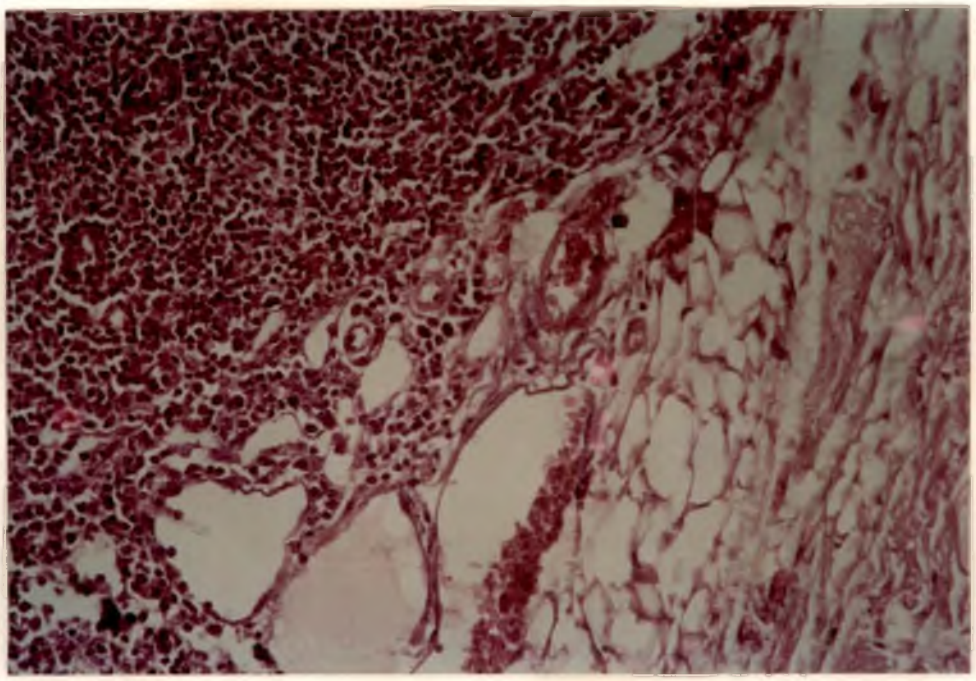
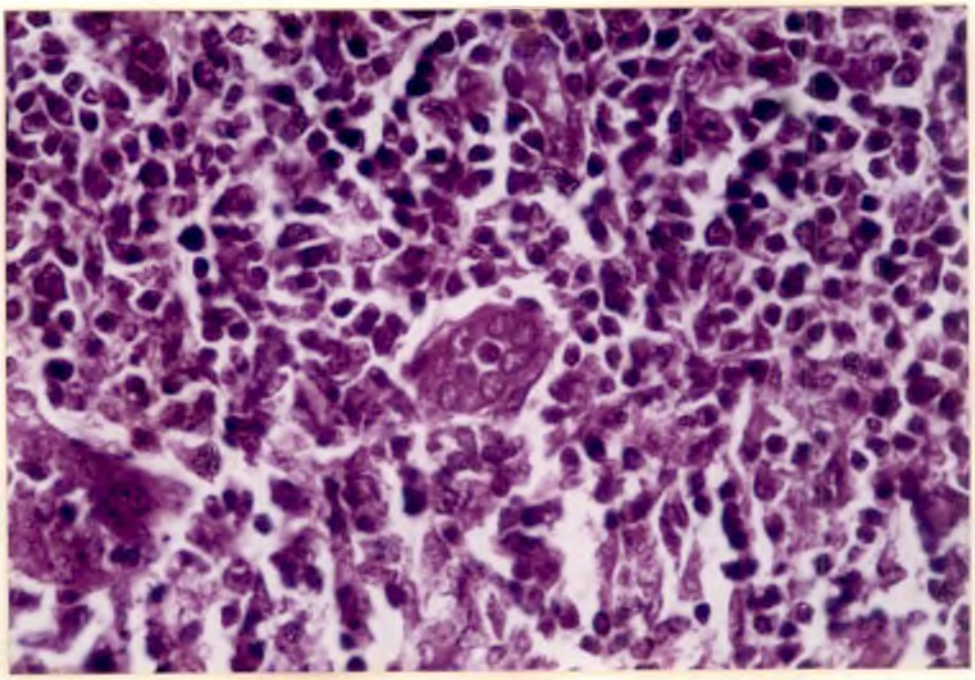


Fig.15 Tumour xenograft in mice - blood vessels within the graft - H&E x 1000

Fig.16 Tumour xenograft in mcie - acini lined by columnar cells - H&E x 1000



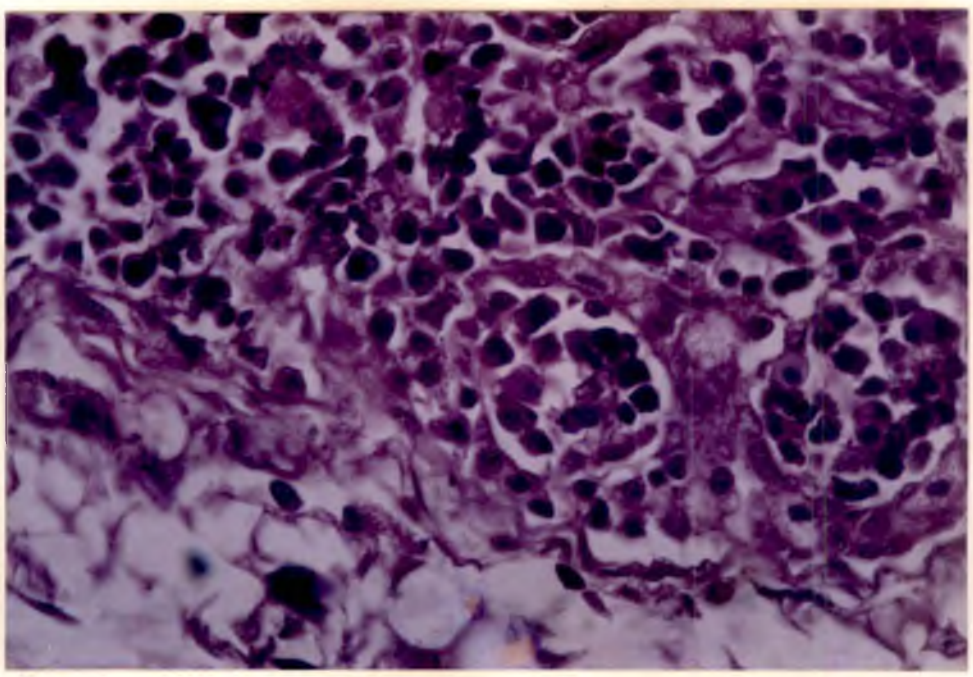
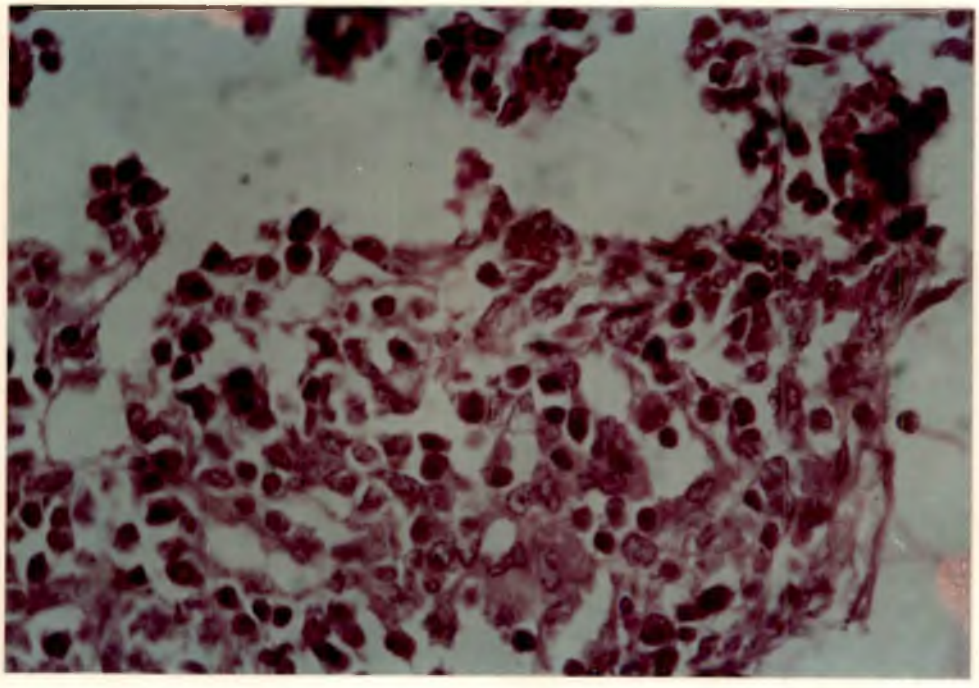
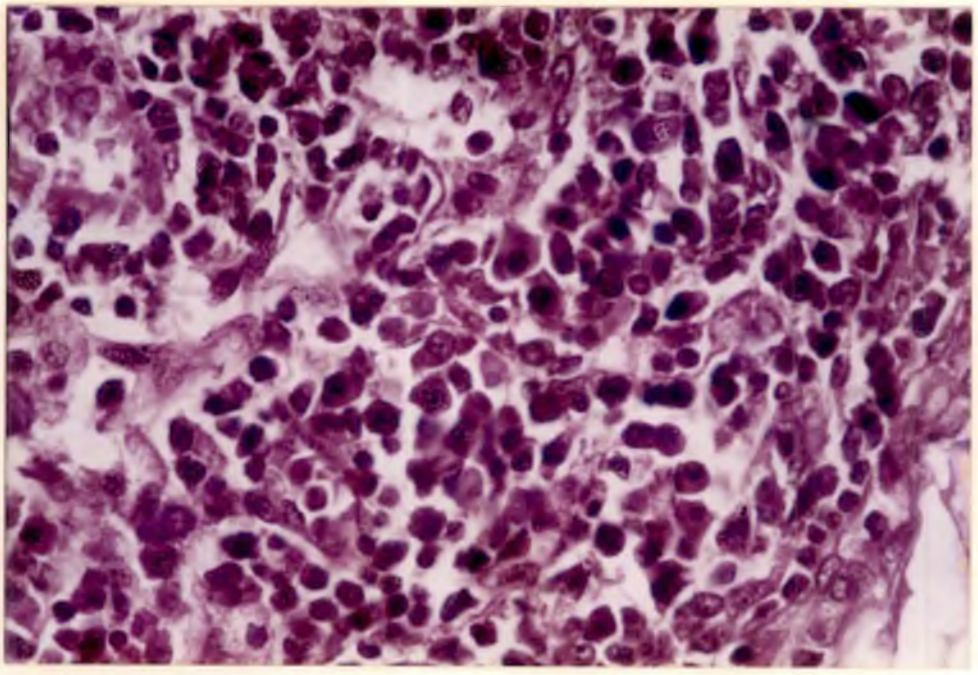


Fig.17 Tumour xenograft in mice - pleomorphic cells with hyperchromatic vesicular nucleus H&E x 1000

Fig.18 Tumour xenograft in mice - binucleated tumour cell - H&E x 1000





Lungs, heart and kidney were free of neoplastic cells in all the animals. Focal areas of fatty degeneration of the liver was seen in mice immunosuppressed with cyclosporine A.

4.7 Haematology

The total leucocyte count, differential leucocyte count and esterase positive lymphocyte count are tabulated. There was significant difference between the control animals and experimental animals in these parameters.

The total leucocyte count in all the animals was very low when compared to the control as shown in Table 6.

The differential leucocyte count of the immunosuppressed animal showed a very low lymphocyte percentage when compared to the control (Table 7) (Fig.19). The lowest was observed in the case of rats immunosuppressed with cyclophosphamide. Among the different animals treated with cyclosporine A, rats had the lowest lymphocyte percentage than mice and rabbits (Fig.20). Among mice treated with different immunosuppressants and inoculated subcutaneously or interaperitoneally with trypsinised single cell suspension, those treated with cyclophorine A and inoculated intraperitoneally with tumour cells showed the lowest lymphocytes percentages (Fig.21). The percentage of neutrophil is tabulated in Table 8. The eosinophil percentage

Table 6. Total leucocyte count (x 10^3/cm mm)

Species	Cyclophosphamide		Hydrocortisone		Cyclosporine A				Control			
	S/C	l/P	S/C	l/P	S/C	l/P	Tumour fragments	Tissue culture	S/C	l/P	Tumour fragments	Tissue culture
	cell suspension								cell suspension			
Rats	4.93± 0.57	4.67± 0.25	4.78± 0.17	4.94± 0.10	2.17± 0.39	2.89± 0.38	2.34± 0.17	2.82± 0.36	13.28± 0.43	12.43± 0.58	12.63± 0.67	12.80± 0.47
Mice	3.54± 0.20	3.52± 0.20	3.78± 0.13	3.58± 0.12	2.65± 0.32	2.40± 0.28	3.10± 0.20	3.04± 0.17	9.72± 0.28	8.89± 0.31	9.14± 0.35	10.00± 0.78
Rabbits	4.32± 0.37		4.95± 0.11				2.97± 0.62		10.65± 0.58			
Calves	4.90± 0.15		5.88± 0.30						9.25± 0.21			

Table 7. Lymphocyte percentage

Species	Cyclophosphamide		Hydrocortisone		Cyclosporine A				Control			
	S/C	l/P	S/C	l/P	S/C	l/P	Tumour fragments	Tissue culture	S/C	l/P	Tumour fragments	Tissue culture
	cell suspension								cell suspension			
Rats	30.50± 0.43	33.00± 0.88	34.80± 1.78	29.00± 0.93	31.70± 0.47	32.70± 0.97	35.80± 1.41	33.70± 0.82	72.60± 1.39	72.80± 1.89	71.60± 1.52	71.50± 1.16
Mice	34.20± 0.76	34.20± 0.56	44.10± 0.6	43.20± 0.94	33.60± 0.52	35.40± 1.06	33.80± 0.95	34.60± 1.04	64.90± 4.75	69.40± 1.00	70.20± 0.79	68.40± 1.00
Rabbits	30.83± 1.14		25.50± 1.02		33.83± 0.79				53.10± 0.54			
Calves	32.00± 0.58		40.00± 0.76						57.30± 0.98			

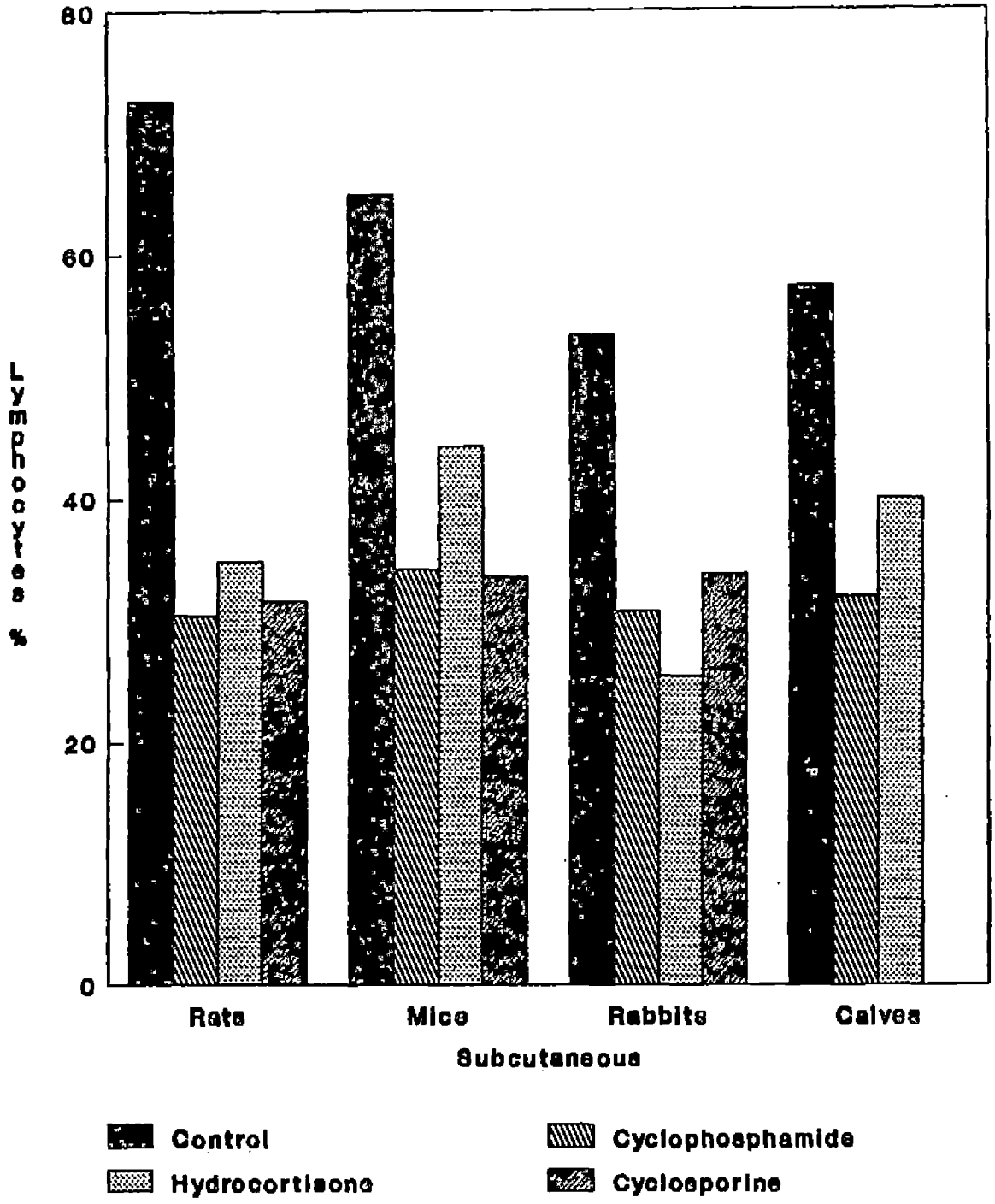


FIG.19 LYMPHOCYTE COUNT OF EXPERIMENTALLY IMMUNOSUPPRESSED ANIMALS (%)

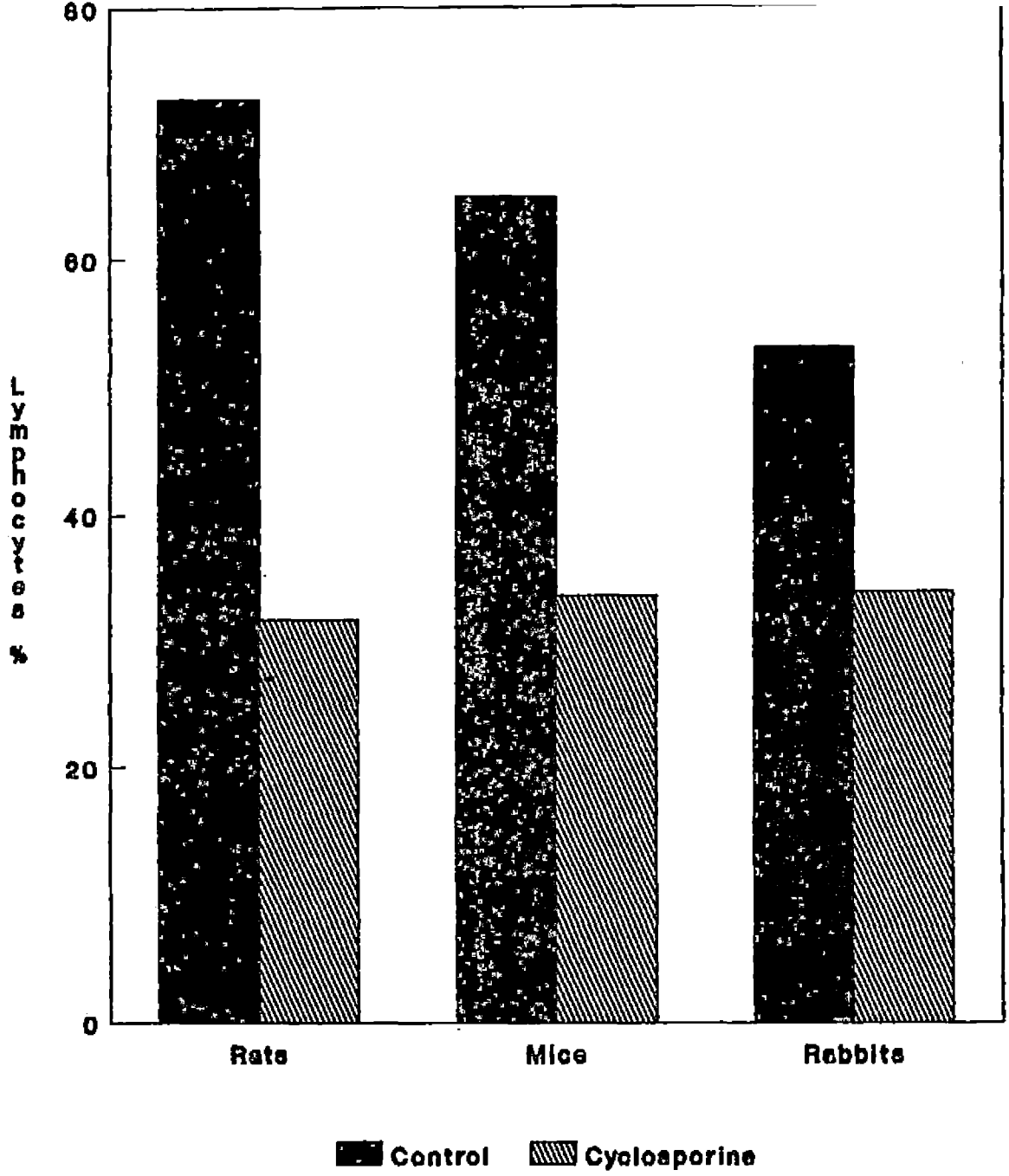


FIG.20 LYMPHOCYTE COUNT OF CYCLOSPORINE TREATED ANIMALS WITH SUBCUTANEOUS TRYPSINISED CELL SUSPENSION (%)

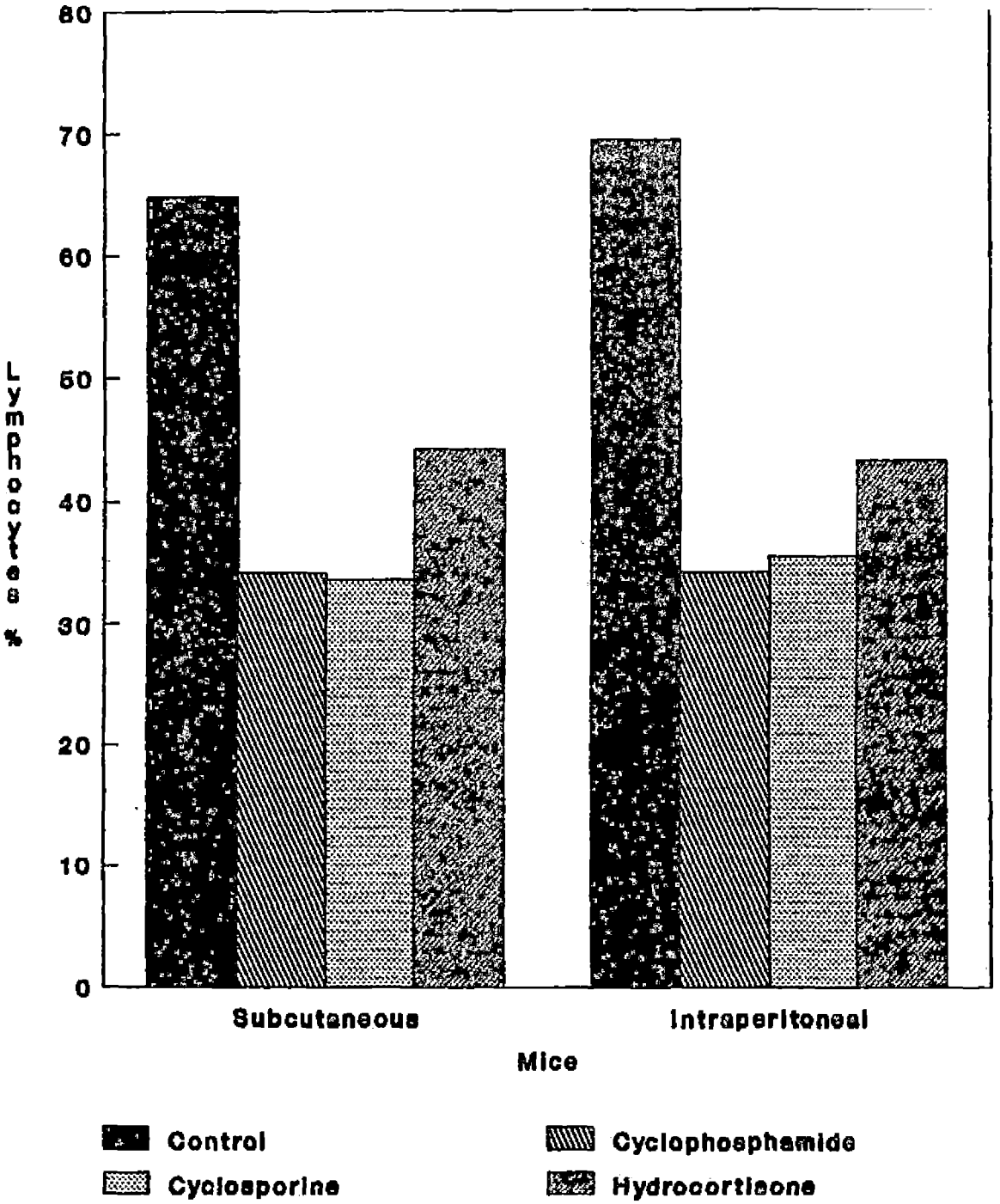


FIG.21 LYMPHOCYTE COUNT OF EXPERIMENTALLY IMMUNOSUPPRESSED MICE (%)

Table 8. Neutrophil percentage

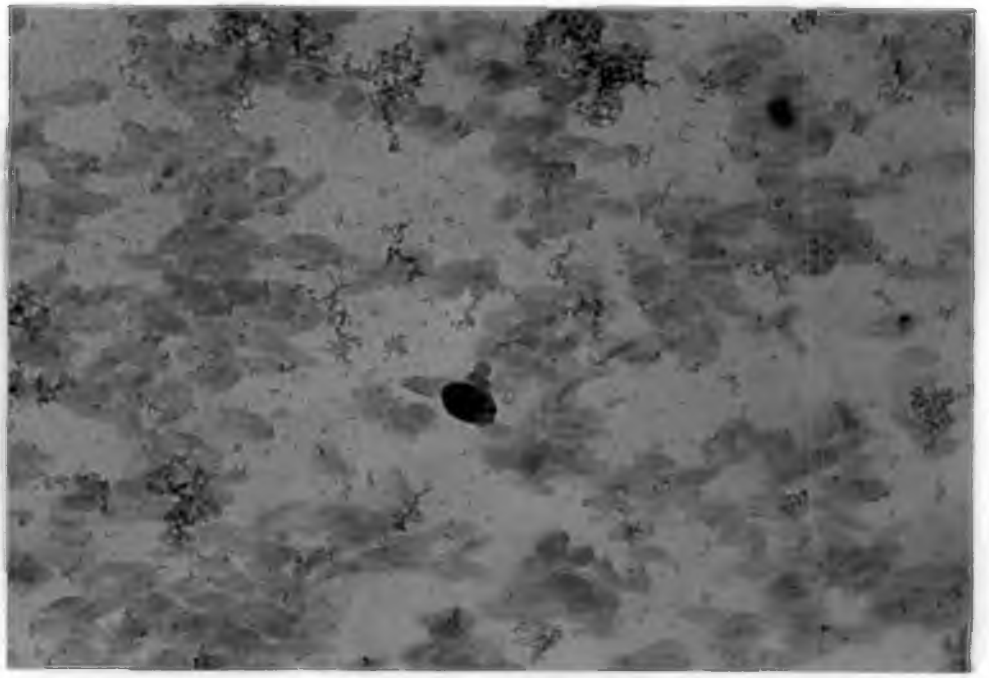
Species	Cyclophosphamide		Hydrocortisone		Cyclosporine A				Control			
	S/C	l/P	S/C	l/P	S/C	l/P	Tumour fragments	Tissue culture	S/C	l/P	Tumour fragments	Tissue culture
	cell suspension								cell suspension			
Rats	68.20± 0.44	66.10± 1.00	61.40± 1.75	63.80± 1.34	67.70± 1.45	65.80± 1.41	64.10± 1.42	65.90± 0.77	24.80± 1.23	24.90± 1.22	25.80± 1.16	24.70± 0.99
Mice	60.50± 0.56	61.80± 0.81	54.10± 0.75	54.40± 0.93	64.90± 0.61	63.30± 1.23	64.90± 1.15	34.60± 1.07	26.30± 0.73	25.30± 0.63	25.40± 0.58	28.40± 1.00
Rabbits	68.17± 1.14		64.17± 0.40		64.17± 1.08				37.50± 1.76			
Calves	46.00± 0.37		47.00± 0.68						35.17± 1.14			

did not reveal any variation from the normal. Basophil count in all the cases were zero.

Alphanaphthyl estease positive lymphocyte of calves, rabbits, rats and mice are shown in Fig.22, 23, 24 and 25. Among mice inoculated with trypsinised single cell suspension with different immunosuppressants those treated with cyclosporine A revealed a moderate decrease in T-lymphocyte percentage (Table 9 and Fig.26).

Fig.22 Blood smear-ANAE positive T lymphocytes of calves x 1000

Fig.23 Blood smear-ANAE positive T lymphocytes of rabbits x 1000



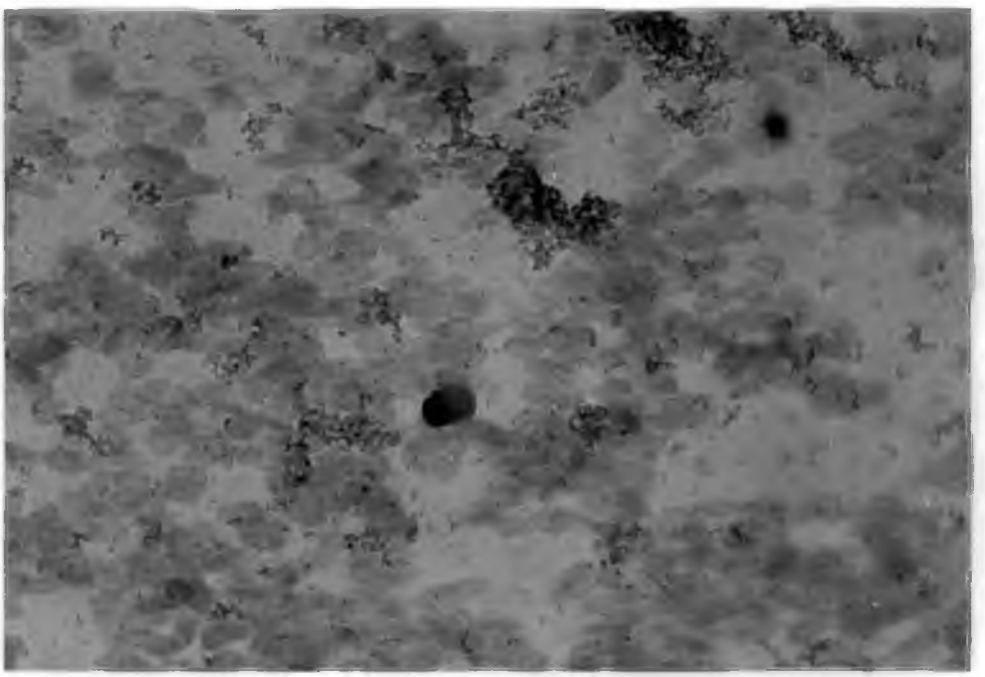
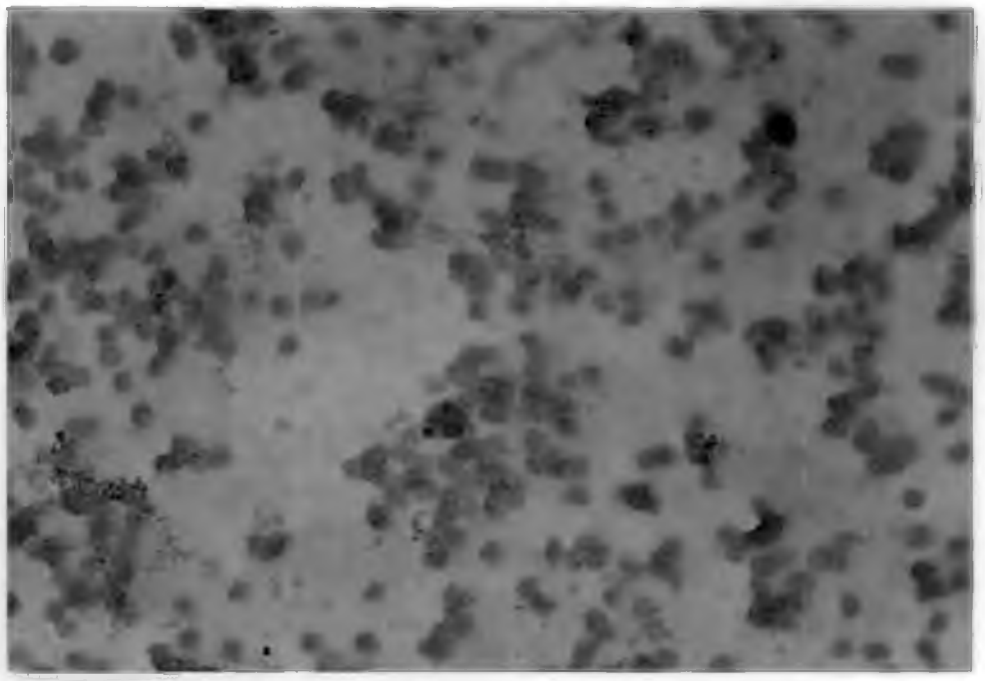


Fig.24 Blood smear-ANAE positive T lymphocytes of rats x 1000

Fig.25 Blood smear-ANAE positive T lymphocytes of mice x 1000



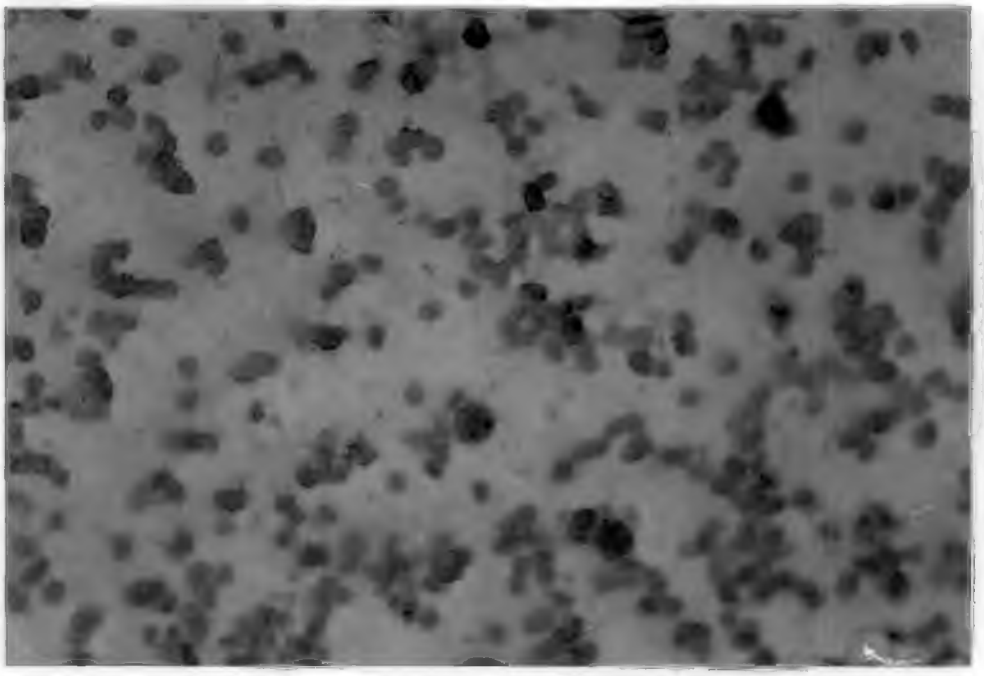


Table 9. T-lymphocyte percentage

Species	Cyclophosphamide		Hydrocortisone		Cyclosporine A				Control			
	S/C	l/P	S/C	l/P	S/C	l/P	Tumour fragments	Tissue culture	S/C	l/P	Tumour fragments	Tissue culture
Rats	40.90± 1.95	39.80± 1.07	39.90± 1.05	38.60± 1.12	19.60± 0.81	18.70± 0.76	18.90± 0.88	19.40± 0.92	15.80± 1.20	13.50± 0.98	12.10± 0.90	15.00± 0.76
Mice	39.80± 1.16	42.90± 1.05	40.90± 0.86	37.00± 1.29	18.40± 1.31	19.20± 1.08	15.20± 1.25	14.80± 1.02	17.90± 0.92	16.80± 1.20	15.20± 1.58	14.30± 0.96
Rabbits	40.17± 2.69				21.00± 0.90		21.33± 1.38		22.50± 0.92			
Calves	40.50± 1.50				21.80± 0.75		16.17± 0.83					

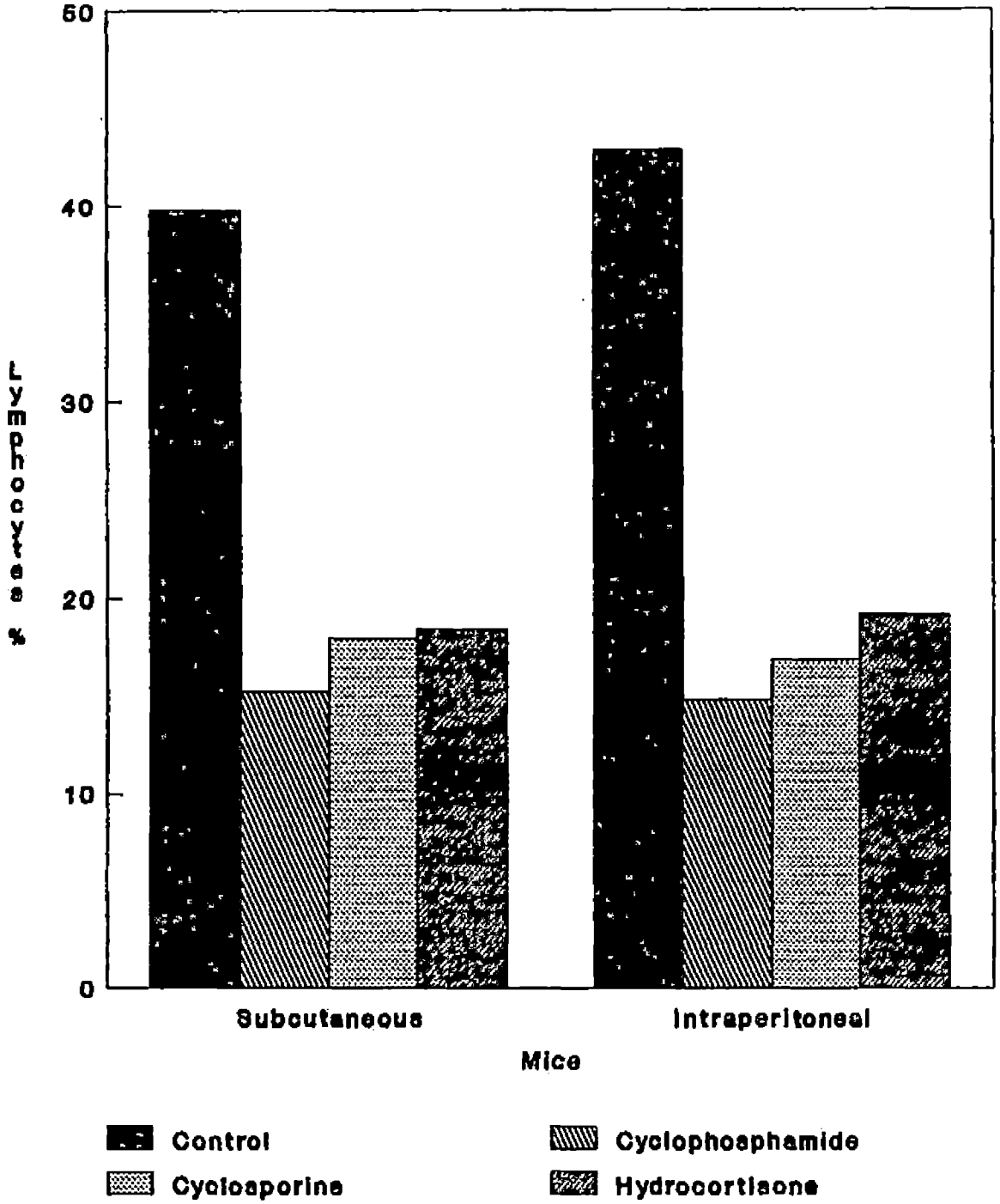


FIG.26 ANAE ACTIVITY OF LYMPHOCYTES OF IMMUNOSUPPRESSED MICE (%)

Discussion

DISCUSSION

The neoplasm arising from the mucosa of the ethmoid in cattle is an important disease problem in Kerala. The results of the interaction between the etiological agent and the neolastic cells might be reflected in the *in vivo* and *in vitro* behaviour of the transformed cells. In order to facilitate evaluation of such biological characters of the cells of ethmoid carcinoma, an attempt was made in this study, to transplant tumour cells and tissue fragments into immunosuppressed homologous and heterologous species of animals.

The tumour tissue for transplantation was obtained from seven affected cows. The clinical diagnosis was confirmed by detailed autopsy. Grossly the neoplastic growth was located in the ethmoid region in firm attachment with the bone. Extension of the tumour mass from the ethmoid region to the paranasal sinus was also seen. Similar gross features of the tumour were observed by (Stenstrom, 1915; Nair and Sastry, 1954; Rajan *et al*. 1972; Nair, 1973; Damodaran *et al*. 1974; Balasubramanian, 1975; Rajan, 1980; Sreekumaran and Rajan, 1983 and Gangadharan, 1992). The tumours encountered were histologically classified as adenocarcinoma, papillary

adenocarcinoma, undifferentiated carcinoma and squamous cell carcinoma.

The common histological type of the tumour was adenocarcinoma. In this the acini were lined by layers of columnar or cuboidal epithelium. Papillary projections lined by columnar or cuboidal epithelium were seen in the papillary adenocarcinoma. Transition from columnar to squamous cells were observed in squamous cell carcinomas. Undifferentiated carcinomas consisted of sheets of proliferating epithelial cells. Similar observations were made by Stenstrom, 1915; Nair and Sastry, 1954; Rajan et al., 1972; Nair, 1973; Balasubramanian, 1975; Rajan, 1980; Sreekumaran and Rajan, 1983; Chakrabarti et al. 1988; Muralimanoharan, 1988 and Gangadharan, 1992.

Therefore, the tumours studied were confirmed grossly and histologically to a primary cancer arising from the mucosa of the ethmoid and their malignant characteristics were established.

Unsuccessful attempts of in vitro cultures of bovine ethmoid carcinoma cells were recorded by many workers (Jayaraman et al. 1979; Sulochana, 1980; Pospischil et al. 1982 and Karki and Rajan, 1986). While analysing the reason for the failure of in vitro studies in which HBSS with 0.5 per

cent lactalbumen hydrolysate and 0.5 per cent yeast extract with antibiotics were used as the growth media, Sulochana (1980) suggested that the deficiency of certain unknown factors required by the tumour cells for growth or possibly the presence of some infectious agent in the tumour cells would be responsible for the lack of cell growth.

In the present study trypsinised single cell suspensions obtained from adenocarcinoma, papillary adenocarcinoma and squamous cell carcinoma were successfully grown in vitro. Active proliferation and monolayer formation of the cells were observed in the culture. Monolayer became confluent within 12 days and first passage could be carried out successfully.

Successful in vitro culture with trypsinised single cell suspension was also achieved by Gangadharan (1992) and Chaudhary (1994). The latter worker employed RPMI and Ham F12 nutrient mixture, insulin, hydrocortisone, 10% foetal calf serum with antibiotics as the culture media for the successful propagation. In the present study it was found that hydrocortisone suggested by Chaudhary (1994) was not essential for the cells to grow in cell culture. The cells in the culture were well spread with epithelial type of morphology. The presence of large number of immature cells with greenish cytoplasm and reddish nucleus seen in the smears made from the

cell suspension taken from the first passage and stained by Papanicolaou's method confirmed their neoplastic character.

Attempts made in the present study for the culture of neoplastic cells from adenocarcinoma, papillary adenocarcinoma and squamous cell carcinoma by explant method were successful. This is in contradiction to the finding of Gangadharan (1992) who failed to obtain explant culture from the tumour, due to heavy bacterial and fungal contamination which could not be controlled even after the addition of high doses of antibiotics and antifungal agents. This possibility was excluded in the present experiment by employing strict aseptic condition.

Chaudhary (1994) could successfully propagate bovine ethmoid tumour cell by explant method employing a medium containing RPMI, HAM F12 nutrient mixture, insulin, hydrocortisone, 10% foetal calf serum and antibiotics. In the present study hydrocortisone was not used as a constituent of the media but still explant cultures could be attained.

A number of workers although made attempts to transplant nasopharyngeal carcinoma of human origin (Heale and Heale, 1985 and Ebbers et al. 1986) and ethmoid carcinoma of bovine origin (Duncan et al. 1967; Rajan et al. 1977; Nair,

1973; Jayaraman *et al*. 1979; Sulochana, 1980; Pospischil *et al*. 1982; Karki and Rajan, 1986 and Chaudhary, 1994) did not succeed in their attempts.

In this investigation on transplantation of bovine ethmoid carcinoma cells, successful transplantation of tissue cells was achieved for the first time in mice. Trypsinised single cell suspension from adenocarcinoma was transplanted subcutaneously in ten mice immunosuppressed with cyclosporine A, out of which in eight cases the tumour growth was evident. Further, the transplantation was successfully repeated in a second attempt using adenocarcinoma cells from a different donor under the same conditions establishing the transplantability of the tumour.

Jayaraman *et al*. (1979) suggested that the failure to transplant bovine ethmoid tumour cells may perhaps be due to the etiological agent not being present in these cells, or remaining in an incomplete form which requires certain exacting condition for maturation and replication. Whereas Karki and Rajan (1986) attributed degraded condition of the tumour tissue and the absence of certain unknown factors required for the growth of the neoplastic cells in the recipient along with the role of infectious agent to the failure of tumour growth *in vivo*.

In addition to the factors described by Jayaraman et al. (1979) and Karki and Rajan (1986) there may be various other factors also like type of host, age of host, immune status of the recipient, type of tissue preparation, type of tumour, inoculation rate, viability of the cells and route of inoculation which would determine the transplantability of tumour.

In the present study the experimental lot consisted of young animals which were naturally at a lower immunological competency. Further, they were immunosuppressed with cyclosporine A, cyclophosphamide or hydrocortisone. Of these immunosuppressants cyclosporine A was found to be more effective in mice than the others as evidenced by the haematological data. The observation that the tumour could be successfully transplanted only in those mice treated with cyclosporine A highlights the effectiveness of this drug in immuno-suppression. This would support the assumption that immunosuppression is a prerequisite for the development of neoplasms. Lowering of the immunological barrier of the host therefore appers to be an important event in establishing neoplastic growth.

Although, different types of tissue preparations were used for the transplantation, growth was established only with trypsinised single cell suspension. The inability of tissue

fragments to grow at the site might be due to some endogenous factors associated with the growth as suggested by Karki and Rajan (1986) or due to a high proportion of other components like fibrous tissue than viable tumour as observed by Yerger et al. (1985) in transplantation studies.

Shin et al. (1975a) demonstrated that by inoculation of in vitro selected anchorage-independent clones of cells from originally non-tumourogenic cell population which form colonies in methylcellulose would produce highly tumourogenic derivatives in nude mice. In the present study the passage of cells in vitro might have eliminated the anchorage - independent cells and selected only anchorage - dependent cells, which may be the reason for the failure to transplant.

From the point of view of differentiation it has been suggested that an adenocarcinoma is a less differentiated neoplasm than a squamous cell carcinoma. In the present study transplantation could only be attained with trypsinised single cell suspension of adenocarcinoma but not with squamous cell carcinoma in the same experimental condition. This observation supports the view that transplantability of neoplastic cells is inversely proportional to its differentiation.

The difference in transplantability could either be due to the difference in the trypsin-cell interaction or due

to the absence of certain factors present in the original host but lacking in the recipient.

The relation of rate of inoculation to the transplantability of tumour cells has been established by Price et al. (1990) with experiments using human breast carcinoma cell lines in nude mice. They observed that at high doses of 10^6 cells and 5×10^6 cells tumour developed in all the experimental mice whereas the lower dose of 10^5 cells produced tumour in 2 of 5 mice only. The results of the present study is in agreement with their observation that a dose rate of 10^6 cells were able to produce tumour growth in 8 out of 10 mice inoculated with trypsinised ethmoid tumour cell suspension and in all the control mice inoculated with Ehrlich's ascites tumour cells.

In the present study, the conditions provided to the recipients were ideal as tumour growth was obtained by intraperitoneal inoculation of Ehrlich's ascites tumour cells in mice and by subcutaneous inoculation of ethmoid tumour cells in mice.

Watanabe et al. (1980) suggested that route of transplantation influences the acceptance rate. They suggested that subcutaneous route was more effective than I/P or I/V routes of inoculation probably due to better blood

supply and presence of connective tissue framework for fixation and proliferation of the inoculum. Although S/C and I/P routes were attempted in the present study, tumour transplantation was successful only by the former route. These findings are in agreement with the observation of Watanabe et al. (1980). The tumour growth was found embedded in the subcutaneous fat which might have protected the cells from the host immune system and promoted the maintenance and growth of the transplant.

Shin et al. (1975) opined that human tumour cells infected in nude mice depend for their growth on their ability to elicit a vascular bed from the host. The presence of angiogenic factor(s) is necessary for the progressive growth of tumour cells.

It has also been reported that some tumours fail to grow when inoculated subcutaneously (Al-Yamen and Wallenborg, 1984) probably due to factors such as lack of proper vascularisation or to the lack of essential factors required for tumour growth, which is present in the original host but not in the recipient mice. The tumour transplant obtained in the present study revealed numerous capillaries indicating a moderate degree of vascularisation which might have facilitated in the proliferation and growth of the tumour cells.

The attempts for intraperitoneal transplantation of bovine ethmoid carcinoma were not successful possibly due to the lack of congenial environment for the growth and differentiation of the tumour cells in the peritoneal cavity. The failure of lymphoma cells to grow intraperitoneally in nude mice was explained by Watanabe et al. (1980) to be due to the activation of the IP phagocytic system. The immunosuppressant cyclosporine A by inhibiting only the T cell system has presented a similar condition in the present study. The peritoneal macrophages might have been instrumental in destroying the transplanted cell.

Yerger et al. (1985) opined that the peritoneal environment provided certain factors which promoted the differentiation and growth of certain components of transplanted tumours as in the case of the tubular component of Wilm's tumour. The failure of intraperitoneal inoculation of ethmoid tumour to grow suggested that such promoting factors were not present in the peritoneal cavity of the recipients.

The attempts for heterologous transplantation of bovine ethmoid carcinoma in rats and rabbits did not succeed. Whereas the transplant was established in mice. The failure of the transplantation attempts may be attributed to the impaired adaptation of the metabolic system of tumour cells to

the altered nutritional environment. Lack of proper vascularisation has been suggested to be one of the major cause for failure of transplantation attempt by Al-Yamen and Willenborg (1984).

Histopthological examination of the site of inoculation of experimental rats and rabbits did not show any evidence of neo-vascularization. At the same time numerous capillaries were observed in the subcutaneous tissue of mice in which the transplantation was successful. The probable reason for this difference in the vascular reaction requires further investigation.

The immune system of the host might not have interfered with the growth of the inoculum since the animals were immunosuppressed with various immunosuppresant drugs. This was in agreement with the suggestion of Fogh and Giovanella (1978) that by applying specific immunosuppressants to temporarily immunoincompetent new born animals would prolong and potentiate the immunodepression. The lack of inflammatory reactions as well as lymphoid aggregation at the site of inoculation, the low total leucocyte count and lymphocyte percentage and the extensive depletion of lymphocytes in periarteriolar region of the spleen of immunosuppressed animals supports the suggestion of suppressed immune system.

The lack of tumour growth in control animals is due to the destruction of the neoplastic cells by the host immune cells as evidenced microscopically by infiltration of inflammatory cells at the site.

Calves were used as homologous hosts for the experimental transplantation of ethmoid carcinoma. Subcutaneous inoculation of the tumour tissue did not result in establishing a growth at the site. The haematological and histopathological data of the animals also demonstrated that the immune system did not influence the elimination of the tumour transplant.

The increase in skin thickness noticed after a period of two weeks in all the animals in which the tumour failed to grow is probably due to the growth of the animal. This was more evident in control animal than in the experimental group. The increase in skin thickness noticed in control animals in the first week might be due to inflammatory reaction.

Ethmoid carcinoma has been reported to be a tumour affecting adult cattle of 6-8 years of age (Sreekumaran and Rajan, 1983). The incidence of the tumour was remarkably high in pregnant animals (Sreekumaran and Rajan, 1983). These observations suggest the probable role of hormones in the promotion of the tumour growth. Cumulative effect of metabolic products of various toxic materials also should be considered. It could be concluded that the absence of the

various factors mentioned might have prevented the growth of the tumour in calves.

The growth obtained in mice was very small. This may be due to the high latent period and low doubling time of the tumour cells. The tumour had a very thin layer of fibrous tissue capsule as the host defence was well compromised. Price et al. (1990) observed reduced fibrous capsule when tumour cells were given into orthotopic site rather than ectopic site. Hence it may be possible to reduce the fibrous encapsulation when inoculated in the respiratory epithelium of immunosuppressed animals.

Many of the human tumours proliferating subcutaneously in nude mice rarely showed metastasis to other organs (Giovanella and Fogh, 1985). Price et al. (1990) suggested that metastasis depends on health and housing and route of tumour cell inoculation in addition to intrinsic properties. They opined that the chance for metastasis could be increased by injecting the cells into anatomically approximate or orthotopic sites rather than ectopic sites.

The successful repeatable transplantation of bovine ethmoid carcinoma cells, subcutaneously into mice has established the transplantability of those cells and can be utilized as an experimental model for the detailed study of the biology and chemosensitivity of these cells.

Summary

SUMMARY

Bovine ethmoid tumour cells were transplanted in calves, rats, mice and rabbits to assess the xenograftic efficiency of the neoplastic cells.

1. Calves were used as the homologous host. For immunosuppression one group was treated with cyclophosphamide and another with hydrocortisone. A separate control group was maintained without immunosuppression. Trypsinised single cell suspension of the tumour was inoculated subcutaneously and the calves were observed for 4 months.

2. Rats, mice and rabbits formed the heterologous hosts. Rats were immunosuppressed with cyclophosphamide, hydrocortisone and cyclosporine A. In each group, subgroups were inoculated with trypsinised single cell suspension subcutaneously and intraperitoneally. In the group immunosuppressed with cyclosporine A two separate subgroups inoculated subcutaneously with tumour tissue fragments and cell suspension from tissue culture were maintained. Separate control groups were maintained for each route and type of inoculum and all animals were observed for 30 days.

Mice in separate groups were immunosuppressed with cyclosphosphamide, hydrocortisone and cyclosporine A. In each group, subgroups were inoculated with trypsinised single cell suspension subcutaneously and intraperitoneally. In the group immunosuppressed with cyclosporine A two separate subgroups inoculated subcutaneously with tumour tissue fragments and cell suspension from tumour tissue culture were maintained. In addition to separate control group for each route and type of inoculum, mice inoculated with Ehrilich's ascites tumour cells were also maintained. All the animals were observed for 30 days.

Cyclophosphamide, hydrocortisone and cyclosporine A were used separately as immunosuppressants in rabbits. A control group without immunosuppression was maintained. All the animals were inoculated subcutaneously with trypsinised single cell suspension and were observed for 30 days.

3. The ethmoid cancer cell were obtained from seven clinically affected cows. Histopathologically the tumours were identified as adenocarcinoma, papillary adenocarcinoma, undifferentiated carcinoma and squamous cell carcinoma.

4. Tumour cell/tissue culture employing trypsinised single cell suspension/tumour tissue fragments were carried out. After the monolayer formation the cells were harvested and subcultured. The cells from the second passage were made into single cell suspension containing $1x10^6$ viable cells per 0.25 ml and was inoculated subcutaneously in rats and mice. There was no evidence of tumour growth either grossly or microscopically at the end of the observation period.

5. Trypsinised single cell suspension of tumour was inoculated in homologous and heterologous hosts. The transplantation was successful in mice as evidenced by the development of a small palpable pin head sized fluctuating firm nodule in 8 out of 10 mice, treated with cyclosporine A. There was progressive growth of the tumour and by the end of the 30th day it could easily be palpated. These was no evidence of tumour growth in calves, rats and rabbits.

6. Histopathologically, the nodule observed in mice were identified as clumps of proliferating tumour cells embedded in the subcutaneous fat. The nodule was well vascularised with characteristic acini formation. Pleomorphic, multinucleated cells showing various stages

of mitosis were also observed. In other animals inoculated subcutaneously or intraperitoneally there was no evidence of neoplastic cells or inflammatory cells, at the site.

7. In animals inoculated with tumour tissue fragments the initial swelling of the fragment subsided gradually and was not palpable after two weeks. There was no evidence of tumour growth grossly or microscopically even after 30th day.

8. In control groups there was oedeomatous swelling at the site of inoculation for four days which later regressed gradually. Ascites was observed by 10-15 days in all mice inoculated with Ehrlich's ascites tumour cells.

9. The interference of hosts immunological mechanisms with the transplantability of tumour was effectively minimised in all the animals using different immunosuppressants, as evidenced by the very low level of total leucocyte count, percentage of lymphocytes and alphanaphthyl esterase positive T lymphocytes. Thus the involvement of extra immunological factors in the growth of transplanted tumour cells was suggested.

10. The results of the present study indicated that neoplastic cells of bovine ethmoid carcinoma could not be transplanted successfully in immunosuppressed calves, rats and rabbits.

11. In mice immunosuppressed with cyclosporine A and inoculated subcutaneously with the neoplastic cells tumour growth was evident. Therefore it was concluded that mice could be used as an experimental model for studies on bovine ethmoid carcinoma.

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ABSTRACT

Homologous and heterologous transplantation of bovine ethmoid carcinoma cells were attempted to assess the xenograftic efficiency of the neoplasm.

Tumour tissue for the study was obtained from seven clinically affected cows. Histopathologically the tumours were classified as adenocarcinoma, papillary adenocarcinoma, undifferentiated carcinoma and squamous cell carcinoma. Tumour tissue/cell culture was successfully carried out using trypsinised single cell suspension and tumour tissue fragments.

Calves were used as homologous hosts and rats, mice and rabbits were used as heterologous hosts. Cyclophosphamide, hydrocortisone and cyclosporine A were used as immunosuppressants. Transplantation was carried out subcutaneously or intraperitoneally.

The inoculum consisted of trypsinised single cell suspension, tumour tissue fragments and cell suspension from tissue culture. Neoplastic cells failed to grow in immunosuppressed calves, rats and rabbits. Whereas, the tumour cells obtained by trypsinisation were successfully transplanted in mice immunosuppressed with cyclosporine A.

The tumour growth was evident from the second week onwards. In immunosuppressed animals the total leucocyte count, differential leuocyte count and alphanapthyl acetate esterase positive lymphocyte count were very low compared to their controls.

The results of the present study indicated that mice could be used as an experimental model for the studies on bovine ethmoid carcinoma.