NATIONAL FACILITY
FOR
ANIMAL TISSUE AND CELL CULTURE

ANNUAL REPORT
1992-93

Ongoing construction of the NFATCC Laboratories
on the University of Poona Campus.

Cover Page : Endothelial Cells from human Saphenous Vein.

CONTENTS

NATIONAL FACILITY
FOR
ANIMAL TISSUE AND CELL CULTURE

DEPARTMENT OF BIOTECHNOLOGY
GOVERNMENT OF INDIA

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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 organisations.
- 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 organise 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 organisations including industries working in the country.
- To collaborate with foreign research institutions and laboratories and other international organisations 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

It has been a year full of memorable events and achievements for NFATCC. The Minister of State for Science & Technology Shri P.R.Kumaramangalam laid the foundation stone of the NFATCC building complex on the Poona University campus in the presence of many distinguished scientists, dignitaries and other well wishers. The construction work is progressing very well under the direction of the Director and chief engineer of C & S Group, Department of Atomic Energy, who are executing the construction work. Our new building "Jidnyasa" became fully functional with the shifting of the activities of Cell Repository and Supply services, Media Preparation, Library & Documentation, Computers, Instrumentation & Maintenance, Photography and Trainee Laboratory from 'Jopasana' where these were housed.

Three hundred and ten cultures were procured to add to the stock repository. Thus the stock of cell lines in the repository has reached 1127. Cell Repository is now geared up to meet the country's need of specialised cell lines for growing viruses including HTLV, manufacturing cell based vaccines and for immunological studies. It also has cells from many endangered species and hybridomas secreting monoclonal antibodies to specific antigens.

There has been increasing demand for the supply of cell lines. However the disruption in air services affected the supply activity. The supply service for vectors, plasmids and genomic libraries has been initiated.

As recommended by the experts, MK medium is being prepared and supplied to eye banks for mid term storage of cornea. Thus eye banks can extend the storage period of cornea upto 5 days instead of 2 days.

The technology for maintenance and cultivation of skin as organ culture and 3-D epithelia from human keratinocytes and its subsequent grafting to burns, nevi and vitiligo cases has been standardised and the results of grafting are promising. The studies on standardisation of technology for banking bone marrow cells and heart valves have been continued. The results are interesting leading to newer approaches towards cryopreservation of tissues.

Experimental studies on filariasis revealed that the monoclonal antibody F46 raised against cuticular antigens of *B. malayi* infective larvae also cross reacts with antigen from setaria,a cattle filarian. These findings pave way for conducting studies in antigenic characterisation and assessing the antigenic efficacy *in vivo* in terms of protection. The *in vitro* method of quantitation of functional antibodies to tetanus toxoid employing Neuro 2a cells was found to correlate well with the neutralization assay. This technique can substitute animal experimentation. An anti-insulin monoclonal antibody-secreting hybridoma CC9C10 was successfully adapted to goat serum and continues to secrete the antibody without loosing its specificity.

The newly established laboratory for molecular biology became functional and the studies on characterisation of cell lines based on genomic (nuclear) and mitochondrial DNA analysis, on immortalization of bone marrow cells by transfecting with oncogenes and on identification and isolation of melanoma specific oncogenes are progressing well. The Restriction Fragment Length Polymorphism (RFLP) pattern of mitochondrial DNA has been found to distinguish mosquito cell lines of *A. albopictus* from those of *A. stephensi*. Melanoma DNA transfected clones have been tested for the presence

of melanoma derived proteins by immunofluorescence. The earlier observation regarding role of erythropoietin leading to stimulation of bone marrow stromal cell proliferation has been confirmed.

The laboratory for cell biology is functional and has been screening some potential anticancer drugs using cell lines. Initiation of cultures from fastidious cells of buccal, oropharyngeal, breast, bone and uterine tissues has been undertaken, yielding good results.

The faculty continued to actively participate in the Biotechnology teaching programme of University of Poona, especially in animal tissue culture Biotechnology. Training was imparted to scientists and technicians deputed from various Indian laboratories in specific areas requested by them. Five students were working for their Ph.D. Degree.

Our greatful thanks to Prof. Sridhar Gupte,Vice Chancellor and other University of Poona officials for their excellent co-operation rendered towards the comming up of the NFATCC's R & D activities and institutes complex on their campus. Clinicians and scientists of various hospitals and institutes in and around Pune have actively participated in NFATCC's R & D activities and deserve our heartful thanks. We also thank Shri. D.K.Afzalpurkar, Additional Secretary, Department of Atomic Energy for his active interest and full support towards the construction and development of NFATCC. Our linkage with the University of Poona in terms of collaborations in Research activities, teaching and co-operating in the construction activity of NFATCC complex continues and got further strengthed during the year.

Taking into consideration various programmes in the field of Bio-Medical and Biotechnology which are being executed and are being initiated in the country, NFATCC has planned newer activities to support these programs to acheive the national goals expeditiously.

REPOSITORIES

- Cell cultures
- Obligate Parasites
- Vectors, Plasmids and Genomic libraries
- Media

REPOSITORIES

CELL CULTURE

NUCLEAR STOCK STORED IN LIQUID NITROGEN

310 cultures were procured as frozen ampoules in liquid nitrogen refrigerators from American Type Culture Collection, Maryland, Food and Drug Administration Cell Repository c/o ATCC,Maryland, AIDS Reagent and Research Program, Baltimore, USA and European Collection of Animal Cell Culture, Porton Down, UK. With this addition to previous stock of 817 cultures, the total number in the stock repository has reached 1127, comprising 594 different cell strains.

Now the repository has cultures in stock :

I. Which can be used for :
   1) isolation of viruses of medical and veterinary importance including HTLV,
   2) humoral and cellular immunological studies and raising hybrid cells of murine and human species,
   3) cell culture based vaccine preparation and quality control

II. Of different types covering
   4) EBV transformed HLA defined B lymphocytes,
   5) hybridoma cells producing monoclonal antibodies (85 hybridomas)
   6) cells from rare and endangered species.

STOCK FOR QUALITY CONTROL AND REDISTRIBUTION

A total of 118 cell lines were expanded for redistribution and quality control.

SUPPLY SERVICE

A total of 401 cultures comprising 118 different cell lines were supplied to different research teams from 84 institutes. (Fig.1 & 2) List of cultures available for supply was published in two Journals, *(Indian J Biochem. Biophys.* October, 1992 and *Indian J Exp. Biol.* October, 1992), so that it is readily available for reference to all the users. In addition about 180 registration forms along with the list of cell cultures available for distribution were sent to different institutions and individuals. A total of 144 individuals/departments from 87 institutes registered their names with NFATCC.

OBLIGATE PARASITES

The cultures from the obligate parasites repository are being revived and maintained to ascertain their viability particularly the *P. falciparum* culture strains. Some of the chloroquine resistant strains of *P. falciparum* were supplied to the researchers.

VECTORS, PLASMIDS AND GENOMIC LIBRARIES

To the stock of vectors, plasmids and genomic libraries a total of 51 new DNA probes and genomic libraries have been added. These were procured from American Type Culture Collection, Maryland Washington, USA. Thus the stock has 15 host strains, 51 plasmid bearing host strains, 4 phage vectors and 4 genomic libraries. Request for supply of these have started coming and two host strains of *E coli* were supplied this year.

CELL LINES SUPPLIED

NUMBER OF CULTURES

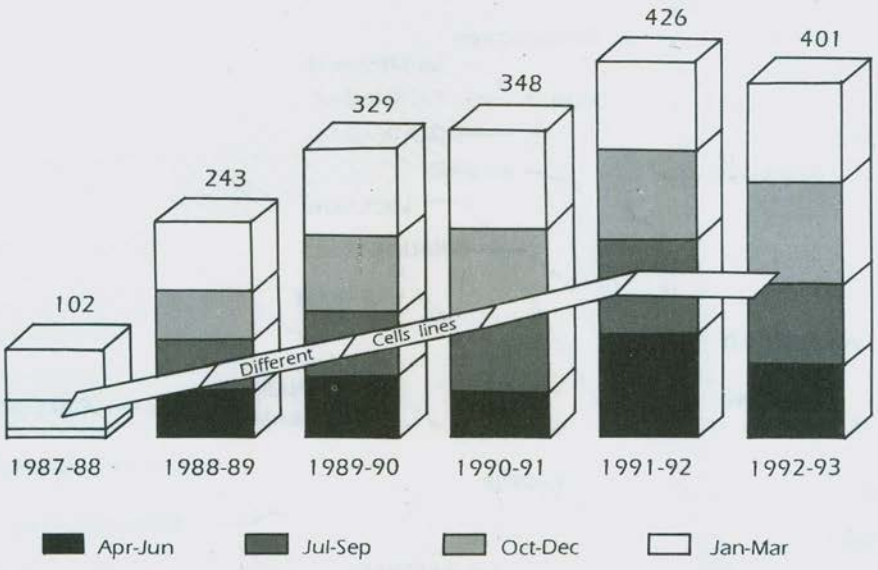
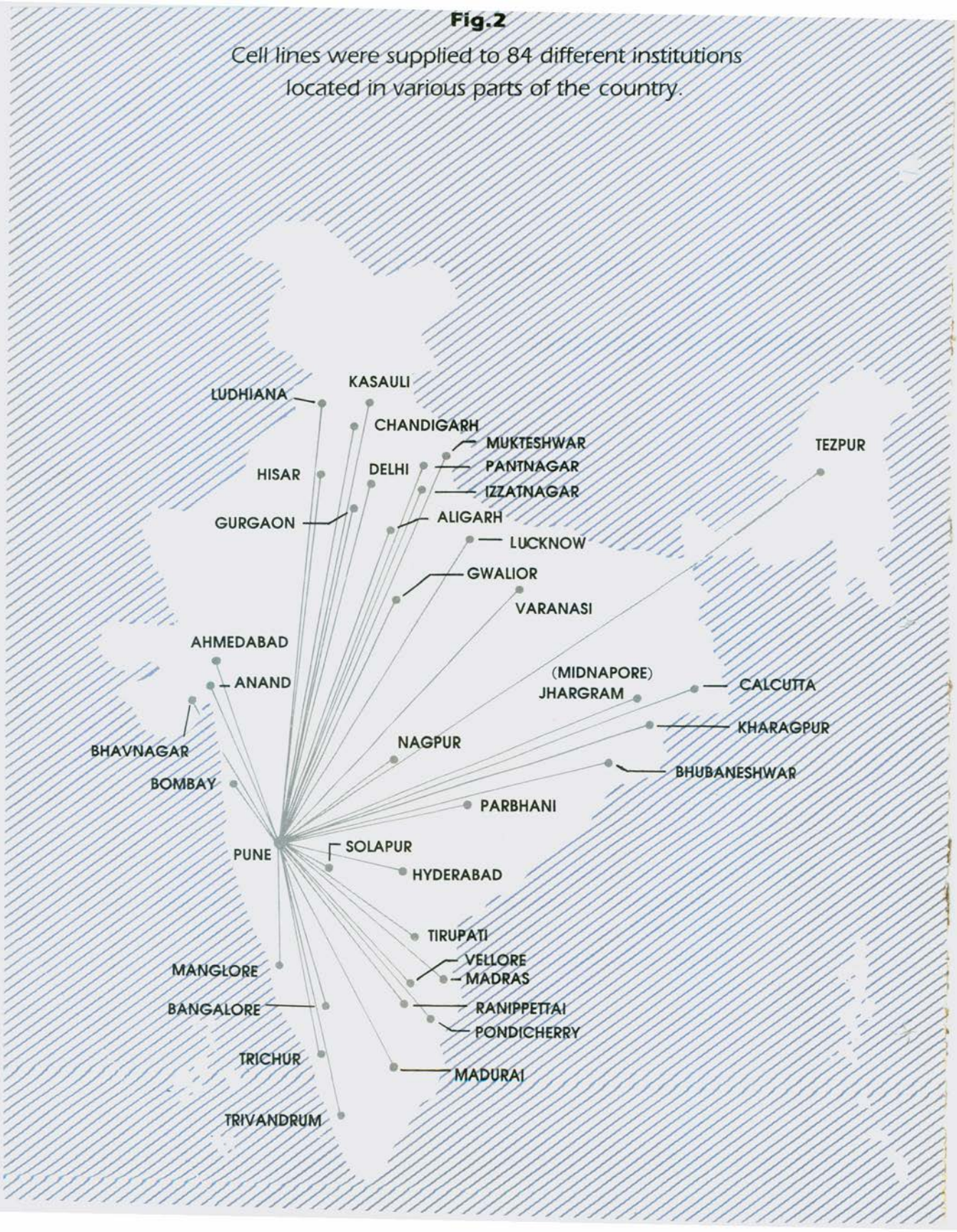


Fig.1 There has been a steady increase in the supply of cell cultures, both in number and variety of cell lines. (In the year 1992, due to disruptions in the Air Services, the supply was affected severely).

Fig.2

Cell lines were supplied to 84 different institutions located in various parts of the country.



MEDIA

Sixteen different synthetic media, various balanced salt solutions and tissue culture reagents were prepared. Goat blood for serum was procured and processed in our laboratories. The reagents and various batches of Foetal Calf Serum (FCS), Horse Serum (HOS), Goat Serum (GS) and New Born Calf Serum (NBCS) were tested for sterility, quality control and supplied to the staff members.

DEVELOPMENT OF TECHNOLOGY FOR TISSUE BANKING

- Cornea
- Skin
- Bone Marrow
- Heart Valves

Development of Technology for Tissue Banking

HIV SCREENING

Human blood samples from individuals whose tissues/cells were obtained for experimental work were screened for HIV-I by the commercially available microagglutination test kit. And from 1992 these samples are being screened both for HIV-I and HIV-II.

CORNEA

Out of 20 million (12 million bilaterally, 8 million unilaterally) blinds in the country, 3.41% of them require cornea transplantation. The eye banks use moist chamber methodology which permits storage of eye ball to a maximum period of 48 hrs. This period is too short to communicate to the expected recipients and in turn for them to reach the hospital for keratoplasty. Hence, there is a dire need to extend the storage period of cornea.

NFATCC has standardised technique permitting storage of cornea without loosing transparency and viability for a maximum period of 35 days. Hence the problem to evaluate these possibilities to implement this technology in the Indian Eye Banks was referred to experts.

The expert committee and the representatives of Indian Opthalmic Associations recommended that mid-term storage of cornea in MK medium (5 days) is the most appropriate technology suitable for implementation by various Indian Eye Banks. Hence this technology be adopted wherever possible replacing the present method of storage in moist chamber, and that provision be made to supply MK medium and transport containers by NFATCC at least until this is made available by commercial organisations. Thus MK medium is being prepared and supplied on request. Steps have also been taken to get a prototype of the transport container made.

STUDIES ON FREE RADICALS DURING FREEZING AND THAWING OF CORNEA

The most effective method for long term storage of cells and tissues is cryopreservation in liquid nitrogen (196°C below zero). There are reports which suggest that during freezing and thawing free radicals are generated which have detrimental effect on the cells. Hence it was felt desirable to undertake studies on this problem.

Cornea is an important yet a simple organ to study. The criteria used to assess degenerative changes of the cornea are loss of its transparency and viability of endothelial cells. Moreover long term preservation of cornea is also an important problem, particularly in the Indian subcontinent. Hence studies on generation of free radicals and its effect on cornea during freezing and thawing were undertaken.

Fresh chicken eye balls were collected from local shops. Corneas were surface sterilized, trephined and one batch was stored at –66° C in an ultra low temperature cabinet and the other batch was maintained at 37°C as an organ culture for different periods of time. The medium used was Minimum Essential Medium (MEM) supplemented with Foetal Calf Serum (FCS) at 10% level and glycerol was added at 10% level as a cryoprotectant. To assess the generation of free radicals, lipid peroxidation was estimated. It was observed that lipid peroxidation levels were higher at 37° C than at –66°C. Even at –66°C lipid peroxidation levels were higher compared to control (zero hour sample). It therefore appears that peroxidation occurs during freezing and thawing also (Fig 3). The role of antioxidants in the freezing medium is being studied.

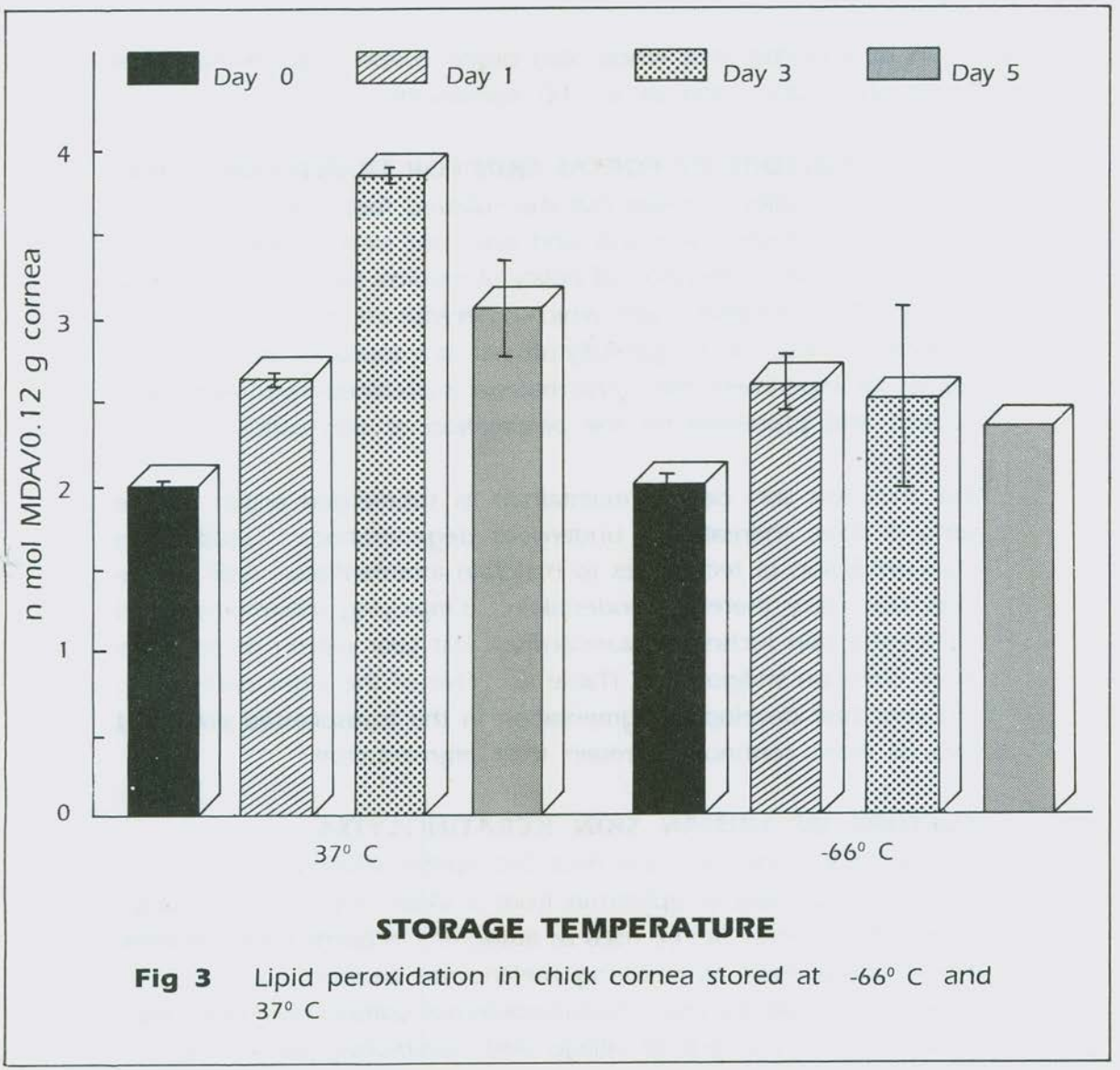


Fig 3 Lipid peroxidation in chick cornea stored at -66° C and 37° C

SKIN

Skin can be cultured as a whole skin organ culture, intermediate split thickness skin culture and as a 3-D epithelium.

ORGAN CULTURE OF FOETAL SKIN FOR TRANSPLANTATION

Our earlier studies revealed that the cultured foetal skin, particularly upto first trimester takes well and does not elicit immune rejective reactions. Due to adoption of policy of medical termination of pregnancy (MTP) the foetal tissues which otherwise are discarded are now available. These can be gainfully utilised as a source of viable human tissues for experimentation. The material is obtained from recognized centres and processed for the preparation of skin culture.

Full thickness skin can be maintained as submerged organ culture upto 8 days, thereafter it undergoes degeneration. Studies on standardisation of techniques to maintain intermediate - split - thickness skin were therefore undertaken. Employing intermediate-split - thickness skin technique standardised last year eleven more grafts were done on vitiligo cases (Table I). Five of the eight cases done last year had developed pigmentation in the transplanted area and two of them continue to retain their pigmentation.

CULTURE OF HUMAN SKIN KERATINOCYTES

Culture of keratinocytes and their 3-D epitheliation enables one to grow a large sheet of epidermis from a small biopsy. Such large sheets of epidermis can be used as autografts in burns cases. Several modifications such as culturing melanocyte bearing epithelia and intermediate split thickness dermis-epidermis composite culture etc., enables the treatment of vitiligo and non-healing ulcer cases.

Keratinocytes obtained from biopsies were cultured and 3-D epithelial sheet was prepared. The media modifications needed for such cultures

TABLE I

Follow up of vitiligo cases grafted with organ cultured foetal skin.

Case No.	Sex/Age	Pigmentation In		Follow up (months) REMARKS
		Grafted Area	Control Area	
1	M/60	Retained	No	(23) lost to follow up
2	F/21	Sepsis	No	(-)
3	M/25	Transient	No	(33) Total depigmentation of the grafted area by 24 months
4	F/19	Retained	No	(20) lost to follow up
5	F/19	Transient	No	(26) Depigmentation started by 16 months. At present the grafted area is totally depigmented.
6	M/54	Retained	No	(24) Continues to retain pigmentation
7	M/16	Transient	Retained	(24) Both areas are now depigmented
8	M/20	Retained	No	(18) Pigmentation is still retained
9	M/17	Transient	No	(9) Depigmentation of the grafted area is going on
10	F/17	Sepsis	Mottled Pigmentation	(9) Mottled pigmentation of control area continues
11	F/17	Transient	No	(8) Depigmentation of the grafted area continues
12	M/17	Retained	No	(7)
13	F/36	Sepsis	No	(7)
14	F/18	Sepsis	No	(6)
15	F/36	Retained	No	(1)
16	F/19	Retained	No	(1)
17	F/33	Retained	No	(1)
18	F/19	Retained	No	(1)
19	F/18	Retained	No	(1)

P.S. 1. Informed consent has been obtained from all the patients.
2. Dermabrasion only served as controls.

were standardised. Methods for melanocyte bearing epithelia and 3-D - dermis from monolayer fibroblasts were standardised and grown successfully. Clinical application of the skin cultivated by these methods has now been initiated.

Grafting of the cultured 3-D epithelia was initiated using burn cases. So far only two cases were treated. Two different methods were used for lifting of the 3-D epithelia from the culture dish and for preparation of the recipient wound bed for grafting. It was found that stretching autoclaved micropore tape over the culture was better than white petrolactum gauze for lifting the 3-D epithelia as a sheet, without disrupting them into individual cells. A minimum of 24 hours wound granulation tissue seems to help successful graft takes as compared to dermabrasion alone.

Culture of nevus free epithelia for transplantation : A case of congenital giant hairy nevus syndrome was taken up for growing nevus free cultured epithelia for transplantation. A 2.5 cm^2 biopsy of the nevi skin was surgically removed. The dermis with nevi cells were removed enzymatically and nevi free epidermis was cultured to make a 25cm^2 epithelial sheet. This was grafted onto the wound bed and keratinocytes were found to migrate from the 25cm^2 graft and fill up the entire 100 cm^2 wound bed. No nevi are seen in the transplanted area upto six months of post operative period. (Fig 4-8).

Melanocyte bearing epithelia : Melanocyte bearing epithelia was grown from a normal skin of a case of vitiligo and has been grafted to vitiligenous patch as autograft. Results are awaited.

GROWTH CHARACTERISTICS OF NORMAL AND ABNORMAL MELANOCYTES AND THEIR RESPONSE TO SYNTHETIC AND NATURAL GROWTH FACTORS

Almost 3% of the world's population suffers from one or the other form of pigmentary disorder. These include vitiligo, melanoma and

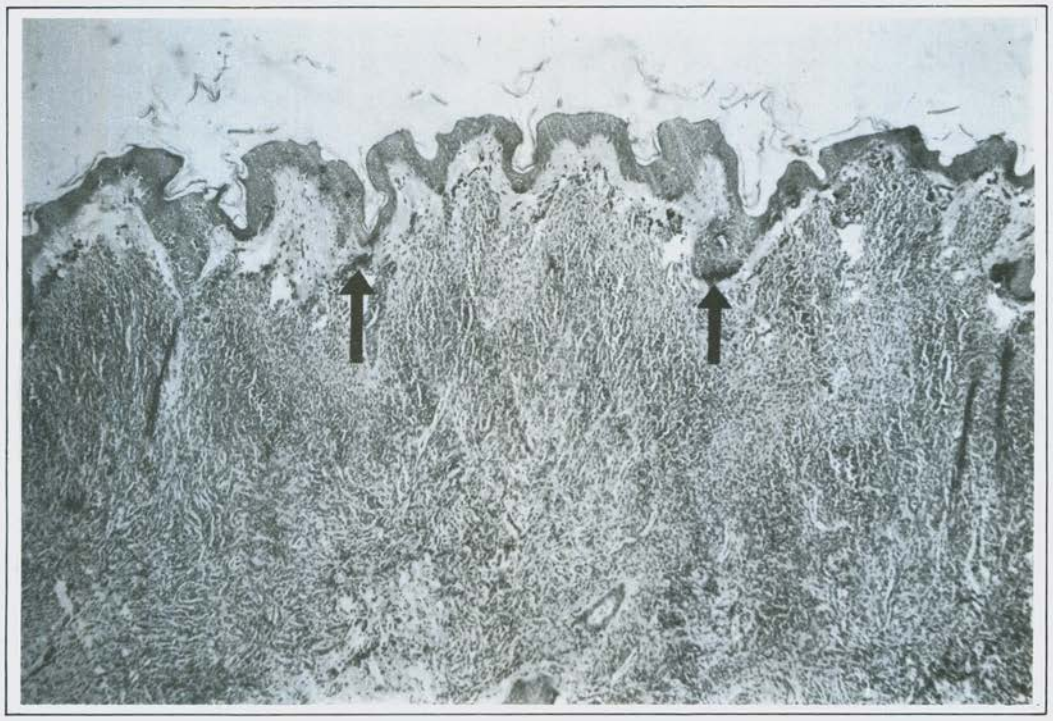


Fig 4 Transverse section of the nevi skin. Arrows indicate nevi. Note that the nevi are intradermal. X 90.

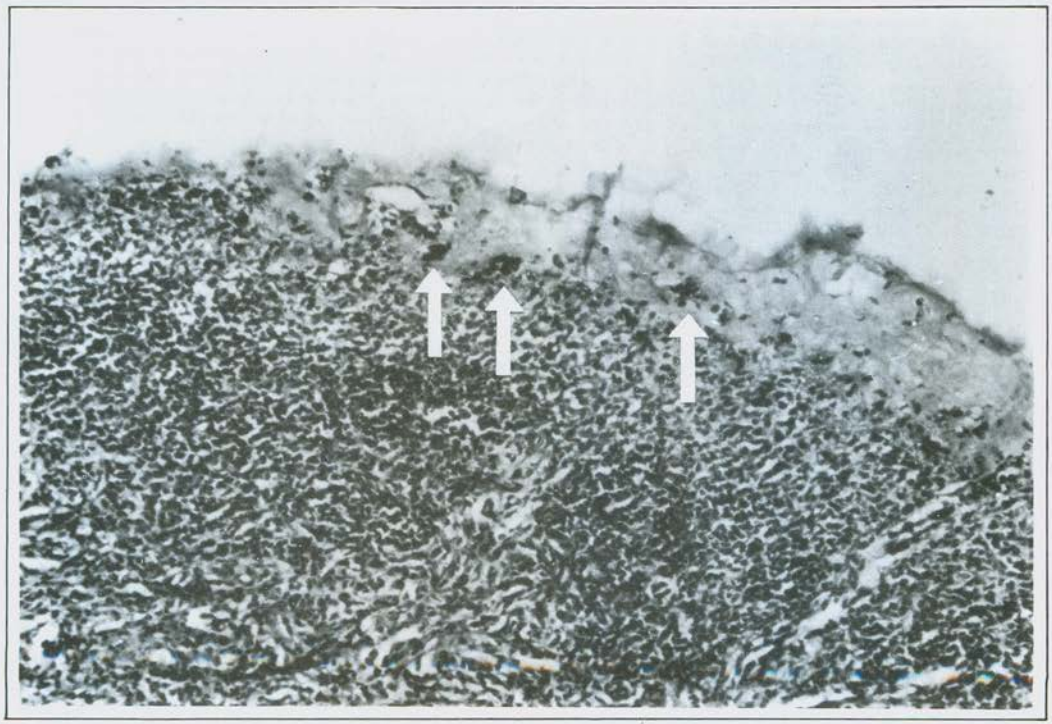


Fig.5 The dermis with nevi is enzymatically separated and only the nevi free epidermis is used for culturing 3-D epithelia. Arrows indicate nevi. X 160

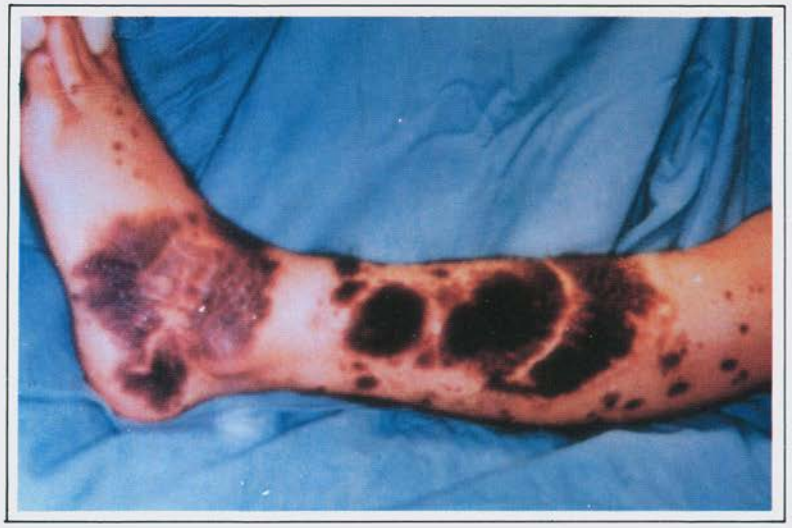


Fig 6
Area of the nevi skin of the patient chosen for cultured epithelia auto-grafting

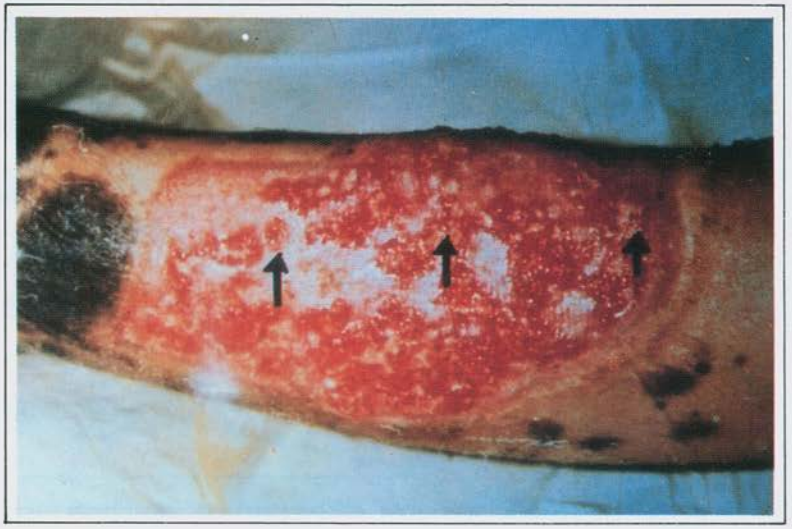


Fig.7
Two weeks post-grafting, pinkish jelly like growth of the cultured epithelia (arrows) is seen on the wound bed.

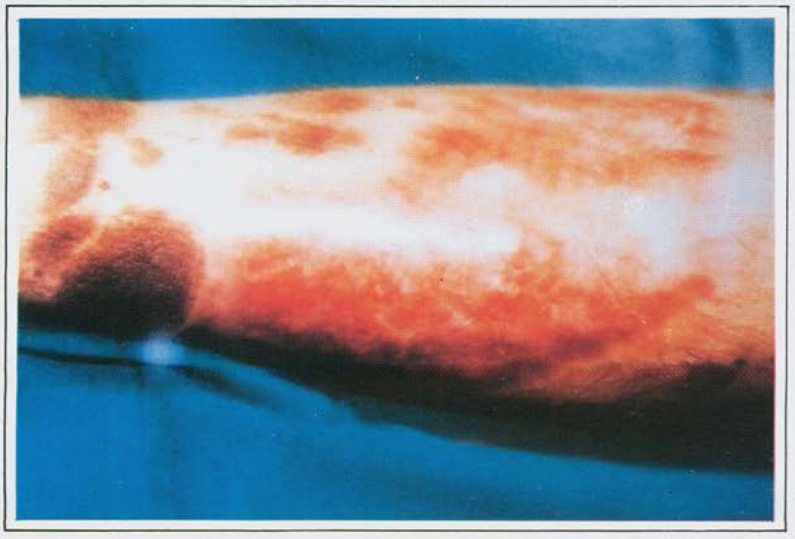


Fig.8
Six months post grafting. The skin shows normal appearance and is devoid of nevi.

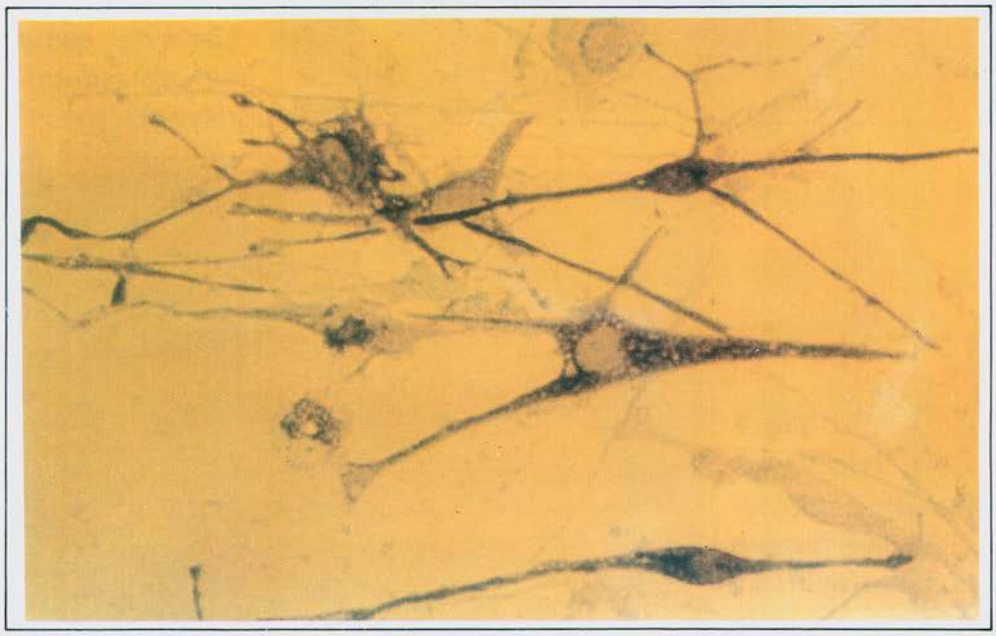


Fig.9 Histochemistry of tyrosinase reaction using dihydroxyphenylalanine (dopa) as a substrate in normal human melanocytes. Note melanin deposition occurs perinuclearly and in the dendrites. X300

various kinds of nevi. Melanomas grow well in normal culture conditions of Minimum Essential Medium (MEM) supplemented with 10% FCS.

Normal melanocytes however require synthetic growth factors like tetra decanoyl phorbol myristate acetate (TPA), a tumor promotor for overcoming their mitotic block. Basic fibroblast growth factor (bFGF) promotes the proliferation rates of normal melanocytes cultured in the presence of TPA. In contrast, the melanoma cell proliferation is inhibited by bFGF. The growth requirements of nevi melanocyte cells remain unknown.

Attempts to grow melanocytes in tumour promoter free media were made. Studies to cultivate normal melanocytes on fibronectin coated plates revealed that TPA concentration could be brought down from 10 to 4 ng/ml without affecting their growth rates. However, TPA continued to be necessary for the growth of melanocytes.

The role of TPA and other growth factors in pigment cell growth and differentiation is thus being investigated. B-16 melanoma cells were grown in the presence of various concentrations of TPA. Dopa reaction (Fig 9) and tyrosinase activity as markers of differentiation and 3-[4,5-Dimethylthiazol - 2yl] - 2,5-diphenyltetrazolium bromide (MTT) reduction as marker of cell proliferation were carried out. Preliminary results indicate that TPA inhibits the proliferation of B-16 melanoma cells at the concentrations of 10 mg/ml or above but inhibits melanin synthesis without affecting proliferation rates at lower concentrations.

BONE MARROW

Radical chemo- and radio-therapy for the removal of wide spread malignancy is being practiced by many Cancer Hospitals. The treatment also destroys the bone-marrow cells. Therefore, attempts to remove and preserve normal bone marrow and reimplant it back after radical therapy

is gaining importance. Technology for long-term cryopreservation of bone marrow with a good revival capacity needs to be developed. This will also be useful for people working in hazardous areas like nuclear reactors to keep their bone marrow safely cryopreserved for latter use if necessary.

STUDIES ON CRYOPRESERVATION AND REVIVAL OF NORMAL HUMAN BONE MARROW

The earlier work carried out indicated that while there is some loss of mature cells, the stem cells and early progenitor cells were not harmed by cryopreservation.

The cell yield from human bone marrow samples is limited. Hence different parameters planned to be studied cannot be studied in every sample. The efficiency of cryopreservation has been tested employing GM and GEMM colony forming abilities as parameters on four samples and presence of CD34 as a parameter on three samples. The results confirm the findings that stem cells have a higher capacity to recover from freezing and thawing as compared to committed cells.

With a view to screen the efficacy of different cryoprotectants such as Hydroxyethyl starch, polyvinyl pyrollidone and study the effect of anti-oxidants like ascorbic acid, catalase, superoxide dismutase, to reduce cryoinjury, experiments with mouse bone-marrow cells are being planned.

HEART VALVES

Every year cases requiring heart valve replacement therapy are increasing. Currently artificial valves and glutaraldehyde-fixed homograft valves are being used and they have their own problems such as life long de-

pendency on anti-coagulant medication in the former and frequency of replacement surgery in the later.

CRYOPRESERVATION AND REVIVAL OF VIABLE HEART VALVES FOR TRANSPLANTATION

There has been a long standing belief that transplantation of viable aortic and pulmonary valves are more durable, as they are capable of repair of wear and tear, than the bio-prosthetic valves currently being used. The mechanical strength of the valves comes from the extracellular matrix laid by the fibroblasts while non-thrombogenic surface is provided by the endothelial cells lining the valves. Endothelial cells are very sensitive to antibiotics and it has been difficult to obtain contamination-free viable valves.

Fibroblast and endothelial cell cultures from heart valve leaflets and conduits were set up from the human heart valves procured from Sion Hospital, Bombay. These were cryopreserved by either slow cooling or rapid cooling method. Last year the studies had revealed that while mechanical strength remained unchanged, no outgrowths of fibroblasts and endothelial cells were seen.

The main reason for the loss of viability of the heart valves in our earlier studies was thought to be due to heavy antibiotic treatment. Therefore viability before antibiotic treatment was sought to be established initially and then optimum conditions for obtaining sterility of the valves be developed later. Therefore, twelve human aortic and pulmonary valves were obtained and studied for viability by the mitochondrial succinate dehydrogenase dependent reduction of MTT to its end product formazan. The results indicate that the valves are viable at the time of procurement (12-24 hours after death) but loose their viability over a period of 24-48 hours. Tests that assay differentially, viability of fibroblast and endothelial cells of the heart valves are being standardized.

DEVELOPMENT OF CULTURES FROM FASTIDIOUS CELLS/TISSUES AND THOSE WHICH HAVE RETAINED THEIR SPECIALISED FUNCTION/CHARACTER

- Bone cells
- Rheumatoid Arthritis
- Oropharyngeal cells
- Turbinate cells and Atrophic Rhinitis
- Breast Cancer cells

CULTIVATION OF BONE CELLS AND THEIR CHARACTERISATION IN TERMS OF OSTEOBLASTIC FEATURES

Bone cell cultures is another system which can be stimulated to undergo differentiation in vitro. Isolated bone cells cultured in vitro as monolayers are fibroblastic in nature and manifest a number of osteoblastic features such as high alkaline phosphatase activity, synthesis of osteonectin and other bone specific proteins etc. that differentiate them from fibroblasts originating from other tissues. In 3-D cultures however, these osteoblasts start ossifying and form bone. A number of growth factors have been shown to stimulate the proliferation of osteoblasts in culture. However little is known about the factors that induce differentiation in these cells. Hence the identification of factors and environmental conditions that modulate proliferation and differentiation of osteoblasts would be valuable.

Femur and tibia of 5 young Balb/c mice and 2 human MTP foetuses were initially subjected to 0.5% collagenase for 2 hrs and then to 0.2% trypsin for 1 hr. The dissociated cells were collected and seeded on plastic petri dishes with MEM+10% FCS as media. The cultures exhibited fibroblastic morphology (Fig.10). Further characterisation of these cells in terms of osteoblastic features like alkaline phosphatase activity, bone specific protein synthesis etc. are being carried out.

HYALURONIC ACID IN RHEUMATOID ARTHRITIS SKIN CULTURES BEFORE AND AFTER AYURVEDIC TREATMENT.

Rheumatoid arthritis (RA) is characterized by impairment in the functioning of joints leading to swellings and deformations in fingers, arms, legs and feet. There is some evidence that extracellular matrix proteins and carbohydrate polymers are involved in the etiopathogenesis of RA.

Ayurvedic treatment seems to improve the conditions of RA patients whereas allopathic treatment gives only symptomatic relief. Hence, studies on hyaluronic acid (HA), a major extracellular carbohydrate polymer, synthesis, secretion and degradation by skin fibroblast and keratinocytes is being carried out.

Skin cultures from 5 untreated RA patient's and 2 normal donors have been established. In early cultures RA patients skin fibroblasts appeared to manifest stronger whorling patterns than those from normal donors. The assay of HA has been standardized using the ^{125}I-HA binding protein and HA in a few skin cultures has been estimated. Fig.11 shows the time course of HA release from cultured skin fibroblasts. Techniques for estimating matrix degrading enzymes and for determining the molecular weight of HA in normal and RA fibroblast cultures are being standardised.

CHARACTERISATION OF CULTURED HUMAN OROPHARYNGEAL MUCOSAL EPITHELIAL CELLS

Human oropharyngeal mucosa is susceptible to the agents that cause upper respiratory tract infections. The mucosal epithelial cells of oropharynx may be exhibiting more propensity to different external stimuli leading to the initiation of disease process. Therefore, an in vitro model consisting of cultured human oropharyngeal mucosal epithelial cells would provide a system to explore the mucosal tissue tropism.

Human oropharyngeal mucosal epithelial cells and buccal mucosal epithelial cells were cultured successfully from four foetuses and from one adult subject. Cultures were set from mucosal epithelial cells derived from laryngopharyngeal region. The cultures of epithelial cells exhibiting continued ciliary activity were maintained for about 37 days. (Fig.12)

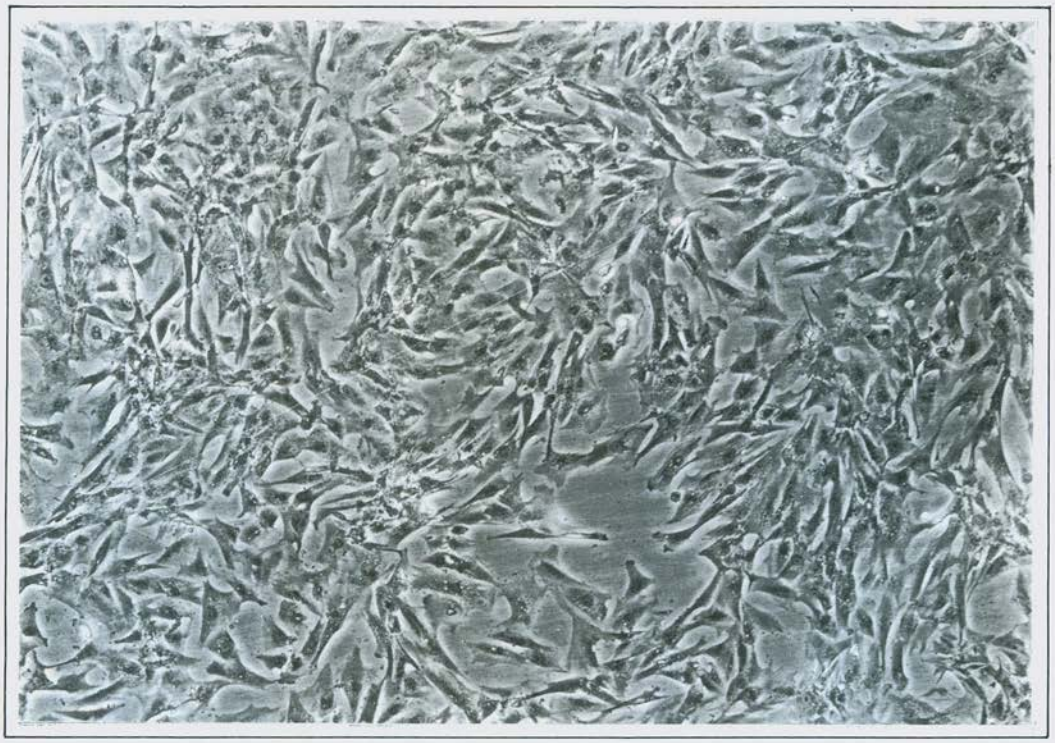


Fig.10 Human bone derived cells exhibiting fibroblastic nature. X 150

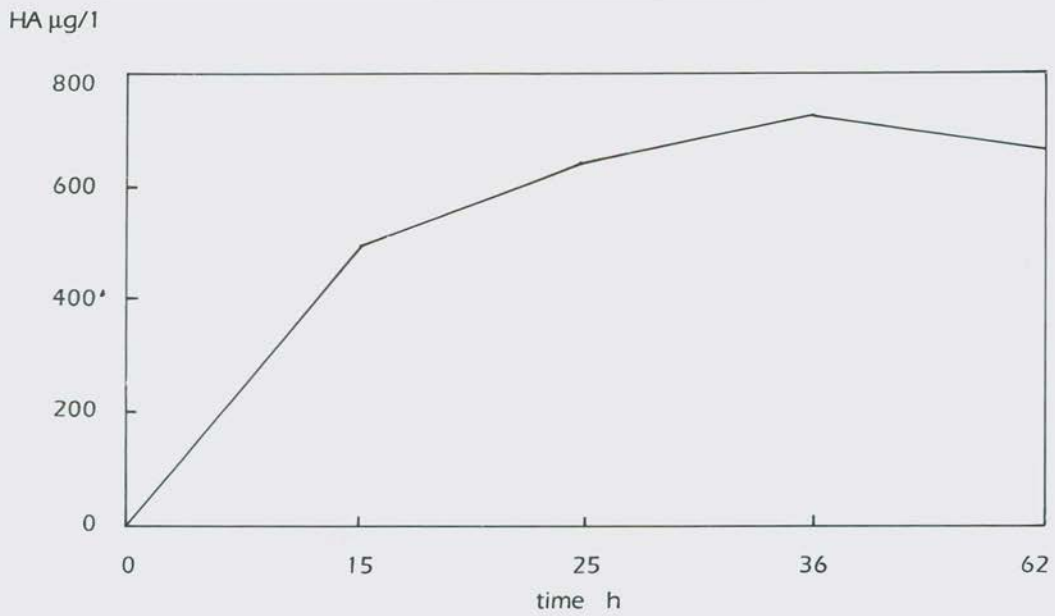


Fig.11 Assay of hyaluronic acid released into the medium by normal human skin fibroblasts. Note the steady increase with time.

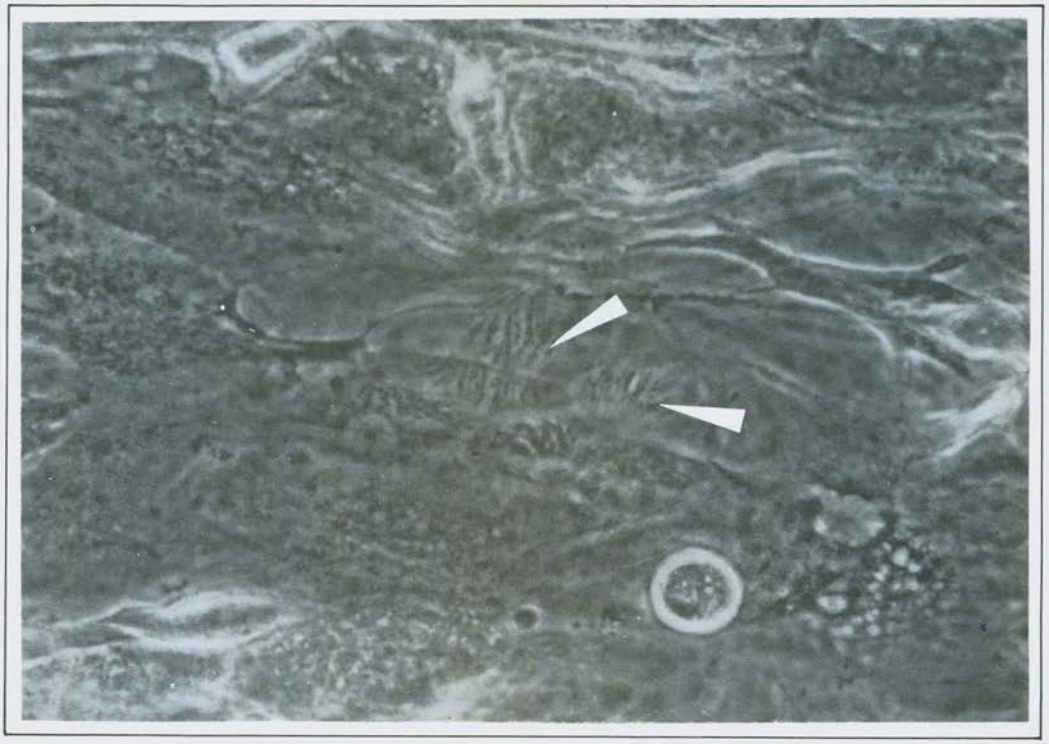


Fig.12 Cultured epithelial cells from the laryngo-pharyngeal mucosa of human foetus exhibiting beating of cilia. 25 days old culture. Arrow heads indicate cilia. X 900.

STUDY ON LOCALIZED CELLULAR CHARACTERISTICS IN PATIENTS WITH ATROPHIC RHINITIS

The pathogenesis of atrophic rhinitis in human is a complex cellular phenomenon which occurs in the nasal mucosal systems. The inflammatory cascade of the disease is due to individual local immune handicap and local epithelial cell functionality which is altered by bacterial and viral infestation. Therefore the study was initiated to investigate the cellular characteristics of nasal mucosal epithelia in cultured cells. Both biochemical as well as immunochemical aspects will be studied to understand the cellular functionality of nasal cells of patients with atrophic rhinitis.

Study was initiated by culturing mucosal epithelial cells from the tissues taken from the inferior and middle turbinate of two adult human subjects. The epithelial cell morphology was compared with cells originating from other mucosal areas. Nasal mucosa from a patient with atrophic rhinitis was also cultured.

ESTABLISHMENT OF BREAST CANCER CELL LINES FROM HIGH RISK ETHNIC GROUPS : STUDIES ON CHARACTERISATION AND EARLY DETECTION :

Cancer of the breast is among those human diseases whose etiology has not been clearly established, although factors such as genetic predisposition, environment and involvement of a retrovirus have been considered to be important. Within the framework of genetic susceptibility and predisposition Parsi women present the world's highest breast cancer incidence. Moreover, Parsis constitute a bare 80,000 individuals living in and around Pune-Bombay region, are closely inter-marrying, urban-living and socio-economically well-to-do families. Breast cancer tissue from Parsi women therefore is an excellent material with which to address

basic questions about carcinogenesis. The present project is therefore initiated to develop and characterize permanent breast cancer cell lines, which will not only be used for experimental studies on carcinogenesis but would also enable preservation of the unique genome with a special trait.

Methods for standardisation of preparation of cell cultures, requirements of media, chromosome preparation for karyotype analysis have been undertaken. For the purpose breast cancer tissues from two cases were collected and cultures were set and lymphocytes of these patients were used for chromosome preparations.

CHARACTERISATION OF CULTURES

- Genomic and mitochondrial DNA analysis

CHARACTERISATION OF CELL LINES BY GENOMIC AND MITOCHONDRIAL DNA ANALYSIS.

Detection of cross contamination of cell lines is a problem in many research laboratories and methods to detect these are karyotype analysis, isoenzyme patterns, immunological specificity, DNA finger-printing etc. DNA finger-printing offers a powerful and a sensitive method for characterization and for checking cross contamination in cell lines.

Genomic DNA was isolated from cell lines of different species such as human, murine, monkey, muntjac and mosquito. This DNA was cut by Hinf I restriction enzyme and size fractionated on agarose gel. After blotting on nitrocellulose paper, the blot was hybridised with radiolabeled VNTR 2(8) probe (kindly provided by Dr.Lalaji Singh, Centre for Cellular & Molecular Biology, Hyderabad). The autoradiogram indicated that the probe could hybridize with DNA from all the species used in the experiment. However the hybridization pattern was complex with murine and human cell lines as the probe hybridized with many fragments which are very close in size. However number of fragments were few and discrete in mosquito indicating that the probe is a good candidate for speciation of mosquito cell lines. This work was temporarily suspended due to non availability of probe.

Mitochondrial DNA, in animal kingdom, is reported to have a very high rate of evolution as compared to nuclear DNA. This property of mitochondrial DNA is being used in studies concerning evolution and speciation. In cell cultures, this property can be used to differentiate between the various cell lines. Probably, it would be a more sensitive method to check the cross contamination occurring between the cell lines derived from closely related species. Hence, mitochondrial DNA from two mosquito cell lines Aedes albopictus and Anopheles stephensi was isolated by CsCl density gradient and was cut with 10 different

restriction enzymes. The results obtained by end labeling experiment showed that the RFLP pattern of these two cell lines are highly specific for each of them and could be used to characterise mosquito cell lines. Preliminary experiments with five different cell lines have indicated that Eco RI RFLP of mitochondrial DNA exhibits distinct patterns to differentiate these cells.

Studies on fingerprinting of cell lines with VNTR and M13 DNA and attempts to clone complete mitochondrial DNA in EMBL phage vector are in progress.

DEVELOPMENT OF INDIGENOUS TECHNOLOGY AND EXTENDING EXPERT SERVICES

- Adaptation of myeloma and hybridoma cells to goat serum
- Studies on lymphatic filariasis
- *In vitro* quantification of tetanus toxoid antibodies
- Genotoxicity of Aureofungin
- Cancer chemotherapeutic drug screening

ADAPTATION OF MYELOMA AND HYBRIDOMA CELLS TO GOAT SERUM

Cell lines are normally grown in the presence of Foetal Calf Serum (FCS). FCS has to be imported at considerable cost. In India goat blood can be obtained rather easily. Thus goat serum (GS) can be prepared in the laboratory from goat blood. NFATCC prepares its own stock of goat serum and has adapted several cell lines to grow in GS. It is of interest to see if myeloma and hybridoma cells can be adapted to grow in goat serum and at the same time maintain their functional properties.

After some initial difficulties, it was found that SP2/0 myeloma cells grow well in 6% GS supplemented with 20 µg/ml of soyabean lipid mixture. These GS adapted SP2/0 cells could also be cryopreserved and revived successfully. Fusion of GS adapted SP2/0 cells with splenocytes of mice immunized with *Leishmania donovani* promastigote antigen was made and 44 hybrids were obtained. Out of these four showed the presence of antibody by ELISA. However, these hybrids lost their secretory activity. Fusion of GS adapted SP2/0 cells with splenocytes of mice immummized with *D. medinensis* was then attempted. None of the hybrids showed presence of specific antibodies to *D. medinensis* antigen.

CC9C10, an anti insulin antibody secreting hybridoma cell line, was then gradually adapted to GS + soyabean lipid mixture. The CC9C10 cells adapted to goat serum continued to secrete immunoglobulin specific for insulin (Table II). These GS adapted cells were cloned twice and three secretary clones were stored frozen. The cryopreserved clones after revival continued to secrete antibodies to insulin. The antigenic specificity of the antibodies secreted by these hybridoma cells grown in FCS and GS remains unchanged. The data on chemical analysis of GS and FCS

revealed that total proteins, cholesterol and globulins were significantly higher in GS than in FCS. The osmolality of the media prepared with GS and FCS did not vary significantly and was between 340-350 mOsm/kg.

TO IDENTIFY AND CHARACTERISE PROTECTIVE ANTIGENS IN LYMPHATIC FILARIASIS

Lymphatic filariasis caused by *W. bancrofti, B. malayi* and *B. timori* affects over 90 million people inhabiting the tropical and sub-tropical areas. A monoclonal antibody 46.08.76 developed against infective larval antigen of *B. malayi* has been found to kill infective larvae of *B. malayi* and *W. bancrofti* under *in vitro* conditions and protect mastomys against challenge by these larvae. However, the antigen to which this antibody binds is not yet fully characterized. Therefore experiments were planned to study these aspects.

Efforts to cultivate human filaria *(W. bancrofti)* infective larvae (L3 stages) from mosquitoes collected from endemic areas were continued. Simultaneously soluble and cuticular antigens were prepared from adult worms of Setaria (Cattle filaria) which are readily available. ELISA test using this fraction as antigens revealed that MAB.46.08.76 cross reacts with soluble and cuticular antigens of Setaria.

IN VITRO ASSAY FOR QUANTITATION OF ANTITETANUS ANTIBODIES: AN ALTERNATIVE TO ANIMAL MODEL

The mouse neutralization assay (MNA) is currently employed to assess the potency of tetanus toxin or the tetanus toxoid vaccine. This assay is time consuming and is expensive. An alternative to animal assays needs to be developed. MNA measures the neutralizing antibodies while the serological assays e.g. ELISA systems developed measure total antibodies

TABLE II

Antibody secretory activity of CC9C10 hybridoma cell line adapted to goat serum

% Replacement of FCS with GS culture medium		ELISA INDEX	
FCS	GS	Control	After adaptation
9	1	1.685	1.600
8	2	1.590	1.512
7	3	-	-
6	4	1.431	1.323
5	5	1.431	1.464
4	6	1.340	1.284
3	7	1.405	1.344
2	8	1.146	1.262
1	9	1.060	1.142
0	10	1.137	0.953

MEM + 10% FCS OD = (-ve control)
C9C10 (1 : 100) OD = (+ve control)

$$\text{ELISA Index} = \frac{\text{OD of unknown - OD of negative control}}{\text{OD of known - OD of negative control}}$$

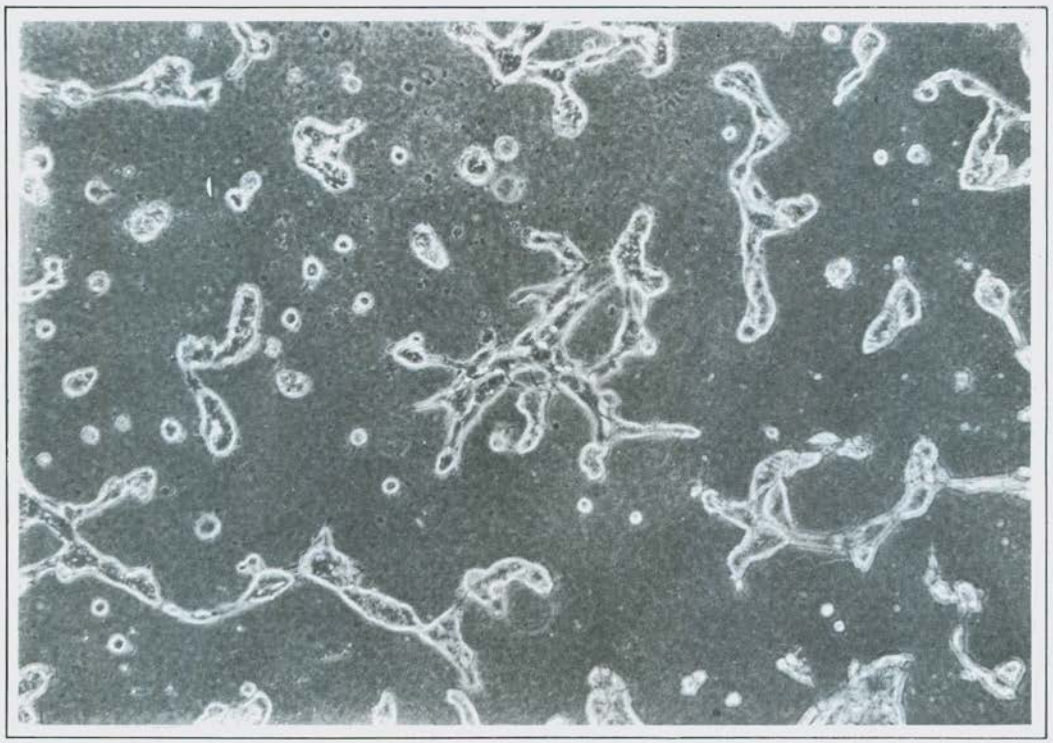


Fig.13 Tubular projections induced in bone marrow stromal cells by conditioned media of erythropoietin stimulated mononuclear cells. X 150.

which include non-neutralizing antibodies that are biologically irrelevant.

Using mouse Neuro 2a cells an enzyme linked assay method for assessing the levels of neutralizing anti-tetanus toxoid antibodies has been developed. The validation of the newly developed enzyme linked system has been done by comparing it with MNA. Horse anti-serum (ATS) is used as a reference standard as recommended by WHO. Therefore the newly developed enzyme linked system was initially assessed with ATS (10IU/ml) and the sensitivity of this method was found to be 0.0007 IU/ml. Using this as standard, serum samples from the tetanus patients were screened. A highly significant correlation between enzyme linked system using Neuro 2a cells, MNA and clinical history was found.

GENOTOXICITY TESTING OF AUREOFUNGIN

Aureofungin, a fungicide antibiotic manufactured and supplied by Hindustan Antibiotics, Pune, is used in agriculture. LD_{50} in mice and rats have been found to be 620 mg/kg and 540mg/kg weight, respectively. Genotoxicity studies on this antibiotic however, remain to be done.

Using Chinese hamster ovary (CHO) cells, the LD_{50} at 24 hr. was found to be somewhere between 0.1 to 1 µg/ml. The doses 0.2 to 0.4 µg/ml inhibited the mitotic index by 50%, and therefore, 0.2 µg/ml was chosen to test genotoxicity employing sister chromatid exchange (SCE) as a mutagenic marker. Auerofungin did not induce any significant increase in SCEs in treated cells compared to the untreated controls, at the concentration used.

MECHANISM OF ACTION OF THIOPHENE DERIVED DRUGS FOR EVALUATION OF THEIR POSSIBLE USE IN CANCER CHEMOTHERAPY

Anti-metabolites of the Hexose Monophosphate (HMP) pathway are one of the preferred drugs for cancer therapy because in malignant cells, this pathway is more prevalent than in their normal counterparts. Thiophene and its derivatives are one such group of HMP antimetabolites that need to be tested as potential anticancer agents.

Out of four thiophene derivatives, viz. thiodiglycolic acid, thiodipropionic acid, dicetol and thiophene 2, 5-dimethylenyl thiouromium chloride, synthesized and supplied by NCL group the last two were found to have antimitotic effect at 10 µg/ml concentration when tested on leukemia cell lines K562 and KG1 and normal human lung cell line WI 38. Two more drugs thiodiglycol sulphoxide and thiodiglycol ester were tested on WI 38 and its transformed counterpart WI38 VA13. The cells continued to proliferate when treated at 1:1000 dilution. However at 1 : 100 dilution these drugs were toxic.

OTHER PROJECTS

- Bone Marrow Immortalization
- Melanoma Oncogenes
- Molecular Biology of Hexokinase
- Stress proteins of mosquito cell lines
- Shear stress and Mass Cultivation of Cells in Bio-Reactors
- Functional characterisation of Granulosa cells
- Effect of cytokines on neuroblastoma cell lines
- Endocytic vesicles and cytoskeleton
- Melanosome transfer from melanocytes to keratinocytes

IMMORTALIZATION OF HUMAN BONE MARROW DERIVED STROMAL CELLS AND STEM CELLS VIA TRANSFECTION OF CLONED ONCOGENES

Bone marrow is a unique tissue which consists of stem cells that give rise to differentiated cells of various lineages. Stromal cells play an important role in this differentiation.

Immortalization of Bone Marrow stromal cells is thus useful not only for studies on leukemogenesis but also to obtain cell lines that can continuously produce hematopoietic growth factors involved in normal hematopoiesis.

Transfection of the bone marrow stromal cells with SV-40LT and Polyoma middle T antigen had given rise to seven different cell lines. Of these two 71-SV and 71-Py have been in continuous passage. At present these cells are in the 20th passage and appear to have entered into crisis state and are expected to give rise to immortalized cell lines.

Two more transfection experiments using the above two oncogenes independently as well as in combination have been attempted. Transfection of stem cells with chimeric constructs of Drosophila myb and V-myb cloned under the inducible promoter such as metallothionein promotor is being planned. At present the construction of such vectors is in progress.

EFFECT OF GROWTH FACTORS ON STROMAL CELLS

While studying the immortalization of bone-marrow stromal cells, it was observed that erythropoietin, a potent inducer of erythrocyte commitment, stimulated the proliferation of stromal fibroblasts. Since normal

TABLE III

Growth promoting ability of erythropoietin (EPO) on Bone Marrow Stromal Cells

Culture No.	Cell Counts/Plate	
	Control	EPO-treated
93	4×10^4	8×10^4
94	4×10^4	9×10^4
96	4×10^4	12×10^4
97	9×10^4	14×10^4
F3	16×10^4	42×10^4

hematopoiesis depends upon the intricate balance between stromal cells and stem cells it is quite possible that erythropoietin also stimulates stromal cells. Experiments were therefore conducted to confirm this observation.

In stromal fibroblasts cultured from five different donors erythropoietin stimulated the proliferation rates by 1.5 to 3 fold (Table III). The mitogenic effect of erythropoietin on stromal cells has also been seen by the Brdu staining method. Whether Erythropoietin directly acts on stromal cells or the effect is mediated through its action on hematopoietic cells is being currently investigated. Preliminary studies have revealed that conditioned media of erythropoietin stimulated mononuclear cells induced network like projections in stromal cells when cultured on agarose matrix as 3-D cultures. (Fig.13) Attempts to identify these conditioned media derived factors are under way.

IDENTIFICATION OF MELANOMA ONCOGENE AND CHARACTERISATION OF THE ONCOGENE PRODUCTS

Melanoma is one of the most malignant cancer and is on the rise all over the world. The change to malignancy with normal melanocyte involves a series of genetic and epigenetic events that involve changes in proto-oncogene expressions. However, it is not known which genes are critical for the transformation of normal melanocyte to melanoma.

Isolation of DNA from melanoma clone M3 and its transfection to NIH 3T3 cells was done and the resultant foci were picked up by cloning. Secondary transfection was done and resultant foci were grown in soft agar.

Eight clones obtained from secondary transfection were stored frozen and from these two clones were selected for further studies. Using antibodies M3-1 and antimelanocyte antibody from vitiligo patients, immunofluorescence studies were done. Intense particulate

immunofluorescence on the cytoplasm was observed in both the clones and in melanotic melanoma M3 cells but was absent in NIH 3T3 cells. The clones and M-3 cells were injected subcutaneously at the cell number of 10^6 cells into three week old Balb/c mice and tumours were obtained after 15 days in the animals indicating that transfected NIH 3T3 cells now have acquired tumorigenic potential. However control NIH 3T3 cells injected simultaneously did not give rise to any tumours.

In order to isolate and characterize the protein/s responsible for inducing tumourigenicity in NIH 3T3 cells, Western blotting and metabolic labelling ^{35}S.methionine, immuno-precipitation and fluorographic and autoradiographic studies are being carried out.

MOLECULAR BIOLOGY OF HEXOKINASE

Hexokinase (HK) catalyzes, the first step in glucose metabolism utilizing ATP for the phosphorylation of glucose to glucose-6-phospate. In mammals there are four HK isozymes which vary in their tissue distribution and kinetic properties. The pattern of expression is known to vary in cancer cells and in various organs of diabetic patients. The molecular basis for tissue specific expression and for abnormal patterns of expression in pathological conditions is not known. Attempts are now being made to answer these questions through molecular biology studies.

To facilitate these studies on HK isoenzyme type I, two putative partial cDNA clones coding for bovine hexokinase I had been isolated from bovine brain cDNA library by using a 41-mer oligonucleotide as a probe. Its sequence was a consensus sequence found in many mammalian hexokinases. These cDNA clones had also been characterised with respect to their restriction sites.

Partial nucleic acid sequencing of the above two putative bovine brain

hexokinase cDNA clones has strongly suggested that these clones are of a HK gene. Sequence data on translation to amino acids revealed more than 70% homology with a sequence of bovine brain HK published recently. Experiments are under way to complete the sequencing of these cDNA clones.

STUDY OF STRESS PROTEINS IN MOSQUITO *A. STEPHENSI*

Many cells respond to stressful conditions by synthesizing a set of proteins. These proteins were first discovered during heat shock and they have been termed as heat shock proteins (hsps). The hsps have been well studied in drosophila and higher organisms including human beings. Mosquitoes are vectors for many viral and protozoan pathogens and as such are heavily exposed to insecticide stress. This provides a rationale for investigating hsps and other stress proteins in these insect cells.

Anopheles stephensi cell line was used as model system for investigating hsp's. When these cells grown in FCS (CS-cells) at 28°C were shifted to 37°C a set of five hsps, hsp 83, hsp 74, hsp 70, hsp 63 and hsp 55 were induced. When cells were grown in goat serum (GS cells), these stress proteins were already expressed at 28°C. Level of heat shock factor (HSF) was also much higher in GS cells than in FCS cells at 28°C. Levels of esterase-A, esterase-B and acetylcholinesterase were much elevated in GS cells than in FCS cells. At normal growth conditions, the hsp levels in GS cells were similar to those exposed to organophasphates or carbamates. GS cells showed induction of acetylcholinesterase when exposed to propoxur, a carbamate insecticide. GS cells show resistance to 50 fold higher concentration of propoxur and their acetylcholinesterase level remained unchanged after exposure. The reasons for these are being investigated.

SHEAR STRESS AND MASS CULTIVATION OF CELLS IN BIO-REACTORS

Cell culture products are becoming increasingly important for basic as well as applied research. Several types of bioreactors for growing cells in large numbers are now available. A flow of liquid nutrients is maintained in these bioreactors and the cells are thus subjected to hydrodynamic shear stress. The effect of shear stress on the growth of animal cells is being investigated.

Initially murine hybridoma secreting anti-Japanese encephalitis virus antibody were grown in hollow fiber bioreactor. A high titer of antibody production was observed.

The relationship between growth of cells and nutritional requirement was then investigated in static and under nutritional flow conditions using glucose uptake as a parameter. One of the important observation made was that the transport of solutes occurs without any hindrance in the hollow fiber bioreactors. The effect of shear stress on suspension and adherent cultures were studied and the major effect was found to be on actin network. When human lymphoblastoid KG1 cells were subjected to high shear regime (300 rpm), there was no change in total cell number, percent viability, cell morphology and metabolic activity as measured by glucose uptake. These cells however failed to grow in fresh medium. This is now being confirmed.

FUNCTIONAL CHARACTERISATION OF HORMONALLY INDUCED GRANULOSA CELL LINES

Granulosa cells present in the follicles of the ovary support the development of germ cells into ova, by secreting steroid hormones. It was thought that a study on the factors involved in the synthesis and secretion of progesterone be planned to understand the considerable variation in steroid secretion that is seen between different follicles. An established cell line from goat granulosa was used as an experimental system and effect of testosterone and pregnenolone on the production of progesterone was studied.

Pregnenolone (0.2 - 2.0 mg) used as a precursor, increased the secretion of progesterone in a dose dependent manner. Supplementation of the culture medium with 0.2 - 1.0 µg/ml of testosterone resulted in increased secretion of progesterone in a dose dependent manner. Additional supplementation of the medium with 1.0 µg/ml of pregnenolone resulted in even lowering the doses of testosterone (0.04l - 0.1 µg/ml) inducing the secretion of progesterone. The levels of estradiol in the medium were unaffected by these additions.

EFFECT OF CYTOKINES ON PROLIFERATION OF NEUROBLASTOMA CELL LINES

The immune system through production and secretion of factors can regulate specific functions of cells of the nervous system. Many types of neural cells express a range of antigens and respond to mediators made by immune cells. The effects of immune cytokines on neural cell growth, survival and differentiation is therefore being investigated.

Supernatants of Neuro 2a cells have been found to stimulate the

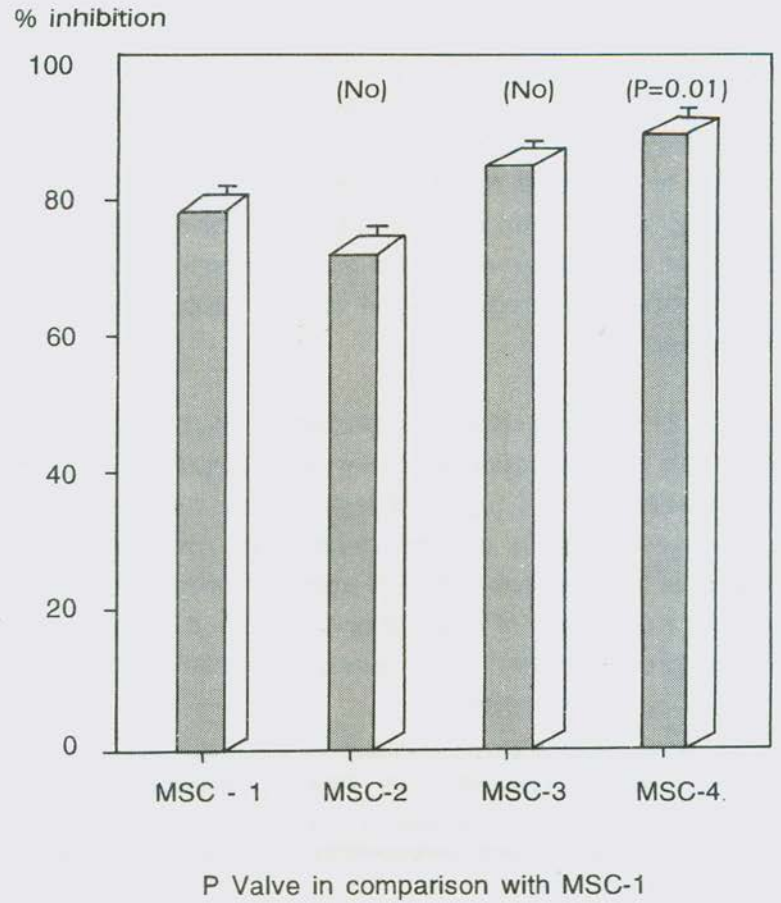


Fig.14 Effect of supernatants of Con A activated splenocytes on the proliferation of Neuro 2a cells. MSC refers to mouse spleenocyte culture.

increased proliferation of mouse splenocytes as investigated by ^{3}H-thymidine incorporation. In contrast supernatants from activated splenocytes inhibited the proliferation of Neuro 2a cells (Fig.14) in a dose dependent manner. These experiments have been repeated five times and consistent results have been obtained.

ENDOCYTIC VESICLES AND THEIR ASSOCIATION WITH CYTOSKELETON

The role played by cytoskeletal elements in the formation, organisation and movement of membranous organelles, such as pinocytosed vesicles, can be directly visualised. For example, in the mouse peritoneal macrophages, the PMA-induced tubular lysosomes and the closely associated microtubules can be simultaneously localised by fluorescence microscopy.

Initial experiments were carried out to search cultured cells that respond to PMA by enhancing their pinocytic activity, as evidenced by the formation of tubular lysosomes. These included chick embryo fibroblasts (CEC), human epithelial cells of tumor origin (MCF-7) and mosquito (C6/36) cell lines. Preliminary observations on C6/36 indicated that a small proportion of cells responded to PMA as judged by the appearance of tubular endosomes.

These structures varied in abundance from a few isolated tubules to branching structures to anastomosing network, similar to those generally seen in stimulated macrophages. Further studies on the nature of these vesicles/tubular network (e.g early vs. late endosomes), and their association with the cytoskeletal elements will be evaluated.

IN VITRO STUDIES ON THE ROLE OF GROWTH FACTORS ON MELANOSOME TRANSFER FROM MELANOCYTES TO KERATINOCYTES IN THE EPIDERMIS

Normal colour of the skin depends upon the interaction of two cell types in the skin. The melanocytes make the melanosomes (melanin synthesizing granules) and the keratinocytes pick these melanosomes and bring them to the topmost cellular layer of skin. The number of melanocytes and keratinocytes do not vary in different individuals and the variation in skin colour is mainly due to type and transfer of melanosomes. However very little is known about how these two types of cells interact with each other in maintaining normal skin colour.

Fibroblast and keratinocyte cultures from Balb/C mice have been established and tissue engineered intermediate split thickness skin is being attempted. These experiments have been repeated twice so far.

COLLABORATORS

NFATCC has established collaborative projects with scientists & clinicians from different research institutes and hospitals the details of which are as follows :

B J Medical College & Sassoon General Hospital, Pune	Prof. A.S. Labhashetwar Prof. A.V. Jamkar Dr. Shailaja Jadhav Prof. Y.W. Risbood Prof. A.R. Bhide
Bharati Vidyapeeth Ayurved College Pune	Prof. R.D. Mhardikar Mrs.GudrunUlrich-Merzenich
Tarachand Hospital, Pune	Dr. Y. Joshi Dr. A. Desai
Janakalyan Eye Bank, Pune Janakalyan Blood Bank, Pune	Dr. R.D. Kolte Dr. D.M. Wani
King Edward Memorial Hospital Pune	Prof. B.B. Gokhale Dr. Y.V. Tawade Prof.. S.B. Kelkar Prof. C.S. Yajnik Prof.(Mrs.) U.D. Sutaria Dr. D. Bhattacharya
Mahatma Gandhi Eye Bank, Pune	Dr. N.Shah
Jahangir Nursing Home , Pune	Dr. R.L. Marathe

National Chemical Laboratory Pune	Dr. R.A. Mashelkar Prof. John Barnabas Dr. B.D. Tilak Dr. C. Sivaram Dr. S.P.Vernekar
University of Poona Pune	Prof. D. N. Deobagkar Dr.(Mrs.)D.D.Deobagkar Dr. N.N. Godbole
University Department of Chemical Technology, Bombay	Prof. J.B. Joshi
Indian Red Cross Society Ahmedabad	Mr. G. Mazmudar
Lokmanya Tilak Muncipal Hospital Bombay	Prof. A.P.Chaukar Prof. Alka Gogate
Cancer Research Institute Bombay	Prof. M.G. Deo Dr. A.N.Bhisey Dr. S.G.A. Rao
Institute for Research in Reproduction, Bombay	Dr.(Mrs.)T. Nandekar
Sophia College, Bombay	Dr.(Mrs.)Medha Rajadhyaksha
Hindustan Lever Research Centre Bombay	Dr. G.Raman
King Edward Memorial Hospital Bombay	Prof. S.R. Tambwekar

Tata Institute for Fundamental Research, Bombay	Dr. L.C. Padhy
Centre for Cellular & Molecular Biology, Hyderabad	Dr. P.D. Gupta Dr. Lalji Singh
Vector Control Research Centre Pondicherry	Dr. V.Dhanda Dr. K.Balaraman
National Filaria Control Unit Thane	Dr. S.B. Dongre
Malaria Corporation Unit, Pune	Mr. J.K. More

PUBLICATIONS/CONFERENCES/ WORKSHOPS/SYMPOSIUM/SEMINARS etc.

1. **J.M.Chiplonkar, D.D.Vandre and J.M.Robinson**
   Stimulus - dependent relocation of the microtubule organising center in human polymorphonuclear leukocytes
   J Cell Sci 102 : 723-728, 1992

2. **D.Prakash, P.B.Parab and R.N.Sharma ✪**
   Immunodiagnosis of Dracunculus by Dot.ELISA
   Annals Trop Med Parasit. 87(2) : 195-200, 1993.

3. **I.D. Arya, T.N. Chakravarty and S.K. Sopory**
   Development of secondary inflorescence, vegetative structures and plantlets in in vitro inflorescence culture of Amaranthus Paniculatus.
   Plant Cell Reports, 12 : 286-288, 1993

4. **Renu Deswal, T.N. Chakravarty and S.K Sopory**
   The glyoxalase system in higher plants : Regulation in growth and differentiation.
   Biochem Soc. Trans, 21(2) : 527-530, 1993.

5. **S.Guha-Roy and A.N. Bhisey**
   Cytoplasmic microtubule assembly is altered in cytoplasts.
   Cells Biol. Int. Rep. 16(3) : 269-281, 1992.

6. **S.Guha-Roy ✪**
   Role of stress fibres in the association of intermediate filaments with microtubules.
   Cell Biol. Int. Rep. In press.

7. **F.D.Dastur, P.Shastry, E.Iyer, V.Awataramani, S.Raut, S.D.Mehta and S.I.Irani**
The fetal immune response to maternal tetanus toxoid immunisation
J Assoc Physicians of India. In Press

8. **K.S.Nandakumar, N.K.Ganguly, Y.Chandrashekar, I.S.Anand and P.L.Wahi**
Salivary specific antibodies in relation to Streptococcus Pyogenes to pharyngeal cells of patients with rheumatic fever.
Recent Advances in Mucosal Immunology, In Press

9. **T.N. Chakravarty and S.K. Sopory**
In vitro studies on cell profileration and differentiation in Amaranthus paniculatus
XIII Plant Tissue Culture Conference India In Press

10. **T.N. Chakravarty**
Tissue Culture for Amarnath Crop improvement.
LEGACY (The official News letter of the Amarnth Institute, USA) In Press

11. **M.Mojamdar**
Invited to the UGC sponsored workshop on Modern methods of teaching Bio-Sciences, Mangalore University and gave three lectures. Dec.20-23, 1992

    1. Basic Tissue Culture Techniques
    2. Biotechnological Applications of Tissue Culture, and
    3. Biomedical Applications of Tissue Culture

12. **Y.Chandrashekar, S.Garg, K.S.Nandakumar, I.S.Anand and N.K.Ganguly**
Relation between streptokinase specific antibodies and reperfusion rates in patients with myocardial infarction.
44th Annual Conference of the Cardiological Society of India, Nov.3-5, 1992.
Abstracted in Ind.Heart J. 44 : p291

13. **S.Garg, Y.Chandrashekar, K.S.Nanda Kumar, I.S.Anan, and N.K.Ganguly**
Incidence and time course of stretokinase specific antibodies in patients with myocardial infarction.
44th Annual Conference of the Cardiological Society of India, Nov.3-5, 1992.
Abstracted in Ind.Heart J. 44 : p338

14. **K.S.Nandakumar, N.K.Ganguly, I.S.Anand and P.L.Wahi**
An Immunomicrobiological study of streptococcal adhesion to Pharnygeal cells of Rheumatic fever and Pharyngitis patients.
"XVI Ind. Ass. of Med. Microbiologists - October 18-21, 1992, AIIMS, New Delhi.

15. **K.S.Lole and D.N.Deobagkar J**
Development of cross-resistance to stress in A.stephensi cells adapted to goat serum
"XVIth All India Cell Biology Conference and Symposia Banaras Hindu University, Varanasi Jan 1993.

16. **R.N.Sharma and D.Prakash**
Plant products for control of Dracunculiasis
IIIrd Asian Congress of Parasitology - Feb 18-21, 1993, CDRI, Lucknow

18. **D.Prakash, P.B.Parab and R.N.Sharma**
"Immunodiagnosis of Dracunculiasis by Dot-ELISA
IIIrd Asian Congress of Parasitology - Feb.18-21, 1993, CDRI, Lucknow

19. **M.Mojamdar**
Biotechnology in Health Care
Science Reporter 28 (ii) : 60-66, 1992

20. **S.Kshirsagar**
"Restriction and modification of DNA."
Mahabaleshwar seminar on modern biology. Nov.5-10, 1992, Bangalore

21. **Dr.R.N. Damle**
Training Course on ``Molecular immunobiology of self and non-self reactivity``, Madurai Kamaraj University, Madurai from 16th-22nd Nov. 1992.

22. **S.N. Satoor**
Short-term technician training course on ``Experiments in Genetics Engineering`` conducted by Dept. of Biotechnology, Madurai Kamaraj University, from Dec. 1992-March 1993.

✪ Work Carried out at NFATCC

OVERSEAS ASSOCIATESHIP

- Dr. R.R. Bhonde was awarded the Department of Biotechnology Overseas Associateship for the period 8th July 92 to 7th July, 1993. During this period he would be working at University of Calgary, Canada.

- Dr. Yogesh Shouche has gone abroad for Post Doctoral Fellowship at Microbiology Division of GBF Institute for Biotechnological Research, Germany for the period 1st March, 93 to 15th April, 1994.

I) The staff of NFATCC continued to participate in the teaching programme of M.Sc. Biotechnology conducted by Zoology Dept, Poona University.

II) Five students are working towards obtaining Ph.D. degree on topics such as stress proteins, cell characterisation by mitochondrial DNA analysis, molecular biology of Hexokinase, antigens of cattle filaria and effect of growth factors on melanocytes etc.

III) The following individuals were deputed for training at NFATCC:

NAME	INSTITUTE	TRAINING PERIOD From	To
Dr. P V L Rao	Division of Pharma-Toxicology, Defence Research & Development Organisation, Gwalior	18.08.92	30.08.92
Dr. R.Bhattacharya	-do-	-do-	-do-
Dr. Y.S. Badhe	Institute of Veterinary Biological Products, Pune	21.09.92	03.10.92
Dr.S D Bhatawadekar	-do-	-do-	-do-

COMPUTERS AND DATA BASE

The computer unit added 3 Dot Matrix printers and upgraded 7 PCXT's to PC AT after shifting to Jidnyasa building.

- Software has been developed for improving the cell supply management system which has been implemented from Jan. 1993.

- User awareness programme has been conducted from time to time to ensure an efficient and effective use of computer facilities available. The Administration, Accounts, Stores and infrastructural units like Instrumentation and Maintenance, Library and Documentation, Research and Training Laboratory (presently nearing completion) have been equipped with PCXT/AT's and various aspects of the activities of the above sections have been computerised.

- Different programmes such as developing central monitoring system for laboratory equipments, Presentation of NFATCC's activities etc are being worked out.

LIBRARY AND DOCUMENTATION SECTION

The NFATCC library augmented the basic infrastructural facility for its users after shifting to Jidnyasa building.

The library has enriched its collection by procuring various types of documents, books, reports, conference proceedings, journals and periodicals. The current collections consist of about 3000 books, reports, conference

Shri P.R. Kumaramangalam
Minister of State for Science & Technology laid the
Foundation stone of NFATCC building.

Lighting the lamp in the presence of Dr. S. Ramchandran, Secretary, DBT, and Dr. S.C.Gupte, Vice Chancellor, University of Poona.

Taking an overview of model of NFATCC complex with Shri S.Beri, Architect, Y.P.Samant, represrentative of the construction company, Shri S.B. Krishnan, Financial Advisor, DBT, and Dr.S.Ramachandran.

Unveiling the Foundation Stone of the new complex coming up on Poona University Campus in the presence of Dr. V.G. Bhide, Ex. Vice Chancellor, University of Poona, Dr. S. Ramachandran and Prof. Sridhar Gupte.

Distinguished guests and scientists at the Foundation Stone laying ceremony

CONSTRUCTION OF NFATCC LABORATORY COMPLEX ON POONA UNIVERSITY CAMPUS

The foundation stone of NFATCC complex at Poona University campus was unveiled by Shri P.R. Kumaramangalam, Hon. Minister of Science & Technology, Government of India, on 25th August, 1992. The construction of the NFATCC complex on the University of Poona campus is progressing as per schedule under the able management of C & S group of Department of Atomic Energy. The salient features in this regard are as under :

- Direct water pipe lines and bore wells have been provided at the site to ensure sufficient supply of water, round the clock for the construction activities.
- Temporary electrical connections have been procured and provision for obtaining HT line from MSEB has been made.
- Construction activities are being monitored as per the PERT chart submitted by C&S Group, DAE.
- Chain link fencing on open boundaries of the plot has been constructed. 1500 trees have been planted and these are being maintained.
- Plans for Residential Complex and ancillary buildings have been prepared and got approved from the University of Poona and submitted to the Pune Municipal Corporation. for approval.

COMPLETION OF REMAINING CONSTRUCTION OF "JIDNYASA" BUILDING

The construction of the unfinished RCC structure purchased from M/s Vanaz Engineers Ltd. has been completed and the completion

certificate for the same has been received from the PMC.

Office activities such as Administration, Accounts, Stores and Infrastructural Support Activities such as Instrumentation and Maintenance, Computer, Photography, Library and Documentation have already been established at this building. The entire stock Cell Culture maintenance, Supply and Media Preparation activities have been shifted to this building. In addition to the above a Laboratory for Training and Quality Control is also being established at this building and is expected to be fully functional by this June end.

COMPOSITION OF STAFF

Following is the staff complement as on 31.3.93 :

1)	Scientific staff	17
2)	Technical staff	
	Laboratory	15
	Inst. & Maint.	07
3)	Administrative staff	12
4)	Auxiliary staff	17
	TOTAL	68
5)	Research Associates and Research Fellows	08
6)	Contractual services	18

DISTINGUISHED VISITORS

Following distinguished persons and dignitaries visited the Institute during 1992 - 93 :

1. Prof. M. G. K. Menon,
   President,
   International Council of Science Unions, Member of Parliament,
   77, Lodi Estate,
   New Delhi

2. Prof. Mukund J. Modak,
   University of Medicine & Dentistry, New Jersey Medical School, U. S. A.

3. Shri Jyotirdhar B. Desai,
   Dean (Television),
   Film and Televison Institute of India, Pune.

4. Dr. M. R. Ven Murthy,
   Biochemistry & Medicine,
   Laval University,
   Canada.

5. Dr. R. Chandrashekar,
   Washington University Medical Centre, St Louis, U. S. A.

6. Prof. L. M. Singh,
   Ayurveda Campus,
   Kalankisthan, Kathmandu,
   Nepal

7. Dr. M. J. Mulky,
   Hindustan Lever Research Centre, Andheri, Bombay - 19

8. Dr. K. M. Cherian,
   Hindustan Lever Research Centre, Andheri, Bombay - 19

9. Prof. Amu Therwath,
   Professor, Molecular Biology,
   University of Paris,
   Paris (FRANCE)

10. Dr. Suresh C. Jhanwar,
    Head, Laboratory of Solid Tumor Genetics, Memorial Sloan - Kettering Cancer Centre,
    New York. (U.S.A.)

11. Prof. Sharad Deodhar,
    Head, Immuno Pathology Section, Department of Clinical Pathology
    Cleveland Clinic Foundation,
    Cleveland, U. S. A.

12. Prof. Raghbir S. Athwal,
    Associate Professor,
    Department of Microbiology & Molecular Genetics,
    New Jersey Medical School,
    New Jersey, U. S. A

13. Dr. P. Rama Rao,
    Secretary, Government of India, Department of Science & Technology,
    New Delhi

14. Dr. Nitin Telang,
Department of Surgery,
Memorial Sloan Kettering
Cancer Centre, 1275, York
Avenue
N. Y. 10021, U. S. A

15. Prof. M. B. Sahasrabudhe,
Agharkar Professor,
Maharashtra Association for
Cultivation of Sciences,
Agharkar Research Institute,
Agharkar Road, PUNE - 411 004

16. Prof. G. Padmanabhan,
Department of Biochemistry,
Indian Institute of Sciences,
Bangalore - 560 012

17. Dr. R. K. Datta,
Director,
Central Sericultural Research &
Training Institute and
International Centre For
Training & Research in Tropical
Sericulture, Central Silk Board,
Shrirampura, Mysore - 570 008

18. Dr. G. Bhaskaran,
Vice President,
Micro Plantae Ltd.
1, Gulmohar Glaze, Nagar Road,
Pune - 411 014

19. Major Gen. D. Raghunath,
Dy. Commandant & Dean,
Armed Forces Medical College,
Stevely Road,
Pune - 411 040

20. Prof. A. K.Gupta,
Director,
CSIR Complex,
Palampur,
Himachal Pradesh

21. Prof. H. M. Pandit,
Emeritus Scientist,
Buffalo University,
New York,
U. S. A

22 Dr. A. B. Joshi,
Vice-President,
Maharashtra Association For
Cultivation Of Science,
Law College Road, Pune - 411004

COMMITTEES OF INSTITUTION

SOCIETY AND GOVERNING BODY

Shri P R Kumaramangalam
Minister of State for
Science & Technology
(President of NFATCC Society)
Govt. of India
New Delhi

Dr.C.R. Bhatia,
(Chairman, Governing Body)
Secretary,
Dept. of Biotechnology,
Ministry of Science &
Technology,Govt. of India
New Delhi

Prof.H.Sharat Chandra
Professor, Dept. of Microbiology
& Cell Biology,
Indian Institute of Science,
Bangalore

Prof.S.C.Gupte
Vice Chancellor,
University of Poona,
Pune

Dr.Smt.Manju Sharma
Adviser,
Dept. of Biotechnology,
Ministry of Science & Technology,
Govt. of India, New Delhi

Shri S.B.Krishnan,
Joint Secretary & Financial Adviser
Govt. of India
Dept. of Biotechnology,
Ministry of Science & Technology,
New Delhi

Dr.Prema Ramachandran
Dy.Director General,
Indian Council of Medical
Research
New Delhi

Dr.K.Banerjee
Director,
National Institute of Virology,
Pune

Dr.B.B.Mallick
Joint Director (Research),
Indian Veterinary Research Inst.
Izatnagar : 243 122 (UP)

Prof.A.S.Kolaskar,
Head, Dept. of Zoology,
University of Poona,
Pune

Dr. U.V. Wagh,
Member Secretary,
Director,
National Facility For Animal
Tissue and Cell Culture,
Pune

SCIENTIFIC ADVISORY COMMITTEE

Prof.H.Sharat Chandra (Chairman),
Professor, Dept. of Microbiology &
Cell Biology, Indian Institute of
Science, BANGALORE

Dr.S.Sriramachari,
Emeritus Scientist
Institute of Pathology,
P.B.No.4909
Safdarjang Hospital campus,
New Delhi : 110 029

Dr.A.N.Bhisey,
Head,
Division of Cell Biology,
Cancer Research Institute,
Parel, Bombay : 400 012

Prof.V.R.Kalayana Raman
Chairman & Managing Director,
Bharat Immunological and
Biological Corpon.Ltd.,
V-13, Green Park Extn.,
New Delhi : 110 016

Prof.(Mrs)Indira Nath,
Head,
Department of Biotechnology,
All India Institute of
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Ansari Nagar,
New Delhi : 110 029

Dr.P.R.Krishnaswamy,
Director,
Vithal Mallya Scientific
Research Foundation,
P.B.No.406, K.R.Road,
Bangalore : 560 004

Prof.K.P.Gopinathan,
Chairman,
Dept. of Microbiology &
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Indian Institute of Science,
Bangalore : 560 012

Dr.Madan Mohan,
Director,
M.M.Eyetech,
Clinic & Microsurgery Centre,
28-29 A Block, Lajpat Nagar III,
Lajpat Raj Marg,
New Delhi : 110 024

Prof. V.R.Muthukkaruppan,
Professor & Head,
Dept. of Immunology,
School of Biological Sciences,
Madurai Kamraj University
Madurai : 625 021

Dr.Rama Mukherjee,
Staff Scientist,
Microbiology Div.,
National Institute of
Immunology,Shaheed Jeet Singh
Marg,New Delhi110 067

Dr.Ashok Khar,
Scientist,
Centre For Cellular &
Molecular Biology,
Uppal Road,
Hyderabad : 500 007

Prof.U.W.Kenkare,
National Facility For
Animal Tissue & Cell Culture,
Pune

Dr.U.V.Wagh,
Director,
National Facility For
Animal Tissue & Cell Culture,
Pune

FINANCE COMMITTEE

Shri S.B.Krishnan,(Chairman)
Joint Secretary & Financial Adviser,
Govt. of India
Dept. of Biotechnology,
Ministry of Science & Technology,
New Delhi

Prof.H.Sharat Chandra
Professor, Dept. of Microbiology Cell
Biology, Indian Institute of Science,
Banglore

Dr.S.H.Iqbal
Head,
Divisional of Technical Services,
National Chemical Laboratory,
Pune

Dr.U.V.Wagh
Director,
National Facility For Animal
Tissue and Cell Culture,
Pune

BUILDING COMMITTEE

Dr.U.V.Wagh (Chairman)
Director,
National Facility For Animal
Tissue and Cell Culture,
Pune

Mrs. Sachi Choudhari,
Director (Finance),
Dept. of Biotechnology,
Ministry of Science & Technology,
New Delhi

Dr.M.K.Goverdhan
Dy.Director,
National Institute of Virology,
Pune

Prof.V.R.Sardesai
Principal,
BKPS College of Architecture,
Pune

Shri B.Bose
Sr. Manager (Admn)
National Institute of
Immunology, New Delhi

Shri M.V. Patil,
Chief Engineer,
PWD, Pune Div.,
Pune

Prof.D.N.Deobagkar
Dept. of Zoology,
University of Poona,
Pune

PURCHASE COMMITTEE

Dr.C.Sivaram (Chairman),
Ex-Director, Gr. Scientist,
National Chemical Laboratory
Pune

Prof.U.W.Kenkare,
National Facility For
Animal Tissue and Cell Culture,
Pune

Prof.D.N.Deobagkar
Dept of Zoology,
University of Poona,
Pune

Adminsatrative Officer
Major P.K.Bapat
National Facility For Animal
Tissue and Cell Culture,
Pune

Accounts Officer,
Shri T.G.R.Pillai
National Facility For Animal
Tissue and Cell Culture,
Pune

Director
Dr.U.V.Wagh
National Facility For Animal
Tissue and Cell Culture,
Pune

INSTITUTIONAL CO-ORDINATION COMMITTEE

Prof.S.C.Gupte (Chairman)
Vice Chancellor,
University of Poona,
Pune

Dr. Mrs. Manju Sharma,
Adviser,
Dept of Biotechnology,
Ministry of Science &
Technology,Govt. of India
New Delhi (Nominee of DBT),

Shri S.B. Krishnan,
Joint Secretary & Financial
Adviser,Govt. of India
Dept. of Biotechnology
New Delhi

Dr. Kalyan Banerjee,
Director,
National Institute of Virology,
Pune
(Nominee of Director General
Indian Council of Medical
Research, New Delhi)

Prof. A. S. Kolaskar
Head, Dept. of Zoology
University of Poona

Dr.U.V.Wagh
Director,
National Facility For Animal
Tissue and Cell Culture
Pune

List of Cell Lines

ARMADILLO
DNI.F *(Foreskin)*
DNI.K *(Kidney)*
DNI.Lu *(Lung)*
DNI.Sk *(Skin)*
DNI.Sp *(Spleen)*
DNI.Tr *(Trachea)*
TN1.LU *(Lung)*

ARMYWORM,FALL
Sf9 *(Ovary)*

BABOON
26CB-1 *(Lymphoblast)*

BAT
Tb 1 Lu (NBL-12) *(Lung)*

BOVINE
BT *(Turbinate)*
Bu (IMR-31) *(Lung)*
CPA *(Artery)*
CPA-47 *(Artery)*
EBTr (NBL-4) *(Trachea)*
EJG *(Endothelium)*
MDBK (NBL1) *(Kidney)*

CAT
AK-D *(Lung)*
CRFK *(Kidney)*

CHICKEN
SL-29 *(Embryo)*

DOG
Cf2Th *(Thymus)*
D1712/30 C3 *(Sarcoma)*
MDCK (NBL-2) *(Kidney)*

DOLPHIN
Sp 1 K (NBL-10) *(Kidney)*

DROSOPHILA
D. MEL. (2) *(Embryo)*

DUCK,PEKING
Duck embryo *(Embryo)*

FISH
BB *(Trunk)*
FHM *(Skin)*
Grunt Fin (GF) *(Fin)*
RTG-2 *(Gonads)*
RTH-149 *(Hepatoma)*

FOX,GREY
FoLu *(Lung)*

FROG,BULL
FT *(Tongue)*

FROG,GRASS
ICR 134 *(Embryo, Diploid)*
ICR 2A *(Embryo, haploid)*

GIBBON
MLA-144 *(Lymphoma)*

GOAT
Ch 1 Es (NBL-8) *(Esophagus)*

GOOSE
CGBQ *(Sternum)*

HAMSTER,ARMENIAN
AHL-1 *(Lung)*

HAMSTER,CHINESE
CHO *(Ovary)*
CHO 1-15 500 *(Kidney)*
CHO-K1 *(Ovary)*
Don *(Lung)*
Lec1 *(Ovary)*
Lec2 *(Ovary)*
Lec8 *(Ovary)*
R 1610 *(Somatic cell)*
UT-1 *(Ovary)*

HAMSTER,SYRIAN
BHK (CLONE 13) *(Kidney)*
BHK TK *(Kidney)*
BHK-21 *(Kidney)*
BHK-21(C-13) *(Kidney)*
BHK21 CLONE 13-3P (Kidn
HIT-T15 *(Pancrease)*
HaK *(Kidney)*
PYY *(Kidney)*
RPMI 1846 *(Melanoma)*
tk-ts13 *(Kidney)*

HORSE
E.Derm (NBL-6) *(Dermis)*

HUMAN
1321 N1 *(Brain)*
143 B *(Sarcoma)*
293 *(Kideney)*
303 L *(Peripheral blood)*
5637 *(Bladder)*
8E5 *(Lymphocyte T)*
A-375 *(Melanoma)*
A-431 *(Skin/Carcinoma)*
AD 14163 *(Peripheral Blood*
AE 12037 *(Leukemia)*
AE 2117 *(Peripheral blood)*
AE 7389 *(Leukemia)*
AV3 *(Amnion)*
BRISTOL 8 *(Lymphoblast)*
BUD-8 *(Skin)*
BeWo *(Choriocarcinoma)*
Bu25 TK *(Cervix)*
C-33A *(Cervix)*
C211 *(Skin)*
CEM-A *(Lymphoblast)*
CEM-CM3 *(Leukemia)*
CEM-T4 *(Lymphoblast)*
COLO 205 *(Colon)*
COLO 320 DM *(Colon)*
COLO 320 DM *(Colon)*
COLO 679 *(Melanoma)*
CRI DU CHAT *(Skin)*
Chang Liver *(Liver)*
D283 *(Medulloblastoma)*
Daudi *(Burkittlymphoma)*
Dempsey *(Skin)*
Detroit 510 *(Skin)*
Detroit 525 *(Skin)*
Detroit 529 *(Skin)*
Detroit 532 *(Foreskin)*
Detroit 562 *(Pharynx)*
E.H.IV (Elaine IV) *(Leukocytes)*
EB-3 *(Burkittlymphoma)*
EB1 *(Maxilla)*
EJ138 *(Bladder)*
F 265 *(Peripheral blood)*

FHs 74 Int *(Small intestine)*
FL *(Amnion)*
G-361 *(Melanoma)*
G-401 *(Kidney)*
GCT *(Histiocytoma)*
GK-5 *(Lymphoblast)*
GO-G-CCM *(Brain)*
GO-G-UVM *(Brain)*
H33HJ-JA1 (Jurkat derivative) *(Lymphoma)*
H9 *(Lymphoma)*
H9/HTLV-IIIB *(Lymphocyte T)*
HBL-100 *(Breast)*
HDCS *(Kidney)*
HEL 299 *(Lung)*
HEL.92.1.7 *(Leukemia)*
HEp-2 *(Larynx)*
HF19 *(Lung)*
HFL 1 *(Lung)*
HFS-9 *(Fibrosarcoma)*
HISM *(Instestine)*
HL-60 *(Leukemia)*
HLS-2 *(Sarcoma)*
HM2 *(Lymphocyte T)*
HMCB *(Melanoma)*
HOS *(Sarcoma)*
HS-1 *(Sarcoma)*
HS-Sultan *(Plasmacytoma)*
HSF *(Skin)*
HT 55 *(Colon)*
HT-1080 *(Fibrosarcoma)*
HT115 *(Colon)*
HUV-EC-C *(Umbilical Cord)*
HeLa *(Cervix)*
HeLa 229 *(Cervix)*
HeLa CD-4-Clone 6C *(Cervix)*
HeLa S3 *(Cervix)*
Hep 3B *(Liver)*
Hep G2 *(Liver)*
Hs 67 *(Thymus)*
Hs 696 *(Adenocarcinoma)*
HuNS-1 *(Lymphoblast)*
HuT 78 *(Lymphoma)*
IM-9 *(Lymphoblast)*
IMR-32 *(Neuroblastoma)*
IMR-90 *(Lung)*
INT407 *(Intestine)*
Intestine 407 *(Intestine)*
JM *(Lymphocyte)*
JURKAT E6.1 *(Leukemia)*
JURKAT J6 *(Leukemia)*
Jiyoye (P-2003) *(Ascitic fluid)*
Jurkat.clone E6-1 *(Leukemia T Cell*
K-562 *(Leukemia)*
K562 C1.6 *(Leukemia)*
KB *(Mouth)*
KG-1 *(Leukemia)*
KG-1A *(Leukemia)*
KHOS/NP(R-970-5) *(Sarcoma)*
L-132 *(Lung)*
LL 29 *(Lung)*
LNCaP.FGC *(Prostate)*
LY-28 *(Nasal)*
MCF-7 *(Breast)*
MDA-MB-231 *(Breast)*
MDA-MB-453 *(Breast)*
MDA-MB-468 *(Breast)*
ME-180 *(Cervix)*
MG-63 *(Osteosarcoma)*
MIA-Pa-Ca-2 *(Pancreas)*
MOLT-3 *(Leukemia T cell)*
MOLT-4 *(Leukemia)*
MRC-5 *(Lung)*
MRC-9 *(Lung)*
Malme-3M *(Melanoma)*
Mo *(Leukemia)*
Mo-B *(Leukemia)*
Molt 4 Clone 8 *(Lymphoblast)*
NC-37 *(Leukocytes)*
NCI-H510A *(Lung)*
NIH:OVCAR-3 *(Ovary)*
Namalwa *(Lymphoblastoid)*
OAW42 *(Ovary)*
P3HR1 *(Ascitis)*
PA-1 *(Ovary)*
PC-3 *(Prostate)*
PESS *(Skin)*
PLC/PRF/5 (Alexander cells) *Liver*
QIMR-WIL *(Lukemia)*
RAJI A *(Maxilla)*
RD *(Sarcoma)*
RDS-15 *(Rhabdosarcoma)*
RPMI 1788 *(Leucocytes)*
RPMI 2650 *(Nasal Septum)*
RPMI 6666 *(Lymphoblast)*
RPMI 7666 *(Lymphoblast)*
RPMI 7951 *(Melanoma)*
RPMI 8226 *(Myeloma)*
Raji *(Maxilla)*
SCC-15 *(Tongue)*
SCC-25 *(Tongue)*
SCC-9 *(Tongue)*
SK-BR-3 *(Breast)*
SK-HEP-1 *(Liver)*
SK-MEL-1 *(Melanoma)*
SK-MEL-2 *(Melanoma)*
SK-MEL-28 *(Melanoma)*
SK-MEL-3 *(Melanoma)*
SK-N-MC *(Neuroblastoma)*
SK-N-SH *(Neuroblastoma)*
SK-NEP-1 *(Kidney)*
SKL-1 *(Leukemia)*
SKO-007 *(Myeloma)*
SU.86.86 *(Pancreas)*
SW 1353 *(Humerus)*
SW 620 *(Colon)*
SW 626 *(Ovary)*
SW 900 *(Lung)*
SW 982 *(Synovial Sarcoma)*
SW-13 *(Adrenal cortex)*
Saos-2 *(Sarcoma)*
SiHa *(Cervix)*
T-24 *(Bladder)*
T-47D *(Breast)*
T24 *(Bladder)*
THP-1 *(Monocyte)*
TK6 *(Lymphoblast)*
TK6 TGR *(Lymphoblast)*
TT *(Thyroid)*
U-373 MG *(Glioblastoma)*
U-87 MG *(Glioblastoma)*
U-937 *(Lymphoma)*
U-Amnion *(Amnion)*
U266B1 *(Myeloma)*
UC 729-6 *(Lymphoblast)*
VRC-4 (HAM-3) *(Amnion)*
WI-26 *(Lung)*
WI-26 VA4 *(Lung)*
WI-38 *(Lung)*
WI-38 VA13 subline 2 RA *(Lung)*
WISH *(Amnion)*
WM 266-4 *(Melanoma)*
WRL 68 *(Liver)*
WS1 *(Skin)*
XP *(Skin)*
XP 12 BE *(Skin)*
Y79 *(Retinoblastoma)*
ZR-75-1 *(Breast)*
ZR-75-30 *(Breast)*
nEM-SS *(Lymphoblast)*

IGUANA
IGH-2 *(Heart)*

LIZARD
Gekko lung-1 (GL1) *(Lung)*

MARMOSET
B95-8 *(Leukocytes)*

MINIPIG
MPK *(Kidney)*

MINK
MiCl1 (S+L-) *(Lung)*
Mv 1 Lu (NBL-7) *(Lung)*

MONKEY, AFRICAN GREEN
BS-C-1 *(Kidney)*
COS 7 *(Kidney)*
COS-1 *(Kidney)*
CV-1 *(Kidney)*
DBS-FCL-1 *(Lung)*
GMK (AH-1) *(Kidney)*
TC-7 *(Kidney)*
VERO 76 *(Kidney)*
VERO C1008 *(Kidney)*
Vero *(Kidney)*

MONKEY,BUFFALO GREEN
BGM *(Kidney)*

MONKEY,OWL
OMK(637-69) *(Kidney)*

MONKEY,RHESUS
FRhK-4 *(Kidney)*
LLC MK-2 *(Kidney)*
LLC MK-2 Derivative *(Kidney)*
MA-104 *(Kidney)*
MS *(Kidney)*

MOSQUITO
ATC 10 *(Larvae)*
ATC 121 *(Larvae)*
ATC 136 *(Larvae)*
ATC 137 *(Larvae)*
ATC 15 *(Larvae)*
ATC 16 *(Larvae)*
ATC 173 *(Larvae)*
ATC 176 *(Larvae)*
ATC 18 *(Larvae)*
ATC 23 *(Larvae)*
ATC 245 *(Larvae)*
ATC 88 *(Larvae)*
Anopheles stephensi *(Larvae)*
M 61 *(Larvae)*
TRA-171 *(Larvae)*

MOTH
Antheraea cells,adapted *(Ovary)*

MOUSE
3T3-L1 *(Embryo)*
3T3-Swiss albino *(Embryo)*
3T6-Swiss albino *(Embryo)*
653-SF: HAZ653-SF *(Myeloma)*
A-3T3 *(Embryo)*
ABE-8. 1/2 *(Lymphoma)*
AtT-20 *(Pitutary)*
B104-1-1 NIH/3T3 *(Embryo)*
B16-F1 *(Melanoma)*
BALB/3T3 clone A31 *(Embryo)*
BC3H1 *(Brain)*
BCL1 clone 5B1B *(Leukemia)*
BW5147.3 *(Lymphoma)*
BW5147.G.1.4.OUAR.1 (Lymphoma)
Balb/C SFME *(Embryo)*
C127I *(Breast)*
C3H/10T1/2,Clone 8 *(Embryo)*
C57/B1 *(Melanoma)*
CCRF S-180 II *(Sarcoma)*
CL-S1 *(Breast)*
CLONE 707 *(Leukemia)*
CNC 127I *(Breast)*
CTLL *(Lymphocyte)*
CTLL-2 *(Lymphocyte,T)*
Clone M-3 *(Melanoma)*
D10.G4.1 *(Lymphocyte,T)*
EJ-6-2-BAM-6A NIH/3T3 *(Embryo)*
EL-4 *(Lymhpma/Leukemia)*
EL4 Bu ou6 *(Lymphoma)*
EL4.IL-2 *(Lymphoma)*
ES-E14TG2a *(Embryo)*
Ehrlich-Lettre Ascites strain E *(Carcinoma)*
F-9 *(Testies)*
FO *(Myeloma)*
FOX-NY *(Myeloma)*
G-7 *(Myoblast)*
G-8 *(Myoblast)*
HSDM1C1 *(Fibrosarcoma)*
HSDM1C1 *(Fibrosarcoma)*
Hepa 1-6 *(Liver)*
I-10 *(Testes)*
IC-21 *(Macrophage)*
J558 *(Myeloma)*
J744A.1 *(Monocyte-macropha)*
Jensen Sarcoma *(Sarcoma)*
KLN 205 *(Carcinoma)*
L-1210 *(Leukemia)*
L-M *(Connective tissue)*
L-M (TK-) *(Connective Tissue)*
LBRM-33-1A5 *(Lymphoma)*
LBRM-33-1A5 *(Lymphoma)*
LBRM-33-1A5 *(Lymphoma)*
LBRM-33-5A4 *(Lymphoma)*
LT-2-E *(Carcinoma)*
M1 *(Myeloblast)*
MC/9 *(Liver)*
MFS-8 *(Fibrosarcoma)*
MOP-8 *(Embryo)*
MOPC-31C *(Plasmacytoma)*
MPC 11 OUA R *(Myeloma)*
MPC-11 *(Myeloma)*
McCoy *(Unknown)*
NB-41A3 *(Neuroblastoma)*
NCTC 2071 *(Connective Tissue)*
NCTC 3749 *(Lymphoma)*
NCTC clone 929 *(Connective issue)*
NIH/3T3 *(Embyro)*
NIH3T3 CLONE D4 *(Embryo)*
NULLI-SSC1 *(Testis)*
Neuro-2a *(Neuroblastoma)*
P1.17 IGG2A *(Myeloma)*
P3.6.2.8.1 *(Myeloma)*
P3/NS1/1Ag4-1 (NS-1) *(Myeloma)*
P388.D1 (CLONE 3124) *(Lymphoma)*
P388D1(IL1) *(Lymphoma)*
P3X63Ag8.653 *(Myeloma)*
P3X63Ag8U.1 *(Myeloma)*
P815 *(Mastocytoma)*
PA317 *(Embryo)*
PCC-4 *(Carcinoma)*
PCC-4-AO *(Carcinoma)*
PCC-4-Aza-R *(Carcinoma)*
PSA-1 *(Carcinoma)*
PU5-1R *(Monocyte-macropha)*
RAG *(Kidney)*
RAW264.7 *(Monocyte-macrioga)*
SCA-9 *(Submandibular gla)*
SSC-PSA1 *(Carcinoma)*
STO *(Embryo)*
STO-1 *(Embryo)*
STO-2 *(Embryo)*
Sarcoma 180 *(Sarcoma)*
Sp2/01-Ag14 *(Myeloma)*
Swiss SFME *(Embryo)*
TM3 *(Testis)*
TM4 *(Testis)*
WEHI 164 *(Fibrosarcoma)*
WEHI-231 *(Lymphoma)*
WEHI-274.1 *(Monocyte)*
WEHI-279 *(Lymphoma)*
WEHI-3 *(Myelomonocyte)*
WGD5 *(Embryo)*
XB-2 *(Keratinocytes)*
Y-1 *(Adrenal Cortex)*
YAC-1 *(Lymphoma)*

MOUSE/HUMAN
F3B6 *(Myeloma)*
SHM-D33 *(Myeloma)*

MUNTJAC
Indian Muntjac *(Skin)*

PIG
LLC-PK *(Kidney)*

LLC-PK1A *(Kidney)*
PK-15 *(Kidney)*
PS *(Kidney)*

PIG,GUINEA
GPBr-5 *(Brain)*
JH400 clone 1 *(Lung)*

POTOROO
Pt K2 *(Kidney)*

RABBIT
HIG-82 *(Synoviocyte)*
RK13 *(Kidney)*
SIRC *(Cornea)*

RABBIT,COTTONTAIL
Sf 1 Ep (NBL-11) *(Skin)*

RACCOON
PI 1 Ut (NBL-9) *(Uterus)*

RAT
6-23 (Clone 6) *(Thyroid, medulla.)*
A7r5 *(Muscle , smooth)*
AR42J *(Pancreas)*
BRL 3A *(Liver)*
C6 Glial Cell *(Glial tissue)*
CRI-10P *(Pancreas)*
FRTL *(Thyroid)*
GH1 *(Pitutary)*
GH3 *(Pitutary)*
H4TG *(Liver)*
H9c2(2-1) *(Heart)*
IEC-6 *(Intestine , Small)*
L6 *(Muscle)*
LC-540 *(Testis)*
LLC-WRC 256 *(Carcinoma)*
MH1C1 *(Liver)*
NMU *(Breast)*
NRK *(Kidney)*
NRK-49F *(Kidney)*
NRK-52E *(Kidney)*
NRK-52E *(Kidney)*
PC-12 *(Pheochromocytoma)*
PHI1 *(Liver)*
R2C *(Testis)*
RR 1022 *(Sarcoma)*
Y3-Ag1.2.3 *(Myeloma)*

SNAIL
Bge *(Embryo)*

TICK
ATC 424

TOAD,S.AFRICAN CLAWE
A6 *(Kidney)*

TURTLE
TH-1 subline B1 *(Heart)*

VIPER,RUSSELL'S
VSW *(Spleen)*

HYBRIDOMA
(BF1) 8A3.31 *(Human T Cell (Antigen Receptor)*
10B9 *(Human Endothelial Cell)*
11-5.2.1.9 *(I-A K)*
12.1 *(Human CD 6)*
13C4 *(Shiga Toxin)*
14E5 *(Human Endothelial Cell)*
158.2 *(Murine Macrophage)*
15F3-1 *(Dengue Type 1 Virus)*
1H10-6 *(Dengue Type 4 Virus)*
2.4G2 *(Mouse Fc Receptor)*
2AB1-IA10 *(Laminin)*
2D12 *(Yellow Fever Virus)*
2E.6 *(Murine CD 18)*
33D1 *(Mouse Dendritic Cells)*
34 *(Murine Leukemia virus p15 gag protein)*
35.1 *(Human CD 2 (T Cell Surface Tp 50))*
3C10 *(Human Monocyte)*
3H5-1 *(Dengue Type 2 Virus)*
3I *(Human Anti DNA Antibody)*
4B2 *(Human CD 45)*
5 F12 AD3 *(Human Erythropoetin)*
51.1 *(Human CD 8)*
5D4-11 *(Dengue Type 3 Virus)*
63D3 *(Human Monocyte)*
71F7 *(Concanavalin A)*
72A1 *(EB Virus)*
76-7-4 *(Porcine B Cell)*
7D4 *(Murine IL-2 Receptor)*
7G7B6 *(Human IL-2 Receptor)*
9-A5 *(Rat Na,K-ATPase)*
9-B1 *(Rat Na,K-ATPase)*
9.3 F10 *(Human HLA DR/DO)*
A1G3 *(Human Thymocyte (Medullary))*
A5A2-F8 *(Human T Helper Cells)*
A5A2-F8 *(Human T Cell- Helper)*
A9 *(HMG-CoA Reductase)*
ACT-1 *(Prokaryotic Actin)*
AE9D6 *(Insulin)*
AF6-120.1.2 *(Human IA B Haplotype)*
ALPHA INT FIL *(Alpha Intermediate Filaments)*
Alpha IR-1 *(Human Insulin Receptor)*
Antibody 2,06 *(Human IA Monomorphic)*
B3/25 *(Human Hematopoietic Cells)*
BE3F9 *(Insulin)*
BF-11 *(Human Erythropoietin)*
Bet-2 *(Mouse IgM)*
CA3-F4 *(Lewis A Blood Group)*
CC9C10 *(Insulin)*
CE9H9 *(Insulin)*
CF4-C4 *(Lewis A Blood Group)*
CG7C7 *(Insulin)*
CH26-1352 *(DNA Double Stranded)*
D1-4G2-4-15 *(Flavi- Virus)*
DB9G8 *(Insulin)*
DII33.1 *(Human Insulin Receptor)*
E5BB3IIA2 *(Human IgE)*
Ep16 *(Human Epithelial Cell)*
GAMMA 2-11.1 *(Human Interferon Gamma)*
GAMMA 3-11.1 *(Human Interferon Gamma)*
H21F8-1 *(Hepatitis B Virus)*
H25B10 *(Hepatitis B Virus)*
HFN 7.1 *(Human Fibronectin)*
HK-PEG-1 *(Influenza Virus)*
HP6000 *(Human Fc regeion)*
HP6023 *(Human IgG4-Fc)*
IFGCP-F1BA10 *(Human Interferon)*
IGG-4A4 *(LDL receptor)*
IL-A11 *(Bovine CD4)*
IL-A30 *(Bovine IgM)*
IL-A42 *(Bovine CD2)*
IV.3 *(Human Fc Receptor)*
J 11D.2 *(Murine B Cell)*
L101 *(Human Melanoma)*
L203 *(Human Ia Monomorphic)*
LI 27 *(Human Melanoma)*
LM2/1.6.11 *(Human MAC 1)*
Lym-1 *(Human B Cell)*
M 144 *(Human Melanoma)*
M-2E6 *(Human IgM)*
M-2E6 *(Human IgM Fab Portion)*
M1/70.15.11.5 HL *(Murine MAC 1 Alpha)*
M17/4.4.11.9 *(LFA 1 Alpha)*
M3/38.1.2.8 HL2 *(MAC-2)*
M3/84.6.34 *(MAC-3)*

M5/114.15.2 *(Human I-A b,d,q Haplotype and I-Ed, K)*
MAR 18.5 *(Rat Kappa Chain)*
MB23G2 *(Murine CD 45)*
MMA *(Human Monocyte)*
MRSS-1 (D2D4) *(DNA Single Stranded)*
MYC 1-9E10.2 *(C-MYC Protein)*
Mab 126 *(Ganglioside GD2)*
OKM-1 *(Human CD 14 (Monocyte))*
OKT-1 *(Human CD 5 (T Cell-Peripheral))*
OKT-11 *(Human CD 2 (T Cell-Peripheral))*
OKT-3 *(Human CD 3 (T Cell-Peripheral))*
OKT-4 *(Human CD 4 (T Cell-Helper))*
OKT-5 *(Human T Cell Subset Suppressor / Cytotoxic)*
OKT-6 *(Human CD 1a (Thymocyte))*
OKT-8 *(Human CD 8 (T Cell Subset Suppressor/Cytotoxic))*
OKT10 *(Human CD 38 (T CEll Precursor))*
OKT9 *(Human Transferin receptor (T Cell Activated))*
PK136 *(Murine NK Cells)*
R4-6A2 *(Murine Interferon Gamma)*
T58/30 *(Human Hematopoetic Cells)*
TS1/18.1.2.11 *(Human LFA 1 Beta)*
TS1/22.1.1.13 *(Human LFA Alpha)*
TS2/18.1.1 *(Human LFA 2)*
TS2/9.1.4.3 *(Human LFA 3)*
W4F.5B *(Human T Cell Antigen Receptor)*
W6/32 *(Human HLA-A,B,C)*
YN1/1.7.4 *(Murine ICAM)*

HLA defined Human B-LYMPHOCYTE

1JHAF *(9030)*
31227ABO *(9061)*
AMALA *(9064)*
ARBO *(9102)*
BER *(9093)*
BM 16 *(9038)*
BTB *(9067)*
DBB *(9052)*
DEU *(9025)*
EHM *(9080)*
EJ32B *(9085)*
EK *(9054)*
FPAF *(9105)*
HO301 *(9055)*
IBW9 *(9049)*
KAS011 *(9009)*
KT 12 *(9072)*
KT 17 *(9024)*
LKT3 *(9107)*
LOO81785 *(9018)*
MZ070782 *(9002)*
OLL *(9100)*
OMW *(9058)*
PE117 *(9028)*
RML *(9016)*
SA *(9001)*
SP0010 *(9036)*
TAB089 *(9066)*
VAVY *(9023)*
WT24 *(9015)*
YAR *(9026)*

CELLS VACCINE PRODUCTION

DBS-FCL-1 Passage 10
DBS-FRhL-1 Passage 101
DBS-FRhL-2 Passage 10
FDA- HeLa Passage 92-94
FDA-FCK-4 Passage 104
FDA-HEP2 Cincinnati
FDA-LLC-MK2 Passage 119
FDA-MRC-5 Passage 16
FDA-WI-38
FDA-WIDR Passage 17

Cultures will be supplied to registered Laborartories and Institutes only.
For registration and procurement of cultures write to The Curator, NFATCC, 85/1, Jopasana, Paud Road, Kothrud, Pune 411 029.
Tel. 335928/335954/369422-2 Telex. 145 7576 NTCF IN FAX 212-369501.

NATIONAL FACILITY ANIMAL TISSUE AND CELL CULTURE
REGISTRATION FORM

1. Institute/Organization

_____

2. Address

_____
_____
_____
_____

Telephone Numbers _____ Facsimile Number _____
Telex Number _____ Gram _____

3. Category (Please √)

- ☐ Govt. Central
- ☐ Govt. State
- ☐ Govt. Autonomous
- ☐ Public
- ☐ Private
- ☐ Any other (Please specify)

- ☐ Research
- ☐ Training
- ☐ Public Health
- ☐ Pharmaceutical
- ☐ Production
- ☐ Any other (Please specify)

4. Laboratories/Departments of your organisation to whom the service for culture supply can be extended.

_____
_____
_____

5. List the ficilities available for handling cultures (including biosafety containment facility)

_____
_____
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Please turn to next page

FAREWELL

NFATCC has grown through different phases - seeding, initiation,metamorphosis, development and is now establishing fast as a new cultivated species of organisation through biotechnological advancements. This has been possible because of the able guidance and unhesitant support extended by many a distinguished professors, eminent scientists, science managers, executives and other erudite academicians. One amongst these is Dr.Ramachandran, who has been not only actively associated with NFATCC's conceptual plan, initiation and establisment but also contributed towards planning its organisational and structural framework of the society. His dynamic leadership, scientific temperament, clear vision and affectionate attitude as a guardian nurtured and fostered NFATCC to achieve the present status. His association catalysed NFATCC to take up new activities to support the biotechnological programmes planned and executed in the country.

The memories of the late burning oil and speedy preparation and movements of file to expedite the progress of NFATCC by the so called bureaucrats Mr. S. Chatterjee and Mr. S.R. Sapra are cherished.

Shri. L. Venkatesh, Director and Chief Engineer, Shri B.S. Ramamoorthy, Project Engineer, and Shri. Krishnamurari, Additional Chief Engineer, of the C&S Group of Department of Atomic Energy, were actively involved in the designing and executing of the NFATCC complex.

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