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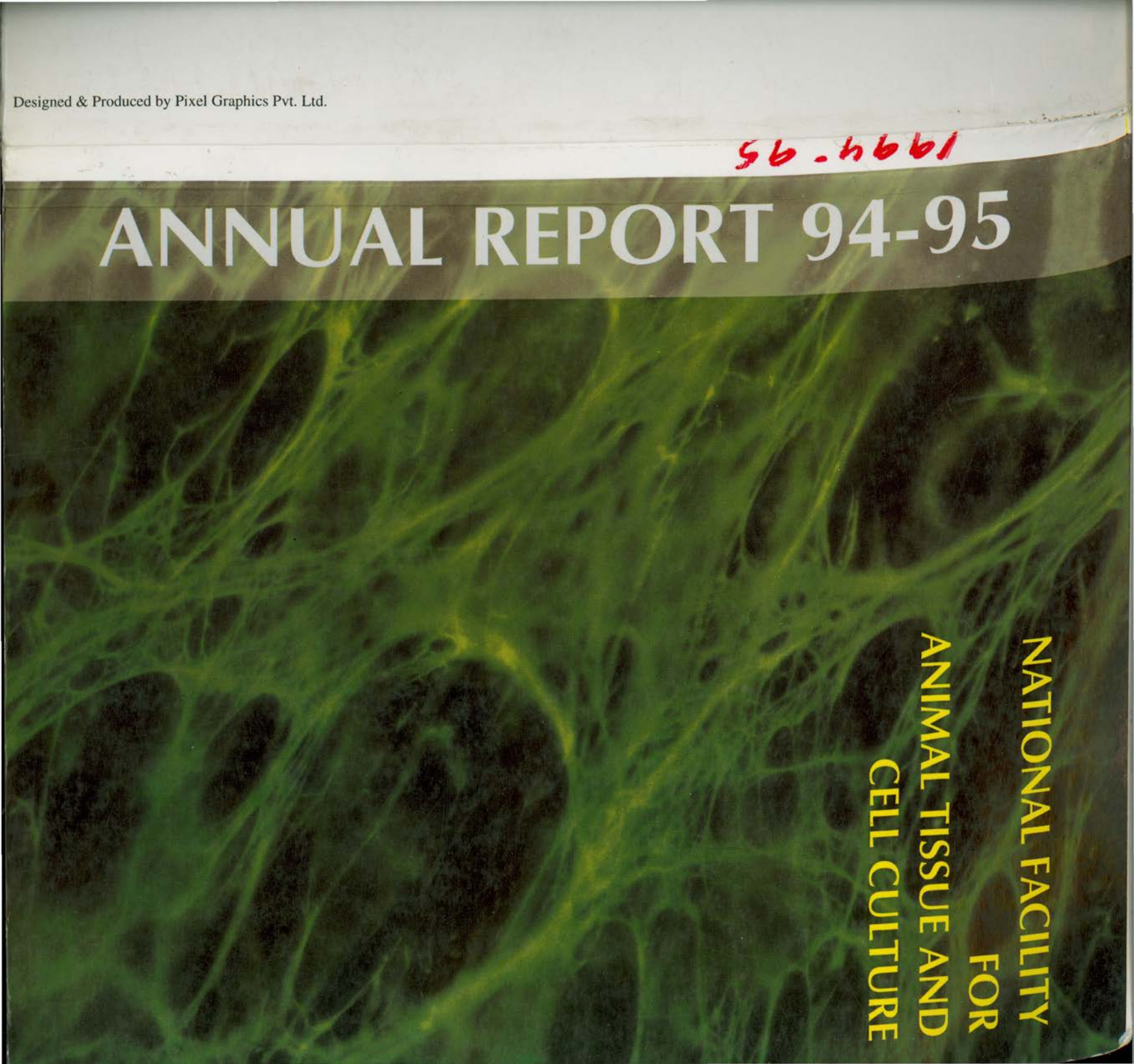

NATIONAL FACILITY FOR ANIMAL TISSUE AND CELL CULTURE

NATIONAL FACILITY FOR ANIMAL TISSUE AND CELL CULTURE

DEPARTMENT OF BIOTECHNOLOGY
GOVERNMENT OF INDIA

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CONTENTS

f) Studies on factors affecting growth of skin fibroblasts *in vitro*.

g) *In vitro* cultivation of erythrocytic stages of *Plasmodium vivax* and large scale cultivation of *Plasmodium falciparum*.

DEVELOPMENT OF TISSUE BANK TECHNOLOGY 11

a) Culture of human skin keratinocytes and their 3-D epitheliation for transplantation to burns, non-healing ulcers and vitiligo cases.

b) Studies on cryopreservation and revival of normal human bone marrow.

c) Cryopreservation and revival of heart valves for transplantation.

d) Endothelialization of homograft valves.

INDIGENOUS TECHNOLOGY AND RENDERING EXPERT SERVICES 17

a) Adaptation of myeloma and hybridoma cells to goat serum.

b) Identification and characterization of protective antigens in lymphatic filariasis.

c) Mechanism of action of thiophene derived drugs for evaluation of their possible use in chemotherapy.

d) Effect of copper derivatized taxol analogues on breast cancer cell line (MCF-7).

BIOMEDICAL AND BIOTECHNOLOGICAL APPLICATIONS.

a) Immortalization of human bone marrow derived stromal cells and stem cells via transfection of cloned oncogenes.

b) Effect of growth factors on stromal cells.

c) *In vitro* studies on the role of growth factors on melanosome transfer from melanocytes to keratinocytes in epidermis.

d) Identification of melanoma oncogene(s) and characterization of the oncogene protein product.

e) Organization, expression and regulation of mammalian hexokinase.

f) Use of mitochondrial DNA restriction fragment length polymorphism for characterization of mosquito cell lines.

g) Endocytic vesicles and their association with cytoskeleton.

DEVELOPMENT OF CULTURES

a) Cultivation of bone osteoblasts and their characterization in terms of osteoblastic features.

b) Characterization of cultured human oropharyngeal mucosal epithelial cells.

c) Establishment and characterization of breast cancer cell lines from high risk ethinic groups

d) Growth characteristics of normal and abnormal melanocytes and their response to synthetic and natural growth factors.

e) Cell models for investigating the molecular mechanisms associated with etiology and pathophysiology for mental disorders.

f) Studies on factors affecting growth of skin fibroblasts *in vitro*.

g) *In vitro* cultivation of aerythrocytic stages of *Plasmodium vivax* and large scale cultivation of *Plasmodium falciparum*.

TERMS OF REFERENCE

* To receive, identify, maintain, store, grow and supply:
    - Animal and human cells / cell cultures, cell lines of both existing (typed) and newly developed, hybrid cells including hybridomas
    - Tissues, organs, eggs (including fertilized), and embryos
    - Unicellular obligate pathogens, parasites and vectors, plasmids, genes and genomic libraries
* Develop, prepare, quality control and supply culture media, other reagents and materials, and cell products independently and in collaboration with industry and other organizations.
* Research & Development in the above and cell culture related materials and products
* To establish and conduct post-graduate courses, workshops, seminars, symposia and training programmes in the related fields
* To organize training programmes for technical personnel in tissue culture technology, tissue banking, cell products and related areas.
* To serve as a National Reference Centre for tissue culture, tissue banking, cell products and data bank etc. and to provide consultancy services to medical, veterinary, pharmaceutical institutions, public health services and industries etc. in the country.
* To provide and promote effective linkages on a continuous basis between various scientific and research agencies / laboratories and other organizations including industries working in the country.
* To collaborate with foreign research institutions and laboratories and other international organizations in the areas relevant to the objectives of the facility.
* To participate in such programmes as required in the country for the betterment of society and advancement in science and technology.

PREFACE

The civil works of the laboratories and infrastructure on the University of Poona campus is completed. The laboratories are being furnished and will be made functional soon.

As a National Facility the stock Cell Culture repository continued to preserve 1165 culture strains. During the year 1994-95, a total of 779 cultures comprising 189 different cell lines were supplied to more than 100 researchers, public health laboratories, academic institutions spread over 48 different geographical locations in the country.

The technology for development of cultures was standardised for fastidious cells - osteoblasts, oropharyngeal mucosal epithelia, breast tumours, keratinocytes, melanocytes, skin fibroblast, endothelial, stromal & foetal liver, beta cells and islets of Langerhans.

Experimental studies planned and executed as collaborative programmes with different organisations revealed interesting results.

Primary cultures of human foreskin fibroblast were found to be the most suitable cell system to serve as a feeder layer for the growth of human skin epithelia for grafting. This is a step forward towards application of this technology as it ensures dependable and consistent results.

The cultures developed from skin of schizophrenic & normal siblings were positive for vimentin expression - specific for fibroblast cells. There was a significant difference in the fibroblast growth pattern, morphology, rate of attachment and senescence between the patient and the normal groups. Storage of these skin fibroblast cultures is an important addition to the Cell Repository.

Studies on the communication of melanocytes and keratinocytes related to transfer of melanosomes revealed that after accepting one dendrite from melanocyte, a keratinocyte does not accept another dendrite from the same melanocyte. The same keratinocyte however is capable of accepting dendrites from the other melanocytes indicating a sort of communication between the melanocyte and keratinocytes, regulating the formation and penetration of dendrites. Further it was observed that

isolated melanocytes in culture expressed two prominent methonine-labelled proteins of M.W. 72 and 24 kDa.

A new approach towards long term performance of heart valves used for grafting by restoring viable endothelial cells has been contemplated. Efforts towards collection and "in vitro" maintenance of endothelial cells from umbilical cord vein have been successful. Further work on defining the conditions for cell proliferation and cell attachment to heart valves is in progress.

Standardization of technology for cryopreservation and revival of bone marrow were continued. Experiments were conducted to examine the protective effect of various additives to freezing mixtures. Improved recovery were observed with alphatocopheryl acetate, catalase and ascorbic acid.

Studies on factors which trigger the interaction between cells of haemopoietic system have lead to the identification of soluble factors of TGF-FGF family and membrane bound receptors such as fibronectin receptor.

After achieving success in standardization of method for isolation and maintenance of islets in culture, studies were undertaken to cryopreserve these islets. The data suggests that micro-encapsulation of islets gives significant protection during cryopreservation retaining their morphological integrity and viability. Addition of glucose to cryopreservation medium not only improved islet viability but also protected the islets from streptozotocin-induced damage.

On successful adaptation of hybridoma CC9C10 (antiinsulin antibody) to goat serum supplemented with soya bean lipid, one more commonly used hybridoma OKT3 has been adapted to goat serum.

A differential diagnosis based on the presence of specific IgG4 antibodies to Setaria digitata antigen is now possible in lymphatic filariasis.

Earlier studies demonstrated the antiproliferative effect of supernatents from stimulated mouse/human lymphocytes on neuroblastoma cell lines.

Partial characterization have demonstrated these factor/s to be thermostable and were not toxic to the cell lines.

Cultures of human feotal pharyngeal epithelial cells and buccal epithelial cells were studied for their interaction with streptococcal adhesin - lipoteichoic acid (LTA). The results demostrated that LTA is not cytotoxic to these cells and that it induces the production of interleukins - IL-4 & IL-6.

Ayurvedic medicine is known to be effective in the rheumatoid disorders. The effect of an Ayurvedic formulation was studied in rheumatoid arthritis (RA) with respect to level of serum hyaluronic acid (HA)- a carbohydrate polymer. It correlates with the degree of inflammation and the immunologic status. Patients with elevated serum HA levels responded by lowering these to near normal range. However, patients with normal serum HA levels showed no alteration in the HA levels. These results suggest the anti-inflammatory action of the treatment. Additional studies on skin fibroblast culture revealed that it is an excellent system to study the extracellular matrix involvement in RA patients.

Studies to understand the events involved in melanocyte transformation using the mouse melanoma cell lines as an experimental model were continued. An expression cDNA library was prepared from mRNA of NIH 3T3 transfected cells and melanoma clone M3 cells. Initially only the M3 cDNA library is being screened for oncogenes. Of the various clones analysed two clones have produced tumors in nude mice.

In vitro studies on screening drugs and compounds has been continued.

Six derivatives sulphones, esters and sulphoxides of thiodipropionic acid (TPDA) were analysed for their anticancer properties using Sarcoma 180, HT1080, MCF-7 and KB Chr8-5 cell lines. Only thiournium and dicetol compounds inhibited cell proliferation in these cell lines.

The cytotoxicity of a copper chelate of taxol analogues designated Cu-Tx was compared with 10 -DAB on a breast cancer cell line MCF-7. The mechanism of action and the effective dose of these two compounds

were found to be different as revealed from data by MTT assay, tritiated thymidine uptake and microtubule studies.

A group of 12 Tyrosinase seeking compounds were screened for their antimelanoma effect using melanotic B-16 melanoma cells and non-melanotic fibroblast in culture . Preliminary studies indicated that two of these compounds have the potential as anti-melanoma compounds.

Molecular biological studies have been undertaken to ascertain the regulatory mechanisms of tissue and cell type specific expression of the 4 isoenzymes of hexokinase, a rate limiting enzyme in the glycolytic pathway. In situ hybridization and Southern blott experiments have indicated the presence of different genes encoding these isoenzymes. Furthermore, reverse transcriptase-PCR and Northern blot assays have revealed over-expression of type II-like isoenzyme in many tumour cell lines.

The faculty members of the NFATCC continued to participate in the teaching programmes in Microbiology, Biotechnology and Zoology in the University of Poona. Training in basic and specialised tissue & cell cultures was imparted to 13 individuals from different institutions and organizations.

NFATCC is looking forward to expand its activities on shifting to the new premises on the campus of the University of Poona - with the continued support and from work enjoyed so far from the collaborators and well wishers.

CULTURE REPOSITORY

a) Nuclear Stock stored in liquid nitrogen

b) Stock for quality control and redistribution

c) Supply sservices

d) Obligate parasites

e) Vectors, plasmids and genomic libraries

f) Media

g) HIV screening

CULTURE REPOSITORY

a) Nuclear stock stored in liquid nitrogen

A total of 1165 cultures comprising 632 different cell strains are in stock at NFATCC.

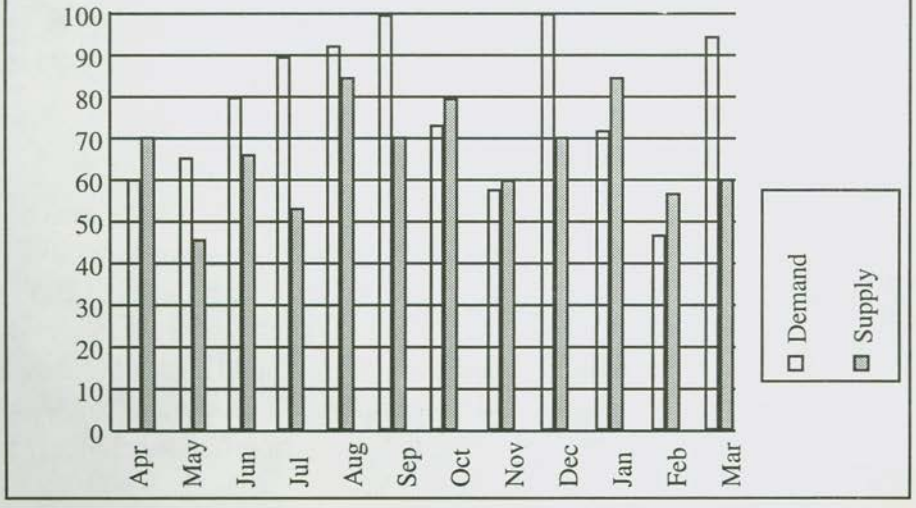
b) Stock for quality control and redistribution

A total of 189 cell lines were expanded for redistribution and quality control.

c) Supply services

Between April 1994 and March 1995, 779 cultures comprising of 189 different cell lines were supplied to 100 research institutes located in 48 cities (Figure 1). Two hundred ninety-one scientists have registered for availing of cell supply facilities. The various institutes availing NFATCC's cell supply services is listed separately.

Figure 1 : Cell cultures supplied to different centres in the country.



d) Obligate parasites

The cultures from obligate parasites repository were periodically revived and maintained.

e) Vectors, plasmids and genomic libraries

A total of 58 vectors, plasmids, DNA probes and genomic libraries are stocked in the repository. Three of the DNA probes were revived and checked for the presence of plasmids and were found to be satisfactory and were re-stored, both, as glycerol cultures and as isolated plasmid DNA.

f) Media

Twenty-six different synthetic media, balanced salt solutions and tissue culture reagents were prepared at NFATCC. The preparation of goat serum from goat blood procured from local abattoirs was continued. Quality control, cell proliferation assays, sterility, etc. were tested for foetal calf serum (FCS), horse serum (HS), goat serum (GS) and new born calf serum (NBCS) and supplied to the staff members.

g) HIV screening

Routine screening for HIV in serum of all human donors whose tissues / cells are procured for experimental work at NFATCC is done by the Immunocomb kit that detects both HIV-I and HIV-II antibodies. Only those tissues of donors are taken up for further study whose serum has been found negative for HIV-I and HIV-II. Wherever necessary, proper ethical clearance is obtained. In all clinical studies informed consent is obtained from each patient by appropriate clinical / hospital authorities.

DEVELOPMENT OF CULTURES

a) Cultivation of bone osteoblasts and their characterization in terms of osteoblastic features.

Bone cells cultured *in vitro* is an excellent tool for investigating the physiological processes of bone formation. Hence, establishment of primary cultures of osteoblasts, their characterization and studies on the factors governing bone formation were undertaken.

Osteoblasts were isolated and primary cultures were prepared using the protocols previously standardized for human and animal bones. Primary cultures of osteoblasts could be established from bones of 12 out of 14 fetuses (MTP cases) received from Sassoon General Hospitals, Pune. Some of these cultures were subcultured through 3 to 7 passages. Four cultures were cryopreserved at different passage levels.

Alkaline phosphatase activity, which is the first detectable histochemical marker of osteoblast phenotype, was found to increase with advancing passages. *In vitro* mineralization could be induced in some of the cultures by ascorbic acid and beta glycerophosphate.

Attempts to induce bone like structures using synthetic and natural biomaterials, such as porous gelatin matrix and sea corals, and detection of bone osteogenic proteins are under way.

b) Characterization of cultured human oropharyngeal mucosal epithelial cells.

Adhesion and colonization of bacteria to human mucosal epithelial

cells is the primary step in the initiation of various diseases of the upper respiratory tract. Earlier studies have shown the association between certain oral bacteria, like streptococci,human oropharynx and the development of various autoimmune complications. Efforts to establish an *in vitro* model of cultured human oral epithelial cells to investigate molecular events in the pathogenesis of such diseases were continued.

Cultures of Pharyngeal epithelial cells (PEC) and buccal epithelial cells (BEC) were prepared from human foetuses obtained from the Sassoon General Hospitals, Pune. Immunocytochemical characteristics of these cells were described previously (see AR 93-94). These cells were further studied by scanning electron microscopy (SEM). SEM micrographs revealed the presence of numerous microvilli and microridges on the cell surface (Figure 2).

It has been shown that the bacterial adhesin, lipoteichoic acid (LTA) promotes attachment and colonization of the streptococcal bacteria to oral epithelial cells. The cultured epithelial cells were, therefore, used to study their interaction with LTA. LDH release and MTT assay performed on the treated cells indicated no cytotoxic effect of LTA. It was further observed that LTA has a dose-dependent proliferative effect on these cells.LTA treatment was found to induce synthesis of IL-4 and IL-6 as detected by indirect immunofluorescence microscopy . These results suggested that the streptococcal adhesin LTA was not cytotoxic to pharyngeal and buccal epithelial cells and that it induced the production of interleukins.

c) Establishment and characterization of breast cancer cell lines from high risk ethnic groups.

Cancer of the breast is one of the commonest malignancy in woemn. In India, the Parsi community has a higher incidernce of breast cancer. Since carcinogenesis is a multi-step process, it is important to establish breast epithelial cell lines from such high-risk groups.

Human breast epithelial cells, both normal and malignant, are difficult to cultivate *in vitro* over extended periods of time. Experimental work was continued to set primary cultures of breast epithelial cells. Breast biopsy specimens were obtained from 15 Parsi patients from Breach Candy Hospital, Bombay, and 6 non-Parsi patients from Jehangir Nursing Home, Pune. For setting the primary cultures, the protocols which have been standardized earlier were followed. Primary cultures, generally accompanied by fibroblasts, could be obtained from 14 specimens (11 Parsi and 3 non- Parsi patients). Three of these cultures could be subcultured. The culture conditions required for extending the life of the cultures both in terms of time and number of passages are being evaluated. These efforts enabled a primary culture to be passaged through 8 subcultures (Figure 3). This 'line' is currently being maintained.

Experimental work has been carried out to immortalize the epithelial cell cultures through transfection by SV-40 large T antigen DNA. Calcium phosphate co-precipitation and microinjection techniques were attempted. However, these did not yield the desired effect.

Efforts to characterize the cultured cells were also continued

using immunocytochemical parameters. These cells express cytokeratins typical of human breast epithelium, *viz.*, cytokeratin peptide 8 and cytokeratin peptide 18.

d) Growth characteristics of normal and abnormal melanocytes and their response to synthetic and natural growth factors.

Vitiligo, melanoma, melasma, piebaldism, nevi etc. are some of the human pigmentary disorders where melanocyte proliferation and differentiation are central to the etiopathogenesis of the diseases. *In vivo* the melanocytes are thought to proliferate once in 6-7 years and under *in vitro* conditions these cell require synthetic growth factors to multiply.

Efforts to grow normal human melanocytes and melanoma cells on dermal equivalents have been made earlier. These studies were continued and it was found that when pure melanocytes were grown on dermal equivalents the melanocytes were less dendritic and produced less melanin as compared to those grown on dermal equivalents on which keratinocytes were seeded along with melanocytes. Melanogenic enzymes such as tyrosinase, Dopachrome tautomerose etc. in melanocytes grown on dermal equivalents are being assayed to understand the roles played by fibroblasts and keratinocytes on the proliferation and differentiation of the pigment cells.

e) Cell models for investigating the molecular mechanisms associated with etiology and pathophysiology of mental disorders.

A recent multinational survey conducted by the WHO indicated that the course and outcome of schizophrenia is favourable

in India as compared to the USA. This variation perhaps may be a reflection of the differences in dietary intake of essential fatty acids: the Indian diet is richer in essential fatty acids, essential polyunsaturated fatty acids and antioxidants which protect brain against the damaging effects of free radicals.

A number of studies have indicated altered lipid metabolism in schizophrenic patients. Similarly, decreased adhesion, abnormal growth properties, and lower plasma membrane phospholipids have been observed in skin fibroblasts obtained from the schizophrenic patients.

Experimental work was therefore undertaken to understand the relation betweeen the dietary essential fatty acids, caloric intake and the outcome of schizophrenia, employing skin fibroblast cultures derived from schizophrenia patients and their normal siblings.

Skin biopsies were obtained from Shree Kripamayee Institute of Mental Health, Miraj and used for setting up explant cultures. Fibroblast cultures were established from skin biopsies from 12 schizophrenia patients and their normal siblings. These were characterized for their adhesive properties, growth patterns and vimentin expression. Although the explants from patients required more time to adhere and spread as compared with those from the normal siblings, there was no difference in the time needed for fibroblast outgrowth from the explants. All these cultures were found to express vimentin which is a characteristic of fibroblasts. Further characterization of the fibroblasts and their use as an experimental system are under way.

f) Studies on factors affecting growth of skin fibroblasts *in vitro*.

Fibroblast cultures are extensively used as research tool for the study of heritable diseases, aging and gene transfer as well as for grafting. For all such application areas, it is of prime importance to be aware of the factors that may influence fibroblast growth.

While setting up 19 mouse skin cultures and 36 human skin cultures from donors of different age, health status and from 5 different biopsy sites the following observations were made: Through the inclusion of a trypsinisation step in the routine procedure, we could successfully develop primary cultures from biopsies from any age group as well as critical and fastidious regions. These cultures, however, showed differences in the time to attain conveyance depending on the biopsy site and the health status of the donors. Such differences are unlikely to be specific for a particular disease, but more of a generalized nature. In-depth studies of the molecular mechanisms responsible for these differences may help in understanding growth and senescence of the cultured fibroblasts and their relation to the respective diseases.

g) *In vitro* cultivation of erythrocytic stages of *Plasmodium vivax* and large scale cultivation of *Plasmodium falciparum*.

1. The incidence of *Plasmodium vivax* malaria is much higher than *P. falciparum*. Although culture strains of *P. falciparum* have been available since 1976, there are no strains of *P. vivax*. It is therefore essential to develop new culture strains of *P. vivax*. Efforts were continued to cultivate *Plasmodium vivax in vitro* using the candle jar method. Enriched parasite blood samples were collected from *P. vivax* malaria infected patients from the Primary Health Centre, Paud village, near Pune. Attempts to cultivate the parasites in O+ RBCs pretreated with hypo-osmotic

solution or proteolytic enzymes were continued. These however did not succeed.

The parasites invade only in the duffy positive reticulocytes. In order to circumvent the limitations in obtaining a regular supply of enriched reticulocytes, alternative methods were evaluated for the cultivation of the parasites.

It has been reported that reticulocyte binding protein Pv(RBC) of *P. vivax*merozoites binds to reticulocyte rich RBCs of rabbit. The enriched parasitized *P. vivax* erythrocytes were incubated in rabbit RBCs in RPMI 1640 supplimented with rabbit serum. However, no invasion or attachment of the parasites to the rabbit RBC was observed. With a view to obtain erythroid differentiation, Leuckemia cell line K 562 was cultured by supplementing spent medium of Human Blood Carcinoma cell line (ATCC-5637), hemin or DMSO. These methods did not yield positive results. Ultra violet (UV) treated human O+ RBCs also failed to support cultivation of *P.vivax.*

2. Large scale cultivation of *Plasmodium falciparum.*

Usually, malarial parasites (*P. falciparum*) are cultivated using RPMI 1640 medium supplemented with human serum (collected separately) and washed RBCs of compatible blood group. This method requires collection of plain blood from one donor for the preparation of serum. The RBC component is wasted as a clot. Another blood sample from a donor of compatible blood group has to be collected in CPD and washed RBCs from these samples are used for the propagation of the malarial parasites. Due to scarcity of donors and to save the precious blood samples, following method was evaluated. The blood samples collected

were defibrinated and the plasma containing RBCs stored. Plasma and washed erythrocytes from the same sample were used for the cultivation of *P. falciparum*. A total of five culture strains were used. It was seen that the *P. falciparum* cultures could be well maintained using this method. However, attempts to revive the cryopreserved plasma-supplemented cultures were not successful.

DEVELOPMENT OF TISSUE BANK TECHNOLOGY

a) Culture of human skin keratinocytes and their 3-D epitheliation for transplantation to burns, non-healing ulcers and vitilligo cases.

b) Studies on cryopreservation of normal human bone marrow

c) Cryopreservation and revival of heart valves for transplantation.

d) Endothelialization of homograft valves

DEVELOPMENT OF TISSUE BANK TECHNOLOGY

a) Culture of human skin keratinocytes and their 3-D epitheliation for transplantation to burns, non-healing ulcers and vitiligo cases.

The need to cover large areas of burn patients has lead to the development of culture techniques for growing human skin epithelia. Modifications in these culture techniques enable the growth of melanocyte- bearing epithelia for grafting onto vitiligo cases.

Procedures for initial skin processing, media formulations, culture conditions and actual grafting have been developed. It was found that outcome of grafting cultured eptithelia was superior to the currently available methods for the treatment of vitiligo, burns and nevi cases. One of the major features that determined the growth of skin epithelial cells, their 3-D epitheliation and their successful grafting for wound coverage was the feeder layers used.

Normally NIH 3T3 cells are used as feeder layers. These cells, however, are immortalized cells and loose their capacities to support the growth of skin epithelia when grown above 10-20 passages. Studies to develop alternative feeder cells was therefore initiated.

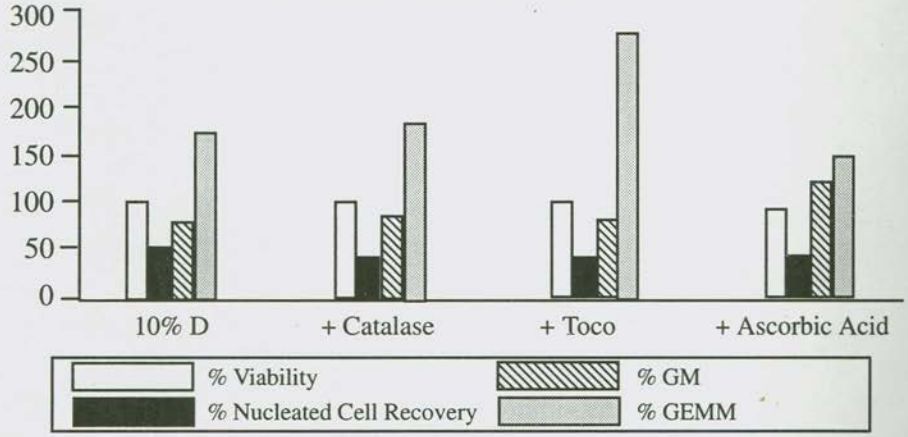
Human foetal lung and dermal fibroblasts were found to support the growth of human skin keratinocytes to a far greater extent than human foreskin fibroblasts, adult human skin fibroblasts, Balb/C mouse embryo fibroblasts and NIH 3T3 cells. However, foetal lung and dermal fibroblasts were found very difficult to be mitotically arrested with mitomycin-C. Human foreskin fibroblasts were found to be relatively easier to be growth-arrested and at the same time support the growth of human skin keratinocyte and are thus the ideal cells to serve as feeder layer for the growth of human skin epithelia for grafting.

b) Studies on cryopreservation of normal human bone marrow

Cryopreservation of bone marrow forms a very important part of Bone marrow transplantation programmes, especially for high risk groups such as workers in the nuclear reactor plants and individuals with family history of leukemia. The efforts to standardize the technology for cryopreservation and revival of bone marrow were continued.

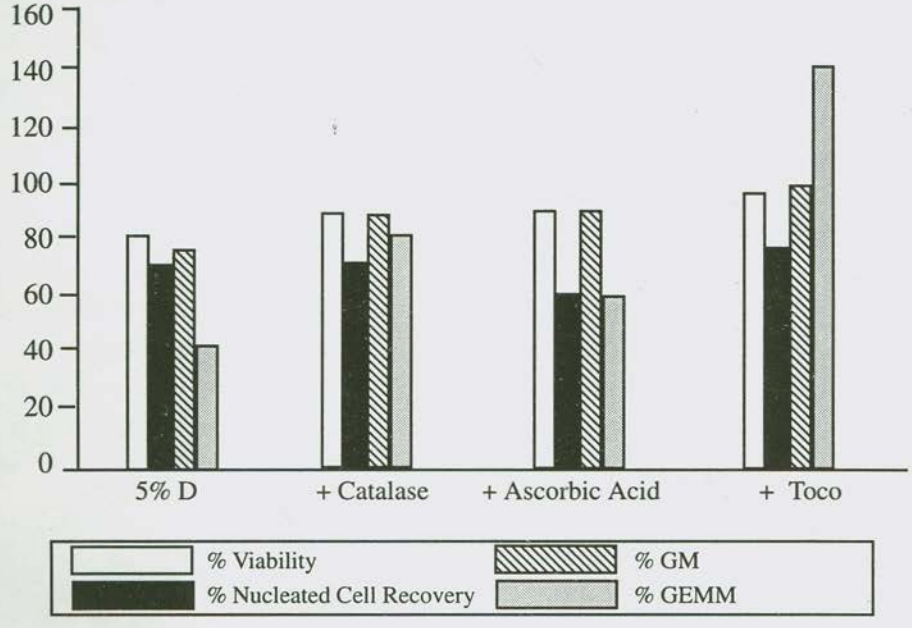
Nineteen human bone marrow samples were collected from the Sassoon General Hospitals, Pune and used for cryopreservation experiments. Various additives to the freezing mixture (containing IMDM, 20% FCS and 10% DMSO) were tested for their ability to protect the bone marrow cells during cryopreservation. These included antioxidants, membrane stabilizers, antifreeze proteins and glycoproteins etc. The results indicated that alpha tocopheryl acetate (40 ug/ml), catalase (100 ug/ml) and ascorbic acid (80 ug/ml) improved the recovery of cryopreserved cells, particularly in terms of GEMM and GM assays (Figure 4). Effects of other additives are being studied. Similar experiments carried out with

Figure 4 : Effect of antioxidants on the cryopreservation of human bonmarrow (F-23)



mouse bone marrow suggested that reduction of DMSO concentration to 5% yielded better cell recoveries in presence the antioxidants (Figure 5).Different cell types were assayed before and after cryopreservation, employing indirect immunofluorescence for CD-34 positive cells and Leishman-Giemsa stained smears. The results indicated significant reduction in the number of fully differentiated cells leading to apparent increase in the erythroid blasts after revival of the cryopreserved cells.

Figure 5 : Effect of antioxidants on the cryopreservation of mouse bone marrow (MBM - 30)



The stimulatory effect of cytokines was studied in bone marrow obtained from 50 Swiss albino mice. Two million cells were treated for 24 hours with various cytokines: recombinant GM CSF (20 U/ml), erythropoietin (EPO, 2 U/ml), IL-3 (20 U/ml), and assayed for GEMM colony formation. EPO did not exhibit stimulatory effect while GM CSF and IL-3 showed two- and three-fold more colonies,

respectively, than untreated control cells.

Eighteen fetal liver samples have been processed and the haematopoietic cells cryopreserved. The direct and indirect interaction of the foetal liver cells with the adult bone marrow cells are being studied.

c) Cryopreservation and revival of heart valves for transplantation.

Donor aortic and pulmonary heart valves that have their fibroblasts in viable condition and their surfaces seeded with recipients endothelial cells offer the best alternative to patients needing heart valve replacement therapy. Such recipients do not have depend upon life long anti-coagulant therapy and need not come for re-replacement of heart valve every 7-10 years.

Earlier, heart valves with a warm ischemic time of up to 11 hours were found to be viable. To confirm these findings, fibroblast outgrowth studies from heart valves were initiated. The heart valves having thick collagenous layers did not allow the fibroblast outgrowths to occur when cultured as explants. Efforts to remove collagen with enzymes such as trypsin and collagenase and allow the fibroblast to come out the explants are in progress.

d) Endothelialization of homograft valves

Homograft valves used in the treatment of cardiac disorders are fixed and stored in glutaraldehyde to retain mechanical rigidity. These treatments, however, result in the denudation and loss of the endothelial cell lining. It is well recognized that endothelium not only provides a non-thrombogenic surface but also plays a role in modulating the microenvironment by producing various mediators. It is postulated that

Figure 6 Processing of human umbilical cord for isolation of endothelial cells.

Figure 7 Primary culture of human umbilical vein endothelial cells.

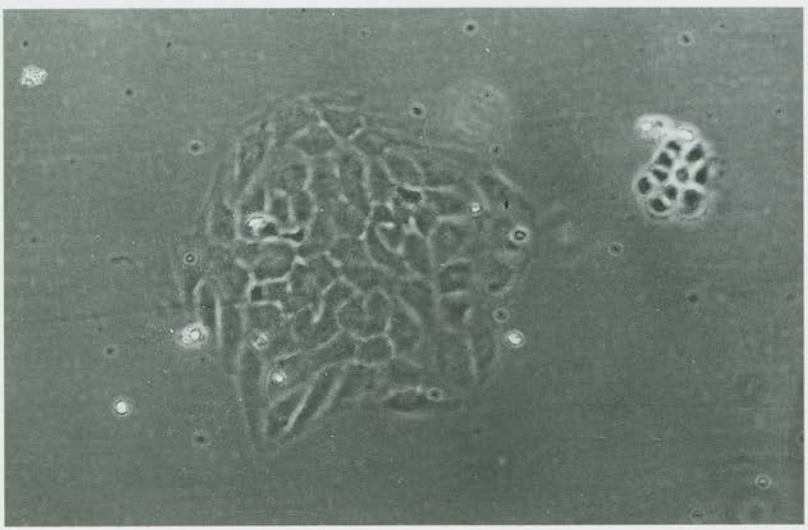
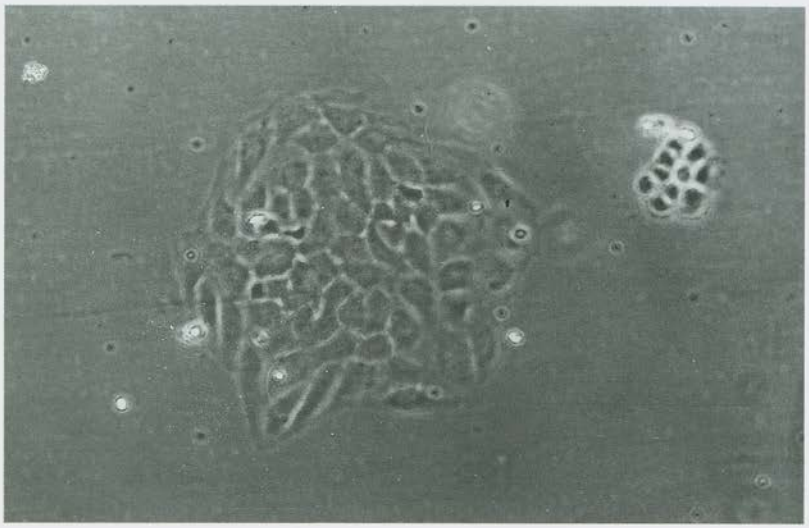


Figure 6 Processing of human umbilical cord for isolation of endothelial cells.

Figure 7 Primary culture of human umbilical vein endothelial cells.



restoring endothelial lining on the valves would increase their long term performance. Experimental studies have therefore been initiated to grow endothelial cells on such valves using tissue culture technology.

Initially, attention was concentrated on sphenous veins which are left over after bypass surgery, and were obtained from Ruby Hall Nursing Home, Pune and Sion Hospital, Bombay. Attempts to isolate viable endothelial cells from these veins (11 samples) by mechanical and enzymatic dissociation were largely unsuccessful. The failure was in part due to high rate of contamination, presence of a large number of RBCs and debris. Moreover, these samples were extensively handled and distended during the surgical procedure. As an alternative source of endothelial cells, umbilical cord veins, which can be obtained without trauma, were used. A total of 25 samples of umbilical cords were obtained so far. Protocols have been standardized for prevention of contamination, minimization of RBCs and debris and collagenase digestion for isolation of endothelial cells. Currently, efforts are directed to define the conditions for endothelial cell attachment and proliferation in vitrto (Figures 6 and 7).

INDIGENOUS TECHNOLOGY AND RENDERING EXPERT SERVICES.

a) Adaptation of hybridoma cell lines to goat serum.

b) Identification and characterization of protective antigens in lymphatic filariasis.

c) Mechanism of action of thiophene derived drugs for evaluation of their possible use in chemotherapy.

d) Effect of copper derivatized taxol analogues on breast cancer cell line (MCF - 7)

INDIGENOUS TECHNOLOGY AND RENDERING EXPERT SERVICES.

a) Adaptation of hybridoma cell lines to goat serum.

Foetal calf serum (FCS) is imported at a considerable cost in foreign exchange. Goat serum (GS) is available in plenty within the country and NFATCC has adapted several cell lines to GS. Myeloma and hybridoma cell lines have industrial applications and if these cells can also be adapted to GS without altering their fusion and antibody secreting properties, it would be possible to lower the production cost of diagnostic kits etc.

CC9C10 (anti insulin antibody secreting hybridoma) and OKT 3 hybridoma cell lines were adapted to goat serum supplemented with soy bean lipid mixture. Secretory activity of these cell lines were monitored.

b) Identification and characterization of protective antigens in lymphatic filariasis.

Lymphatic filariasis is an important vector borne disease prevalent in tropical and sub tropical regions (Figure 8). In India, *Wuchereria bancrofti* and *Brugia malayi* nematodes are the causative agents. Studies on identification and characterization of the protective antigens in lymphatic filariasis were continued.

A field survey was carried out in endemic areas of filariasis at Tahsil - Palghar in Thane district. Twenty five sera samples were collected from these areas.

Our earlier studies have shown that the bovine filarial worm (*Setaria digitata*) antigen cross reacts with human filarial sera, although no distinction was possible between various groups such as endemic normals, microfilaraemics and clinically diagnosed filarial subjects.

Therefore, total Ig and IgG4 subclass antibodies in the sera of lymphatic filariasis reactive to soluble adult*S. digitata* antigen were analyzed using enzyme linked immunoassay. Fifty sera from endemic area and ten from non-endemic area were tested (Table 1). *S. digitata* reactive IgG4 antibody levels were higher in the microfilaraemics as compared to the endemic normals and elephantiasis sera. Moreover, the frequency of a positive reaction in the microfilaraemics was much higher (93.3%) than the endemic normals and elephantiasis sera. Thus, a differential diagnosis based on the presence of specific IgG4 subclass in filarial sera is now possible.

A mouse monoclonal antibody developed against *B. malayi* infective larvae (F46) was found to react with cuticular antigens of both infective larvae and the adult worm. The target epitopes of F 46 could be destroyed by pronase and not by trypsin, collagenase and metaperiodate as assessed by ELISA, indicating that F 46 binds to polypeptide determinants.

Table - 1 Crossreactive total immunoglobulins (A) and *IgG4* antibodies (B) to *Setaria diaitata* soluble antigen in lymphatic filarial sera : Differential detection of microfilaraemic individuals.

A

Sr. No.	Sera	No. of Cases	Elisa Index (E.I.) Mean ± S. E.
1.	Normals (NEN)	7	0.054 ± 0.031
2.	Endemic Normals (ENS)	13	0.523 ± 0.119
3.	Elephantoid (ELS)	10	0.328 ± 0.077
4.	Microfilaraemics (mf + ve)	22	0.645 ± 0.118

insert sizes (ranges 1.3 to 3.5 kb) and five clones were selected for subcloning and partial sequence analysis. Preliminary results showed that 2 of the clones were full length *Brugia* paramyosin and one clone each of myosin and tropomyosin. One clone seems to be novel and it has similarity to *C. elegans* Calmodulin like genes. Further studies are in progress.

Antifilarial compound (PT) was received from the Department of Chemistry, University of Pune. Its antifilarial activity was assessed *in vitro* over 48 hours using different stages of *B. malayi*. At 10mM concentration of the drug, adult worms and infective larvae were killed. However no deleterious effect was observed on *W. bancrofti* microfilariae.

An animal model (*Mastomys*) has been established to facilitate availability of *B. malayi* parasites and *in vivo* drug screening.

c) Mechanism of action of thiophene derived drugs for evaluation of their possible use in chemotherapy.

Thiophene derivatives are antimetabolites that have anticancer properties. Four of such compounds were previously tested in vitro for their anticancer properties using K-562, a leukemia cell line. In continuation with these studies, six more derivatives were analysed viz. sulphones, esters and sulphoxides of thiodipropionic acid (TDPA) and thiodiglycollic acid (TDGA).

The following cell lines were used: Sarcoma 180 (mouse sarcoma), HT1080 (human sarcoma), MCF-7 (human breast carcinoma), KB (human oral carcinoma) and KB Chr8-5 (multidrug resistant cell line derived from KB, resistant to 10 ng/ml of colchicine). The test compounds

were added at various concentrations and the effect on growth of the cells was estimated by uptake of tritiated thymidine.

It was found that the thiournium compound and dicetol (which were previously shown to be antimitotic) were active compounds even in case of sarcomas and carcinomas. The other compounds were unable to inhibit cell proliferation. The fifty percent inhibitory concentration (IC50) of the thiouronium compound was determined for different cell lines (Table 2).

Table 2 IC 50 of thiouonium compound on different cell lines.

Cell lin	IC 50 (ug / ml)
K-562	12
Sarcoma 180 (Mouse sarcoma)	17
HT 1080 (Human sarcoma)	14
KB Chr 8-5 (Multigdrug resistant)	40

Multidrug resistance (MDR) is characterized by increased expression of a 170 kD glycoprotein (p-gp) on the cell membrane. which actively pumps a wide range of compounds including anticancer compounds out of the cells. The increased levels of p-gp can be demonstrated by immunolocalization, by Western blot, and also by Northern blot. Alternatively, the efflux of a fluorescent tracer compound can be directly observed.

In KB Chr8-5 (a clonal line of KB, expressing multidrug characteristics) pgp was localized at the cell surface by immunolocalization; further, efflux of Rhodamine 123 could also be observed in these cells. On the other hand, neither pgp, nor Rhodamine 123 efflux could be detected

in wild type KB cells.

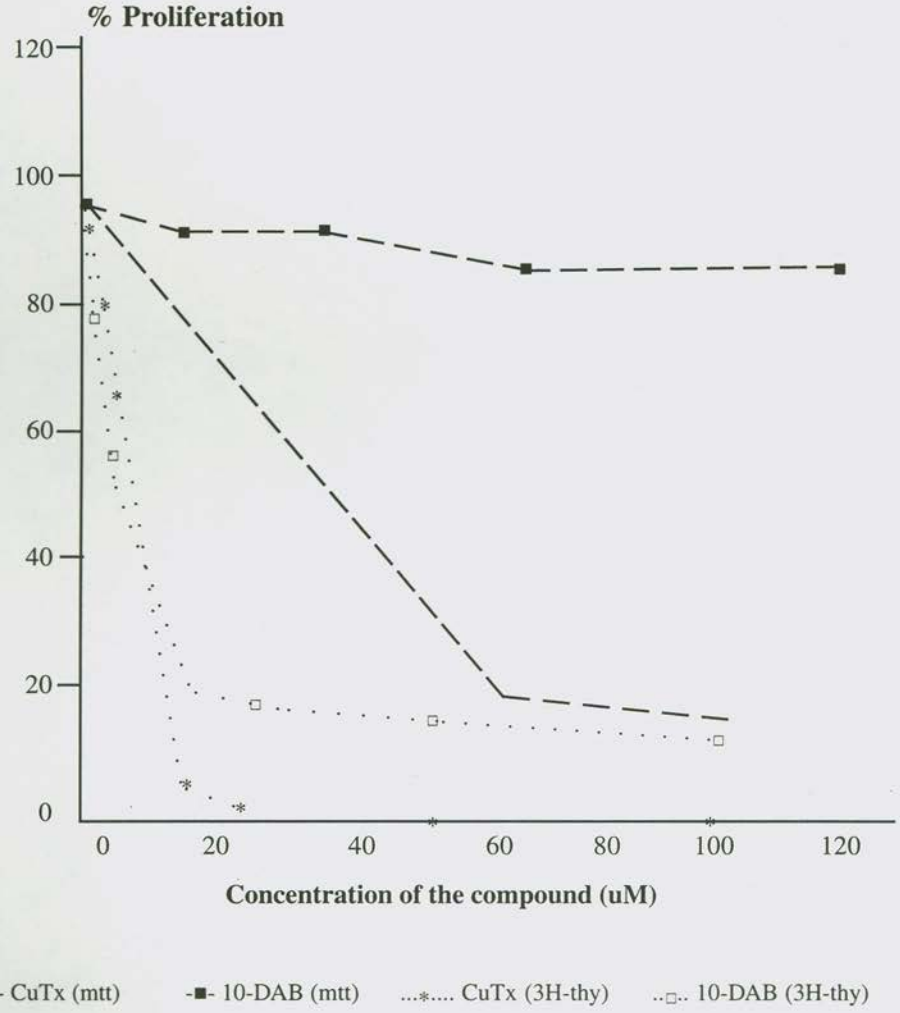
RNA isolation, formaldehyde gels, blotting and hybridization was standardized on K-562 and OVCAR 3, a drug resistant ovarian carcinoma cell line.

d) Effect of copper derivatized taxol analogues on breast cancer cell line (MCF-7).

Taxol is one of the most promising drugs used in the treatment of breast and ovarian cancers. Taxol is isolated from the trunk bark of the Pacific yew trees of the *Taxus* species. Since the yield is very low, other sources of taxol are being explored in order to meet its growing demand. Total and partial synthesis of taxol related compounds have been done. 10-Deacetylbaccatin isolated from the leaves is one of the most accessible taxol precursor. As total synthesis of taoxl isn't a simple procedure, partial synthesis of the ring structure was done. Copper chelates of taxol analogues designated CuTx was prepared in order to enhance the cytotoxicity of the parent compound. The cytotoxicity of this copper chelate was compared to that of 10-DAB.

Two methods were used: The MTT assay was used to determine the viability of cells and tritiated thymidine uptake was done to estimate the percentage of proliferating cells. 10-DAB treated cells were growth arrested but viable, whereas Cu-Tx was lethal to the cells at comparative doses (Figure 9). Taxol is a microtubule stabilizing drug which brings about mitotic arrest. Staining cells treated with 10-DAB by antitubulin antibodies showed that a large percentage of cells were arrested in metaphase. This was not observed in CuTx treated cells. Thus the toxicity of these two compounds were different both in the mode of action as well as the effective dose.

Figure 9 : Effect of CuTx on the Viability of MCF - 7 Cells (MTT Assay) Comparison with 3H thymidine uptake data



BIOLOGICAL AND BIOTECHNOLOGICAL APPLICATIONS

a) Immortalization of human none marrow derived stromal cells and stem cells via transfection of cloned oncogenes.

b) Effect of growth factors on stromal cells

c) *In vitro* studes on the role of growth factors on melanosome transfer from melanocytes to keratinocytes in the epidermis

d) Identificaation of Melanoma oncogenes and characterization of the oncogene protein product.

e) Organization, expression and regulation of mammalian hexokinases

f) Use of mitochondrial DNA Restriction Fragment Length Polymorphism for characterization of mosquito cell lines.

g) Endocytic vesicles and their association with cytoskeleton

h) Hyaluronic acid in rheumatoid arthritis patients and skin cultures before and after Ayurvedic treatment.

i) Effect of soluble factors produced by mitogen activated imnune cells on proliferation and differentiation of Neuroblastoma cells.

j) Investigation on Beta cell protective mechanisms as means of reducing incidence of diabetes

k) Studies on microencapsulation of cells and istlets of Langerhans in alginate-gelatin beads.

l) Fetal Liver infusion : Its mode of action, efficacy of cryopreserved cells and its potential application in the management of cancer

m) Screening of antimalarial compounds.

n) Structure activity relationship of tyrosinase seeking compounds : potential chemotherapeutic agents for melanoma

BIOLOGICAL AND BIOTECHNOLOGICAL APPLICATIONS

a) Immortalization of human bone marrow derived stromal cells and stem cells via transfection of cloned oncogenes.

Adult bone marrow stromal cells can be grown in culture through a limited number of passages, generally about 4 to 5. It was shown earlier that transfection of these cells with SV-40 large T-antigen and Polyoma middle T-atntigen allowed these cells to be passaged through about 20 passages. These experiments were continued further.

Besides these viral oncogenes, suitability of cellular oncogenes, such as activated and normal ras-H and oncogeneic derivatives of myc and myb genes is being evaluated. For this purpose, two plasmid DNA constructs are under development where derivatives of V-myb oncogenes will be placed under a viral promoter such as MuLv or SV-LTR.

b) Effect of growth factors on stromal cells

Although the hemopoietic cell differentiation pathways originating from the pluripotent stem cell and culminating in fully differentiated blood cells are broadly understood, factors that determine cell-cell interactions have not been established.

Erythropoietin (EPO)-dependent stromal cell adhesion was detected in long term cultures of bone marrow derived mononuclear cells (see AR 93-94). Further experimental work was undertaken to identify the adhesion signals that may be present in the conditioned medium, using heparin agarose binding, Mink lung fibroblast proliferation inhibition (Mv1Lu) and antibody neutralization assays and immunofluorescence. These experiments enabled the identification of fibroblast growth factor (FGF) and transforming growth factor (TGF) family of molecules in the conditioned medium. Exposure to FGF or TGF could induce stromal cell

aggregation in absence of EPO.

The stromal cells treated with EPO-conditioned medium could generate up to 3 times more colonies as compared with those treated with conditioned medium without EPO. These colonies however were heterogeneous and were not restricted to erythrocytic lineage alone.

Integrins play an important role in the interaction of hemopoietic cells. The stromal cells were therefore tested by indirect immunofluorescence for the presence of various integrins following exposure to conditioned media. Preliminary results indicate that expression of B3 integrin subunit in the stromal cells was elevated while fibronectin receptor (alpha 5 B) showed only a modest increase. However, aggregation assays using anti B3 integrins did not inhibit cell aggregation; on the other hand, anti-fibronectin receptor antibody was found to be a potent inhibitor of stromal cell aggregation. These results suggest that alpha 5 B integrin may be involved in the stromal cell aggregation.

c) *In vitro* studies on the role of growth factors on melanosome transfer from melanocytes to keratinocytes in the epidermis

Normal skin pigmentation depends on the production of melanin pigment in the melanocytes and their transfer to keratinocytes. Co-culture experiments of melanocytes and keratinocytes at normal physiological (1 : 16) and reverse physiological ratio (16 : 1) have revealed that melanocytes have on an average 4 dendrites in early passages and in latter passages become more dendritic. Further studies on similar lines have revealed that after accepting one dendrite from a melanocyte, the keratinocyte does not accept another dendrite from the same melanocyte. The same keratinocyte however, is capable of accepting dendrites from other melanocytes. These studies reveal that there is a sort of communication between the melanocytes and keratinocytes regarding

the formation and penetration of dendrites. To further understand the nature of this communication, ^{35}S. methionine labelling experiments with pure cultures of melanocytes and keratinocytes and their co-cultures were done. Preliminary results indicate that melanocytes in pure cultures express two prominent proteins with M. W. of 72 and 24 kDa respectively, labelled with methionine.

Further studies to understand if there are any dendrite inducing and dendrite-extension factors made by keratinocytes are in progress.

d) Identification of Melanoma oncogenes and characterization of the oncogene protein product.

The incidence of melanoma is on the rise throughout the world. The normal diploid melanocyte undergoes progressive stages of carcinogenesis that can be also identified clinically. In addition, melanoma genesis and melanin synthesis seem to occur in a concurrently progressive manner and therefore is an ideal system to decipher critical factors that push the cells towards proliferative and differentiative stages.

Studies to understand the events involved in melanocyte transformation using the mouse melanoma cell-line as an experimental model were continued. Observations on NIH3T3 cells transfected with DNA isolated from the melanoma clone M3 cells were described earlier (see AR 93-94).

An expression cDNA library was prepared from mRNA of the transfected cells and Clone M3 cells using a phagemid pCEV27, following the automatic directional cloning approach. A titre of 4×10^7 pfu/ml was achieved for both the libraries.

Initially, only the M3 cDNA library is being screened for oncogenes. For this purpose the cDNA library has been amplified in liquid culture, DNA extracted and used for transfection of NIH3T3 by lipofection. Each 5 mg of the transfected DNA yielded about 50-60% geneticin resistant (G418 positive) clones (Figure 10). These were expanded in culture and further characterized for transforming ability by phenotypic appearance, growth in low serum, soft agar assay and tumorigenicity in nude mice. Of the various clones analyzed two clones have produced tumors in nude mice (Figure 11). The tumor tissue is being analyzed histochemically for the tumor type.

e) Organization, expression and regulation of mammalian hexokinases

The initiation of carbohydrate metabolism by the conversion of glucose to glucose-6-phosphate is carried out by the enzyme hexokinase (HK). Four HK isoenzymes exist which have different kinetic properties and exhibit tissue-specific expression. The molecular mechanism that determines tissue specificity of the different isoenzymes in normal conditions and its impairment in pathological conditions such as cancer and diabetes remains to be elucideated.

In continuation of our efforts to understand the molecular mechanisms which determine tissue specificity of hexokinase isozymes, role of methylation pattern of hexokinase gene(s) in different tissues was undertaken. Genomic DNA isolated from different tissues was digested with Hpa II and Msp I restriction isoenzymes (if internal cytosine residue is methylated Hpa II cannot cleave that DNA). Digested DNA from rat brain, kidney, muscle, liver and (C6 cancer cell line) were blotted onto nylon membranes. Hybridization of the blot using all the 4 cDNA sequences is in progress.

To characterize isozymes patterns during carcinogenesis, expression at RNA level was estimated by reverse transcription PCR in the following cell lines and tissues: c6 glioma (brain tumor), L6, (muscle), GH1 (pituitary tumor), NMU (Breast cancer), NRK (Kidney tumor), RIN (pancreatic tumor), Regenerating liver, Brain and Normal liver. PCR primers specific for each isozymes have been designed to minimize cross hybridization between such extensively homologous DNA sequences. Total RNA isolated was reverse transcribed to cDNAs and PCR amplified with isozyme specific primers.

All the cancer cell lines expressed type II isozyme at RNA level irrespective of the tissue of origin. NRK and c6 glioma expressed very low level of type I while RIN showed basal level of type IV expression. Interestingly, regenerating liver showed type II isoenzyme but not type IV seen under normal conditions. Normal brain and liver shows typical pattern of predominant expression of type I and type IV respectively.

Experiments are in progress to complete restriction mapping of the amplified fragmented to verify authenticity of the amplified products.

f) Use of mitochondrial DNA Restriction Fragment Length Polymorphism for characterization of mosquito cell lines.

Various cell lines derived from *Anopheles*, *Culex* and *Aedes* mosquitoes cannot be readily distinguished morphologically or by isoenzyme patterns. Previous studies using ethidium bromide staining and end labeling of restriction enzyme digested fragments indicated that enzymes Cla I, Hind III and Hinf I can distinguish the *Anopheles stephensii* and *Aedes albopictus* cell lines. Mitochondrial DNA (mt-DNA) is small (~17kb), maternally inherited, has a faster rate of evolution than the nuclear DNA and does not undergo genetic recombination. These characters make it an ideal tool for these studies. Attempts are therefore being made to develop

mt-DNA RFLP as a parameter for the characterization and identification of mosquito cell lines.

Five clones of mt-DNA from *Anopheles quadrimaculatus* were kindly provided by Prof. A. F. Cockburn. These clones are being used to probe digests of total DNA from mosquito cell lines after Southern transfer of DNA. The clones were revived, retransformed in *E. coli* DH 5 alpha and plasmid DNA was prepared.

Since the mitochondria code for their own ribosomal RNA molecules, RFLP of ribosomal RNA genes has been used as a tool in many taxonomic, epidemiologic and population genetics studies. Using the published sequence information of mitochondrial ribosomal RNA genes, primers were designed which specifically amplify a portion of insect 16S ribosomal RNA gene. When used in Polymerase Chain Reaction, these primers specifically amplified a 500 bp fragment from all the mosquito cell lines tested (*A. stephensi*, ATC-10, ATC-15, ATC-448 and *Culex thileria*) and also from the total larval DNA of *Aedes aegypti*.

g) Endocytic vesicles and their association with cytoskeleton

It was reported earlier that a small proportion of mosquito cells (ATC 15 and its clonal line, C6/36) exhibit formation of tubular endosomes, besides spherical endocytic vesicles. These were visualized by loading a membrane impermeant dye, Lucifer Yellow (LY). The spherical vesicles as well as the tubular endosomes varied in abundance. The tubules ranged from a few isolated tubules to branched structures to anastomosing networks. Attempts to simultaneously visualize these endocytic organelles loaded with LY and the cytoplasmic microtubules by indirect immunofluorescence for tubulin were continued. It has been possible to preserve only a few of these membrane bound organelles

Figure 8 Elephantitis patient.

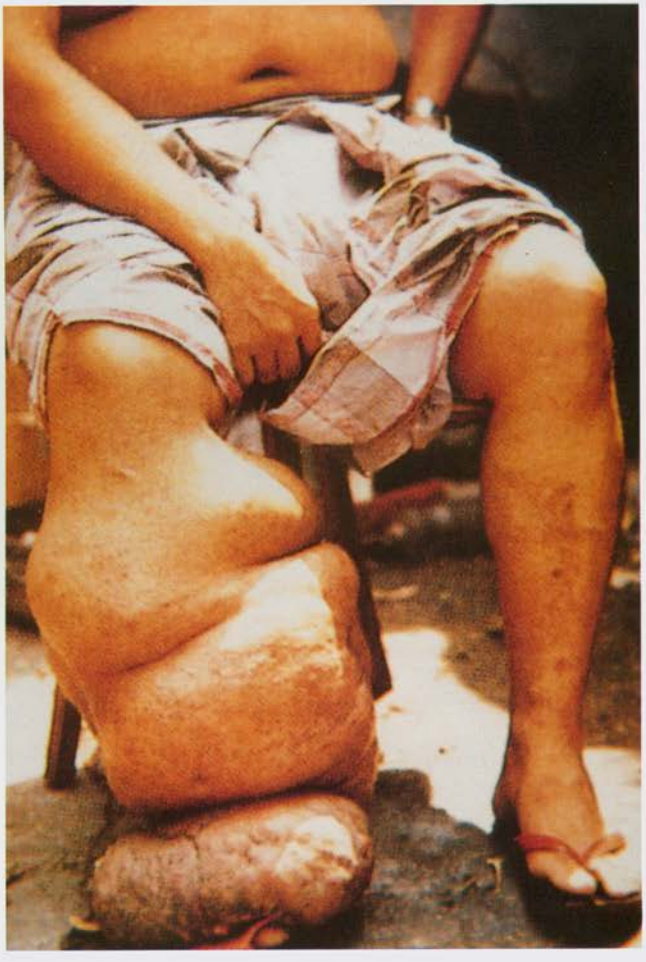


Figure 10 Geneticin resitant clone of NIH 3T3 cells generated by transfection with M3 cDNA library.

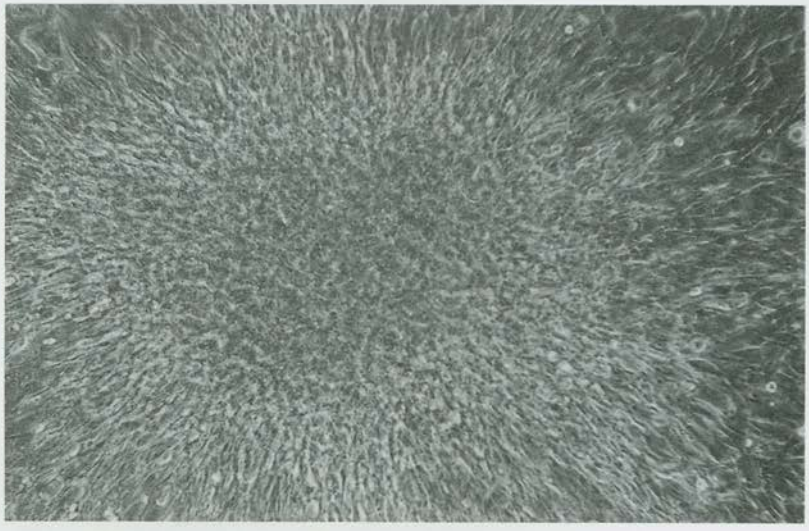
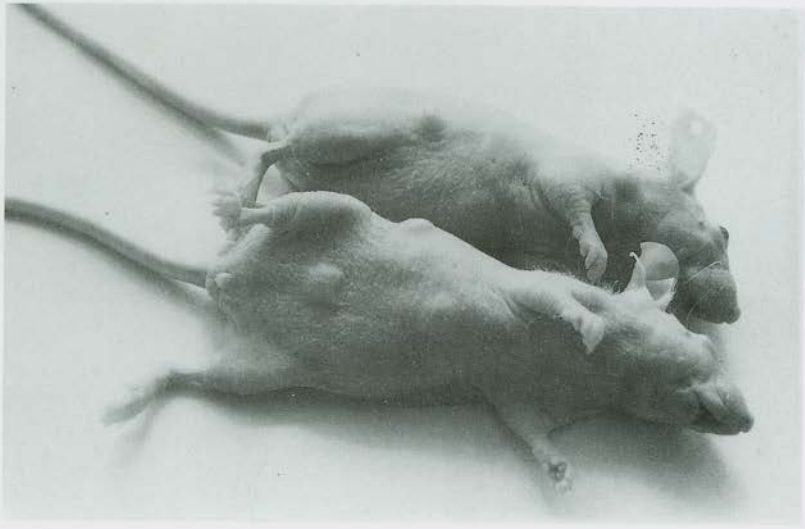


Figure 11 Tumours developed in nude mouse atter injecting the geneticin resistant NIH 3T3 cells.



through fixation and immunostaining protocols. Further refinements are being carried out for better visualization of these structures in the mosquito cells.

h) Hyaluronic acid in rheumatoid arthritis patients and skin cultures before and after Ayurvedic treatment.

Rheumatoid arthritis (RA), an inflammatory joint disease leading to severe deformities, has a uniform prevalence of approximately 1% all around the world. Its pathogenesis is thought to involve environmental as well as genetic factors. While Allopathic treatment has been merely symptomatic, Ayurvedic medicine is known to be effective in the rheumatoid disorders.

Extracellular matrix of the joints is the major source of hyaluronic acid (HA), a carbohydrate polymer. Increased serum levels of HA are correlated to the degree of inflammation and to the immunological status. Consequently, HA is a sensitive parameter for the assessment of joint degeneration and progress of the disease. In a clinical trial of a defined course of proprietary Ayurvedic formulations against RA, serum HA levels were estimated at regular intervals, in addition to the standard parameters. In 26 out of 45 RA patients who completed the course of the treatment showed good response with respect to the conventional parameters. Patients with elevated serum HA levels also responded by lowering these to near-normal range. On the other hand, patients with normal serum HA levels showed no alteration in HA levels. The Ayurvedic treatment as such appears to be antiinflammatory.
Since skin is another important source of HA, studies were undertaken to monitor HA synthesis in skin fibroblast cultures. Fibroblast cultures from 13 RA-patients and 12 healthy individuals were established. The cultures were harvested at various intervals and HA, uronic acid (Table 3) and N-acetylglucosaminidase (Table 4) were estimated in the spent

media, cell surface associated fractions and cells. These results indicate that skin fibroblast cultures can be used to study the extracellular matrix involvement in inflammatory processes in RA patients.

Table 3 Estimation of HA and Uronic acid in skin fibroblast cultures.

Serum HA ul/l	Culture samples	HA release into the medium ug / l	Cell Surface associated HA ug /1 g	Uronic acids ug/ ml
102 - 380	n = 8	413.2 ± 119.3	251.9 ± 48.7	4.42 ± 2.5
15 - 78	n = 3	260.8 ± 39.3	183.5 ± 64.7	(n = 6)
10 - 81	Controls : n = 11	235.6 ± 91.4	218.28 ± 67.3	7.95 ± 3.2

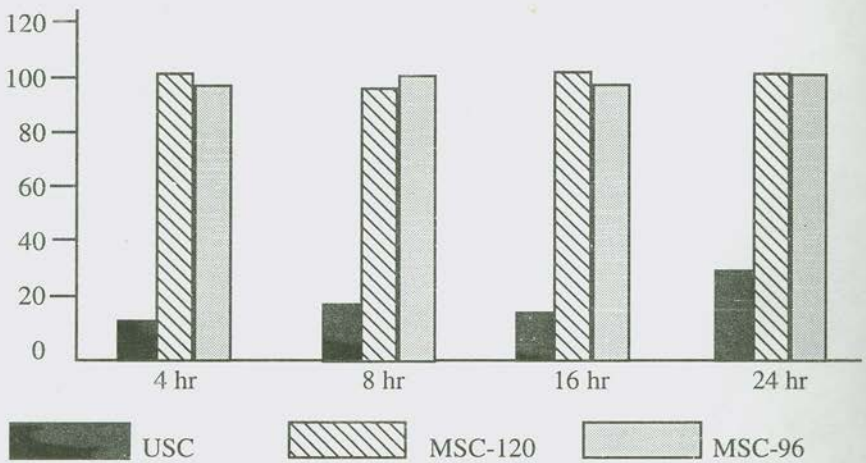
Table 4 Estimation of N-Acetylglucosaminidase in skin fibroblast cultures

Culture Samples	N-Acetylglucosaminidase nkat / mg Protein
Patients : n = 10	11.24 ± 0.836
Controls :	8.73 ± 0.727

Mannose binding protein (MBP) is a c-type lectin, which recognizes carbohydrate structures and thereby activates complement. It has been recently proposed that MBP plays a role in the pathogenesis of RA through binding to the carbohydrate associated with the IgG immunoglobulin. Serum levels of MBP were therefore estimated in the RA patients and were found to correlate with the inflammatory process (ESR) and the rheumatoid factor: patients with nominal inflammation showed lower MBP levels.

Human peripheral blood lymphocytes derived from 6 healthy donors and 1 NB patient were stimulated with Phytohaemagglutinin (PHA), and supernatants were collected between 8 and 120 hours . Supernatants were also collected from parallel cultures which did not receive PHA. Estimation of uptake of tritiated thymidine and MTT assay were carried out to assess the effect of these supernatants on proliferation of human NB cell line SK-N-MC. Results obtained thus far, indicate the presence of a significant (as high as 95-98 % in all individual experiments) anti-proliferative potential of these culture supernatants. The MTT assays indicated that the supernatants had a cytotoxic effect on the SK-N-MC cells. The supernatant treated cells displayed marked chromatin condensation (a characteristic feature of apoptotic cells) as visualized by staining with acridine orange.

Figure 12 : Time course study on the effect of supernatants on N - 2a cells.



Sups collected from - USC - Unstimulated, MSC-96, MSC-120 stimulated for 96 & 120 hr. reap

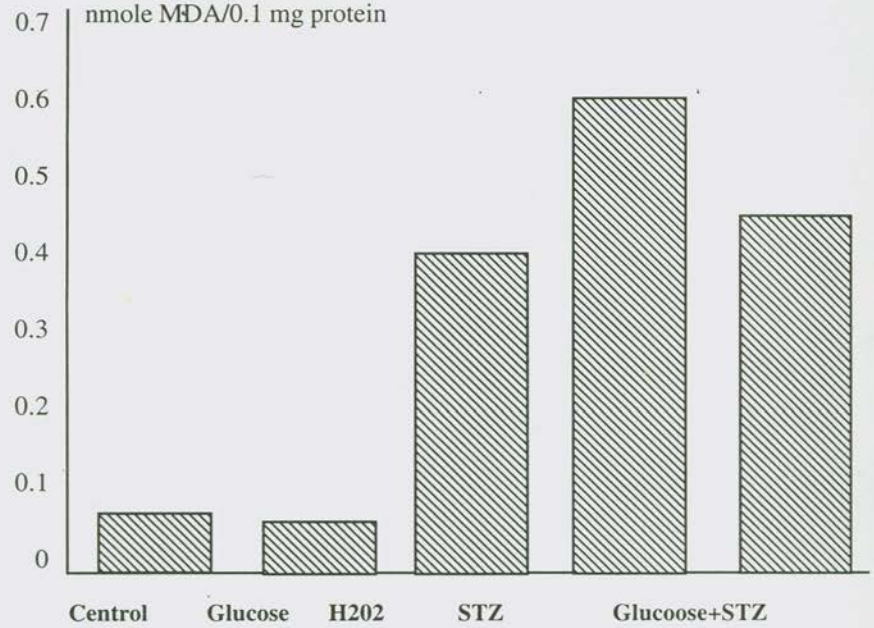
The levels of cytokines IL-1-alpha, IL-2, IL-4, IL-6 and TNF-alpha in activated and resting lymphocyte culture supernatants were quantitated using ELISA and RIA kits. The levels of cytokines did not correlate with the observed inhibition of proliferation. Recombinant IL-6 and TNF-alpha displayed a dose-independent inhibition of proliferation (approximately. 24% and 41% respectively). When used in combination IL-6 enhanced the growth inhibitory effect of TNF-alpha. Partial characterization of these supernatants revealed the thermostable nature of the components contributing largely to the inhibition of proliferation.

j) Investigation on Beta cell protective mechanisms as means of reducing incidence of diabetes

Streptozotocin, an anti-neoplastic drug, is often used for experimental induction of diabetes in laboratory animals. It has been recently observed that sugars like D-Glucose and 5-Thio-D-Glucose prevent the STZ-induced diabetes. Mechanisms involved in the diabetes induction by STZ and its prevention by sugars are not understood.
Experimental work was therefore undertaken to ascertain these mechanisms using freshly isolated islets from 4-6 week Balb/c male mice and rat insulinoma cell line, RIN 5mF (RIN). Malondialdehyde (MDA) levels were measured as an index of lipid peroxidation.

It was observed that STZ induced lipid peroxidation in RIN cells in a dose dependent manner and exposure of RIN cells to D-Glucose, prior to STZ treatment resulted in marked decrease in lipid peroxidation (Fig. 13). Similarly marked decrease in lipid peroxidation was also observed in case of freshly isolated islets (Fig. 14). Similar experiments conducted using 1% bittergourd extract also showed decreased lipid peroxidation

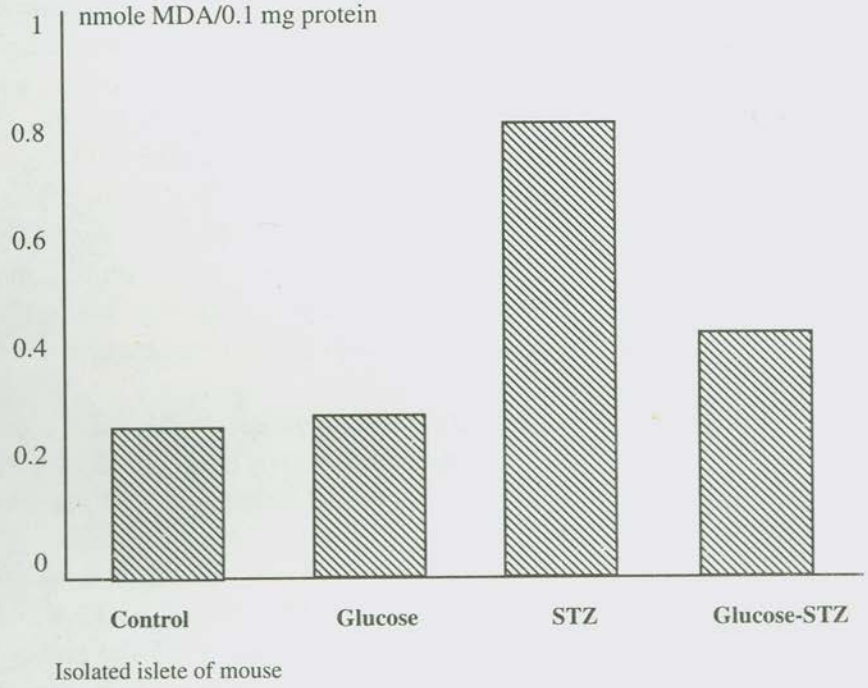
Figure 13 : Effect of Glucose on STZ - induced lipid peroxidation in RIN cells



in STZ treated RIN cells. These results suggested that D-Glucose as well as bittergourd extract modified the toxic effect of STZ by scavenging the free radicals, thereby reducing the levels of lipid peroxidation.

To ascertain the effects of STZ on other cell types, a series of cell lines were treated with STZ and MDA levels were estimated. These included Hela, Hela-229 (carcinoma of human cervix), MCF-7C (human breast cancer), L-929 (mouse connective tissue), BHK-21 (hamster kidney), MDBK (bovine kidney) and ATC-15 and C6/36 (mosquito). Increase in MDA levels was observed in all these cell lines, indicating non-specific nature of STZ toxicity.

Figure 14 : Effect of D-glucose on STZ-induced lipid peroxidation in



k) Studies on microencapsulation of cells and islets of Langerhans in alginate- gelatin beads.

Microencapsulation of cells and tissues in alginate or gelatin shells protect the cells and retain their growth properties. The shell permits passage of low molecular weight substances such as nutrients and oxygen but not of high molecular weight proteins. Limited revival of cryopreserved pancreatic islets of Langerhans is attributed to the injuries inflicted during cryopreservation. Hence, studies were undertaken to evaluate suitability of microencapsulation for protection of the islets through cryopreservation.

Islets from mouse pancreas isolated by collagenase digestion were hand-picked under a dissecting microscope and suspended in 3% sodium alginate. Average diameter of the islets was 200 to 300 u. Microencapsulation was carried out by dropping the suspension into 2 mM $CaCl_2$ solution. The alginate beads so formed measured 0.5 to 1.0 mm and contained 10-15 islets per bead. These beads were washed, suspended in RPMI 1640 supplemented with 10 % FCS and incubated at 37 C. It was observed that the calcium alginate beads disintegrate readily in the phosphate containing medium. Hence, gelatin was incorporated in the sodium alginate solution to obtain firm beads.

The encapsulated and unencapsulated islets cultured over a period of 7 days were viable as detected by beta cell-specific stain Dithizone (DTZ). Insulin secretion in both the cases was comparable as estimated by RIA.

Encapsulated as well as unencapsulated islets were cryopreserved for 15-30 days in a mixture of RPMI 1640, 10% FCS and 10 % DMSO. It was observed that after revival, 80 % of the encapsulated islets showed morphological integrity and viability. On the other hand, unencapsulated islets disintegrated and were not viable. Microencapsulation thus protects the islets during cryopreservation. Further studies are in progress to estimate insulin production by encapsulated and cryopreserved islets after revival.

l) Fetal Liver infusion : Its mode of action, efficacy of cryopreserved cells and its potential application in the management of cancer

Foetal liver cells infused into patients of aplastic anemia and acute leukemia have been known to stimulate the patient's stem cells, leading to bone marrow recovery. Although the mechanism of this autologous

recovery is not known fetal liver cell infusion holds promise for cancer management. Experimental work has therefore been initiated to study the proliferative potential, cryopreservation and revival of the fetal liver cells and to develop a co-culture model to understand the role of fetal liver cells in autologous bone marrow recovery.

Thirty five MTP fetuses were obtained from the Sassoon General Hospitals, Pune. Liver was removed aseptically and dissociated mechanically to obtain single cell suspension. These cells were cryopreserved using a progammable freezer. After revival, there was no significant loss of cell viability, cell number, number of colony forming cells and CD34-positive cells. Further experimental work is in progress to evaluate the retention of functionality of the cryopreserved cells.

Extracts of fetal liver tissues were prepared. These are being analyzed for the presence of any secretory diffusible factor(s) responsible for activation and/or stimulation of the stem cells, using normal and leukemic bone marrow stem cells as targets.

m) Screening of antimalarial compounds

Antimalarial action(s) of a drug or plant extract *in vivo* may depend on its metabolism and internal milieu of an infected animal. Such conditions cannot be reproduced *in vitro* to the fullest extent which does not permit development of an *in vitro* test system. Attempts are therefore being made to develop a model combination of *in vivo* and *in vitro* approaches for screening antimalarial compounds. Here, the test substance is routed through a model animal (rabbit) and subsequently tested for its efficacy *in vitro.*

In a preliminary study two strains of *Plasmodium falciparum* parasites were cultured in RPMI-1640 medium supplemented with rabbit serum

(10 % and 90%) for five days. The results showed that high serum supplement also support growth of the parasites.

Samples of rabbit blood were collected before the administration of drug 0 hr (control) and 12 hr after administration of the drug and serum separated. These sera were used as supplements with RPMI 1640 for maintaining strains of *P. falciparum* (initial parasitaemia, 0.2 to 0.25 %), as described above. Control set with chloroquine-administered serum could eliminate the parasites completely. However, sera collected after administration of *Artemisia valgaris* extract and *Mamjwe Gan Vati* (an ayurvedic drug) did not inhibit growth of the parasites. Further studies are in progress.

n) Structure activity relationship of tyrosinase seeking compounds: potential chemotherapeutic agents for melanoma

Melanogenesis involves the conversion of mono-and diphenols such as tyrosine and dihydroxyphenylalanine to quinones. This metabolic pathway can be exploited for the preparation of chemotherapeutic agents for the treatment of melanomas and other pigmentary disorders. A group of 12 Tyrosinase seeking compounds have been prepared and are being screened for their anti-melanoma effects using melanotic B-16 melanoma cells and non-melanotic fibroblasts in culture. Preliminary studies have indicated that two of these compounds have the potential to become good anti-melanoma therapy agents and are being investigated further.

COLLABORATORS

COLLABORATIORS

A. Projects submitted for financial support from other agencies:

Plasticware for use in Biological research - surface modification of plastics for biomedical applications.	Dr. S. Sivaram, NCL, Pune Dr. S. P. Vernekar, NCL, Pune Dr. A. N. Bhisey, CRI, Bombay Dr. S. G. Gangal, CRI, Bombay Dr. Ulhas V. Wagh
Development of column type bioreactors	Prof. J. B. Joshi, University of Bombay Dr. M. S. Patole
Cryopreservation and revival of viable heart valves for trans-plantation	Dr. A. P. Chaukar, Sion Hospital, Dr. Alka Gogate, Bombay Dr. M. V. Mojamdar Dr. Kamala Gopalkrishnan, IRR, Bombay.
Fetal liver infusion - its mode of action, efficacy of cryopreserved cells its application in manage-ment of cancer	Dr. V. Kochupillai, AIIMS Dr. Lalit Kumar, Delhi Dr. (Mrs.) V. P. Kale
An integrated approach for development and production of anticancer compounds; Taxol and its analogues	Dr. T. Ravindranathan Dr. B. A. Nagasampagi Dr. K. V. Krishnamurthy, NCL Dr. M. C. Srinivasan Dr. S. K. Rawal Dr. Sujata Guharoy Dr. R. R. Bhonde

In vitro studies on the role of growth factors on melanosome transfer from melanocytes to keratinocytes in the epidermis	Dr. G. Raman, HLRC, Bombay Dr. M. V. Mojamdar
Molecular oncology of breast cancer in high risk ethnic groups.	Prof. A. Therwath, University of Paris, France Dr. Vatsala Doctor, Breach Candy Hospital and Research Centre, Bombay. Dr. Ulhas V. Wagh
Establishment of permanent breast cancer cell lines from high-risk ethnic groups: in vitro studies of breast epithelial cell carcino-genesis	Prof. A. Therwath, University of Paris, France Dr. F. Feuilhade Dr. J. M. Chiplonkar
Dietary essential fatty acids, caloric intake and outcome of schizophrenia	Dr. Ulhas V. Wagh Prof. Sahebrao P. Mahadik Dr. Vijay Debsikdar Dr. Sukhdeo Mukherjee Dr. Prabhakar Ranjekar Dr. Harbans Lal Dr. R. R. Bhonde Dr. (Mrs.) V. S. Gupta Dr. Mohan Wani

B. NFATCC has established linkages with scientists and clinicians from different institutes and hospitals. The following list summarizes these collaborations:

ARMED FORCES MEDICAL COLLEGE,
Stavely Road, Pune 411 040.
Col. Ranga Rao, Command Hospital.

B. J. MEDICAL COLLEGE AND SASSOON GENERAL HOSPITALS,
Jay Prakash Narayan Road, Pune 411 001.
Prof. V. M. Bapat,
Department of Pathology,
Prof. A. V. Jamkar,
Department of Surgery.

BREACH CANDY HOSPITAL AND RESEARCH CENTRE,
60, Bhulabai Desai Road, Bombay 400 026.
Dr. Vatsala Doctor, Pathologist.

BHARATI VIDYAPEETH AYURVED COLLEGE,
Near More Vidyalaya, Paud Road, Pune 411 029.
Prof. B. R. Mardikar, Principal,
Prof. and Head, Department of Pharmacology (Ayurvedic and Allopathy)

CANCER RESEARCH INSTITUTE
Dr. Borges Marg, Parel, Bombay 400 012.
Dr. S. G. A. Rao, Scientist, Stem Cell BioloNgy Division.

HINDUSTAN LEVER RESEARCH CENTRE,
Chakla, Andheri (E), Bombay.

Dr. G. Raman, Scientist.
Dr. Virendra Sheorain, Scientist.

INDIAN RED CROSS SOCIETY,
Dholka Branch, Dholka, Ahmadabad.

Mr. G. Mazumdar.

JANAKALYAN EYE BANK,
Narayan Peth, Pune 411 030.

Dr. R. D. Kolte
Dr. D. B. Wani.

JEHANGIR HOSPITAL,
Sassoon Road, Pune 411 001.

Dr. R. L. Marathe, Pathologist.

K. E. M. HOSPITAL,
Sardar Mudliyar Road, Rasta Peth, Pune.

Prof. B. B. Gokhale,
Department of Dermatology,
Dr. Y. V. Tawade,
Department of Dermatology.

LOKMANYA TILAK MEDICAL COLLEGE
Sion, Bombay 400 022.

Prof. A. P. Chaukar, Prof. & Head,
Department of Cardio-Vascular
and Thoracic Surgery.
Prof. Alka Gogate, Prof and Head,
Department of Microbiology.

LABORATOIRE D'ONCQLOGIE MOLECULARE
UNIVERSITITE PARIS 7,
2, PLACE JUSSIEU, PARIS 75005, FRANCE

Prof. A. Therwath.

MALARIA CONTROL UNIT, Pune.

Mr. J. M. More, Officer-in-Charge.

NATIONAL CHEMICAL LABORATORY,
Pashan, Pune 411 008.

Dr. P.K. Ranjekar, Department of Biology and Genetic Engineering, Biochemical Division.
Dr. K. N. Ganesh, Department of Organic Chemistry,
Dr. Shivaraman, Head, Polymer Chemistry Division,
Dr. S. P. Vernekar, Polymer Chemistry Division.
Dr. B. D. Tilak, National Filaria Control Unit, Papdi, Vasai, Thane.

NATIONAL FILARIA CONTROL UNIT
At Post Papdi, Vasai, Thane.

Dr. S. B. Dongre, Filaria Officer.

RUBY HALL CLINIC,
D.P. Road, Pune 411 001.

Dr. A. R. Bhide, ENT Surgeon,
Dr. D. Ghosh, Department of Gynaecology.
Dr. (Mrs.) Sangeeta Wagh, Department of Ophthalmology.

SHREE KRIPAMAYEE INSTITUTE FOR MENTAL HEALTH,
P. O. Wanlesswadi 416 414.

Dr. Vijay Deb Sikdar,
Asst. Director.

SCHOOL OF MEDICINE,
Augusta, Georgia 30912-3800.

Prof. Sahebrao P. Mahadik,
Department of Psychiatry.

TATA INSTITUTE FOR FUNDAMENTAL RESEARCH,
Homi Bhabha Road, Colaba, Bombay 400 005.

Dr. L. C. Padhee,
Department of Molecular Biology.

UNIVERSITY OF POONA,
Ganeshkhind Road, Pune 411 007.

Prof. D. N. Deobagkar,
Department of Zoology.
Prof. Subhash Padhye,
Department of Chemistry.

UNIVERSITY DEPARTMENT OF CHEMICAL TECHNOLOGY,
Kidwai Marg, Matunga, Bombay 400 032.

Prof. J. B. Joshi, Department of Chemical Technology.

VATSAL HOSPITAL
Pune.

Dr. N. Yardi.

PUBLICATIONS / CONFERENCE / WORKSHOPS / AWARDS

PUBLICATIONS / CONFERENCES / WORKSHOPS / AWARDS

PUBLICATIONS:

1. GARG S, Y CHANDRASHAKHER, **K S NANDA KUMAR**, N K GANGULY AND I S ANAND.
Antistreptokinase antibodies before and after streptokinase therapy in patients with acute myocardial infarction from areas endemic for streptococcal infection and influence on reperfusion therapy.
*Am. J. Cardiology, **74**: 187-189 (1994).*

2. **P B PARAB**, G R RAJASEKHARIAH, N WEISS AND D SUBRAMANYAM. *In vitro* protection against *B. malayi* infection by monoclonal antibody MAb 46.08.76.
Proc. CSIR Golden Jubilee Symposium on Trop. Diseases Mol. Biology and Control strategies. Published by Publications and Information Directorate, CSIR. Edited by Sushilkumar, A K Sen, G P Datta and R N Sharma. p. 77. April 1994.

3. **PADMA SHASTRY** *In vitro* Neuro 2a cell assay with enzyme-linked toxin for quantitation of antitetanus antibodies. A comparison with *in vivo* mouse neutralization assay.
In vitro Toxicology 7(4): 321-327 (1994).

4. N RAJARAM, **R N DAMLE** S H ADVANI S G GANGAL.
T cell hyporesponsiveness in Hodgkin's disease: Frequency distribution of Interleukin-2 producing cells and quantitation of IL-2 produced per cell. *Indian J. Biochem. Biophys. 31: 225-230 (1994).*

5. **P B PARAB**.
Anti insulin hybridoma retains functional characteristics in goat serum and soybean lipids.

In vitro cellular and animal developmental biology ***31 A:*** *1-3 (1995)*

6. **K S NANDA KUMAR**, N K GANGULY, I S ANAND, Y CHANDRASHAKHER AND P L WAHI
Salivary specific antibodies in relation to adhesion of *Streptococcus pyogenes* to pharyngeal cells of patients with rheumatic fever and rheumatic heart disease
Advances in Mucosal Immunology Part A, pp. 677-679. Plenum Publication Corporation, New York. (In press).

CONFERENCES / SEMINARS:

1. **P B PARAB**
IVth National Symposium on Vectors and Vector Borne Diseases - 1994. Regional Medical Research Center, Bhubaneshwar , Orissa. 19th to 22nd September 1994.
Presented a paper entitled "Detection of *Setaria digitata* specific IgG4 subclass antibodies in lymphatic filarial sera".

2. **R R BHONDE** AND V SHEORAIN
16th International Congress of Biochemistry and Molecular Biology. New Delhi. 19th to 22nd September 1994.
Presented a paper entitled "Comparative testing of oral hypoglycaemics - development of an in vitro method".

3. **K S NANDA KUMAR**
VIIIth National Congress of Indian Association of Medical Microbiologists. Armed Forces Medical College, Pune from 12th - 14th November 1994.
Presented the work at the conference (work carried out NFATCC)

3. **P B PARAB** AND D P THAKUR

VIIIth National Congress of Indian Association of Medical Microbiologists. Armed Forces Medical College, Pune from 12th - 14th November 1994.

Presented a paper entitled "Evaluation of a rapid Dot-ELISA test for diagnosis of cystic hydatid disease" (presented by Dr. D. P. Thakur of B. J. Medical College, Pune).

4. **PADMA SHASTRY**

VIIIth National Congress of Indian Association of Medical Microbiologists. Armed Forces Medical College, Pune from 12th - 14th November 1994.

Presented a paper entitled "A *in vitro* test measurement of neutralizing antibodies to tetanus toxin (TT) - an alternative to animal model".

5. **DIPSHIKHA CHAKRAVORTTY**

VIIIth National Congress of Indian Association of Medical Microbiologists. Armed Forces Medical College, Pune from 12th - 14th November 1994.

Presented part of the research work.

6. **PRAKASH DESHPANDE**

IVth symposium on vectors and vector borne diseases. Bhubaneshwar. 12th to 15th November 1994.

Presented a paper entitled "Effect of some biotic and abiotic factors on the population density of cyclops faunal complex transmitting guinea worm disease".

7. **R R BHONDE**

LIFE 2000 International Symposium and Exhibition, World Trade Centre, Bombay. 22nd to 25th November 1994.

Delivered Key-note Address on "Biotechnological Advances".

8 **R R BHONDE**
National Symposium on Healthy Animals, Safe Foods and Healthy Man, Bombay Veterinary College, Bombay. 27th to 29th December 1994.
Presented a paper entitled "Recent advances in modulation of tissue immunogenicity prior to transplantation - role of tissue culture and cryopreservation".

9. **L. Limaye**
XVIIIth All India Cell Biology Conference and Symposia, NBRI, Lucknow, 13th to 15 February 1995.
Presented a paper entitled "Cryopreservation of human bone marrow: Improved recovery due to various additives i the freezing solution".

WORKSHOPS:

1. **ANJALI SHIRAS**
International Training Workshop on Gene Cloning organized by SAARC at the Centre for Excellence in Molecular Biology, University of Punjab, Lahore, Pakistan. 30th April to 13th May 1994.

2. **R N DAMLE**
Workshop of confocal laser scanning microscopy at CCMB, Hyderabad. 31st August to 1st September 1994.

3. **PRAKASH DESHPANDE**
Workshop on cultivation of parasites of biomedical importance. CDRI, Lucknow. 28th November to 10th December 1994.

4. **DIPSHIKHA CHAKRAVORTTY**
Workshop on Biotechnological detection of enteric diseases. All India Institute of Medical Sciences, New Delhi.

AWARDS:

1. **K S NANDA KUMAR**
Received V. K. Saini Gold Medal in Cardiovascular Diseases from the Post Graduate Institute of Medical Education and Research, Chandigarh on 29th November 1994.

2. **P B PARAB**
Awarded Membership of the New York Academy of Sciences, New York, U. S. A.

3. **P B PARAB**
Awarded a Short Term Overseas Fellowship from the Department of Biotechnology, Government of India. Visited the Infectious Diseases Division, Jewish Hospital at Washington University Medical Center, St. Louis, MO, U. S. A. February 1995 to May 1995.

HUMAN RESOURCE DEVELOPMENT

HUMAN RESOURCE DEVELOPMENT

i) The staff of NFATCC continued to participate in the teaching programme of M. Sc. Biotechnology conducted by Dept. of Zoology, University of Poona. The staff also delivered lectures and conducted practicals at the Refreshers' Course in Zoology, organized by the Department of Life Sciences, North Maharashtra university, Jalgaon, during 19th December 1994 to 7th January 1995.

ii) Five students are working towards obtaining Ph. D. degree on topics such as stress proteins, cell characterization by mitochondrial DNA molecular biology of hexokinase, effect of cytokine on nerve cells, etc.

iii) NFATCC and Department of Zoology, Goa University jointly conducted a workshop on "State of Art - Animal Tissue Culture" and the Goa University, during 2nd to 4th February 1995.

iii) Following individuals were deputed for training at NFATCC:

No.	Name of the trainee	Deputing institute	Period of training
1.	Mrs. Shubhangi Koparkar	Garware College, Pune.	13-6-94 to 23-6-94
2.	Dr. M. M. Rajan	IIT, Bombay	15-6-94 to 15-7-94
3.	Mr. S. Dhammarajan	Toticorin Research Centre for CMFRI Tuticorin.	4-7-94 to 8-7-94

4.	Mr. Mahadevi Mustafa	Krishi Mitra Bioproducts, Pune.	12-7-94 to 23-7-94
5.	Ms. S. S. Wagh	Hindustan Lever Ltd., Bombay	29-8-94 to 2-9-94
6.	Ms. Anupama Sharma	Hindustan Lever Ltd., Bombay	29-8-94 to 2-9-94
7.	Mr. M. M. Patel	Vaccine Institute Vadodara.	7-11-94 to 21-11-94
8.	Mr. P. R. Rawal	Vaccine Institute Vadodara	7-11-94 to 21-11-94
9.	Dr. L. S. Lakra	Central Institute of Fisheries, Bombay.	4-12-94 to 23-12-94
10.	Ms. Truypti Goankar	L. T. M. Medical College, Bombay.	12-12-94 to 22-12-94
11.	Ms. Shashirekha Shetty	L. T. M. Medical College, Bombay.	12-12-94 to 22-12-94
12.	Ms. Manisha Joshi	North Maharashtra University, Jalgaon.	15-12-94 to 31-12-94
13.	Dr. P. Karmarkar	Institute of Post-graduate Medical Education and Research, Calcutta.	16-2-95 to 28-2-94

SUPPORTING UNITS

a) Computers and data base

b) Library and documentation

c) Animal house

d) Instrumentation and maintenance

SUPPORTING UNITS

a) Computer and database

Computer unit upgraded 6 PC's to PC 486 SX and added two Nos. 486 and a Dot Matrix Printer. Multimedia sound blaster kit from Creative Lab was procured for multimedia Computer aided learning development. Many areas identified for computerization and software developed. Statistical and plotting software were added for statistical analysis and plotting of scientific data.

Planning for computerization at NFATCC University campus taken up. 100 node LAn designed and cabling work is in last stages of completion. User awareness programs conducted from time to time. Special training program for Windows, MS-Office etc. conducted.

b) Library and documentation

Library has continued to offer computerised services such as CAS/SDI and providing information consolidation/repackaging services viz. Biovision, Press Vision etc. to provide precise information having relevance in NFATCC activities. Apart from the renewal of MEDLINE & BIOTECHNOLOGY CD-ROM databases for the year 1995, it has continued to provide On-Line access to the major bibliographical databases via. DIALOG information system.

The ERNET facility has been introduced, which will enable the library to access INTERNET through IUCAA, Pune node for accessing to worldwide scientific information instantly.

c) Animal house

The Animal House for small laboratory animals became fully functional in the 'Jidnyasa' building. Humidity ventilation and temperature is

regularly monitored and maintained. Health monitoring of animals is done regularly by faecal sample examination for parasite load, blood examination for haemoprotozoans and skin and hair is examined for mites and other ectoparasites. All the animals used for various experiments were healthy and were free from the above infection.

The details of the animals procured from National Institute of Virology, bred in animal house and supplied for various R & D activities is given below.

1. RAT			
Wistar	28	12	51
C.F	8	--	8
2. MICE			
C578L	--	--	6
Balb/c	163	228	330
Swiss	30	111	122
nu/nu	23	--	23
3. MASTOMYS	9	--	9
4. RABBIT		2 --	2
5. GERBILS		4 --	4

d) Instrumentation and maintenance

Day to day required services to the instruments installed at Jopasana & Jydnyasa maintenance & upkeeping of allied services.

Production of Liquid Nitrogen

Services (Sale of) Liquid Nitrogen to research oriented institution. Co-ordiantion with DAE/Architect Interior designer, scientist users for organizational set up of new building at Pune University campus related to Civil / Electrical / Mechanical / Scientific / Computer / Horticulture / Furniture etc.

The building of Animal house civil works is nearly complete, laboratory building B & C wings civil work 90% completed. Installation of compressor and chilling plant over trials awaited. Procurement of DBS, panel transformer, elevators, bumbwaiters are delayed and will be over within 3 months period. MSEB has laid single cable to charge the transformer additional feeder is assured within due course. Internal approach roads are under construction and BM is over- asphalting awaited.

In want of clearance of site horticulture job is yet to start. Tendering of Interior & furniture work is in progress.

Tendering of additional water tanks Solar water system, underground rising pipelines from bore well etc. is in progress with department of Atomic Energy.

ADMINISTRATIVE DESK

a) Construction of NFATCC laboratory complex on Poona University campus :

b) Composition of staff :

ADMINISTRATIVE DESK

Construction of NFATCC laboratory complex on Poona University campus:

Prescribed time schedule is being adhered to, for the construction activities of the NFATCC Laboratory complex on Poona University campus and the construction is nearing completion.

Meetings were arranged every month of the Co-ordinating Committee consisting of the representatives of C & S Group, DAE, NFATCC and the architect. The Committee periodically reviewed, discussed, controlled and monitored the progress of construction activities.

A brief outline of the progress of the construction activities during the year is as follows:

1. The construction of the Animal House building has been completed and the taking over formalities are being completed.

2. The construction of the main laboratory is almost complete. Flooring, tiling etc. at the complex are in progress, on completion of which final touches would be given to electrical wiring, power points and connection.

3. The construction of the Hostel and Guest House buildings is fast nearing completion. The slab work and brick work of the residential buildings and the Director's Bungalow has been completed and the internal and external plastering of the walls is in progress.

4. The landscaping and the interior designing (including the furniture) works to be undertaken have been tendered and awarded. The works are progressing with good momentum and are expected to be completed as per the envisaged time schedule.

5. The equipments and other infra-structural facilities for the laboratory and Animal House buildings have been planned, tendered and ordered. The delivery of these items is being dovetailed with the completion schedule of
the buildings.

6. The 320 KVA D. G. Set has been shifted from the interim facility and has been mounted on the foundation at the complex site. The electricity supply for the buildings would be obtained from MSEB on commissioning of the new sub-station established for the purpose.

7. Various support activities / arrangements such as canteen, garbage disposal, transport with the campus has been meticulously planned and their execution is being monitored.

Composition of staff:

Following is the staff complement as on 31st March 1995:

No.	Category	Number of persons
1.	Scientific Staff	17
2.	Technical Staff Laboratory	14
	Instrumentation and Maintenance	08
3.	Administrative Staff	13
4.	Auxiliary Staff	21
	Total	73
5.	Research Associates and Research Fellows	03
6.	Contractual Services	
	Jopasana and Jidnyasa	19
	University Construction Site	25

DISTINGUISHED VISITORS

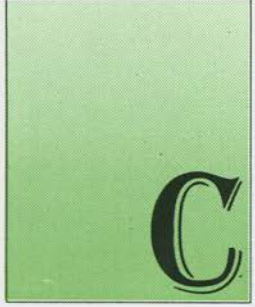
1. Dr. H. Y. Mohan Ram	Professor, Department of Botany, University of Delhi, Delhi 110 007.
2. Dr. Yair Degani	Head, Life Sciences Division, Ministry of Science, Israel.
3. Dr. Ashok B. Vaidya	Medical Research Director, CIBA Research Centre, Bombay 400 063.
4. Dr. Vinod Patel	Torrent Pharma Ltd., Ahmadabad 9.
5. Dr. D. G. Hapse	Director, Dnyaneshwar Foundation, Pune.
6. Dr. S. F. Patil	Dean, Science Faculty, University of Poona, Pune.
7. Dr. Mukund J. Modak	Professor, New Jersey Medical School, Newark, NJ, USA.
8. Dr. M. Rajashekhar	Project co-ordinator, ICAR Project on Disease Survillence, Hebbal, Bangalore.
9. Dr. Ramakant Pilani	Ganesh Benzoplast Ltd., Bombay.

10. Dr. A. N. Inamdar	Professor and Head, Biochemistry Department, Institute of Science, Bombay.
11. Dr. S. Subramaniam	Professor Emeritus, Microbiology, Madras University, Madras.
12. Dr. Ranajit Ray	St. Louis University, St. Louis, MO, USA.
13. Dr. M. Shoyab	University of Washington, Seattle, WA, USA.
14. Dr. Satish K. Gupta,	National Institute of Immunology, New Delhi 110 067.
15. Dr. Manorama Bhargava	Professor and Head, Department of Haematology, All India Institute of Medical Sciences, New Delhi.
16. Dr. K. Nachimuthu	Professor and Head, Department of Animal Biotechnology, Madras Veterinary College, Madras.
17. Dr. Ferzaan N. Engineer	R & D Manager, Core Pharmaceuticals, Ltd., Ahamadabad.

Figure 15 LIFE 2000 exhibition.

Figure 16 National Science Day celebration.

Figure 17 Staff Welfare Society Annual picnic to Rahuri.



CELL SUPPLY SERVICE

CELL SUPPLY SERVICE

During the current year, cell lines were supplied to the following institutes. There has been an increase in the number of users this year.

1	Astra Research Centre, Bangalore	1
2	Air Force Hospital, Kanpur	1
3	Armed Forces Medical College, Pune	2
4	Amala Cancer Research Centre, Trichur	6
5	AICRP on Animal Disease Monitoring, Bangalore	8
6	All India Institute of Medical Sciences, Delhi	68
7	Banaras Hindu University, Varanasi	1
8	Bharat Immunological & Biologicals, Bulandshahr	2
9	Breach Candy Medical & Biological, Bombay	2
10	B. J. Medical College, Ahmedabad	3
11	Bombay Veterinary College, Bombay	6
12	BAIF Development Research Foundation, Pune	12
13	College of Veterinary Sciences, Hissar	2
14	Centre for Biotechnology, Chandigarh	2
15	Central Leather Research Institute, Madras	4
16	Centre for Biochemical Technology, Delhi	4
17	Chittaranjan National Cancer Institute, Calcutta	5
18	Central Agricultural Research Institute, Port Blair	5
19	Central Drug Research Institute, Lucknow	6
20	Centre for Cellular & Molecular Biology, Hyderabad	10
21	Cancer Institute (WIA) Madras	12
22	College of Veterinary Sciences, Pantnagar	12
23	College of Veterinary Sciences, Tirupati	13

24	Cancer Research Institute, Bombay	48
25	Defence Institute of Physiology and Allied Sciences, Delhi	4
26	Defence Research & Development Est., Gwalior	13
27	Dabur Research Foundation, Gazziabad	16
28	Dr. Reddy's Research Foundation, Hyderabad	18
29	Gujarat Veterinary College, Anand	2
30	Guru Nanak Dev University, Amritsar	8
31	Haffkine Institute for Training, Bombay	3
32	Himachal Pradesh Krishi Vishva, Himachal Pradesh	3
33	Hindustan Lever Limited, Bombay	4
34	Hoechst India Ltd., Bombay	6
35	Indian Veterinary Research Institute, Bangalore	1
36	Institute of Veterinary Preventive, Tamilnadu	1
37	Indira Gandhi Centre for Atomic Research, Kalpakkam	1
38	Indian Institute of Technology, Kanpur	2
39	Institute for Research in Reproduction, Bombay	2
40	Institute of Microbial Technology, Chandigarh	2
41	Indian Immunologicals, Hyderabad	2
42	Institute of Animal Health & Veterinary, Calcutta	2
43	Indian Institute of Toxicology, Bombay	2
44	Institute of Pathology, Delhi	3
45	Institute of Toxicology, Bombay	3
46	Institute of Immunohaematology, Bombay	3
47	Institute of Nuclear Medicine, Delhi	5

48	Indian Institute of Chemical Biology, Calcutta	8
49	Indian Veterinary Research Institute, Bombay	11
50	Indian Institute of Science, Bangalore	35
51	Jawaharlal Institute for P. G. Medi. Edu & Research, Pondicherry	3
52	Jamia Hamdard University, New Delhi	4
53	Jawaharlal Nehru University, New Delhi	5
54	Kendriya Sainik Pashuchikitsa Prayogshala, Meerut	4
55	King Institute of Preventive Medicine, Madras	5
56	Kasturba Medical College, Manipal	15
57	L.T.M. Medical College, Bombay	1
58	Maulana Azad Medical College, New Delhi	2
59	Malaria Research Centre, ICMR, Delhi	10
60	Marathwada Agricultural University, Parbhani	10
61	Madurai Kamaraj University, Madurai	15
62	Madras Veterinary College, Madras	18
63	National Institute of Nutrition, Hyderabad	1
64	National Research Centre on Equines, Haryana	1
65	National Centre for Biological Science, Banagalore	1
66	National Institute of Occupational Health, Ahmedabad	2
67	Nizam's Institute of Medical Science, Hyderabad	3
68	National Environmental Eng. Research, Nagpur	6
69	National Institute of Virology, Pune	10
70	National Institute of Mental Health and Neural Science, Bangalore	15

71	National Institute of Cholera & ENT Diseases, Calcutta	19
72	National Institute of Immunology, New Delhi	25
73	Osmania University, Hyderabad	2
74	Punjab Agricultural University, Ludhiyana	15
75	P. G. Institute of Medical Education, Chandigarh	16
76	Regional Cancer Centre, Thiruvanthapuram	4
77	Ranbaxy Laboratories, Ltd., Delhi	9
78	Shantha Biotechnics Pvt. Ltd., Hyderabad	1
79	Shree Chitra Tri. Inst. of Med. Sciences, Thiruvanthapuram	4
80	Sanjay Gandhi P. G. Inst. of Medical, Lucknow	13
81	Seth G. S. Medical College and K.E.M. Hospital, Bombay	13
82	The Foundation for Medical Research, Bombay	1
83	The Gujarat Cancer and Research Institute Ahmedabad	1
84	Tata Institute of Fundamental Research, Bombay	1
85	Tuberculosis Research Centre, Madras	3
86	Tribunal Institute of Medical Science, Thiruvanthapuram	4
87	Tropical Botanic Garden & Research, Trivandrum	5
88	Tata Institute of Fundamental Research, Bangalore	15
89	Tata Memorial Hospital, Bombay	19
90	University of Kerala, Thiruvanthpuram	1
91	University of Agricultural Science, Bangalore	2

92	University of Calcutta, Calcutta	3
93	University of Madras, Madras	5
94	University of Hyderabad, Hyderabad	6
95	University of Haryana Agricultural, Hissar	7
96	University of Delhi, Delhi	14
97	University of Poona, Pune	20
98	Visva Bharati, Santiniketan	4
99	Vaccine Institute, Vadodara	6
100	Vallabhbhai Patel Chest Institute, Delhi	9