

93 - 94

# ANNUAL REPORT



NATIONAL FACILITY  
FOR

ANIMAL TISSUE AND CELL CULTURE

# **NATIONAL FACILITY FOR ANIMAL TISSUE AND CELL CULTURE**

DEPARTMENT OF BIOTECHNOLOGY  
GOVERNMENT OF INDIA

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**ANNUAL REPORT  
1993-94**

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## **T**ERMS 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

The laboratories and the infrastructural set up established at the 'Jopasana' and 'Jidnyasa' buildings as an interim facility are very active. Most of the functions of NFATCC have been initiated and some have excelled even in this small interim facility. The pace of construction of the laboratories, residential and support facilities on the University of Poona campus has been maintained as per time schedule. Considering the progress which is in keeping with the planned schedule, these new laboratories could be made functional in the early 1995. Thanks to the architect, DAE group, the contractors and the University of Poona for this progress.

There is a 46% increase in the supply of cell lines over the last year. Technology for the culture of new and fastidious cells such as from bone, oropharyngeal mucosal epithelia, breast cancer, fibroblasts from people with schizopherenia etc., have been developed. Attempts to cultivate *Plasmodium vivax*, the common malarial parasite in India and other obligate parasites were made.

Development of Tissue Bank Technology continued with the cultivation of skin, bone marrow, heart valves and cornea. Studies in cryobiology indicate that free radicals which cause damage to the tissues during freezing-thawing cycles which be minimised by supplementing foetal calf serum in the cryopreservation medium.

To facilitate the studies on protective antigens from filaria an animal model consisting of *Brugi malayi* infected *mastomys* and *Aedes aegypti* mosquito is being developed within our facilities. Studies on adaptation of myeloma and hybridoma cells to Goat serum were continued and screening of anticancer compounds on various cell lines is on the rise.

Immortalization of bone marrow cells, studies on melanoma oncogenes, rheumatoid arthritis skin fibroblast cultures etc. are being undertaken to understand the disease processes. Effect of growth factors on bone marrow stromal cells, mechanisms of melanin transfer from melanocyte to keratinocytes, the role

of cytokines on neuroblastoma cells, stress proteins in mosquito cells in response to insecticides and development of *in vitro* pancreas models for testing hypoglycemics are some of the projects with potential biotechnological and biomedical applications that are being worked out. Other projects such as shear stress on cells cultured in bioreactors are likely to lead to immediate improvements in bioreactors design employed for collection of cell products.

The faculty continued to actively participate in the Biotechnology teaching programme of the University of Poona. NFATCC's Human Resources Development programme is popular and a large number of Scientists and technicians are deputed from various Indian Institutes for training in general and specialized areas of tissue culture. Over the year 24 persons received training and working facilities at NFATCC.

## **CULTURE REPOSITORY AND CHARACTERIZATION OF CULTURES**

- a) Nuclear stock stored in liquid nitrogen
- b) Stock for quality control and redistribution
- c) Supply services
- d) Obligate parasites
- e) Vectors, plasmids & genomic libraries
- f) Media
- g) HIV screening

# **C**ULTURE REPOSITORY AND CHARACTERIZATION OF CULTURES

## **CELL CULTURE**

### **NUCLEAR STOCK STORED IN LIQUID NITROGEN**

A total of 1127 cultures comprising of 594 different cell strains are in stock at NFATCC.

### **STOCK FOR QUALITY CONTROL AND REDISTRIBUTION**

A total of 156 cell lines were expanded for redistribution and quality control.

## **SUPPLY SERVICES**

Between April 1993 and March 1994, 765 cultures comprising of 156 different cell lines were supplied to 107 Research teams of various institutes located in 37 cities (Fig.1). 216 scientists have registered for availing of cell supply facilities. The various institutes availing NFATCC's cell supply services is listed separately.

## **OBLIGATE PARASITES**

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

## **VECTORS, PLASMIDS & GENOMIC LIBRARIES**

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

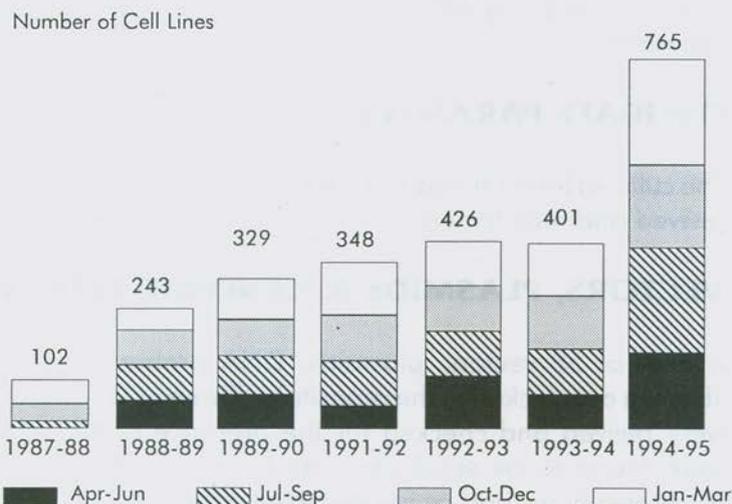
## MEDIA

Twenty-two different synthetic media, balanced salt solutions and tissue culture reagents were prepared at NFATCC. The preparation of goat serum from goat blood procured from local abattoirs was continued. Quality control, cell proliferation assays, sterility etc. were tested for Fetal Calf Serum (FCS), Horse Serum (HS), Goat Serum (GS) and New Born Calf Serum (NBCS) and supplied to the staff members.

## HIV SCREENING :

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

## Cell Lines Supplied



**Fig. 1** There has been an increasing trend in the demand of cell lines.

## DEVELOPMENT OF CULTURES

- a) Isolation and cultivation of Bone cells and their characterization in terms of osteoblastic features
- b) Characterisation of cultured human oropharyngeal mucosal epithelial cells
- c) Establishment of breast cancer cell lines from high risk ethnic groups : Studies on characterization and early detection
- d) Cell models for investigating molecular mechanisms associated with etiology/pathophysiology of mental disorders
- e) In vitro cultivation of erythrocytic stages of Plasmodium vivax and large scale cultivation of Plasmodium falciparum

# **D** **VELOPMENT OF CULTURES**

## **ISOLATION AND CULTIVATION OF BONE CELLS AND THEIR CHARACTERIZATION IN TERMS OF OSTEOBLASTIC FEATURES**

Normally Bone cells under *in vivo* conditions ossify 3-dimensionally to form bone. Under *in vitro* conditions however, they grow as fibroblast monolayers. These fibroblasts differ from other organ-derived fibroblasts in some osteogenic features such as expression of alkaline phosphatase activity and secretion of some osteogenic proteins. It is therefore of interest to culture bone cells and identify factors that induce full bone-like differentiation in these cells.

Earlier, bone cells from human foetuses and young Balb/C mice obtained by trypsin and collagenase digestion were cultured in MEM(E) and Ham's F-12 supplemented with 10% FCS.

Attempts to increase cell yields were made by modifying the enzyme treatment of human foetal and mice bones. It was found that a crude collagenase treatment (2 mg/ml) for 1 hr for foetal bones and 2 hr for mice bones yielded far more cells than the sequential collagenase - trypsin method followed earlier. In Ham's F12 supplemented with 10% inactivated FCS the cells grew for more than five passages. Histochemical detection of alkaline phosphatase activity was done and the cells were found to be positive for this enzyme indicating their osteogenic nature. Some of these cells have been cryopreserved at various passages. 3-D growth of these cells on Nylon bolting matrices is being attempted.

## **CHARACTERIZATION OF CULTURED HUMAN OROPHARYNGEAL MUCOSAL EPITHELIAL CELLS**

The oropharyngeal mucosa is exposed to many external agents which are responsible for upper respiratory tract infections. Whether the mucosal epithelial cells exhibit any specific trophism

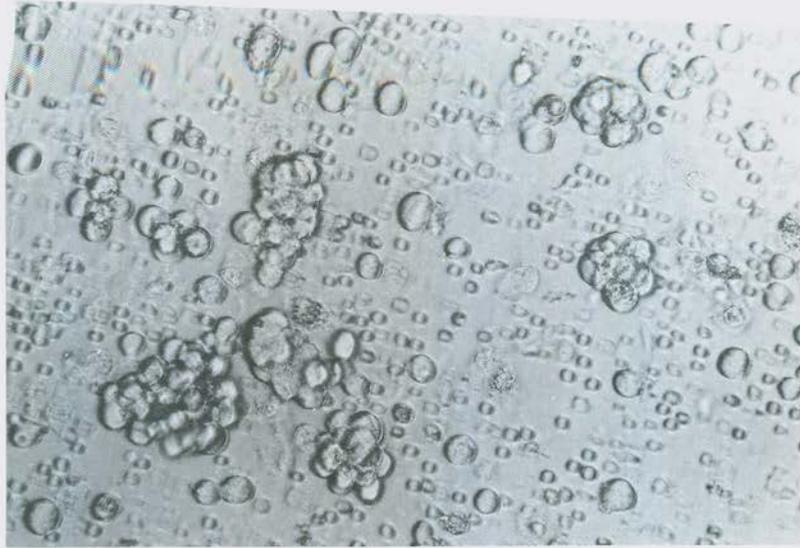
initiating the disease process can be investigated in a bio-mimetic model using cultured mucosal epithelial cells.

Pharyngeal and buccal mucosal epithelial cells from 21 fetuses were cultured. These cells have been subcultured upto three passages. The plating efficiency decreased with passage. These cells were also characterized on the basis of cytokeratin and vimentin expression.

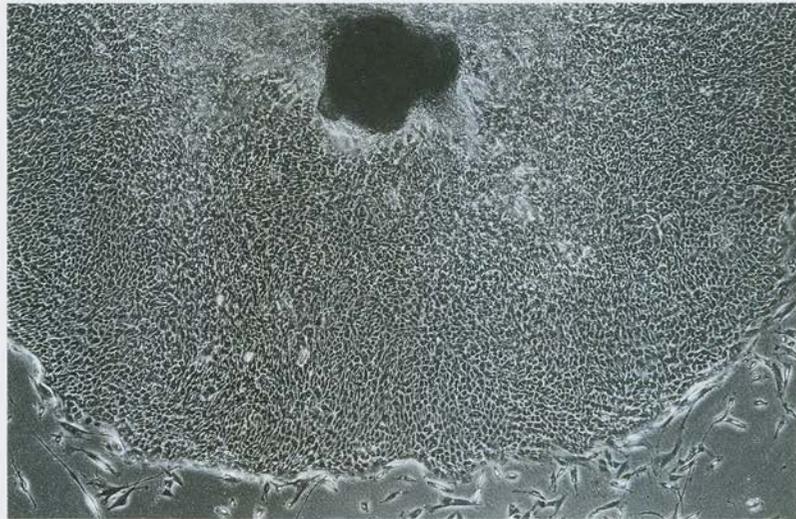
### **ESTABLISHMENT OF BREAST CANCER CELL LINES FROM HIGH RISK ETHNIC GROUPS : STUDIES ON CHARACTERIZATION AND EARLY DETECTION**

Cancer of the breast is one of the commonest malignancy in women. Although the etiopathogenesis of breast cancer is not known, there is a general agreement that a genetic predisposition exists as is also reflected in existence of breast cancer prone families. In India, the Parsi community has a higher incidence of breast cancer as compared to other communities. Parsis in India are a near homogenous population and recent studies have indicated that Parsi women with breast cancer show different ECO-RI restriction patterns for neu and int.2 oncogenes as well as for p53 tumour suppressor gene. Since carcinogenesis is a multi-step process it is of importance to establish normal, precancerous and cancerous breast cell lines from Parsi women.

Primary cultures of breast epithelial cells from 14 cases of infiltrating ductal carcinoma were successfully established (Fig.2 & 3). Attempts to passage these cells were not successful except in 2 cases. Contaminant fibroblast cells were removed by mechanical scraping or by selective detachment with EDTA. Metaphase chromosome spreads prepared from lymphocytes of the patients were trypsin-Giemsa stained and photographed. Cytokeratin peptides PAN, 8 and 18 were localized in some primary cultures of breast cancer cells. These cells also stained positive for vimentin.



**Fig. 2** Breast tumour biopsy subjected to protease digestion gives rise to organoids x 125



**Fig. 3** These organoids attach to the culture dish and give rise to epithelial cells x 45

## **CELL MODELS FOR INVESTIGATING MOLECULAR MECHANISMS ASSOCIATED WITH ETIOLOGY/PATHOPHYSIOLOGY OF MENTAL DISORDERS :**

Fibroblasts from skin biopsies are easy to culture and maintain their metabolic and growth properties over a long period of time. Recent studies have indicated that some of the neuronal abnormalities seen in nerve tissue are also manifested in cultured fibroblasts. These include enzymes of neurotransmitter mechanisms, ion channels, monoamine oxidase, catechol-o-methyltransferase, choline uptake and muscarinic receptors. Further abnormal growth properties, decreased adhesive properties and lower plasma membrane phospholipids and cholesterol have been reported for fibroblasts from schizophrenia patients. Hence fibroblast cultures from these patients can be used to study neuronal functional abnormalities as well as to enrich human genetic mutant cell repository.

A total of twenty skin biopsies from schizophrenia patients and their normal siblings were obtained from Shree Kripamayee Institute of Mental Health, Miraj and fibroblast explant cultures were set up. Of these, cultures from 6 patients and 6 normal individuals grew as monolayers and could be passaged to 4-5 passages. These are being used for morphological, growth kinetic and chromosomal studies. Some of these cultures have been stored frozen in liquid nitrogen.

## **IN VITRO CULTIVATION OF ERYTHROCYTIC STAGES OF PLASMODIUM VIVAX AND LARGE SCALE CULTIVATION OF PLASMODIUM FALCIPARUM**

Malaria caused by *P. falciparum* and *P. vivax* has become drug resistant and is thus a major cause of worry throughout the world. In vitro culture system has been developed for *P. falciparum* and several species of primate malarias. However, the worldwide efforts to develop in vitro culture system for *P. vivax* have not been successful so far. The two reasons which hamper in vitro culture of *P. vivax* are

- a) the parasite invades only reticulocytes,
- b) infected reticulocytes rupture during schizont stages in vitro.

Methods for large scale culture of erythrocytic stages of P. falciparum have been developed. These procedures require daily change of medium either by manual or by semi automated methods.

In vitro cultivation of Plasmodium vivax erythrocytic stages has been tried by candle jar method. O<sup>+</sup> RBC were treated with different enzymes and solutions. The treated RBC were inoculated with P. vivax infected RBC's obtained from patients. However, the enzymes did not bring about any changes which facilitated parasite growth. The hypo-osmotically treated O<sup>+</sup> RBC's too, did not support the parasite growth.

The PIGPA (50 mM sodium pyruvate, 50 mM inosine, 100 mM Glucose, 500 mM sodium phosphate, 5 mM adenine in normal saline) solution was used to restore ATP levels in RBC's and used for large scale in vitro cultivation of P.falciparum. This has not significantly enhanced the parasitemia compared to controls. These experiments are being modified and repeated.

## **DEVELOPMENT OF TISSUE BANK TECHNOLOGY**

- a) Organ culture of Foetal skin for transplantation
- b) Culture of skin keratinocytes and their 3-D epitheliation for transplantation to burns, non-healing ulcers and vitiligo cases
- c) Growth characteristics of normal and abnormal melanocytes and their response to synthetic and natural growth factors
- d) Studies on cryopreservation and revival of normal human bone marrow
- e) Cryopreservation and revival of heart valves for transplantation
- f) Studies on free radicals during freezing and thawing of cornea



Bhoomi Puja being performed at the site of residence quarters for NFATCC staff.



NFATCC Staff enjoyed their first monsoon picnic. Photographs shows Table land, Panchgani, Maharashtra

## **D** **VELOPMENT OF TISSUE BANK TECHNOLOGY**

The technology for storing cornea, skin, bone marrow and heart valves is being developed at NFATCC. These are detailed under the respective projects.

### **ORGAN CULTURE OF FOETAL SKIN FOR TRANSPLANTATION**

Foetal tissues do not elicit classical immune reactions and at the same time have a tremendous potential for growth. These features make them a good source of transplantable material. Development of organ culture systems allows the retention of functional and structural integrity of the foetal tissues till the recipient is readied for transplantation.

Intermediate-split thickness skin organ culture system that allows the retention of structural integrity as revealed by histological criteria for upto 35 days, was developed. Thus cultured skin, was transplanted onto 19 cases of vitiligo with dermabrasion only on the contralateral region serving as controls. In order to assess the physiological functional state of the organ culture, periodically, pieces of organ cultured foetal skin were taken and cultured for the outgrowth of fibroblasts and keratinocytes.

Pieces of skin obtained from the mother organ culture after 10,20 and 30 days manifested fibroblast and keratinocyte outgrowth. On the whole, the repigmentation of the vitiligo patches was found to be retained for upto 2 years. Thus intermediate-split thickness organ culture of foetal skin has been shown to retain its functional and structural integrity for 30 to 35 days. When transplanted it helps repigmentation of vitiligenous skin which is retained for upto 2 years.

### **CULTURE OF HUMAN SKIN KERATINOCYTES AND THEIR 3-D EPITHELIATION FOR TRANSPLANTATION TO BURNS, NON-HEALING**

## **ULCERS AND VITILIGO CASES**

Proliferation of human skin keratinocytes *in vitro* and their 3-D epitheliation allow the growth of large sheets of epidermis that can be used for the treatment of large burns, non-healing ulcers etc. The technique involves :

- i) Separation of epidermis from the dermis
- ii) Optimizing the media and culture conditions for the selective growth of keratinocytes
- iii) Induction of 3-D epitheliation in these monolayers
- iv) Removal of this newly formed epithelial sheet without disrupting it into single cells
- v) Preparation of a smooth granulated wound bed for grafting and
- vi) Development of actual grafting procedures.

Culturing of keratinocytes, their 3-D epitheliation and grafting of the epithelia for the treatment of burns, nevi and vitiligo were standardized. Two cases of burns and three cases of giant nevi were taken up for autologous cultured epithelia transplantation. The biopsies were successfully grown as 3-D epithelial sheets and grafted onto 25 to 100 cm<sup>2</sup> granulation wound bed. In all the cases the graft took well.

Cultured-epithelia-grafting appears to be superior to all the currently available methods such as biological substitutes, porcine skin grafts and homografts for treating burns, nevi and ulcer cases.

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

Skin pigmentation varies from individual to individual and between races. The pigment cell, melanocyte, is a center of many pigmentary disorders such as vitiligo, melanoma, melasma, piebaldism and various kind of nevi. Therefore an understanding of factors involved in melanocyte proliferation

and melanization is a necessary prerequisite to unravel the etiopathogenesis of these disorders.

Melanocytes in contrast to melanoma cells require a synthetic tumour promotor, tetradecanoyl phorbol acetate (TPA) for their proliferation *in vitro*. Earlier the role of TPA in melanoma cell growth and differentiation was investigated and it was demonstrated that low concentration of TPA (1-10 ng/ml) stimulates the proliferation of melanoma cells while their tyrosinase activity, a marker enzyme of pigment cells, is reduced.

Since TPA is a synthetic growth factor and a potent carcinogen, the search for natural growth factors that could be operating through keratinocytes and fibroblasts is being made. In mixed cultures of melanocytes and keratinocytes, melanocytes were found to undergo controlled proliferation maintaining the ratio of 1:16 to 1:32 with keratinocytes. Similarly, dermal equivalents were prepared from cultured human fibroblasts and  $10^4$  melanocytes were seeded and maintained in TPA free media. At various intervals these 3-D dermis equivalents were cryofixed and cryocut sections were taken. These are being analysed by histochemical and immunofluorescence methods.

Tyrosinase and Dopachrome tautomerase, the enzymes involved in melanogenesis, were assayed in different samples of B-16 F10 melanomas and were respectively found to vary from 11.5 to 21 m moles of dopachrome formed/min/mg protein and 32-45 m moles of dopachrome converted/min/mg protein. The effect of natural and synthetic growth factors on these enzymes is now being investigated in B-16 melanoma cells.

### **STUDIES ON CRYO-PRESERVATION AND REVIVAL OF NORMAL HUMAN BONE MARROW**

Bone marrow banking is fast developing as an important aspect of Tissue Banking to meet the requirements of patients with several types of anemias and for those receiving radical chemo- and radio-therapy for widespread malignancy. Development of long term cryopreservation technology would also be useful to people working in hazardous areas like nuclear reactors, as they will be able to bank their bone marrow for later use, if it

becomes necessary.

Our earlier studies had indicated that a large percentage of these stem cells and early progenitor cells could be recovered after cryopreservation.

Stem and committed cells are vital for bone marrow transplantation and further efforts to improve their recovery after cryopreservation were made. The ability of ascorbic acid, tocopherol acetate, superoxide dismutase, catalase and reduced glutathione were tested to protect mouse bone marrow cells from oxygen radical induced injuries during freezing and thawing. Superoxide dismutase and reduced glutathione did not appear to protect cells from oxidative injury while catalase and ascorbic acid were found to confer enhanced protection (Fig 4). Trehalose, a membrane protective disaccharide was also found to protect the cells from oxidative injury. The results of other agents are awaited.

Pretreatment of revived stem cells with lymphokines to enhance their survival and the use of anti-freeze protein and anti-freeze glycoprotein for bone marrow cryopreservation is now being undertaken.

## **CRYOPRESERVATION AND REVIVAL OF HEART VALVES FOR TRANSPLANTATION**

Biomechanical valves used for replacing diseased human heart valves have the drawback of not being able to offer a non-thrombogenic surface. As a result the recipients have to be on anti-coagulant therapy throughout their life. Donor homografts, as currently used after glutaraldehyde fixation, are incapable of repair of wear and tear. Under these conditions the alternative appears to be homografts that have their fibroblasts viable to take care of wear and tear and the valve surface seeded with recipient's own endothelial cells that will offer a non-thrombogenic surface without eliciting an immune response.

Heart valves cryopreserved by the rapid cooling and by the slow cooling methods were revived and tested for mechanical strength and viability. While mechanical strength was not affected by

cryopreservation, the valves were not viable at the time of procurement itself. Hence a rapid test using the mitochondrial succinate dehydrogenase dependent reduction of 3-[4,5-dimethylthiazol-2yl] - 2,5-diphenyl tetrazolium bromide (MTT) was developed to test the viability status of the donor heart valves with various warm ischemic times. The results indicate that heart valves with a warm ischemic time of less than 11 hours are viable. This is now being confirmed by explant outgrowth of fibroblasts from valves collected after various warm ischemic times.

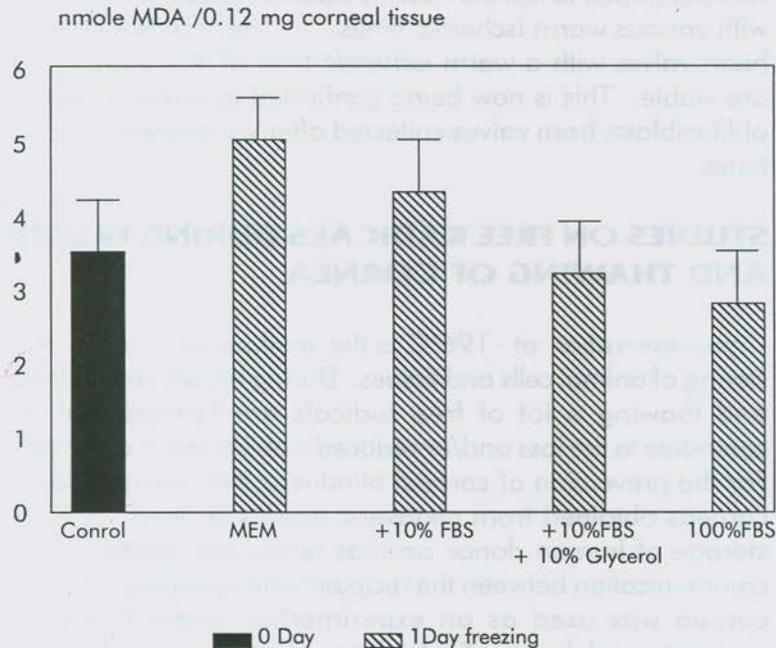
### **STUDIES ON FREE RADICALS DURING FREEZING AND THAWING OF CORNEA**

Cryopreservation at  $-196^{\circ}\text{C}$  is the method of choice for the storing of animal cells and tissues. During the process of freezing and thawing a lot of free radicals are formed that might contribute to the loss and/or reduced viability status of the tissues. For the prevention of corneal blindness, the transplantation of corneas obtained from cadaveric donors is done. Long term storage of human donor corneas allows the establishment of communication between the recipient and eyebanks. Therefore, cornea was used as an experimental model to study the generation of free radicals during freezing and thawing of biological samples.

Using trephined chicken corneas as an experimental system, it was established that lipid peroxidation levels are higher when corneas are stored at  $37^{\circ}\text{C}$  than at  $-66^{\circ}\text{C}$ . Even at  $-66^{\circ}\text{C}$ , it was found that lipid peroxidation levels were higher at 1,3 and 5 days post-storage as compared to 0 day.

The effect of storage media, foetal bovine serum (FBS) cryoprotectants, pre-incubation in cryopreservation medium and pre-treatment with antibiotics on the generation of free radicals by cornea during freezing and thawing was investigated. Addition of cryoprotectants, such as glycerol to the freezing media and the use of 100% FBS as a storage medium prevented the increase in lipid peroxidation levels that occurs in the freeze-thaw cycle (Fig 5). Liver homogenates are expected to generate higher levels of lipid peroxidation because of their large pool of

### EFFECT OF MEDIA COMPOSITION ON LIPID PEROXIDATION OF CORNEA



**Fig. 5** FBS appears to protect the free radical induced damage occurring during freezing and thawing of Cornea.

unsaturated fatty acids. Therefore in further experiments, the free radical protectant properties of FBS and other agents were investigated using liver homogenates. Again, FBS was found to be a very potent suppressor of lipid peroxidation. The studies thus indicate that FBS may be a useful additive to organ preservation solutions.

## **INDIGENOUS TECHNOLOGY AND RENDERING EXPERT SERVICES**

- a) Adaptation of myeloma and hybridoma cells to goat serum
- b) To identify and characterize protective antigens in lymphatic filariasis
- c) In vitro assay for quantitation of antitetanus antibody an alternative to animal model
- d) Mechanism of action of thiophene derived drugs for evaluation of their possible use in chemotherapy

# **I**NDIGENOUS TECHNOLOGY AND RENDERING EXPERT SERVICES

## **ADAPTATION OF MYELOMA AND HYBRIDOMA CELLS TO GOAT SERUM**

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

It was found that SP2/0 myeloma cells and an anti-insulin antibody secreting hybridoma CC9C10 can grow well without changing their fusion and secreting properties in 10% GS supplemented with 20 µg/ml of soybean lipid mixture.

CC9C10 adapted to GS + Soyabean lipid mixture was monitored for secretory activity and antigen epitope specificity by ELISA, Western blotting and inhibition studies. The results indicate that, after adaptation to GS + Soyabean lipid mixture, CC9C10 cells continue to secrete anti-insulin antibodies with the same characteristics as those growing in FCS. The technique for adaptation of myeloma and hybridoma cells to GS + Soyabean lipid mixture has been submitted for obtaining a patent.

## **TO IDENTIFY AND CHARACTERIZE PROTECTIVE ANTIGENS IN LYMPHATIC FILARIASIS**

Approximately 100 million people living in tropical areas are affected with filariasis caused by W.bancrofti and B.malayi. 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 also to protect mastomys against challenge by these larvae. Whether the antigen recognised by this monoclonal antibody can be used as a protective antigen against human filarial infections is therefore being investigated.

**TABLE 1 CROSS REACTIVITY OF FILARIAL SERA WITH CATTLE FILARIAL ANTIGENS**

Sera	No.	ELISA INDEX	
		Soluble antigen	Cuticular antigen
Normal	6	0.20 ± 0.1	0.14 ± 0.18
Endemic normal	15	0.61 ± 0.33	0.62 ± 0.37
Microfilaraemic	23	0.58 ± 0.43	0.54 ± 0.33
Elephantoid	11	0.45 ± 0.29	0.63 ± 0.22

Cultivation of human filarial worm *W. bancrofti* infective larval stage (L3 stages) from mosquitoes collected from endemic areas was carried out with partial success. Readily available cattle filarial worms (*Setaria digitata*) were used to prepare soluble and cuticular antigens. ELISA tests revealed that MAb 46.08.76 identified some cross-reactive proteins from cattle filarial worms.

Cross reactivity of the soluble and cuticular antigens of *Setaria* was then tested against serum obtained from normal, microfilaraemic and elephantoid sera (Table 1). Blood and urine samples of people living in endemic areas were collected. *Microfilaria* from the blood of these individuals were maintained in short term cultures to isolate excretory/secretory antigens and attempts to develop monoclonal antibodies against these antigens were made. An animal model consisting of *B. malayi* infected animals and eggs of *A. aegypti* mosquitoes obtained from CDRI, Lucknow is being developed to investigate filaria protective antigens.

### **IN VITRO ASSAY FOR QUANTITATION OF ANTITETANUS ANTIBODY - AN ALTERNATIVE TO ANIMAL MODEL**

Estimation of antibodies to tetanus toxoid is currently being done by method of mouse neutralization assay. This assay is cumbersome, time consuming and expensive. There is an urgent need to develop a simple quantifiable method that can measure neutralizing antitetanus toxoid antibodies.

Using mouse Neuro-2a cells a two-step enzyme linked method for assessing the levels of neutralizing antitetanus toxoid antibodies was developed. The sensitivity achieved by the assay was found to be at the level of 0.002 IU/ml and correlation coefficients obtained for different set of experiments were found to be 0.85-0.95.

Serum samples from 55 individuals were screened for quantitation of anti-tetanus antibodies by the newly developed Neuro-2a cell assay and compared with those found by the mouse neutralization assay (MNA). There was a significant correlation ( $r = 0.82$ ) between values obtained by these two

methods. The effect of storing fixed cells at - 20°C for 3 and 6 months on the detection of levels of anti-tetanus toxoid antibody was tested. It was found that optical density readings were lower than those of fresh cells. However, the correlation between binding of labelled toxin and concentration of antibody was comparable in both fresh and stored cells. The sensitivity of the assay was found to be 0.00016 IU. These results indicate that this Neuro-2a cell-anti-tetanus toxoid assay system can be used in place of MNA for testing the potency of tetanus vaccines and toxoids.

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

Cancer cells have the hexose monophosphate pathway (HMP) of carbohydrate metabolism functioning at higher levels than in normal cells. Thus anti-metabolites affecting HMP would be ideal drugs for cancer chemotherapy.

Thiophene and its derivatives are one such group of HMP anti-metabolites that are being evaluated in our laboratory.

Four thiophene derivatives viz, thiodiglycolic acid, thiodipropionic acid, dicetol and thiophene 2, 5-dimethylenyl thiouronium chloride were screened on myeloid leukemia (K562), lymphoblastoid (KG1) and human lung fibroblast (WI38) cell lines. The last two were found to have anti-mitotic effects. Two more drugs, thiodiglycol sulfoxide and thiodiglycol ester were tested on WI38 and its transformed counterpart WI38 VA13. These chemicals were found to be toxic at 1:100 dilution.

Multidrug resistance (MDR) is a vexatious problem encountered in cancer chemotherapy. This phenomenon is characterised by increased expression of a 170 KD glycoprotein on the plasma membrane which functions as an efficient effluxer of drugs from the cells. In order to screen the MDR in cells continuously exposed to thiophene drugs, a probe specific to MDR-1 gene was used to transform *E. coli*. The plasmid has been purified on a large scale and labelled with <sup>32</sup>P-CTP. This will be used to screen for MDR in cancer cells exposed to thiophene drugs.

## BIOMEDICAL AND BIOTECHNOLOGICAL APPLICATIONS

- a) Immortalization of human bone marrow derived stromal cells and stem cells via transfection of cloned oncogenes
- b) Effect of growth factors on stromal cells
- c) Identification of melanoma oncogene and characterization of the oncogene products
- d) Shear stress and mass cultivation of cells in Bioreactors
- e) Molecular Biology of Hexokinase
- f) Endocytic vesicles and their association with cytoskeleton
- g) Hyaluronic acid in rheumatoid arthritis skin cultures before and after ayurvedic treatment
- h) In vitro studies on the role of growth factors on melanosome transfer from melanocytes to keratinocytes in the epidermis
- i) Effect of cytokines on neuroblastoma cells
- j) Study of stress proteins in mosquito A. stephensi
- k) Development of in vitro model for testing hypoglycaemics and screening of diabetogenic agents.
- l) Studies on amylase secretion in organ culture of pancreas
- m) Regeneration of pancreatic  $\beta$ -cells in vivo and proliferation of  $\beta$ -cells in vitro

# **BIOLOGICAL AND BIOTECHNOLOGICAL APPLICATIONS**

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

Hematopoiesis is a highly regulated process that occurs within bone marrow cells. Stromal cells and stem cells interact to produce various blood cells in a normal individual and its impaired regulation leads to various kinds of haematologic malignancies. Immortalization of stromal and stem cells is therefore being undertaken to further study the interaction between these two cell types.

Transfection of stromal cells with SV-40LT and polyoma middle T antigen was done and seven different cell lines were made. Of these 71-SV and 71 Py cells grew for more than 20 passages.

The possibility of using V-myb related oncogenes in immortalization of myeloblasts is being explored. Presently, eukaryotic expression vectors known to express chicken V-myb gene under inducible promoters such as SV-40 (constitutive), MMTV-LTR (Hydrocortisone inducible) and metallothionien (heavy metal inducible) are being constructed. Since V-myb induces the transforming phenotype in transfected cells, a series of chimeric-myb molecules are being constructed for use in immortalization of the cells without transformation.

## **EFFECT OF GROWTH FACTORS ON STROMAL CELLS**

A large number of factors that induce stem cells to commit themselves to different lineages in the bone marrow are known. However, the effect of these factors on stromal cells themselves is not known.

Erythropoietin (EPO) a potent hormone that induces

differentiation of stem cells to erythrocyte lineage was found to stimulate the proliferation of stromal cells. EPO was also found to induce network-like structures in stromal cells cultured in 3-D agarose matrix.

Whether the above observed effect was proliferation or adhesion and whether this result is a direct effect of EPO on stromal cells or is mediated through EPO's action on haematopoietic cells was investigated. It was found that EPO acted on mononuclear cells which in turn released some factors that act on stromal cells. This factor was found to be a heparin-binding and heat labile. Fibroblast growth factor (FGF) and transforming growth factor- $\beta$  (TGF $\beta$ ) were also found to induce aggregation in stromal cells cultured in agarose matrix. Conditioned medium (CM) of EPO-treated mononuclear cells also induced the expression of a new glycosylated protein on stromal cells. Both cycloheximide and tunicamycin inhibited the synthesis of this new glycosylated protein. This new protein appears to be a fucosylated glycoconjugate as revealed by UEA-1 lectin fluorescence studies. The expression of this protein in stromal cells exposed to CM was found to be negative at 5 hours and gradually increased between 10-24 hours after treatment. However FGF and TGF- $\beta$  did not induce the expression of this glycoprotein indicating that the stromal cell aggregation induced by CM is probably independent of its activity to induce the glycosylated glycoprotein.

## **IDENTIFICATION OF MELANOMA ONCOGENE AND CHARACTERIZATION OF ONCOGENE PRODUCTS**

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

DNA isolated from melanoma clone M3 cells was transfected to NIH 3T3 cells and resultant clones were picked. Secondary

transfection of DNA from these clones was done and eight clones were developed. Two clones were chosen for further studies. These clones were found to grow in soft agar and also grew as tumours in Balb/c mice. Immunofluorescence studies with M3-1 serum and vitiligo serum revealed intense particulate fluorescence on the M3 cells as well as on the clones.

Efforts to identify the proteins expressed by the transfected genes were initiated using metabolic labelling with  $^{35}\text{S}$ -methionine, immunoprecipitation and SDS-PAGE autoradiography. These studies have revealed that vitiligo serum identifies a 32 KD protein on M3 cells and 32 KD, 28 KD and 110 KD proteins on SK-MEL 28 cells. These differences could be due to different species origin of the melanoma cells. M3-1 serum however identified a 120 KD protein on clone M3 as well as in the M3 DNA transfected clone. In order to facilitate further analysis of these proteins, preparation of a cDNA library from normal human melanocytes, transfected clones and from M3 cells is being undertaken. For this purpose total RNA and mRNA has been isolated.

### **SHEAR STRESS AND MASS CULTIVATION OF CELLS IN BIOREACTORS**

Mass cultivation of cells to produce required biological products is an area in cell culture that is rapidly progressing. The commonly used mass culture systems are the hollow fibre type and the spinner type bioreactors. In these reactors a flow of liquid nutrients has to be maintained and as a consequence the cells are subjected to hydrodynamic shear stress. The effect of shear stress on the growth of animal cells is being investigated.

Initially, a murine hybridoma secreting anti-Japanese encephalitis virus antibody was grown in hollow fiber bioreactors and a high titer of antibody was obtained. Using glucose, it was found that transport of solutes occurs without hindrance. The major effect of shear stress on both suspension and adherent cultures appears to be on the actin network.

The effect of turbulent shear stress on the lymphoblastoid cell line KG1 exposed to 2.5, 5 and 7.5 revolutions per second (r/s)

was studied using total and viable cell counts and actin immunofluorescence. Cells exposed to 5 and 7.5 r/s were unable to proliferate, while cells exposed to 2.5 r/s grew at the same rate as they grew in non-turbulent conditions (Fig 6). The actual turbulence measured by laser Doppler anemometer was found to be two fold higher at 5 r/s as compared to the values obtained at 2.5 r/s. Depolymerization of actin network was observed in cells exposed to high r/s and serum was found to protect stress- induced changes in the cells. The residence time distribution of high molecular weight protein in the Extracapillary space under various flow conditions has been measured using the Hollow Fiber Bioreactor (HFBR). The implication of these findings on the product recovery from cells cultivated in HFBR is being investigated.

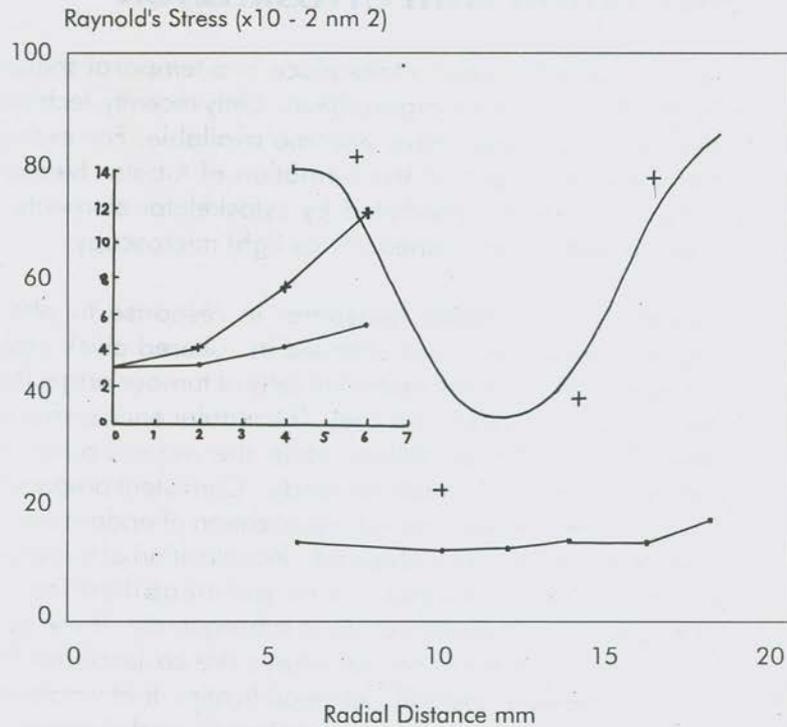
### **MOLECULAR BIOLOGY OF HEXOKINASE**

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

Two putative cDNA clones coding for bovine Hexokinase were isolated from bovine brain cDNA library using 41 -mer oligonucleotide as probe. These cDNA clones were characterised with respect to their restriction sites and partial nucleic acid sequencing was done. Translation of DNA sequence data to amino acid sequence revealed that the isolated clones had 70 % homology with the sequence published for bovine HK.

Efforts at sequencing were continued using the 41-mer as primer and 1.7 Kb clone as a template. The amino acid sequence extrapolated from the translation of the nucleic acid sequence revealed that, for a stretch of 81 amino acids from the carboxyterminal end there was 80% homology with published sequences for HK-I.

**FETAL CALF SERUM REDUCES TURBULENT  
STRESS & SHEAR SENSITIVITY OF CELLS  
IN STIRRED BIOREACTORS**



**Fig. 6** Radial profile of Reynold's stress at an agitation speed of 2.5 r/s for medium without FBS (+) and with 10% FBS (.)

**Inset :** The cells agitated at 2.5 r/s for a period of 120 min and allowed to proliferate in RPMI 1640 supplemented with 10% FBS. The cells agitated in FCS grew well.

*In situ* hybridization of fluorescein dUTP labelled rat Type I and II cDNA probes of HK followed by a single immunological amplification was done. The fluorescent signals were found on different chromosomes of the rat metaphase preparations indicating that type I and type II hexokinases are different single copy genes.

### **ENDOCYTIC VESICLES AND THEIR ASSOCIATION WITH CYTOSKELETON**

Cellular functions normally take place in a temporal sequence within the 3-Dimensional organisation. Only recently, techniques to study these changes have become available. For example, the temporal sequence of the formation of tubular lysosomes and their movements mediated by cytoskeletal elements can now be visualized simultaneously by light microscopy.

The formation of tubular lysosomes in response to phorbol myristate acetate (PMA) was checked in cultured chick embryo fibroblasts, (CEF), human epithelial cells of tumour origin (MCF-7) and mosquito (C6/36) cell lines. The tubular endosomes were localised using Lucifer Yellow while the microtubules were visualized using anti- $\beta$  tubulin antibody. Consistent preparations that would permit simultaneous visualization of endosomes and microtubules could not be obtained. Visualization of endosomes was done before fixation and permeabilization and immunostaining of tubulin was done subsequently. These studies have revealed that these two structures are co-localized in the cells. Conditions of fixation/permeabilization that would allow simultaneous localization of tubular endosomes and microtubules are being standardized.

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

Rheumatoid arthritis (RA) is a crippling disorder of the joints, with unknown etiopathology. Allopathic treatment is merely symptomatic whereas Ayurvedic treatment seems to improve the condition of the patient. Extracellular matrix proteins and

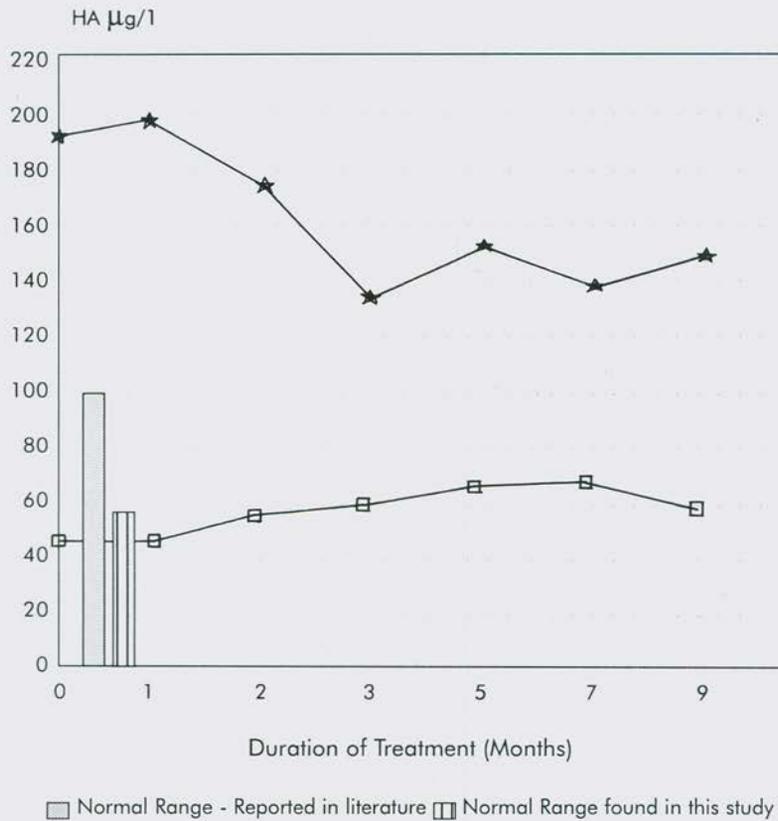


**Fig. 7** Skin fibroblasts from Rheumatoid arthritis patients manifest intense whorling patterns x 140



**Fig. 8** In comparison skin fibroblasts from normal donor do not manifest such intense whorling patterns x 140

### HA Levels of RA Patients Changes under Ayurvedic Treatment



**Fig. 9** Serum HA levels were high in some of the RA patients. There was a tendency in these patients to have lower HA levels following Ayurvedic treatment (★). In others the HA levels remained at normal levels (□). Clinically all the patients showed improvements.

carbohydrate polymers have been shown to play a role in the etiopathogenesis of the disorder and therefore a study of the changes in hyaluronic acid (HA, a major extracellular carbohydrate polymer) in RA patients before and after Ayurvedic treatments was initiated.

Fibroblast cultures were established from 13 RA patients and 13 normal individuals. The fibroblasts from RA patients manifested intense whorling as compared to those from normal donors (Fig.7 & 8). Media and cells were harvested at various intervals and HA was estimated. The levels of HA in fibroblast culture medium ranged from 298.70 to 527.30 ug/l in 5 RA patients whereas in normal individuals the values were between 174.00 to 279.50 ug/l. These values correlated with the serum levels of HA in the normal donors as well as in RA patients. The serum HA levels have been followed up at various intervals after Ayurvedic treatments (Fig 9) and a tendency for HA levels to come down to normal range was found in those RA patients who had higher serum HA levels. In addition to these experiments, the techniques for estimation of uronic acid and N-acetylglucosaminidase in fibroblasts, media and extracellular matrix have been standardized.

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

Melanin is synthesized within a specialised organelle called as melanosome in the melanocyte. Initially these melanosomes are localized within the perinuclear area and gradually move outward into the dendrites and from there into the keratinocytes. The keratinocytes distribute melanins throughout the epidermis during the process of differentiation and cornification. Thus, skin colour depends upon the interaction of these two cell types.

Five human truncal and foreskin samples were procured and pure cultures of melanocytes, fibroblasts and keratinocytes were set up. The fibroblasts were used to prepare 3-D dermal equivalents. Melanocytes were seeded onto these 3-D dermal equivalents, allowed to grow for 10 days and fixed with cold p-

formaldehyde. Cryo-cut sections were taken and stained for dopa-oxidase activity. Melanocytes appeared to be growing on these 3-D dermal equivalents. The next step would be to seed keratinocytes on these melanocyte-bearing dermal equivalents to study melanosome transfer. In another set of experiments, melanocytes were grown as co-cultures with keratinocytes at the ratio of 1:16 (normal physiological ratio) and 16:1 (reversed physiological ratio). Microscopic pictures of these cultures are being analysed by image analysis system. Preliminary results however indicate that melanocytes at earlier passages have an average of 4 dendrites with their lengths varying from 1.4 to 3.03 microns in both pure and co-cultures with keratinocytes.

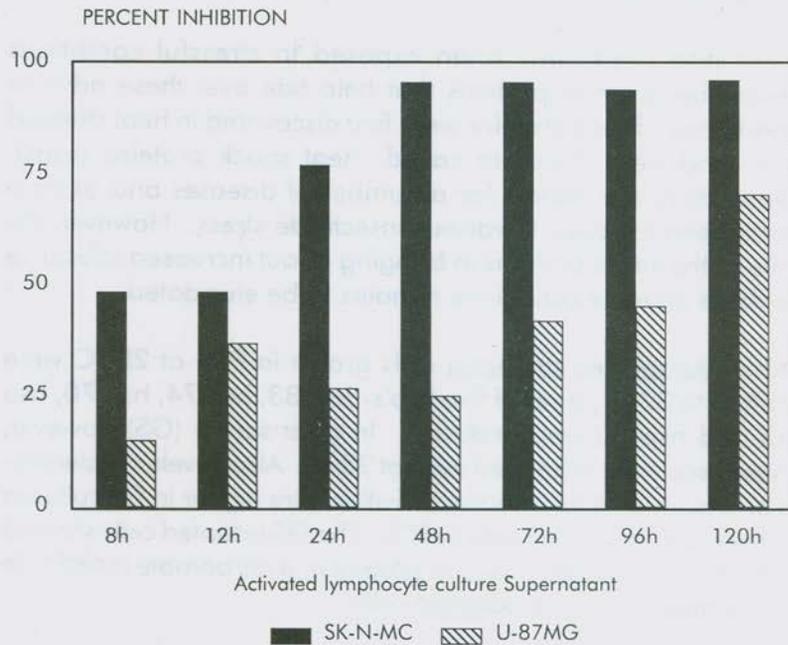
### **EFFECT OF CYTOKINES ON PROLIFERATION OF NEUROBLASTOMA CELLS**

Various types of neural cells that respond to mediators produced by immune cells express a range of antigens on their cell surface. Neuroblastoma (NB) is a good model system for investigating the mechanisms of cell differentiation in neurobiology and cancer. Therefore the effect of immune cytokines on neural growth, survival and differentiation would be of great interest.

Conditioned media of splenocytes activated with Concanavalin A and adherent macrophage/monocytes activated with lipopolysaccharide were found to inhibit the proliferation of a murine neuroblastoma cell line Neuro-2a. Among the cytokines IL- $\alpha$  and TNF  $\alpha$  were found to inhibit the proliferation of Neuro-2a cells, whereas IL-2 and IL-6 did not have any significant effect.

Another murine neuroblastoma cell line NB 41 A3 (a clone of Neuro-2a) was found to be more susceptible to culture supernatant of activated splenocytes and macrophage/monocyte cultures. The degree of inhibition varied with time of collection of supernatant after activation of splenocyte and monocyte/macrophage cells indicating that there would be qualitative and/or quantitative differences in the synthesis and secretion of different cytokines. These studies were extended to human neuroblastoma cell line SK-N-MC and compared with U-87MG,

**EFFECT OF ACTIVATED LYMPHOCYTE (PBL)  
CULTURE SUPERNATANTS ON PROLIFERATION  
OF NEUROBLASTOMA AND GLIOBLASTOMA CELLS**



SK-N-MC-Neuroblastoma cell line.  
U-87MG-Glioblastoma cell line.

**Fig. 10** Neuroblastoma cell proliferation is affected to a far more extent than Glioblastoma cells by supernatants of activated lymphocytes.

a central nervous system derived human Glioblastoma cell line. Results obtained so far indicate that antiproliferative effect of the supernatants is higher on neuroblastoma cells as compared to glioblastoma cells (Fig.10). Further, it was also found that in case of human lymphoblasts the anti-proliferative potential of culture supernatant reaches a peak at 48 hr and remains at a steady level for upto 120 hours.

### **STUDY OF STRESS PROTEINS IN MOSQUITO *A. STEPHENSI***

Cells and organisms when exposed to stressful conditions synthesize a set of proteins that help tide over these adverse conditions. These proteins were first discovered in heat stressed cells and were therefore called heat shock proteins (hsps). Mosquitoes are vectors for a number of diseases and as such have been exposed to various insecticide stress. However, the role of the stress proteins in bringing about increased tolerance to these adverse conditions remains to be elucidated.

When *Anopheles stephensi* cells grown in FCS at 28° C were shifted to 37° C, a set of five hsp's- hsp 83, hsp 74, hsp 70, hsp 63 and hsp 50 were induced. In goat serum (GS) however, these hsps were expressed even at 28° C. Also, levels of esterase-B and acetylcholine esterase activities were higher in GS cultured cells as compared to those in FCS. The GS adapted cells showed a 50 fold higher resistance to propoxur, a carbamate insecticide as compared to FCS adapted cells.

The stress proteins were found to be induced in response to other stressful conditions such as addition of ethanol, 8 - hydroxyquinoline and dexamethasone. These cells and different developmental stages of *A. stephensi* organisms were given heat shock at 39° C for 1 hour and the RNA were screened in Northern and dot blots with *A. albimanus* hsp 70 gene probe. GS maintained cells showed higher levels of hsp 70 expression at 28°C as compared to FCS maintained cells. The stress agents induced the synthesis of hsp 70 in FCS maintained cells. While IV instar larvae and pupae responded to heat shock by synthesizing hsp 70, in I and II instar larvae there was no induction. Normal as well as heat shocked III instar larvae

however, expressed elevated levels of hsp 70, indicating that the heat shock proteins may have a specific developmental role to play. A 3.5 kbp and a 2.5 kbp DNA transcript from *A. stephensi* hybridizing with the 2.4 kbp hsp 70 transcript of *A. stephensi* has been used to prepare a partial genomic library in plasmid bluescript.

### **DEVELOPMENT OF IN VITRO MODEL FOR TESTING SECRETAGOGUES AND SCREENING DIABETOGENIC AGENTS**

There is a need to develop an *in vitro* assay system for testing secretagogues so as to reduce animal experimentation, to study the direct action of drugs on islet cells and to get results quickly and economically. The first step for the development of such *in vitro* model is the isolation of islets from the exocrine pancreas.

Methods were standardized to obtain large number of islets from mouse and rat pancreas. Batches of 40, 60 and 80 islets in triplicate were incubated in 1 ml L-15 medium and stimulated with 16 mM glucose for one hour at 37°C. The insulin in the culture medium was estimated by ELISA. Glucose stimulated islets exhibited higher levels of insulin and the amount of insulin secreted was proportional to the number of islets. This stimulation reached a peak at 60 min and reached the basal level by 120 min.

Three known hypoglycaemics viz. Tolbutamide, Glybenclamide and Linogrinide were tested on isolated islets and were found to stimulate insulin secretion in a dose- dependent manner. These findings indicate that isolated islets are suitable for testing hypoglycaemics.

### **REGENERATION OF PANCREATIC $\beta$ -CELLS IN VIVO AND PROLIFERATION OF $\beta$ -CELLS IN VITRO**

Diabetogenic influences are continuously active in the organism and are normally balanced precisely by adjustments of the beta cell function. It is conceivable that long-term adaptation

involving changes in the total beta cell mass, probably, play an important role in response to chemical injury or immune assault.

Streptozotocin (200 mg/kg body wt.) induced diabetic mice. Pancreata were processed for the estimation of lipid peroxidation. It was found that lipid peroxidation levels were higher in the pancreas from the diabetic mice as opposed to that from non-diabetic control pancreas.

Mice fed with karella (Momordica chirantia) aqueous extract before injecting streptozotocin manifested lower levels of lipid peroxidation. The culture of beta cells from karella plus streptozotocin treated mice is in progress.

### **STUDIES ON AMYLASE SECRETION IN ORGAN CULTURE OF PANCREAS**

Acinar tissue surrounding the islets are larger and are loaded with zymogen granules. Diabetic and rendered diabetic animals and patients with Diabetes mellitus are known to have abnormal patterns of pancreatic amylase secretion.

Pancreatic organ cultures of adult mice were found to secrete amylase in vitro upto 12 days in 10% FCS and serum free medium supplemented with soyabean trypsin inhibitor and insulin.

Amylase secretion patterns of normal pancreatic explants and those derived from recently rendered diabetic (within a week of streptozotocin injection) were comparable. However, a significant difference was observed in amylase secretion of pancreatic explants obtained from diabetic mice (more than one month after induction of diabetes). The finding clearly indicates that normal acinar cell function depends upon the functional integrity of the islets. Further work on islet acinar interaction is in progress.

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**PUBLICATIONS/CONFERENCES/  
WORKSHOPS/AWARDS**

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- 7) R.R.Bhonde  
Attended International Symposium on Cellular and  
Molecular aspects of developmental regulation, Dept. of  
Zoology, University of Poona. Nov. 27 - Dec.1, 1993, Pune

- 8) M.Mojamdar
- a) Invited to the UGC sponsored Refresher Course on "Innovations in Teaching Biosciences"  
for two lectures
    - i) Animal Tissue Culture Biotechnology
    - ii) Tissue EngineeringMangalore University, Nov 24, 1993
  - b) Participated as a member of Scientific Committee and as an expert for the award committee for the selection of the best poster at "International Symposium on Cellular and molecular Aspects of Development Regulation". Department of Zoology, University of Poona Nov.27-Dec.1, 1993
- 9) S.Sitasawad  
Attended workshop on "Safety aspects in Research Applications of Ionizing radiations", Bhabha Atomic Research Centre Aug.2-10, 1993, Bombay.
- 10) P.B.Parab  
Attended workshop on "Safety aspects in Research Applications of Ionizing radiations", Bhabha Atomic Research Centre Aug.2-10, 1993, Bombay.
- 11) V.P.Kale  
Attended workshop on 'Non-radioactive DNA detection' Boehringer-Mannheim Apr. 24, 1993, Bombay
- 12) M.R.Wani  
Attended symposium on 'Recent advances in Production and testing of Veterinary biologicals' Jan. 29-31, 1994, NCL, Pune
- 13) R.N.Damle  
Attended Workshop on New Directions in flow cytometry sponsored by Wipro at CCMB, Nov.22-24, 1993, Hyderabad

- 14) S.GuhaRoy  
Attended short term training course on 'Transfection and transformation of eukaryotic cells' Madurai Kamaraj University 7-25 Feb. 1994 Madurai.
- 15) A.Shiras  
Participated in Indo-US workshop on 'Gene Therapy' sponsored by Fogarty International Association at CRI, Feb.23-March 12, 1994, Bombay.
- 16) The paper on "Heart Valve viability testing by 3-[4,5-dimethylthiazol-2 yl] - 2,5 - diphenyl tetrazolium bromide (MTT) assay" was awarded the First Prize in the Young Investigators Awards Session of 'Annual Conference of Association of Thorasic and Cardio-vascular Surgeons of India', 24-26 Feb.1994. Agra

**HUMAN RESOURCE DEVELOPMENT**

# HUMAN RESOURCE DEVELOPMENT

- i) The staff of NFATCC continued to participate in the teaching programme of M.Sc. Biotechnology conducted by Dept. of Zoology, Poona University.
- ii) Five students are working towards obtaining Ph.D degree on topics such as stress proteins, cell characterization by mitochondrial DNA molecular biology of hexokinase, effect of cytokine on nerve cells, etc.
- iii) Following individuals were deputed for training at NFATCC

	Name of the trainee	Deputing Inst/ Univ	Period
1.	Dr R K Singh	Dept of Pharmacology Inst of Med Sciences BHU,Varanasi	1.4.93-17.4.93
2.	Dr Shivaraman	Dept of Biotechnology GIBCOL,Bulandshahar	10.4.93-17.4.93
3.	Dr Anil Balapure	Central Drug Res Inst Lucknow	11.4.93-17.4.93
4.	Ms Jyothi Karunakaran	School of Biomed Eng IIT,Bombay	13.5.93-2.6.93
5.	Ms Meenal Sawant	Dept of Marine Biotech Goa University,Goa	2.5.93-5.6.93
6.	Dr P S Dubey	Inst of Anim Health & Vet Biologicals MHOW	10.6.93-20.6.93
7.	Mr T Rabi	Dept of Shalya-Shalakya Inst of Med Sciences BHU,Varanasi	11.6.93-22.6.93
8.	Ms Sushama Marathe	Food Tech & Eng Centre BARC,Bombay	9.7.93-29.7.93

- |                                |  |                   |
|--------------------------------|--|-------------------|
| 9. Dr M V<br>Mandkhalikar      | Dept of Microbiol,<br>Coll of Vet & Animal<br>Sciences, Parbhani | 19.7.93-29.7.93   |
| 10. Ms Varsha<br>Likhite       | Hoechst India Ltd<br>Bombay                                      | 6.9.93-16.9.93    |
| 11. Ms Uma<br>Gopalakrishnan   | Hoechst Ind Ltd<br>Bombay  | 6.9.93-16.9.93    |
| 12. Ms Sushama<br>Wagh         | Hindustan Lever Ltd<br>Bombay                                    | 11.10.93-14.10.94 |
| 13. Dr K C Gurnani             | Inst of Vet Biol<br>Prod, Pune                                   | 18.10.93-20.10.93 |
| 14. Mr J V Pathak              | Bohring-Hoechst Ind<br>Ltd, Bombay                               | 8.11.93-12.11.93  |
| 15. Mrs Sulabha<br>Subramaniam | Bohring-Hoechst Ind<br>Ltd, Bombay                               | 22.11.93-27.11.93 |
| 16. Dr V V Limaye              | Dis Prevention Sect<br>Direct of Animal Health<br>Aundh, Pune    | 3.1.94-10.1.94    |
| 17. Dr N V Kurkure             | Dis Prevention Sect<br>Direct of Animal Health<br>Aundh, Pune    | 3.1.94-10.1.94    |
| 18. Ms Kurshid<br>Master       | School of Life Sci<br>Univ of Bombay,<br>Bombay                  | 17.4.94-29.4.94   |
| 19. Ms Durga<br>Chadalavada    | School of Life Sci<br>Univ of Bombay<br>Bombay                   | 17.4.94-29.4.94   |

20. Mr H G Mazumdar	Ind Red Cross Soc Dholka Branch, Dholka	1.2.94-5.2.94
21. Mr D A Acharya	Ind Red Cross Soc Dholka Branch Gujarat	1.2.94-5.2.94
22. Dr Janaki Ram	CIFA, Natl Centre for Pearl Culture, Kausalyaganga	2.2.94-11.2.94
23. Mr S K Adiga	Dept of Radiobiology K.M.C., Manipal	21.2.94-26.2.94
24. Ms Vijayashree	Dept of Radiobiology K.M.C., Manipal	21.2.94-28.2.94

## **SUPPORTING UNITS**

- a) Computer and Database
- b) Library and Documentation
- c) Photography and Illustration
- d) Animal House
- e) Instrumentation and Maintenance

## **S**UPPORTING UNITS

### **COMPUTERS & DATA BASE**

The computer unit is equipped with one 386 AT, nine 286 AT's and 6 PC's and 10 Dot Matrix printers and one laser printer.

#### **Highlights of year 1993-94**

- Softwares for office automation have been developed and implemented.
- The media utilisation system has been computerised for keeping a track of media supply and consumption.
- User awareness program was conducted from time to time to ensure effective and efficient use of computer facilities.
- Modem was installed which has widened to access foreign and Indian databases.
- E. Mail service was set up.

### **LIBRARY AND DOCUMENTATION FACILITY**

The collection and development of library documents has continued to grow during the last one year and now has approximately 3500, which consists of Books, Reports, Conference Proceedings, Reprints/Photocopies, Journals and other type of documents. It subscribes 42 Scientific journals and receives 18 other periodicals on Gratis basis. Resource Sharing through shared library documents within Pune area among bio-medical libraries is being continued.

It has continued to offer CAS/SDI services and providing information consolidation/repackaging services such as Bio-Alerts, Press Vision etc. to provide pinpointed information having relevance to NFATCC activities. Apart from the renewal of MEDLINE database for the year 1994 it has subscribed Biotechnology Abstract from 1982 to the present. A good number of literature searches have also been carried out for other libraries on request. A compendium on AIDS and GATT (December 1993 - January

1994) was compiled from the various news papers subscribed at NFATCC.

Library has started a new service named *BIO-VISION* a bi-monthly review publication on biotechnology to disseminate information on front line R&D activities in addition to computer related services.

NFATCC Library is identified as one of the authorised user of Genome Database.

Various value added services have been introduced during the year by acquiring INET pass word. The following services are now available at the library

ACCESS through E-mail (national and international) Biotechnology related information systems

- interactive message and file transfer
- remote login
- On-line access to Dialog information system

## **PHOTOGRAPHY & ILLUSTRATIONS**

The Photography Unit continues to provide services in preparation of 35 mm projection slides, colour photographs, Black and White photographs, written matter, graphs etc. Other services include layout of continuous tone slides and half tone slides, for photographs from both Siva Gel and Protein Gel, copying from X-ray films etc. The Unit also assists in preparation of dummy for Annual Report, Artwork, layout of colour photographs, Black and White photographs etc, size and type of font, type, paper etc. and final printing of annual report.

## **ANIMAL HOUSE**

The Animal House for small laboratory animals became fully functional in the "Jidnyasa" building. Humidity, ventilation and temperature is regularly monitored and maintained. Health monitoring of animals is done regularly by faecal sample examination for parasite load, blood examination for haemoprotozoans and skin and hair is examined for mites and

other ectoparasites. All the animals used for various experiments were healthy and were free from the above infestations.

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

Animal	Procured from NIV	Bred at NFATCC	Supplied for R & D
Rats (Wistar)	32	19	30
Mice C57BL/6J	10	29	10
Balb/C	282	251	361
Swiss albino	151	216	263
Mastomys	17	-	8
Rabbit	1	-	1

### **INSTRUMENTATION AND MAINTAINANCE**

Regular routine and preventive maintainance of scientific instruments, electrical equipments, Refrigeration and air-conditioning units, vehicle and civil structure of Jopasana, Jidnyasa and guest house is done.

In addition the following activities were undertaken :

1. Installation, erection and commissioning of liquid nitrogen plant.
2. Erection of a canteen in Jidnyasa building
3. PCC flooring was accomplished for Parking and road to animal house
4. Equipments such as clean benches, CO<sub>2</sub> incubators, microscopes, xerox machines etc. were commissioned.
5. Landscaping at Jidnyasa.

Further, the following civil and electrical work needed for the Laboratory Complex being built up in University of Poona Campus was co-ordinated with C & S group of DAE.

- Central A/C plant and local A/C units
- Electrical and energy audit system
- Water utility system
- Fire alarm system
- CCTV and Telephone systems
- Computer cabling and central monitoring systems
- False ceiling, furniture, interiors etc.
- Installation of Incinerator and Generators.



Liquid nitrogen plant was installed in the basement of Jidnyasa building. I & M staff are all smiles at the first flow of liquid nitrogen.



The members of the Assessment committee for recognition of NFATCC as a Post Graduate Study centre are seen discussing with NFATCC staff.



Members of Armed Forces Medical College and Command Hospital visited the laboratories and discussed with the Scientists.



Deliberations of the Governing Body of NFATCC

## **ADMINISTRATIVE DESK**

- a) Construction of NFATCC laboratory complex, recruitments etc.
- b) Distinguished visitors
- c) Invited Lectures
- d) Committees of Institution

## **A**DMINISTRATIVE DESK

### **Construction of NFATCC Laboratory complex on Poona University campus :**

The construction activities of the NFATCC Laboratory complex at Poona University campus are progressing as per the prescribed time schedule.

A Co-ordinating Committee consisting of the representatives of C&S Group, DAE, NFATCC and the architect, is regularly meeting once every month, to discuss, review, control and monitor the progress of construction activities.

The salient features with regards to the progress of construction activities during the year are as follows :-

1. The RCC slabs and columns work of the main laboratory building has been completed and the brick work is in progress.
2. The excavation at the site and laying of slabs and column for the Animal House building has almost been completed.
3. The tender for construction of Residential Buildings, Guest House, Hostel and Director's Bungalow has already been finalised, the work order issued and excavation work has already commenced at the site.
4. The civil and electrification works are being taken up, for the extension laboratories on the second floor of the main laboratory building.
5. The tenders for elevators to be installed in the main laboratory complex have been finalised and work has been awarded.
6. The process of inviting tenders for procurement of L.T. power cables, H.T. panels, fluorescence lights, fittings and fixtures, fire alarm system, incinerator etc. has been taken up and advertisement has been published in news papers for the purpose.
7. The work contract for transplantation of trees and their maintenance at the complex site has been finalised and awarded to M/s Garden Decor.

### **Establishment of Research and Training**

## Laboratory at "Jidnyasa" building :

The "Jidnyasa" building has now become fully functional with the setting up of a Research, Training and Quality Control laboratory on the second floor of the building.

The infrastructural facilities at the building were further augmented with the establishment of an Animal House for small laboratory animals required for experiments, Washing and Sterilisation facilities and a shed for Canteen. A Liquid Nitrogen plant procured from M/s Philips, Holland, has been installed and commissioned at the building during the year.

The support activities such as cell and stock supply and media preparation, Administration, Accounts, stores, Instrumentation & Maintenance, Computer, Photography and Library & Documentation have been set up and became fully functional at this building.

## Composition of staff :

Following is the staff complement as on 31.3.1993,

	Category	No. of Persons
1.	Scientific Staff	17
2.	Technical Staff	
	- Lab.	16
	- I & M	08
3.	Administrative staff	13
4.	Auxiliary staff	21
	<b>Total</b>	<b>75</b>
5.	Research Associates and Research Fellows	07
6.	Contractual services	23

In addition to the above, 16 persons for Housekeeping, Security, Driver and Tradesmen have been engaged on contractual basis for construction of NFATCC complex on Poona University campus. The expenditure incurred in this respect is being directly debited to the project account.

## **DISTINGUISHED VISITORS**

Following distinguished persons and dignitaries visited the Institute during 1993-94.

C.R.RAMACHANDRAN  
CHIEF OF DIVN. OF NCD,  
INDIAN COUNCIL OF MEDICAL  
RESEARCH  
NEW DELHI

V.GNANAPRAKASAM  
VICE CHANCELLOR  
TAMIL NADU VETERINARY &  
ANIMAL SCIENCES UNIVERSITY,  
MADRAS

MRS.R.D.SATHE  
CHAIRMAN,INDIAN RED CROSS  
3, SUYOJANA SOCIETY,  
KOREGAON PARK,  
PUNE : 411 001

R.D.SATHE  
EX-SECRETARY,  
FOREIGN AFFAIRS,  
GOVT. OF INDIA,  
3, SUYOJANA SOCIETY,  
KOREGAON PARK, PUNE : 411 001

LALIT KANT  
DY.DIRECTOR GENERAL  
INDIAN COUNCIL OF MEDICAL  
RESEARCH,  
NEW DELHI

J.M.MEHTA,  
MANAGING DIRECTOR  
SERUM INSTITUTE OF INDIA LTD,  
PUNE

S.G.KALE  
MUNICIPAL COMMISSIONER,  
GREATER BOMBAY  
BOMBAY

RAMESH S.KAMAT  
ASST.DIRECTOR,  
DEPT. OF IMMUNOLOGY,  
HAFFKINE INSTITUTE,  
PAREL, BOMBAY.

P. GOSWAMI  
DIRECTOR,  
INSTITUTE OF ADVANCED STUDY  
IN SCIENCE & TECHNOLOGY  
GUWAHATI, ASSAM

N.R.SUBBARAM  
ADVISER (PATENTS)  
COUNCIL OF SCIENTIFIC AND  
INDUSTRIAL RESEARCH (HQ)  
NEW DELHI

K.C.SRIVASTAVA,  
DIRECTOR,  
V.M.NATIONAL INSTITUTE OF  
CO-OP. MANAGEMENT  
PUNE

S.P. TRIPATHY  
DIRECTOR-GENERAL  
INDIAN COUNCIL OF MEDICAL  
RESEARCH,  
NEW DELHI

J.V.NARLIKAR  
DIRECTOR  
IUCAA,  
PUNE

GORO EGUCHI  
PROFESSOR,  
DEPT. OF DEVELOP.BIOL.  
NATIONAL INSTITUTE FOR BASIC  
BIOLOGY,  
MYODOUJI CHO,  
OKAZAKI 444 (JAPAN)

SAHEBARAO P. MAHADIK  
PROFESSOR,  
DEPT. OF PSYCHIATRY  
MEDICAL COLLEGE OF GEORGIA  
15/5 POPE AVE, AUGUSTA, GA  
30912

MICHEL CREPIN  
IOCMH INSTITUTE D'ONCOLOGIE  
UNIVERSITY OF PARIS NORTH  
93000 BABIGUY

ANNE COMPEL  
HOTELPIC HOSP  
1 PLACE DUR PARIS, NOTRE- DAME  
PARIS 75004  
FRANCE

NITIN TELANG  
STRANG CORNELL CANCER  
RESEARCH LAB., CORNELL UNIVERSITY,  
510 E 73 ROAD,  
STREET NEW YORK,  
NY 10021 USA

KRISHNA V.KESARI  
HOWARD HUGES MED.INST.  
UNIV. OF MICHIGAN,  
ANN ARBOR  
MI 48105 USA

A.R.KULKARNI  
PROFESSOR & HEAD,  
DEPT.OF LIFE SCIENCES,  
UNIVERSITY OF BOMBAY  
BOMBAY

A.S.SALANKI  
PROFESSOR,  
DEPT. OF BOTANY,  
KARNATAK UNIVERSITY,  
PAVATE NAGAR,  
DHARWAD

K.KANNAN  
GENERAL MANAGER,  
(BIOTECHNOLOGY)  
RANBAXY LABORATORIES  
NEW DELHI

P.L.SHARMA,  
CONSULTANT R & D (MEDICAL)  
RANBAXY LABORATORIES,  
NEW DELHI

MINORU OKUDA  
PROFESSOR & HEAD,  
DEPT. OF ENT,  
NIPPON MEDICAL SCHOOL  
TOKYO

VINEY JAIN  
DIRECTOR,  
INST.OF NUCLEAR MEDICINE  
& ALLIED SCIENCES  
NEW DELHI

ARUN.R.WAKADE  
DEPT. OF PHARMACOLOGY,  
WAYNE STATE UNIVERSITY,  
DETROIT,  
MI 48322, USA

G.M.PILLAI  
COMMISSIONER  
(ANIMAL HUSBANDRY)  
MAHARASHTRA STATE  
CENTRAL BLDG.  
PUNE

P.K.UPPAL,  
MANAGING DIRECTOR,  
BIBCOL,  
MIN.OF SCIENCE & TECHNOLOGY  
V-13, GREEN PARK EXTN.  
NEW DELHI

L.K.KOOLWAL  
COMMISSIONER INCOME-TAX  
AMHEDABAD

S.N.SAMARTH  
COMMANDANT,  
ARMED FORCES MEDICAL COLLEGE,  
PUNE

V.PSHARMA,  
DIRECTOR,  
MALARIA RESEARCH CENTRE,  
NEW DELHI

U.C.CHATURVEDI,  
DEPARTMENT OF VIROLOGY,  
KING GEORGE MEDICAL COLLEGE,  
LUCKNOW

PRADEEP SETH,  
DEPT. OF MICROBIOLOGY,  
ALL INDIA INSTITUTE OF  
MEDICAL SCIENCES,  
NEW DELHI

R.S.SHARMA,  
DY.DIRECTOR  
(EPIDERMINOLOGY)  
NICD  
DELHI

MRS. INDERJEET KAUR,  
DEPARTMENT OF BIOTECHNOLOGY  
MIN.OF SCIENCE & TECHNOLOGY  
NEW DELHI

M.A.VIJAYALAKSHMI  
DIRECTOR  
MOLECULAR INTERACTIONS &  
SEPERATIONS TECHNOLOGY  
LIM TECH. S. U.T.C.,B.P.649  
60206 COMPIEGNE  
FRANCE

K.B.GRANT  
MANAGING TRUSTEE,  
RUBY HALL CLINIC,  
PUNE

BILL HEILMAM  
AMERICAN CYANAMID  
PRINCETON,  
NJ, USA

N.NATARAJAN,  
DEAN.  
MADRAS VETERINARY COLLEGE  
MADRAS

S.V.APTE  
DEPUTY DIRECTOR-GENERAL,  
INDIAN COUNCIL OF MEDICAL RE  
SEARCH,  
NEW DELHI

SUBHASH PADHYE  
PROFESSOR,  
DEPT. OF CHEMISTRY,  
UNIV. OF POONA,  
PUNE

MAJ.GEN.D.RAGHUNATH  
DEAN  
ARMED FORCES MEDICAL COLLEGE,  
PUNE

G.P. PHONDKE  
CHIEF EDITOR,  
PUBLICATION & INFORMATION  
DIRECTORATE,  
CSIR  
NEW DELHI

SHOBHA BROOR  
DEPT. OF MICROBIOLOGY,  
ALL INDIA INSTITUTE OF  
MEDICAL SCIENCES,  
NEW DELHI

HANKS VONKEMAN  
DEPUTY HEAD OF THE LAB,  
HINDUSTAN LEVER RESEARCH  
CENTRE,  
CHAKALA, ANDHERI (E)  
BOMBAY

## **Invited Lecturers from Distinguished Scientist/ Persons**

The following distinguished Scientist/Persons delivered lecture at NFATCC on specified subject.

<b>NAME</b>	<b>SUBJECT / DATE</b>
MR.N.R.SUBHARAM, ADVISOR (PATENT) CSIR, NEW DELHI	WHAT TO PATENT AND HOW TO PATENT 22.09.1993
PROF.SAHEBRAO P. MAHADIK DEPT. OF PSYCHIATRIC SCHOOL OF MEDICINE AUGUSTIA, GEORGIA:30912-380	RESEARCH ON SCHIZOPHERINA 01.12.1993
DR.NITIN TELANG MEMORIAL SLOAN- KATTERING CANCER CENTRE, USA	BIOMARKERS FOR MAMMARY CARCINOGENESIS: IDENTIFICATION AND VALIDATION IN AN EPITHELIAL CELL CULTURE 07.12.1993
DR.K.KESARI UNIVERSITY OF MICHIGAN USA	ALLOGENICITY OF MHC CLASS I MOLECULES POTENTIAL ROLE OF IMMUNOTHERAPY OF MALIGNANT TUMORS 11.12.1993
DR.PS.KHANDEKAR NATIONAL INSTITUTE OF IMMUNOLOGY, NEW DELHI	PREPARATION OF KITS FOR LABORATORY DIAGNOSIS 12.12.1993
DR.M.OKUDA NIPPON MED. SCHOOL JAPAN	CHARACTERISTICS OF NASAL MAST CELLS 01.01.1994

DR.D.JANAKI RAM CIFA, BHUBANESHWAR	STORY OF FRESH WATER PEARL CULTURE 13.02.1994
DR.SHOBHONA SHARMA TATA INSTITUTE OF FUNDAMENTAL RESEARCH	IMMUNOSCREENING OF NOVEL PROTEINS OF MALARIA PARASITES 08.03.1994
DR.S.M.ZINGDE CANCER RESEARCH INSTITUTE BOMBAY	FUNCTIONAL ABRRATIONS IN LEUKEMIC GRANULOCYTE -A BIOCHEMICAL AND MOLECULAR ANALYSIS 20.05.1994
DR.VINEETA BAL NATIONAL INSTITUTE OF IMMUNOLOGY NEW DELHI	CD28-B7 INTERACTIONS : POTENTIAL ROLE IN TRANSPLANTAION 04.07.1994
DR. DHARMARAJ CMFRI, TUTICORIN	RESEARCH ON MARINE PEARL CULTURE 07.07.1994

## COMMITTEES OF INSTITUTION

### SOCIETY AND GOVERNING BODY

Shri Bhuvanesh Chaturvedi,  
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  
Prof. & Head,  
Dept. of Cell & Molecular Biology,  
Indian Institute of Science,  
Bangalore

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

Dr.Smt.Manju Sharma  
Advisor,  
Dept. of Biotechnology,  
Ministry of Science & Technology,  
New Delhi

Shri S.B.Krishnan,  
Financial Advisor, 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),  
Prof. & Head,  
Dept. of Cell & Molecular 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,  
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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 &  
Cell Biology,  
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 Kamaraj University  
Madurai : 625 021

Dr.Rama Mukherjee,  
Staff Scientist,  
Microbiology Div.,  
National Institute of Immunology,  
New Delhi 110 067

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)  
Financial Advisor, Govt. of India  
Dept. of Biotechnology,  
Ministry of Science & Technology,  
New Delhi

Prof.H.Sharat Chandra  
Prof. & Head,  
Dept. of Cell & Molecular Biology,  
Indian Institute of Science,  
Bangalore

Dr.S.H.Iqbal  
Head,  
Division 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

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

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

Prof.D.N.Deobagkar  
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