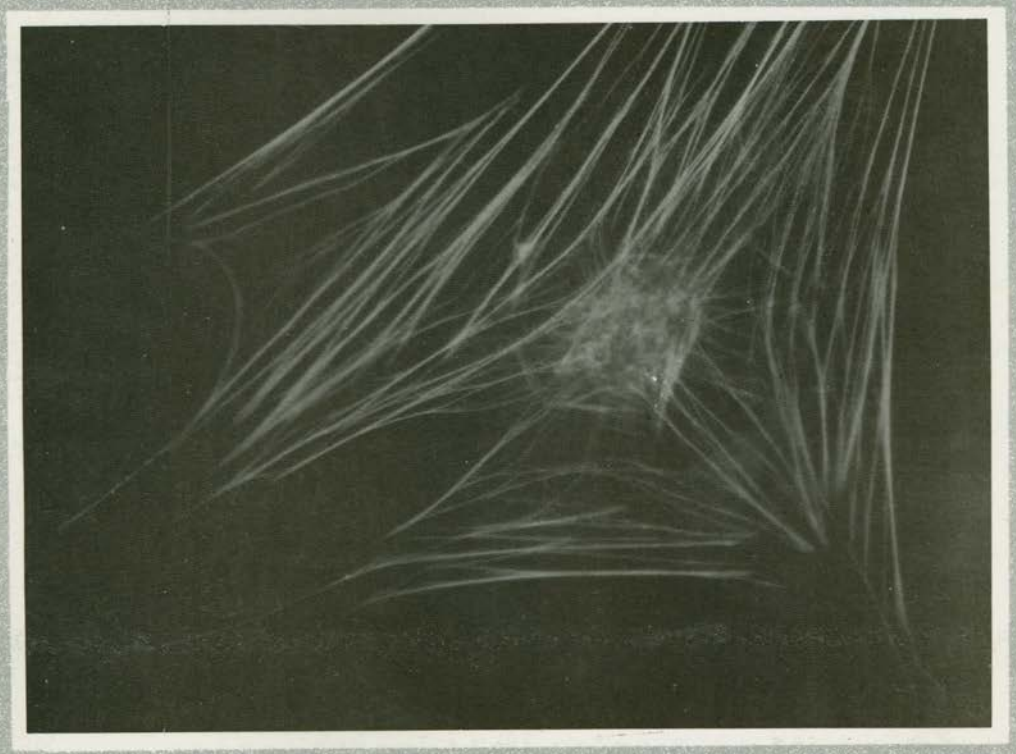
NATIONAL FACILITY FOR ANIMAL TISSUE AND CELL CULTURE



ANNUAL REPORT
1990 - 1991

NATIONAL FACILITY FOR ANIMAL TISSUE AND CELL CULTURE

DEPARTMENT OF BIOTECHNOLOGY
GOVERNMENT OF INDIA

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PREFACE

The NFATCC has been actively involved in Cell Repository and supply of cell lines since its inception and was located at the Dept. of Zoology, University of Poona. This interim facility for the core activities was shifted to the new building "Jopasana" at Kothrud on 14th April, 1990. The infrastructure for Cell Repository and Research & Development activities was established in this new building. This was accomplished by augmenting basic amenities like water storage & supply and electricity supply including back up of a diesel Generator. Facilities were created for clean sterile work, handling of biohazardous material and radioisotopes, microscopy and for storage of liquid nitrogen for cryopreservation. Supporting units – library & documentation and computer systems were also set up.

The availability of space in the new premises permitted induction of additional scientific, technical and supporting staff. This had a synergistic effect towards advancements in the established activities of cell repository, tissue banking and in stimulating work in newer areas like molecular biology and biotechnology. The repository for cell culture was enriched by addition of new cultures. During the period 1990-91, 191 consignments comprising about 348 cell lines were supplied to 171 research teams. The figures from year to year indicate a steady increase in the supply service. A moderate expansion has been made in the repository for obligate parasites. Foundation for Tissue Bank activity has been strengthened. Rapport has been established with clinicians and hospitals and research institute staff for procurement of human and animal material and for further implementation of clinical application and collaborative research & developmental programmes. The technology for collection and maintenance of human cornea for an extended period has been standardised. Procedures for collection and cryopreservation of heart valves are being worked out. Work on similar lines for skin and bone marrow is in progress.

The team efforts of the faculty members has led to successful development of cell cultures from human foetal tissues, bone marrow, melanocytes, keratinocytes and rat hepatocytes and pancreatic cells. These cell cultures are being studied for their characteristic features. Hollow fibre technology (endotronics system) is being standardized and used for obtaining cell products from functionally active cell lines and hybridomas. Monoclonals against insulin and filaria B. malayi are being characterized. Experimental work has been undertaken to investigate the cytoskeletal patterns, study the DNA finger printing and analysis of mitochondrial DNA for characterisation of cells.

Studies on screening antimalarials against chloroquine resistant falciparum strains have been continued. Experimental work on electrofusion of mosquito cells for studying premature chromosome condensation and salivary gland cells of drosophila for induction of polytenisation in other cells is in progress. Projects on characterisation of cell adhesion molecules from BHK cells, stress proteins in Ano stephensi and molecular biology of brain hexokinse, which may bring new scientific information have been continued.

The faculty members actively participate in the post graduate teaching programmes, relating to biotechnology in particular, of the University of Poona. In addition, individuals deputed from different organisations have been trained in the specialised areas related to Animal Tissue Culture.

The NFATCC has been fortunate in having distinguished scientists/visitors from home and abroad. Dr. M.G. Deo, Director, Cancer Research Institute, Bombay, Dr. Sandeep Basu, Director, IMTECH, Chandigarh, Dr. Banu Coyajee, Chief Medical Officer, K.E.M.H, Pune, Dr. V.N. Rao, Director, K.E.M.H,Pune, Dr. V.P. Sharma, Director, Malaria Research Centre, Delhi and Dr. V. Dhanda, Director, VRCR, Pondicherry, visited the Facility. Seminars/lectures were delivered by Dr.Robert Hay, Head Cell Culture Dept., ATCC, USA, Prof. George Martin, Director Alzheimer's disease Institute, USA and Prof. Sahebrao Mahadik, Columbia University, USA. The stimulating seminars followed by enthusiatic discussions have been the impetus for exploring the potential for collaborative projects and to establish liaison for execution of scientific programmes with the visiting teams. A Mongolian team headed by H.E. Dr. J. Batsuuri, Minister for National Development of Mongolia visited the facility.

Activities towards development and construction of the NFATCC laboratory complex on the University of Poona campus are at an advanced stage.

TERMS OF REFERENCE

- To receive, identify, maintain, store, grow and supply :

  i) Animal and Human Cells/Cell cultures, cell lines of both existing (typed) and newly developed.
  ii) Tissues, organs, eggs (including fertilized), and embryos.
  iii) Hybrid cells including hybridomas.
  iv) Unicellular obligate pathogens, parasites and vectors.
  v) 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 and 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.

3-A) INFRASTRUCTURAL ACTIVITIES

a. REPOSITORY

Efforts are underway to enrich the repository by procurement of additional cultures from ATCC, U.S.A, E.C.C, U.K and four Indian laboratories.

(1) CELL CULTURES

- Nuclear stock stored in liquid nitrogen: The cell repository now has a stock of 688 cell lines which includes primary cultures, established cell lines and hybridomas.
- Stock for quality control and redistribution: So far, 116 cell cultures have been expanded on small scale for quality control tests and for redistribution.
- Supply services: Different Cell lines and cultures are supplied to research teams and public health laboratories in various parts of the country (Fig 1). One hundred ninty one consignments comprising 348 cell lines were despatched to 171 research teams from 99 laboratories during the period April 1990 to March 1991.

(2) OBLIGATE PARASITES

Stock position: The following table enlists the stock of obligate parasites available in the repository.

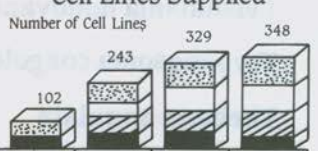
TABLE 1
Obligate Parasites in the repository

	SPECIES	NO. OF STRAINS
1.	**Plasmodium falciparum**	33
2.	**Leishmania donovani**	1
3.	**Trypanosoma congolensis**	1
4.	**Theileria annulata**	1
5.	**Toxoplasma gonidi**	1
6.	**Viruses**	17

CULTURE SUPPLY SERVICE

Cell Lines Supplied

Number of Cell Lines



	1987-88	1988-89	1989-90	1990-91
Jan-Mar	55	78	99	110
Oct-Dec	37	52	89	92
Jul-Sep	7	58	69	96
Apr-Jun	3	57	72	50

■ Apr-Jun ▨ Jul-Sep □ Oct-Dec ▩ Jan-Mar

(3) VECTORS, PLASMIDS, AND GENOMIC LIBRARIES

Stock position: The repository maintains a stock of vectors, plasmids and genomic libraries (Table 2).

TABLE 2

Number of vectors, plasmids and genomic libraires in the repository

Cultures	Number available
Host strains preserved as glycerol cultures	12
Host strains available as lyophilised stock	3
Vectors as plasmid containing hosts	7
Vectors as purified plasmid DNA	4
Phage vectors	5
Clones as plasmid containing host	14
cDNA libraries	3
Strains with special properties	2

(4) MEDIA

Fifteen different synthetic media , salt solutions and tissue culture reagents are being prepared. These reagents and various batches of Foetal calf serum (FCS), Horse serum (HoS) and Goat Serum (GS) are being tested for their sterility, quality control and supplied to the staff members.

MEDIA	QUANTITY (LITRES)
BME	2.00
DMEM	93.20
Ham's F-10	2.00
Ham's F-12	8.00
IMDM	11.00
L-15 medium	2.00
M199 (with Earle's salt)	1.00
M199 (with Hank's salt)	3.50
McCoy's 5A	3.00
MEM (with Earle's salt)	140.50
MM medium	7.20
RPMI 1640	44.20
Schnider's medium	1.00
Waymouth medium	7.00
Willam E (liquid medium)	1.00
TPVG	25.00
Trypsin (0.2%)	6.00
PBS	65.00

SERUM	QUANTITY (LITRES)
Foetal Calf Serum (FCS)	9.00
Horse Serum (HoS)	1.00
Goat Serum (GS)	12.00

b. TISSUE BANK ACTIVITIES

Technology for collection and maintenance of cornea, skin, bone marrow and heart valves is being established and improvised. Efforts are being made to standardise methods to optimise conditions for preservation and maintenance and assessment of the functionality of the tissues following revival. This part of work is at present being carried out as a research and development activity.

HIV SCREENING

Blood samples are being collected from individuals whose tissues are obtained for any type of study done at the facility and screened for HIV. A rapid 2 hour microagglutination test kit by Serodia is being used for this purpose.

(1) CORNEA

Methods standardised for short and intermediate terms of storage are being followed. The logistic was worked out with a view to assess the feasibility of transportation of preserved corneas to different regions in the country. Two eye balls in MK medium were transported by air from Pune to Delhi. The experiment was successful and both these corneas were found in good condition on receipt which could be used for keratoplasty.

(2) BONE MARROW

It is proposed to undertake the development of bone marrow banking technology for autologous transplantations. This will be of great value for people working in high-risk areas, like nuclear reactors or hazardous industries. Therefore, the optimization of long-term preservation of bone marrow cells is being done.

Fifteen normal human bone marrow specimens were obtained from Sassoon General Hospital, Sancheti Hospital and Naik Hospital. The cells were counted and viability was assessed by trypan blue dye exclusion test. They were then suspended in IMDM with 20% FCS and 10% DMSO or glycerol. The vials were immediately chilled to -10° C and put into a biofreezer for further cooling at the rate of 1° C/min upto -130° C. After 2 hrs. they were transferred to liquid nitrogen. Three samples were revived on day 7, day 15 and day 60 after cryopreservation and assessed for their functionality by the viability, cell count, CFU-GM assay and capacity to be cultured (Fig. 1) . While all three samples showed good count and viability after revival, only one sample gave good results with CFU-GM assay (Fig. 2) .

(3) SKIN

Normally trypsin is used to separate epidermis from the dermis. Trypsin is known to bring about changes in the cell surface proteins and is suspected to affect the proliferation of melanocytes. Attempts were therefore made to separate epidermis from dermis using Dispase-II. Use of 0.4% Dispase-II in PBS at 4°C for 12-14 hrs has been found to give a cleaner separation of epidermis and dermis of foreskins and adult skins. The clean separation of epidermis and dermis appears to also lead to improved yield of undamaged melanocytes and keratinocytes. This was revealed by an increased number of melanocytes and keratinocytes attached to the dish and the reduced time period required for attaining confluency. Since the biopsy size is usually small, comparison between yeilds following trypsin or dispase II treatment in the same sample has not been possible.

Human keratinocytes grown as monolayers have been induced to differentiate and multiply as sheets (Fig. 3-8) suitable for transplantation. It is proposed to start transplantation of cultured epithelia initially to non- healing ulcer cases.

(4) HEART VALVES

Studies on cryopreservation of heart valves is being continued. A set of four aortic and pulmonary valves have been cryopreserved by the rapid freeze method, while three sets of aortic and pulmonary valves have been frozen by the slow cooling method employed for embryos. Preliminary studies have revealed that none of these were viable at the time of freezing Hence mechanical strength of these valves are being tested after specific periods of storage.

3-B) RESEARCH AND DEVELOPMENT

a) DEVELOPMENT OF CULTURES

1) DEVELOPMENT OF NEW CELL LINES FROM FOETUSES, FORESKIN AND ADULT SKIN

Fourteen foetuses from Medical Termination of Pregnancy (MTP) cases and 11 samples of foreskin were obtained from KEM and Sassoon General Hospital and processed to develop new cell lines. Six cell lines have been developed, 2 each from foetal skin, foetal lung and from foreskin. These cell lines have been cryopreserved at different passages. They are being continuously passaged and their capacity for serial propogation is being assessed. The fibroblast cell lines are being used as feeder layers to assess their ability to support the growth and epithelization of epithelial cells derived from various organs.

2) LONG TERM CULTURE OF HEPATOCYTES FROM ADULT RAT LIVER

A simple *in situ* perfusion method has been standardised for obtaining high yield of viable hepatocytes. The method instead of using a perfusion pump as reported by others, makes use of a simple glass Cornwall autodispenser syringe. The perfusion is performed at 37°C using a buffer system. The cell yield obtained by this method corresponds to 5×10^6 cells/gm of excised liver tissue, with a viability of about 99% as checked by trypan blue dye exclusion test. Various media used in this study were MEM (E), DMEM, Ham's F-12, DMEM + Waymouth (WaM) and William E to optimise the conditions for the long term culture of hepatocytes. Modified Waymouth medium (containing ornithine instead of arginine, low glucose concentration and enriched with proline), supplemented with FCS seems to be the most suitable medium for the purpose. The effect of insulin, dexamethasone and EGF (Epidermal growth factor) were tested for studying the responsiveness of the hepatocyte cultures. Cultures containing EGF have been found to promote growth of liver epithelial cells instead of parenchymal cells (Fig. 9) . These cultures rich in epithelial cells have undergone 3 passages so far and have been cryopreserved using DMSO.

Hepatocyte cultures from human foetus obtained from MTP cases have also been set up using Waymouth medium with FCS. These cultures demonstrate higher proliferative capacity in comparison with adult rat tissue.

3) *IN VITRO* CULTIVATION OF PANCREATIC CELLS

In vitro culture of rat pancreatic cells is an ideal model for studying the exocrine - endocrine interaction.

Studies have been initiated using rat pancreatic cells. The objectives of the study are as follows:

(i) To establish organ culture of whole pancreatic tissue and long-term preservation of acinar functions *in vitro* with reference to secretion of amylase, trypsin and lipase.

(ii) To study the interaction of islets and acini in relation to retention of their functionality.

So far, 6 rat pancreatic cultures and organ cultures were set up (Fig. 10) Of the different media used, viz DMEM, RPMI 1640 & Ham's F-12, the Ham's F-12 with 20% FCS and 0.1 mg/ml soybean trypsin inhibitor was found to support the cell growth, in comparison with DMEM, and RPMI 1640 media. It was possible to maintain both islets and acinar cells in cocultures up to two weeks . The isolated islet cells aggregated and acquired the morphology of true islets (Figs. 11, 12). Amylase was estimated qualitatively using a rapid spot assay in starch-agar. The acini were tested for presence of amylase up to ten days in culture and were found to be positive.

4) DEVELOPMENT OF BONE MARROW AND STROMAL CELL LINES

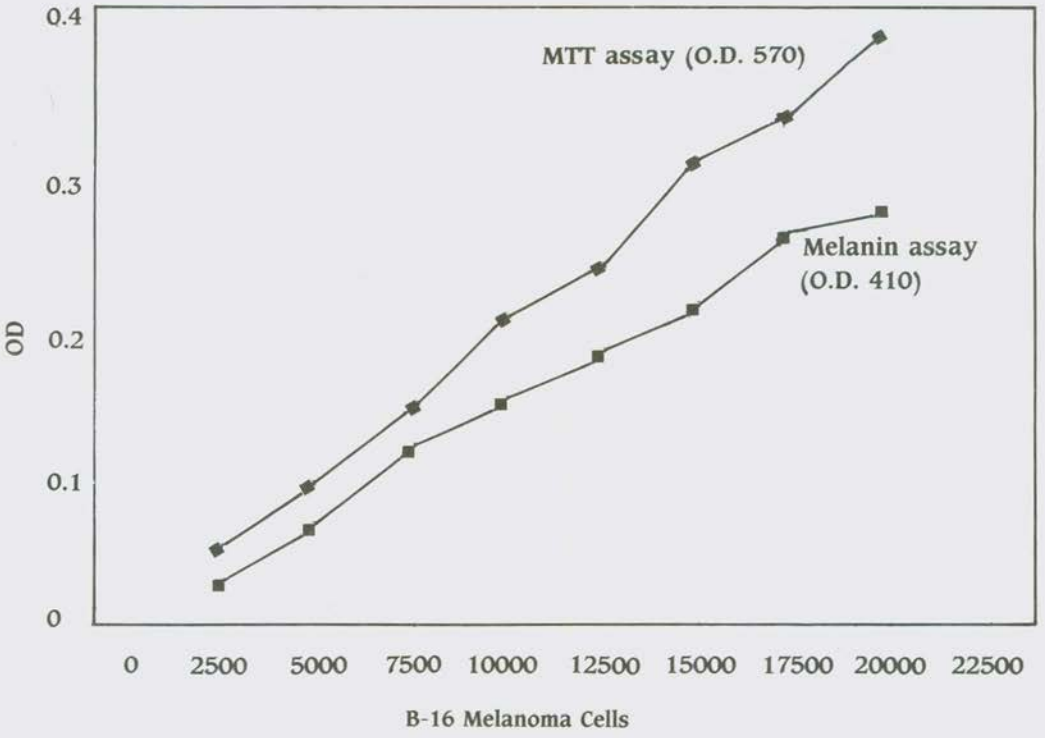
A total of 35 bonemarrow specimens were obtained and processed. Mononuclear cells (MNC) were separated from bone marrow aspirates by ficoll-hypaque separation method. Long term culture of myeloid type (Dexter system) were set up using these MNC. Cultures were incubated at 37° C for 4-5 days and subsequently at 33° C at 5% CO_2. Early detachment of stroma was observed when cultures were maintained continuously at 37° C. CFU-GM assay and observation for cluster formation were checked on 7th and 14th post culture days. The capacity of these cells to grow in cultures was confirmed when they could be maintained upto atleast 4 weeks with confluent stroma.

Development of stromal cell lines is being attempted for facilitating clonal growth of stem cells and progenitor cells. MNC were seeded at low density in Dexter medium and fed every week by demidefoliation. Seven stromal cultures were set up, of these 3 cultures could not be transferred. Two cultures showed an endothelial cell like morphology. Two cultures showed a mixed population of endothelial cells, fibroblasts, macrophages and adipocytes, typical of stromal appearance.

5) CULTIVATION OF MELANOCYTES IN TUMOR PROMOTER- FREE MEDIA

Attempts to grow melanocytes in tumor promoter-free media and thus enable them to be suitable for transplantation was continued. Procedures were modified and standardised to cultivate melanocytes on fibronectin coated plastic dishes. The modified procedure was found to be suitable for eliminating the variability of different batches of sera and to effectively bring down the concentration of tumor promotor level from 10 ng/ml to 4 ng/ml for the optimum growth of melanocytes from normal and vitilgo subjects.

The number of melanocytes from a small skin biopsy, especially those from vitiligo subjects is very low and hence proliferation assays such as 3H-thymidine uptake, or even counting of cells in Neubar chamber or Coulter counter cannot be employed. Therefore studies on proliferation and differentiation in normal melanocytes are rare. Hence it was felt necessary to develop a micromethod for assaying proliferation and differentiation in cultured melanocytes. The assay for proliferation consists of using the ability of live cells to convert MTT to formazon that can be dissolved in DMSO and the absorbance read at 540 nm. The differentiation assay consists of removal of proteins etc. from the cells, dissolving melanin in hot 2N NaOH, and reading the absorbance at 400 nm. Using this micromethod a preliminary experiment with B.16 melanoma cells seeded in 96 well plates at various densities was done. A good correlation between cell number and melanin content was observed (Fig.).



6) ADAPTATION OF SP2/O CELL LINE TO GOAT SERUM

Efforts to adapt SP2/O cell line to goat serum were continued and these cells have now been successfully adapted to goat serum. During the course of the adaptation procedure which was spread over twenty passages, it was invariably observed that the cells were slightly shrunkun during 24-48 hour period following transfer. Attempts were made to track down the cause for this behaviour and it was found that normal cellular morphology and growth of cells were maintained when goat serum concentration was reduced to 6% from 10% (Table 1).

Having achieved the adaptation, experiments were carried out to confirm the mutant status of the HGPRTase locus in these cells. For this purpose, one batch of the cells was maintained in the presence of 8-azaguanine (20 μg/ml) and another batch of adapted cells were maintained in HAT media. Cells in 8 azaguanine showed normal growth and cells in HAT medium were found to be dead within 48 hours (Table 2). For revival studies, adapted cells were frozen in different freezing media. Cells in freezing media with composition of 70:20:10 of DMEM :G S : DMSO showed better viability in comparison with other freezing media. Two fusion experiments carried out using the goat serum adapted SP2/O cells did not yeild successful results.

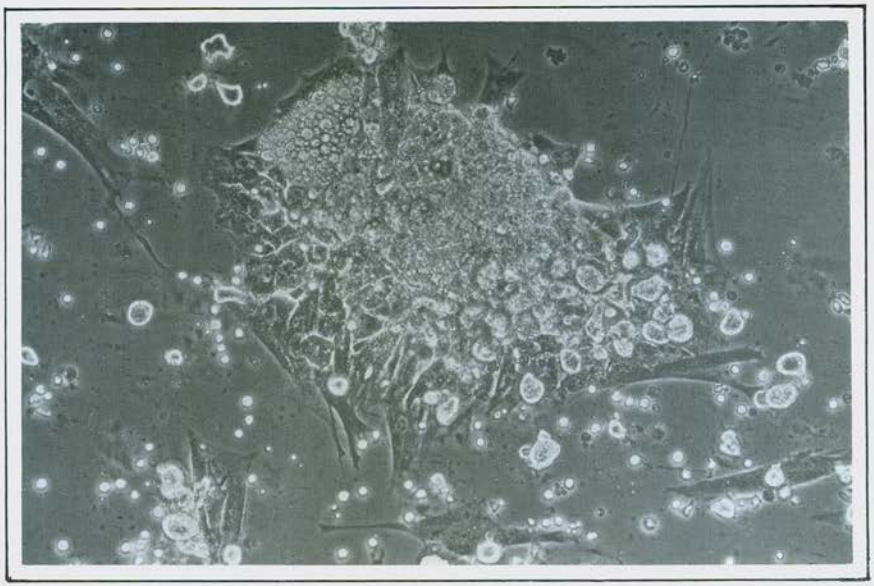


FIG. 1 : Human bone-marrow cells in Dexter type culture system showing adherent layer of stromal cells with hematopoietic cells growing in close association. (Phase contrast, x 250)

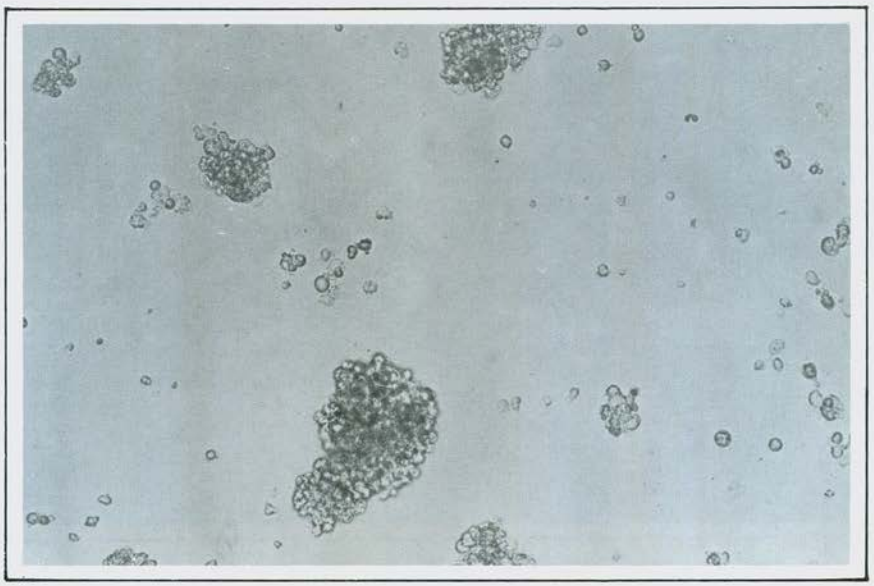


FIG. 2 : CFU-GM assay in soft agar. (x 250)

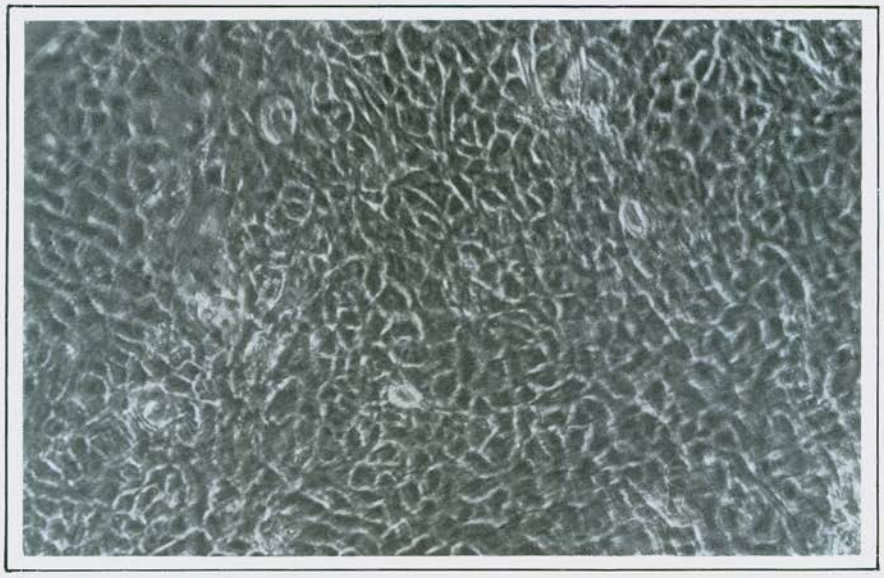


FIG. 3 : Monolayer of human keratinocytes ready for Induction of 3 dimensional epithelia formation (Phase contrast x 250)

FIG. 4 : Bilayer of human keratinocytes (Phase contrast x 250)

FIG. 5 : Multilayer sheets of epithelia growing on dermis equivalent fibroblasts. (Phase contrast, x 250)

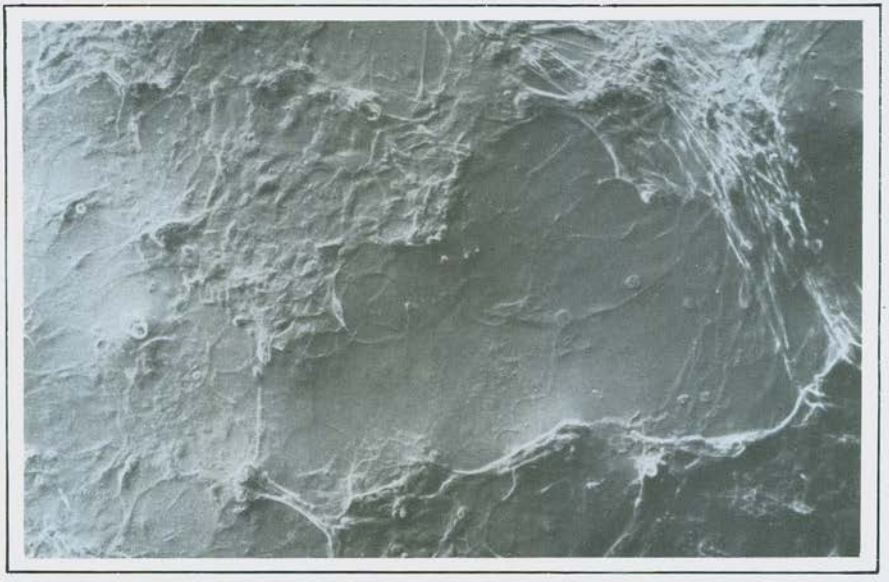


FIG. 6 : Cornification taking place in cultured 3-D epithelia (Phase contrast, x 250)

FIG. 7 : More areas of 3-D epithelia are cornified (Phase contrast, x 250)

FIG. 8 : Fully cornified cultured epithelia ready for transplantation in burns, non-healing Ulcer and vitiligo case (Phase contrast, x 250)

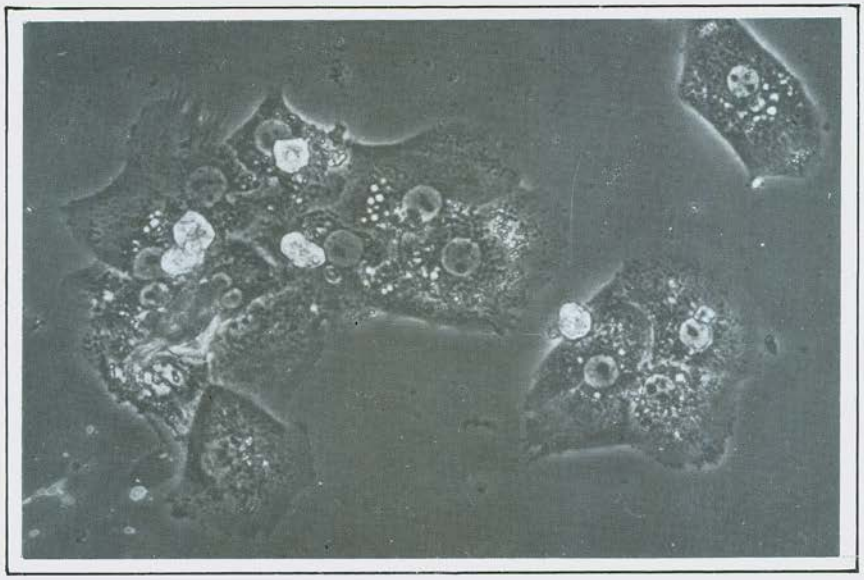


FIG. 9 : Adult Rat Hepatocytes 4th day in culture (Phase contrast – 400 x)

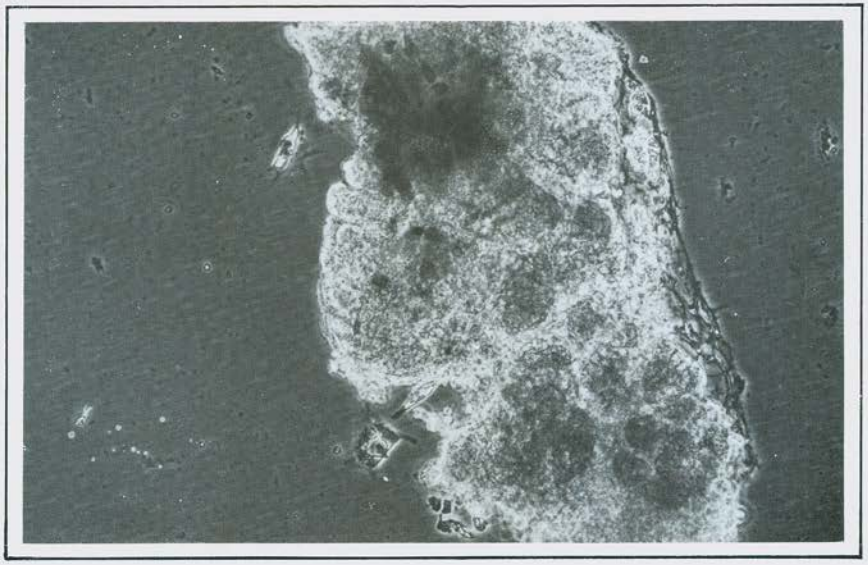


FIG. 10 : Rat Pancreas 7th in Organ Culture (Magnification – 120 x)

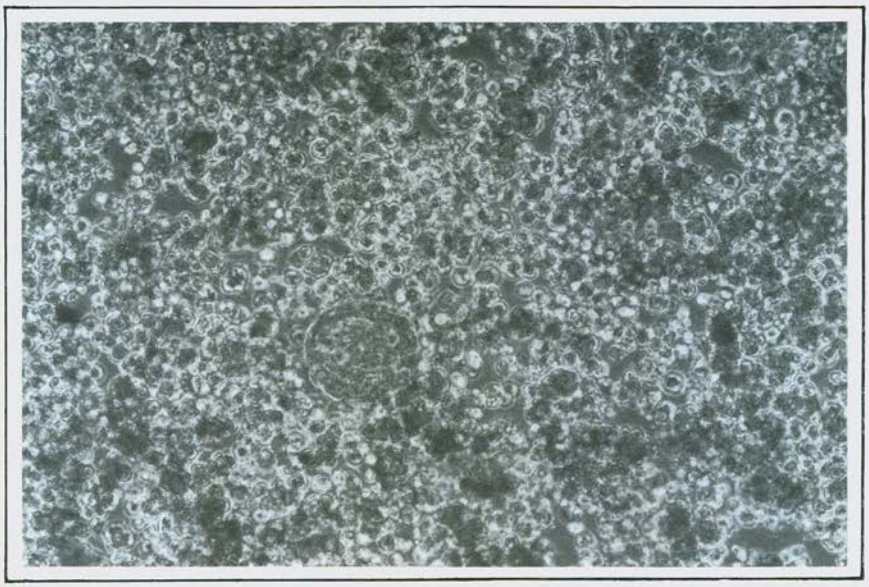


FIG. 11 : Isolated islets acinar cell in 8th day co-culture.

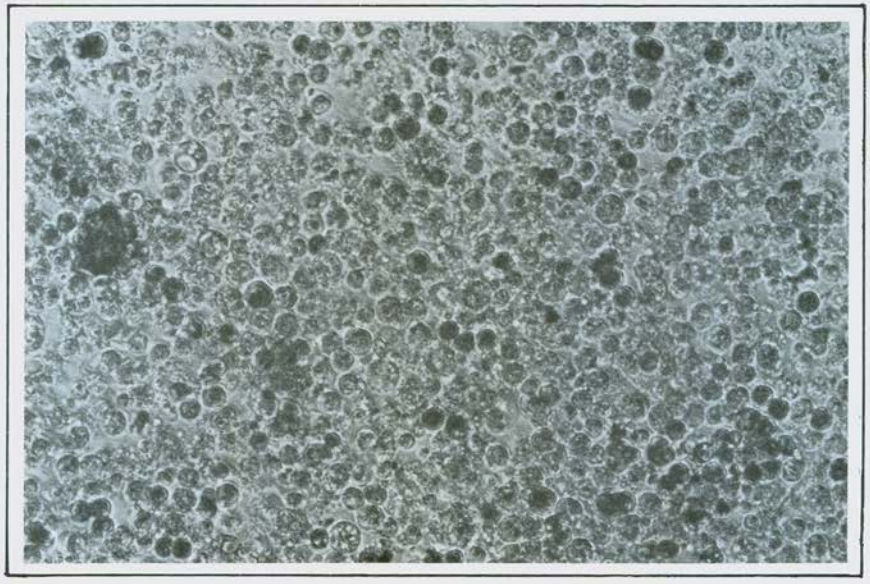


FIG. 12 : Pancreatic acinar cells 10th day in culture.

TABLE 1

Effect of goat serum on rate and viability

Goat serum %	cell counts/ml	Viability
3	5.75×10^5	86
4	3.00×10^6	90
5	3.40×10^6	93
6	1.3×10^7	97

TABLE 2

Sensitivity to of goat serum adapted SP2/0 myeloma cells to Aminopterin and 8 - azaguanine

Initial counts/ml	HAT		8 Azaguanine	
	cell count	Viability	cell count	Viability
0.1×10^5 (FCS adapted, viab. 99% SP2/0)	0.16×10^5	0%	1.25×10^5	100%
0.1×10^5 (GS adapted, viab. 95% SP2/0)	0.1×10^5	0%	0.6×10^5	94%

7) ADAPTATION OF *P. FALCIPARUM* STRAIN- SO-HS TO GOAT SERUM

P. falciparum SO-HS a cloroquine resistant strain maintained on human serum was adapted to grow on goat serum by gradual replacement for human serum. The cultures have been successfully maintained on 10 % goat serum for 5 passages. These culture strains have been cryopreserved and these cultures have been sucessfully revived.

b) CHARACTERISATION OF CULTURES

8) CHARACTERISATION OF HYBRIDOMAS SECRETING ANTIINSULIN ANTIBODIES

Two hybridoma cell lines viz.CC9C10 and AE9D6 were revived and the supernatents were screened for their antibody activity. CC9C10 is a cell line produced against beef insulin and the antibody has reactivity to insulin from beef, pork, rabbit, rat, sheep and human species. AE9D6 is produced against human insulin (A8-10 residues) and is known to react with human insulin only. An indirect ELISA using HRP system and with bovine and human insulin as the coating antigen was set up. Quantitative estimation of antibody secreted by the cell lines was done at different passages. To economise on the amount of insulin use, titrations were done using varying concentrations of insulin (0.075 - 50 μg/ml). A concentration of 1.25 μg/ml was the least concentration required for satisfactory results. The specificity of antibody activity to insulin was ascertained using an inhibition assay. Antibody (IgG) from CC9C10 supernatent was purified and labelled with HRP. The labelled antibody was incubated with varying concentrations of insulin and then added to plates coated with insulin. There was a good correlation between the percent inhibition and concentration of insulin as a inhibiting antigen. Comparison of the species specificity of the antibody from the 2 clones was done. Though antibodies from the 2 cell lines were reactive to both bovine and human insulin, as expected the CC9C10 and AE9D6 demonstrated higher reactivity to their immunising antigen viz. bovine and human insulin respectivity.

9) CHARACTERISATION OF CELL LINES BY DNA FINGER PRINTING AND ANALYSIS OF MITOCHONDRIAL DNA

DNA finger printing technology has been successfully used as a tool to characterize cell lines by ECACC employing Alec Geffrey's probes. A finger print is specific for a cell line and would be a useful marker to ascertain the purity of the cell line and detect any cross contamination . DNA preparations were made from Hela, Hep2, K562, L929, Neu2a, YAC-1, BHK-21,Indian Muntjac, Vero and Culex cell lines. Cells from monolayer were washed in Tris buffered saline (TBS pH 8.0) and suspended in EDTA. This was followed by proteinase K digestion for 5-7 hrs. at 50°C and extraction with phenol chloroform and final precipitation with isopropanol. Preparations from each of the cell lines yielded approximately 100-200 μg DNA. This will be subsequently digested with suitable restriction enzymes and used for finger printing with various probes. Dr. Lalji

Singh, at CCMB has kindly agreed to provide the probes to carry out these studies.

Restriction endonuclease cleavage pattern of mitochondrial DNA is a characteristic feature of a cell line and can be used to check it's purity. No such study has been done on mitochondrial DNA from insect cell lines. Mosquito cell lines from *A. albopictus* and *Ano. stephensi* were grown in large scale and mitochondrial DNA was purified by CsCl-EtBr equilibrium density gradient. Supercoiled mitochondrial DNA purified from these species had mol. wt. in the range of 13-15 Kb. At present, this DNA is being digested with a battery of restriction endonucleases to generate a restriction fragment pattern characteristic of this cell line.

10) STUDIES ON CYTOSKELETON OF ARMADILLO CELLS

Armadillo cells exhibit an unique cytoskeleton which is evident even under phase optics. They are fibroblast cells with a variety of shapes and unusually large in size. They have prominent stress fibres as well as a reticulate structure in the central region of the cell. These structures prompted a study into the cytoskeleton of these cells. Two armadillo fibroblast cell lines DNI-K - derived from the kidney and DNI-Tr derived from the trachea were studied. Using antibodies to cytoskeletal proteins such as tubulin, actin and vimentin these cells were stained by the method of indirect immunofluorescence. The organization of microtubules was typical of fibroblast cells: an extensive array of microtubules arising from the perinuclear region and terminating at the cell periphery (Fig. 13) . Vimentin type of intermediate filaments were present as a dense mass of wavy filaments which is characteristic of these. It was the actin filament system that was unusual. There were several hierarchies of these ranging from granular aggregates to the prominent stress fibers. At several regions there were foci of short stress fibers arising from a single point (Fig. 14) . It is proposed to study the pattern of actin polymerization in cells recovering from the actin disrupting drug, cytochalasin B. Secondly, the interrelation between the three cytoskeletal structures viz. microfilaments, microtubules and vimentin filaments was investigated by treating the cells with a microtubule inhibitor - nocodazole, and the effect of this on the microfilament and vimentin filaments were studied by indirect immunofluorescence. It is known that the depolymerization of microtubules leads to the collapse of vimentin filaments in the cell centre. Treatment of DNI-K cells with nocodazole did not lead to the collapse of vimentin filaments. Instead, they showed marked association with the stress fibers. Cross links between adjacent stress fibers were formed by the wavy vimentin filaments. These filaments also formed bundled coils along the prominent stress fibers (Fig. 15) . These interesting observations were unique to DNI- K cells. Other cells like BHK and NIH 3T3 did not show stress fiber association of vimentin filaments. The experiments are being repeated for confirmation.

11) CHARACTERISATION OF HEPATOCYTES CULTIVATED *IN VITRO*

It is a well established fact that mammalian liver is a mass of highly differentiated epithelial cells. During differentiation the epithelial cells lose the capacity to synthesise

their marker protein - keratin. These cells then express a new specific marker protein - albumin. During embryogenesis instead of albumin, alfa-feto protein is expressed. Though the gene(s) for alfa-feto protein is shut-down in the adult animal, it is expressed on partial hepatectomy or during malignancy of liver . In such cases, adult liver cells dedifferentiate and express the embryonic liver cell marker alfa-feto protein but not keratin. Hence experiments were designed to study the dedifferentiation of liver cells upto a stage where keratin is expressed by these cells.

Liver cells were isolated from embryo and adult rats. These cells were grown in culture for various time periods (5 to 75 days). They were screened for expression of both liver and epithelial cell markers such as albumin, alfa-feto protein and keratin, by indirect immunofluroscence test and immunoperoxidase staining. The presence of keratin and its polypeptides in 75 day cultures was demonstrated. At this stage albumin or alfa-feto protein were not expressed. Keratin polypeptides (58 kDa) were localized in the nucleus whereas other polypeptides (63 and 47 kDa) were seen only in cytoplasm of hepatocytes. The data is summarized in tables 1, 2 and 3. It is clear from this data that adult hepatocytes in culture undergo dedifferentiation in a stepwise manner.

Experiments to investigate the keratin patterns in hepatocytes at different days in culture are being conducted using SDS-PAGE technique. It is planned to extract mRNA from hepatocytes and examine the keratin gene expression, which may serve as an excellent marker for liver cell differentiation.

Table 1

Expression of liver cell markers

Experiment	Alb	AFP	P150
Mouse embryonic liver cells (20)	–ve	–ve (human x FP antibodies)	–ve
Mouse neonate liver cells (30)	+++	–ve	N.D
Rat neonate liver cells (45)	++	++	+++
Rat neonate liver cells (75)	±	±	N.D
Rat adult liver cells (5)	+++	–ve	+++

ALB → ALBUMIN. AFP →ALFA FETO PROTEIN
FIGURES IN PARANTHESIS REFER TO POST CULTURE DAYS.

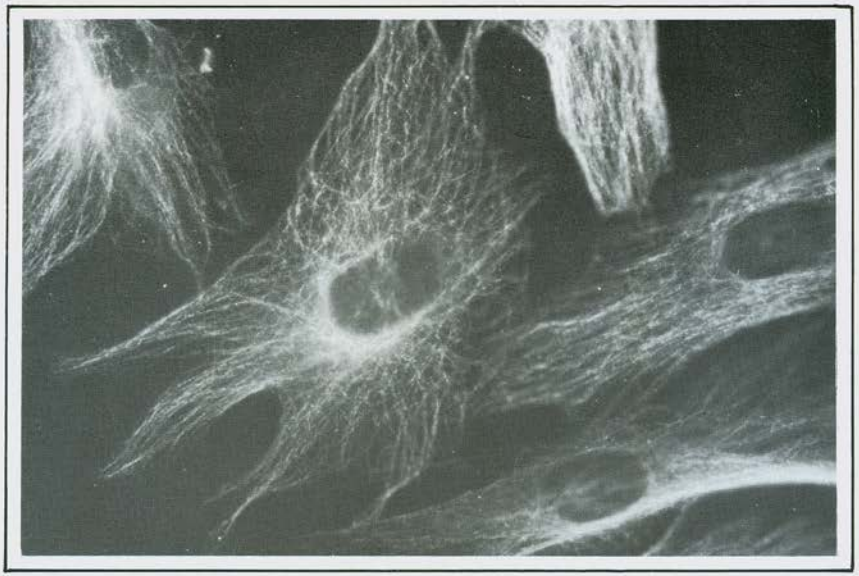


FIG. 13 : Indirect immunofluorescence staining of armadillo cells (DNI-K) cytoskeleton (x 630) cytoplasmic microtubules stained with antiß-tubulin.

FIG. 14 : Indirect immunofluorescence staining of armadillo cells (DNI-K) cytoskeleton (x 630). Actin cytoskeleton showing prominent stress fibres – stained with anti ∝-actin.

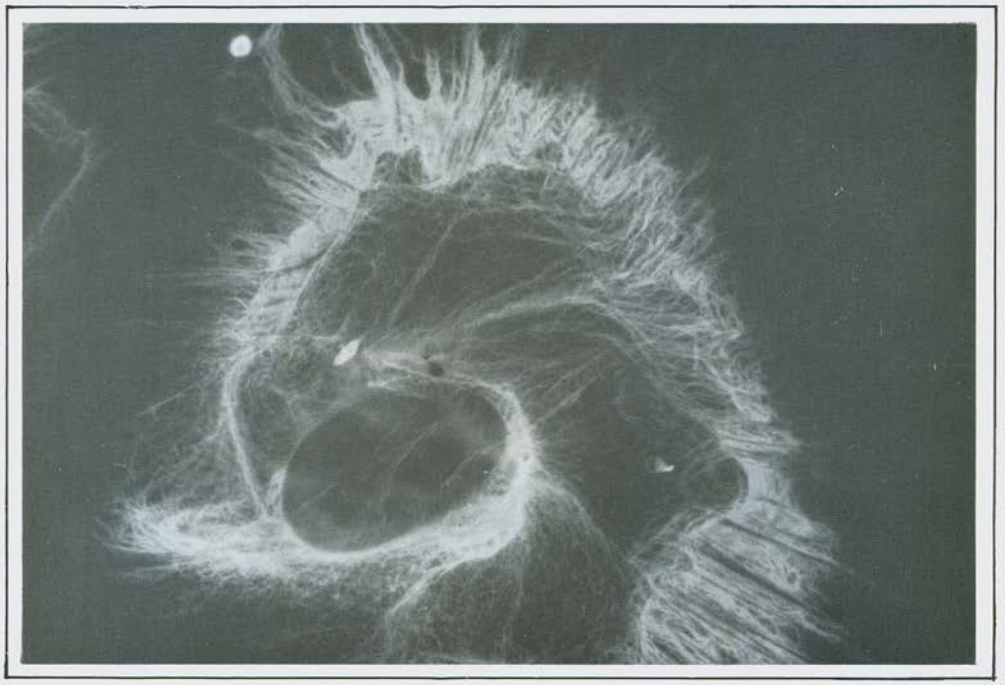


FIG. 15 : Coils of vimentin filaments in cells treated with microtubule inhibiting drug, nocodazole – stained with anti vimentin (x 630).

Table - 2

Expression of liver cell markers by immunofluorescence technique

	TK	K63	K58	K47	Alb
Set I : 3 weeks embryonic	–ve	+	–ve	–ve	++
Set II : 2 weeks embroynic	+++	+++	+	++ filaments	+
Set III : 1 week	+	+	+ Haemopoietic cells intensly + ve		

+ → SLIGHTLY POSITIVE, ++ → MODERATELY POSITIVE
+++ → INTENSELY POSITIVE, –VE → NEGETIVE, TK → TOTAL KERARTIN
ALB →ALBUMIN.

Table 3

Expression of liver cell markers by Peroxidase staining

	TK	K63	K58	K47	Alb	P150
Set I	++ ve all over	+ ve all over	– ve	– ve	faintly + ve	+ ve all over
Set II	no cells	+ ve all over	+ intense cytoplasm/ all over in some	+ve nucleus & general	++ all over	++ all over
Set III	nucleus + ve all over	++ ve intense all over	+ ve intense in nucleus only	+ ve more intense in nucleus	+ ve faintly	+ ve nucleus only

Set I embryonic cultured for 3 weeks
Set II adult cultured for 2 weeks
Set III embryonic cultured for 1 week

c) OTHER RESEARCH PROJECTS

12) MAINTENANCE OF HUMAN CORNEA FOR KERATOPLASTY

Experiments were carried out to determine the optimal conditions for long term preservation of donor cornea. Organ cultures of corneo-scleral buttons and trephined cornea of specific diameter were maintained in nutrient media such as MEM or M 199 with or without dextran and cultured at 37° C, 33° C and 8° C. Parameters used for assessment of cornea included (a) Endothelial cell integrity (b) Viability and (c) Transparency.The results are summarised in Table 1. A total of 42 specimens were processed for these studies. It was observed that trephined corneal buttons cultivated in media containing dextran and maintained at 33° or 8° C remained thin and survived for a longer period (up to 35 days) than the whole corneo-scleral buttons cultivated in media without dextran and maintained at 37° C (up to 20 days). Cultures maintained in MEM expressed marginal benefit over those in M 199. No significant difference was observed in cultures maintained at 33° C and 8°C. It appears from these results that trephined corneal organ cultures used in present studies permit long term preservation of donor cornea. This would help in replacing cutting of cornea-scleral button from the endothelial side, thus reducing chances of endothelial damage and thereby increasing its suitability for eye banking.

TABLE 1

Effect of culture conditions on maintenance of viability and transparency of cornea in organ culture

MEDIA	MAXIMUM PERIOD (IN DAYS) OF VIABILITY AND TRANSPARENCY AT DIFFERENT INCUBATION TEMPERATURES					
	C.S.B.	T.C.	C.S.B.	T.C	C.S.B.	T.C
	37°C		33°C		8°C	
MEM (E) + 10% FCS (INACTIVATED)	17	18	24	23	26	25
MEM(E) + 10% FCS + 5% DEXTRAN *	20	22	28	30	30	35
M 199(H)+10% FCS	15	17	18	22	20	24
MEM 199 + 10% FCS + 5% DEXTRAN *	18	20	20	25	24	26

C.S.B. → CORNEO SCLERAL BUTTONS.

T.C. → TREPHINED CORNEA.

Corneal buttons exhibiting more than 90 % viable endothelial cells were considered as viable.

***Cornea remained thin without swelling as compared to those cultured in media without dextran.**

Data represents average of three experiments.

13) DEVELOPMENT OF AN ASSAY SYSTEM FOR QUANTITATION OF FUNCTIONAL ANTIBODIES TO TETANUS TOXOID IN AN *IN VITRO* MODEL.

The aim of the project is to develop an assay system to quantitate antitetanus antibody in an *in vitro* model. Neuro 2a has been used in the assay. Initial experiments were carried out employing the indirect immunofluorescent test to demonstrate the presence of T.Toxin receptors on neuro 2a cells. A commercial preparation of antitetanus antibody (TIG) was labelled with FITC for this purpose. In a parallel experiment the ELISA using HRP labelled TIG was employed. From the preliminary experiments carried out, it was found that labelled TIG bound to the cells even in control sets in the absence of toxin. We speculate that such a nonspecific binding by FITC/ HRP labelled TIG may be mediated via Fc receptors.

To overcome the problems of nonspecific binding in an indirect ELISA, for demonstration of T-toxin receptors, HRP-labelled T- toxin was used in a direct assay with neuro-2a cells. Standard procedure for labelling with HRP was followed. For cellular ELISA, neuro-2a cells grown in 96 well plates (NUNC) were treated with serial dilutions (1:100 to 1:3200) of labelled toxin and OPD (Ortho Phenyl Diamine) was used as substrate. Titration curve was drawn with dilution vs OD. There was a good correlation between OD and dilutions of labelled toxin.

Effect of temperature and buffers was studied to standardise optimal conditions for receptor binding of labelled toxin. Cells were treated with labelled toxin and incubated at 37° C, 22° C or 4°C for 2 hours. Experiments were done with PBS (pH 7.4) or Tris acetate buffer(TAB). There was no difference in the results when studies were done with PBS or TAB. The readings were significantly high when experiments were done at 37° C in comparison with 22 °C and 4°C.

Inhibition studies were carried out to confirm the specificity of toxin binding. Serial dilutions of labelled toxin were added after the cells were treated with varying concentrations of unlabelled toxin (10, 5, 2.5 and 1.25 μg). 2.5 μg gave optimal results with % inhibition of 53.35, 31.3 and 19.86 for 1:800, 1:400 and 1:200 dilutions of labelled toxin.

Experiments were set up to demonstrate the inhibitory effect of anti T. T. antibody on toxin binding to neuro 2a cells. A two step assay was done for this purpose. In the first step, labelled toxin in dilutions (1:100, 1:200) were incubated with varying concentrations at 37° C for 2 hours. In the following step the treated mixture of toxin and TIG were added onto neuro 2a cells grown in 96 well tissue culture plates. Incubation was done at 37° C for 2 hours for binding of toxin to receptors on neuro 2a cells. The plates were washed and treated with substrate OPD. The preliminary results with TIG inhibition of toxin were as follows:

Dilution of toxin	Percent inhibition			
	0.125 IU/ml	0.0625 IU/ml	0.0151 IU/ml	0.0078 IU/ml
1:100	75.81	ND	57.22	48.37
	42.82	ND	24.76	23.57
1:200	64.86	22.97	ND	ND

(ND - not done)

14) MASS CULTIVATION OF ANIMAL CELLS IN HOLLOW FIBER BIOREACTORS

There is an increasing demand for biologically active products of animal cell origin eg. human growth factors, interferons, interleukins, monoclonal antibodies etc. Although the technology for large scale cultivation of microbial cells is well developed, the same cannot be applied directly to the cultivation of animal cells. This is due to the basic differences in the physiology as well as growth requirements of animal cells. It is relatively easy to cultivate cells as suspension cultures as existing reactor configurations can be used for the purpose. Hollow fibre bioreactors (HFBR) are being increasingly put to use for hybridoma cultivation. The cells are enclosed in the shell of the HFBR and the nutrients are supplied by perfusion through the lumen of the capillaries.

This study therefore aims to obtain a clear understanding of hybridoma cultivation in HFBRS and also to gain basic information on the physiological and environmental factors that affect cell growth and metabolism. An anti -Japanese encephalitis virus (JEV) hybridoma (obtained from National Institute of Virology, Pune) was cultivated in a HFBR (Acusyst R, Endotronics Inc. USA) for a period of 30 days. Samples were collected at regular intervals and analysed for production of anti-JEV antibodies which showed a progressive increase till the end of the run. During the course of the run it was observed that some mass transfer limitations exist in the reactor. This was reflected in the difference in pH of the two compartments i.e. the ICS (Intra Capillary Space) and ECS (Extra Capillary Space) which was observed visually by the change in the color of the indicator. The tracer studies further support this observation.

A murine hybridoma CC9C10 - secreting antiinsulin antibodies was used to study the kinetics of growth and antibody production. The rate of growth of cells and product formation was studied at different glucose and glutamine concentrations. The cells have a lag period of about 4 days after which their number was found to increase in an exponential manner till day 7. There was no further increase in the cell number till day 10. Antibody production was found to occur after the period of growth had been completed. The titre was found to increase from day 6- day 9, after a constant titre was observed. Lactate production was found to be related to the cell number. The initial concentration of glucose present does not significantly affect the rate of growth of the cells and the antibody production. The rate of growth as well as the antibody production

was found to be dependent on the initial glutamine concentration. These observations however need further investigation when both the substrates are in limiting concentrations.

15) STUDIES ON FILARIASIS

Hybridomas secreting antibodies to *B. malayi* infective larval antigen have been revived. Antibody secretory activity was confirmed by immunodiffusion. Specificity of this antibody to *B. malayi* antigen was checked by ELISA. In subsequent experiments, F-46 hybrid was cloned twice. Loss of antibody secreting activity was observed during the maintenance of the clones. Further cloning of this hybrid was not done due to lack of *B. malayi* larval antigen.

Dot blot assay for detection of *B. malayi* larval antigen was developed and standardized using F46 antibody (1 μg/ml). Preliminary studies were carried out using normal sera spiked with *B. malayi* larval antigen . The assay has a sensitivity to estimate upto 1 ng of antigen in sera. The workability of this assay system will be assessed using infected sera from endemic area.

16) SCREENING OF ANTI-MALARIAL COMPOUNDS *IN VITRO*

A synthetic compound with code number 87/209 was supplied by Central Drug Research Institute, Lucknow. This compound is known to increase chloroquine retention time by the chloroquine- resistant strains of *P. falciparum.* Experiments were conducted to analyse it's action by using a chloroquine resistant strain of *P. falciparum* viz., SO-HS. This strain is resistant to chloroquine up to a concentration of 1.25 μM, while the in vitro lethal dose of chloroquine for a sensitive strain is 1.25 nM. The results of the study are shown in table 1. Initial experiments in our laboratory have demonstrated that at a concentration of 75 μg/ml of the compound the effective chloroquine concentration is brought down to as low as 0.125 nM in the resistant strain. Further experiments are being carried out to determine the lowest effective concentration of chloroquine in combination with this compound. Toxicity studies were carried out using WISH, HELA and TC-7 cell lines. Preliminary results indicate that the compound is toxic at 25 μg/ml to these cells.

TABLE 1

Combined effect of compound 87/209 and chloroquine on chloroquine resistant strain 'SO-HS'

Dose of compound (μg/ml)	Dose of Chloroquine (nM)	Average percent parasitemia at 72 hrs
Nil	Nil	4
500	Nil	–ve
Nil	1.25	2.24
500	1.25	–ve
250	1.25	–ve
125	1.25	–ve
100	1.25	–ve
75	1.25	–ve
50	1.25	0.78
25	1.25	1.05
75	1.00	–ve
75	0.5	–ve
75	0.25	–ve
75	0.125	–ve

17) *IN VITRO* CULTIVATION OF *P.VIVAX*

Efforts have been initiated to cultivate erythrocytic stages of *P.vivax*. Arrangements were made for the collection of blood samples from untreated *P.vivax* cases for preparation and maintainence of specific cell cultures. So far only two attempts were made which did not meet with any success.

18) STUDIES ON MOSQUITO CELL FUSION AND INDUCTION OF PREMATURE CHROMOSOME CONDENSATION (PCC)

Experimental conditions for obtaining fused cells were standardized employing C6/36 *(Aedes albopictus)* clone. Further experimental work was undertaken to obtain premature chromosome condensation in these fused cells. For this purpose, c-mitotic cells were accumulated in exponentially growing cultures. These were fused with non-dividing cells obtained from exponential cultures situated randomly around the cell cycle.

It was observed, however, that the extent of cell lysis following fusion event reduced the available fused cells. Furthermore, since random population was employed for fusion, the relative proportion of cells with induced PCC was low. Nevertheless, from several experiments carried out, it was possible to obtain PCC at various stages of the cell cycle. A full view of the chromosome decondensation-condensation cycle could be constructed from such experiments. The stage specific morphological characteristics of the PCC could be clearly plotted.

19) INDUCTION OF POLYTENIZATION IN DROSOPHILA CELLS *IN VITRO* BY FUSION WITH SALIVARY GLAND CELLS

Factors that regulate the polytenization of salivary gland cells in drosophila cells were investigated by employing the techniques of somatic cell hybridization and microinjection.

Salivary glands from third instar larvae of drosophila were dissected out in Schneider's medium. These glands were maintained as intact glands as organ cultures upto a week in Schneider's medium containing 10% fetal calf serum. Although the gland morphology was normal, the chromosomes did not demonstrate normal morphology. The cells from these glands could be dissociated using collagenase treatment. The isolated cells did not adhere to the substratum. Somatic cell hybridization by electrofusion was undertaken to investigate the factors in these cells that could induce diploid somatic cells of drosophila cells to undergo multiple chromosome replication. The parameters for fusion were standardized using H33 cells which are from a drosophila cell line. However when salivary gland cells were used for this procedure the cells underwent lysis at these feild strengths. Fusion by polyethylene glycol was attempted. This led to shrinking of salivary gland cells. Therefore other avenues were tried to introduce the cell contents of salivary glands into insect cell lines of drosophila (H33) and mosquito origin (ATC- 15). Salivary gland cells were lysed by repeated freeze thaw method to release their contents into the surrounding medium. The cells were spun down and the extract was microinjected into H33 and ATC 15 cells. These cells were too small for this procedure and tended to lyse or detach from the substratum. It is now proposed to use larger and more adherent fibroblast cultures.

20) STUDIES ON CELL ADHESION MOLECULES

Cell adhesion molecules of different specificities play a major role in morphogenesis, histiogenesis and regeneration. BHK-21 cells have calcium dependent cell adhesion molecules which are released in the medium by trypsin action. This trypsin solubilized fraction was obtained by standard trypsinisation procedure and used in present studies. It was observed that the solubilized fraction when added to single cell suspension of BHK- 21 cells in MEM (E) brings about aggregation of cells. When the solubilized fraction was obtained from BHK-21 cells at passage 27 after initiation of these experiments there was an abrupt change in the property of the fraction. It was observed that the fraction could bring about aggregation of cells only if the medium was free of calcium or equimolar quantity of calcium chelator was added to the reaction mixture These experiments indicate that aggregation brought about by solubilized fraction was sensitive to calcium after passage 27, and subtle changes in cell membranes and membrane proteins during passage.

21) STUDIES ON STRESS PROTEINS IN *ANO. STEPHENSI* CULTURE

The aim of the study is to make substracted cDNA library from heat shock induced mRNA from Anopheles stephensi cell line. For this, the total RNA was isolated from induced as well as uninduced cells. To separate polyA+ mRNA fraction, total RNA was passed through oligo-dT column. cDNA was prepared from induced polyA+ mRNA. Liquid hybridization of polyA+ mRNA from uninduced cells and single stranded cDNA was done. Separation of hybridized and unhybridized products was done on hydroxy apatite column. Selected single stranded cDNA was tailed with dA using terminal transferase and second strand by using oligo-dT as a primer and reverse transcriptase. This cDNA will be ligated with EcoRI linkers and finally cloned in EcoRI sites of the vector.

3-C) EXTRA MURAL ACTIVITIES (C S I R PROJECT)

REGULATION IN BRAIN HEXOKINASE BIOCHEMISTRY AND MOLECULAR BIOLOGY

Attempts to clone the bovine brain hexokinase gene using bovine brain cDNA library was continued. For this purpose, the library was screened using single stranded 41mer synthetic DNA as a probe. Two clones viz clone 15 and clone 8 were amplified and used for DNA isolation. A dot blot of the DNA from these two clones was probed with the 41mer synthetic probe. It showed positive signals on an autoradiograph after high stringency washes of 0.2 x SSC, 1% SDS at 42°C.The DNA from the two clones was digested with EcoRI to remove the insert and this digested DNA was run on a 0.8 %

agarose gel. Clone 15 showed the presence of two arms of the vector λgt11 m and three bands of sizes 1.5 kb, 1.2 kb and 0.9 kb. Clone 8 showed a positive signal for the 1.2 kb band and clone 15 for the 1.7 kb band, but not for the other two bands.

(a) Subcloning in plasmid bluescript

The DNA extracted from clone 15 and 8 was digested with EcoRI and run on a 0.8 % agarose gel. The insert band was cut out and eluted from the gel. Plasmid bluescript was digested using EcoRI and alkaline phosphatased. 600 ng of the insert DNA and equal molar concentration of the vector DNA were ligated. This ligated mixture was used to transform E. coli DH 10 B. The clones were selected on Luria agar with ampicillin (100 μg/ml) plates containing Xgal (5-bromo-4-chloro-3-iodoyl ßD galactoside) and IPTG (isopropyl BD thiogalactopyronoside). Three white colonies were visible on the plates. Plasmids were isolated from these 3 clones. The electrophoretic migration pattern of the plasmids isolated from the 3 clones was identical to that of blue script, indicating that the vector did not contain the 1.7 kb insert. The cloning was repeated and experiments are in progress to confirm the results.

(b) Restriction mapping of the cloned fragment

Experiments were carried out to map the restriction enzyme sites on the cloned fragment. The DNA was end labelled using terminal transferase and digested using various restriction enzymes and run on a 1.4 % agarose gel. The gel was dried and set for autoradiograph. The autoradiograph showed bands against a smear in the background. Further experiments to standardize this technique are in progress.

COLLABORATORS

NFATCC has established collaborative projects with scientists and clinicians from different research institutes and hospitals.

King Edward Memorial Hospital, Pune	Dr. B.B. Gokhale Dr. Y.V. Tawade Dr. S.B. Kelkar Dr. C.S. Yajnik
B.J Medical College & Sassoon General Hospital, Pune	Dr. A.S. Labhashetwar Dr. P.P. Sambary Dr. A.V. Jamkar Dr. Shailaja Jadhav Dr. Y.W. Risbood
Janakalyan Eye Bank, Pune	Dr. R.D. Kolte
National Chemical Laboratory, Pune	Dr. R.A. Mashalkar Prof. John Barnabas
University of Poona, Pune	Prof.D.N. Deobagkar Dr. N.N. Godbole
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Marathe Clinic, Pune	Dr.R.L. Marathe
Mahatma Gandhi Eye Bank, Pune	Dr. N. Shah
Naik Hospital, Pune	Dr.S.N. Naik Dr.(Mrs.) S.S. Puntambekar
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Hindustan Lever Research Centre, Bombay	Dr. V. Sheorain

Tata Institute for Fundamental Research, Bombay	Dr. L.C. Padhy
Centre for Cellular & Molecular Biology, Hyderabad	Dr. P.D. Gupta
Central Drug Research Institute, Lucknow	Dr. A. Bhaduri
Indian Red Cross Society, Ahmedabad	Mr. G. Mazmudar

PUBLICATIONS

1. P. A. CARVALHO, G.R. RAJASEKHARIAH, L. DESHPANDE, P. B. PARAB, U. R. RAO AND D. SUBRAHMANYAM. Evaluation of *in vitro* released *Wuchereria bancroftii* thrid stage larval antigens for detection of bancroftial filariasis. Trop. Med. Parasit. 41: 71-72 (1990).

2. D. SUBRAHMANYAM, P. B. PARAB, G. R. RAJASEKARIAH, W. RUDIN, B. BETSCHART AND N. WEISS. "Interaction of monoclonal antibodies with cuticular antigens of filarial parasites, *Brugia malayi* and *Wuchereria bancrofti*". Acta Tropica, 47: 381-390 (1990).

CONFERENCES/WORKSHOPS

BHONDE R. R.	"LONG TERM PRESERVATION OF HUMAN CORNEA FOR EYE BANKING". Presented at the International Symposium on Occular Pharmacology and therapeutics held at New Delhi during Aug 8- 10, 1990. (Co-authors: Kolte, R. D. and Kelkar S. B.)
	Visited Centre for Cellular and Molecular Biology, Hyderabad, as a part of the collaborative project on hepatocyte cultures in the study of dedifferentiation, to carry out peroxidase staining and SEM of rat hepatocytes. October 8 to 10, 1990.
	Visited Cancer Research Institute, Bombay, as a part of the collaborative project on enhancement factor studies on intestinal organ cultures from mouse.
MOJAMDAR M. V.	"*IN VITRO* RESPONSE OF NORMAL AND VITILIGO MELANOCYTES TO NATURAL AND SYNTHETIC GROWTH FACTORS" (Co-authors: R. R. Bhonde and M. R. Wani). Accepted for presentation at the First APOCB Congress, Shanghai, China, held during Nov. 4th to 7th 1990.
	"A MICRO METHOD FOR ASSAYING PROLIFERATION AND DIFFERENTIATION IN MELANIN CONTAINING CELLS". (Co-author: R. R. Bhonde). Accepted for presentation at the XIVth International Pigment Cell Conference, Kobe, Japan, held during October 31st to November 4th, 1990.
	"ETIOLOGY OF VITILIGO: A NEW HYPOTHESIS" (Co- authors: A. Ramaiah and N. Puri). Accepted for presentation at the XIVth International Pigment Cell Conference, Kobe, Japan, held during October 31st to November 4th, 1990.
PARAB P. B.	Attended the Annual Conference of the Indian Immunology Society, held at the Sanjay Gandhi Post-Graduate Institute of Medical Science, Lucknow. November 28 to December 3 1990.

KALE V. P.	Attended the international Symposium on "Autologous Bone-marrow Transplantation" held at AIIMS, New Delhi, February 15 1991.
SHASTRY PADMA	Participated in the National workshop on "Cytotoxic Effector Mechanisms" held at CRI Bombay, May 21 to May 30, 1990.
WANI M. R.	"SPLIT THICKNESS AUTOGENOUS FREE SKIN TRANSPLANTATION IN CANINES". (Co-authors: M.D. Narkhede and P.E. Kulkarni). Presented at the 14th Congress of I.S.V.S. and symposium held at Ludhiana during 18th - 20th Feb. 1991.
	"PINCH SKIN GRAFTING IN THE GRANULATING WOUNDS OF CANINE". (Co-authors: M.D. Narkhede and P.E. Kulkarni). Presented at the 14th Congress of I.S.V.S. and symposium held at Ludhiana during 18th to 20th February 1991.

TRAINING COURSES

DR. SUJATA GUHA ROY	Attended UGC Seminar on Cell Engineering, held at the Dept. of Zoology, University of Poona, Pune. December 27 to 29, 1990.
MISS SUPRIYA KSHIRSAGAR	Attended UGC Seminar on Cell Engineering, held at the Dept. of Zoology, University of Poona, Pune. December 27 to 29, 1990.
MRS. M. V. GODE	Attended the Practical Training Course in Molecular Parasitology, held at the International Centre for Genetic Engineering and Biotechnology, New Delhi. November 5 to 30, 1990.
MR. S. S. DAVARE	Attended the Short Term Training Course on Gene Delivery and Targeting, held at the Madurai Kamraj University, Madurai. November 21 to December 11, 1990.

Teaching and training

(Participation in workshops, seminars, etc.)

i. DBT-Sponsored Popular Lecture Series was organised and conducted by the NFATCC .

ii. UGC-sponsored Seminar on Cell Engineering, held at the Department of Zoology, University of Poona,Pune. December 27 to 29, 1990.

iii. Participation in the University of Poona Teaching and training.

Diploma in Medical Virology course at the National Institute of Virology, Pune.

iv. Individual training for deputed candidates.

- DR. TAHER UZ-ZAMAN, Osmania University, Hyderabad. (9-7-1990 to 26-7-1990).
- Mr. NIRAJ ARORA, Indian Vaccines Corporation Ltd., New Delhi. (17-9-1990 to 13-10-1990).
- Mr. SHIV KUMAR, Indian Vaccines Corporation Ltd., New Delhi. (17-9-1990 to 13-10-1990).
- Miss DEWANSI AWASIA, Indian Vaccines Corporation Ltd., New Delhi. (17-9-1990 to 13-10-1990).
- Mr. M. N. LIMAYE, Poultry Diagnostic Research Centre of Venkateshwara Hatcheries Pvt. Ltd., Sholapur Road, Loni Kalbhor, Pune. (25-5-1990 to 5-6-1990).

COMPUTER

Minicomputer-386 based machine and 286 systems (2 Nos.) and 11 stand- alone PCs were installed. The 386 machine is used as a file server for network with PCs. One 286 machine is dedicated for library work and the other for scientific analysis and data storage and for generating reports. Operating Systems used are SCO UNIX and DOS 4.01.

II. System Identification for Computerisation and Software Development

Various Scientific, Administrative and Library activities were identified for computerisation for efficient and easy functioning.

Areas Computerised:

- Personal Management
- Library Management
- Office Automation Systems
- Cell Line Catalogue
- Cell Line Supply System
- Statistical Analysis
- Accounts
- Inventory Control (Under Implementation)
- Equipment Health Management (Under Implementation)
- Education Aids.

III. Compilation of Internal Publications

- Wordstar Release 4: A Command Reference
- Computer Virus: A Write-up
- Floppy Care (Forthcoming)

IV. Users' Awareness And Guidance to Staff

Training courses were conducted for the staff in

- Computer Fundamentals
- Spread Sheets
- Database Management Systems
- Word Processing

Total of 35 staff were given training in two batches. This training was conducted by M/s Computer Point, Pune. In-house training by the staff of computer section was given from time to time on awareness, latest developments, word processing, spread sheets, computer virus etc.

A PC based telex system for telex communication has been installed.

V. Plans for Computerisation Next Year

- Connecting up NFATCC to DIC's VAX through Dial up type modems.
- Global database access (Dialog etc.)
- CD-ROM
- To make our database (Cell Lines) globally available
- Management Information Systems (MIS)

LIBRARY AND DOCUMENTATION

The main activities of the Library and Documentation have been focussed on Collection, Collation and Dissemination of Science and Technology information. The areas covered for this purpose are animal tissue and cell culture and its allied subjects.

The library became a full fledged functional unit in 1990. Earlier 370 books and 4 journals were available in the library. During the year 1990, 200 books were acquired and subscribed to 33 Scientific journals and general periodicals. At present it has a collection of 1000 documents which includes books, reports, conference proceedings, journals, newsletters and reprints.

A meeting was convened by the NFATCC documentation unit in January 1991 to discuss the possibilities of resource sharing between various libraries and information centres in Pune. The communication and interaction initiated via this meeting has made such "a resource sharing" a reality. The NFATCC library now provides inter-library loan co-operation by obtaining books/journals/photocopies from centres in Pune.

Bibliographies, Reading Lists, Compendiums on various topics were compiled on requests. Long and short term reference services were rendered to users at NFATCC and other institutions.

Publications During 1990-91

- A Reading List on Virology
- A Bibliography on Tissue Culture
- A Bibliography on Malaria
- A Bibliography on Corneal Research
- Path-Finder: A Guide to Library & Information Services
- Current Additons (Monthly)
- Technical Report Writing
- Catalog of Holdings - Reprints

Bio-Alerts:-

It is a monthly information service-a product of the database on Biotechnology Literature developed in-house by selecting relevant references from secondary periodicals. It aims at creating an awareness and providing access to the latest technical literature published in those fields in which NFATCC Scientific Community is interested. The coverage of articles are confined to Current Contents, Biotechnology Abstracts & Government Research Announcement and Index (GRA&I). This service is being generated by augmenting the CDS-ISIS Software developed by UNESCO and is being supplied in India by NISSAT.

Library is in the process of generating Selective Dissemination of Information (SDI) to its users by constructing Users' Profiles by manipulating certain features of CDS-ISIS from the existing database created for In-house monthly publication BIO-ALERTS.

Certain reprints/pre-prints etc are not available through normal channels in India. In order to bridge-up this gap and easy procurement NFATCC library has purchased British Library Document Supply Centre (BLDSC) coupons.

Computerisation of library activities, Information retrieval system for books, Circulation monitoring system, Generating current additions list etc. have been done to provide pin pointed exhaustive and expeditious information. Library is in the process of computerisation of acquisition of documents.

INSTRUMENTATION & MAINTENANCE

The instrumentation and maintenance services have been well organised which looks after the following activities :-

1) Receipt , scrutiny and installation of new equipments.
2) Services for rectification of trouble shooting and preventive maintenance round the clock , particularly for electric power supply, diesel generator, water storage and supply, air conditioning units, refrigerators, deep freezers.
3) Ready availability and transportation of Liquid Nitrogen .

New equipments such as microscopes, centrifuges, spectrophotometers, fraction collectors, electrophoretic apparatus, weighing balances, vacuum pumps, ovens, autoclaves, electrofusion set up, CO_2, B.O.D. and other incubators, Liquid Nitrogen refrigerators, Laminar flow cabinets, hollow fibre reactors, projection, recording & P.A. Systems, water filtration units, etc. have been installed and maintained in operational state. Various aspects of designing, planning and construction of laboratory buildings and execution of jobs connected with these activities are in progress.

Expenditure Finance Committee approved the budgetary provisions of NFATCC for the 8th Five Year Plan.

Agreement with the Architects, M/s.Beri Architects & Engineers Pvt. Ltd. appointed for the construction of Laboratory and Residential complex of NFATCC on Poona University campus was signed. The Department of Atomic Energy (C & S) group, Bombay was entrusted with the actual construction work of Laboratory and Residential complex of NFATCC. The Memorandum of Understanding to be signed with Department of Atomic Energy is being finalised. Various meetings between the Architects, M/s.Beri Architects & Engineers Pvt. Ltd., the experts from the Department of Atomic Energy and representatives of NFATCC were held to co-ordinate the construction activity. The building plans were discussed in details and finalised and submitted to Poona University for approval and onward submission to Pune Municipal Corporation for sanction. The borewell has been drilled at the site and temporary electric connection required for the construction activity has been obtained. Maharashtra State Electricity Board has been approached towards the requirement of high tension power supply. Demarcation of site, contour survey and sub-soil investigation has been completed. Plantation of trees at site is in progress.

As it would take another 2-3 years for the completion of main Laboratory and Residential complex on Poona University campus, to cope up with the increased activities of the institution, a plot admeasuring about 16000 sq.ft. and an unfinished RCC structure admeasuring about 6000 sq.ft. on the same plot was purchased from M/s.Vanaz Engineers Ltd, Paud Road, Kothrud, Pune. This plot and an unfinished RCC structure is located just about 150 yards from the existing interim laboratories of NFATCC at 'Jopasana', Paud Road, Kothrud, Pune. An unfinished RCC structure shall be completed to provide additional laboratory space and also to accommodate stores. Animal house shall be constructed separately on this plot. Architect has been appointed to complete the remaining work of this unfinished RCC structure as per the requirements of the institution.

A multiuser computer system using 386 based machine has been installed at the Laboratories located at 'Jopasana', Paud Road, Kothrud, Pune.

The second meeting of the Scientific Advisory Committee and the third meeting of the Governing Body was held on 3rd and 4th of Aug.90 and on 17th Aug.90 respectively.

Octroi exemption was obtained from Pune Municipal Corporation.

A four member Mangolian Delegation headed by H.E Dr.J.Batsuuri, Minister for National Development and Chairman of the State committee for Technical Programmes and Standardization of Mangolian People's Republic, Dr.George Martin, Professor of Pathology and Director of Alzheimer's Diseases Research Centre, University of Washington, Seattle, Washington, USA and Dr.Robert Hay, Head Cell Cultures, ATCC, USA were among the distinguished guests at the institution.

The staff complement of the institution as on 31.3.91 is as follows :

a)	Scientific Staff	12
b)	Technical staff	
	i) Laboratory	13
	ii) Instrumentation & Maintenance	6
c)	Administrative staff	9
d)	Auxilliary Staff	16
e)	Ad-hoc Staff	2
	Total :	58
f)	Trainees	4
h)	Contractual Service	9

Laboratory equipments worth Rs.93.5 lakhs were purchased during the year. The equipments have been installed and are used regularly.

PACKING

AUTOCLAVING & STERILISATION

COLLECTION OF CLEAN WATER

CRYOPRESERVATION

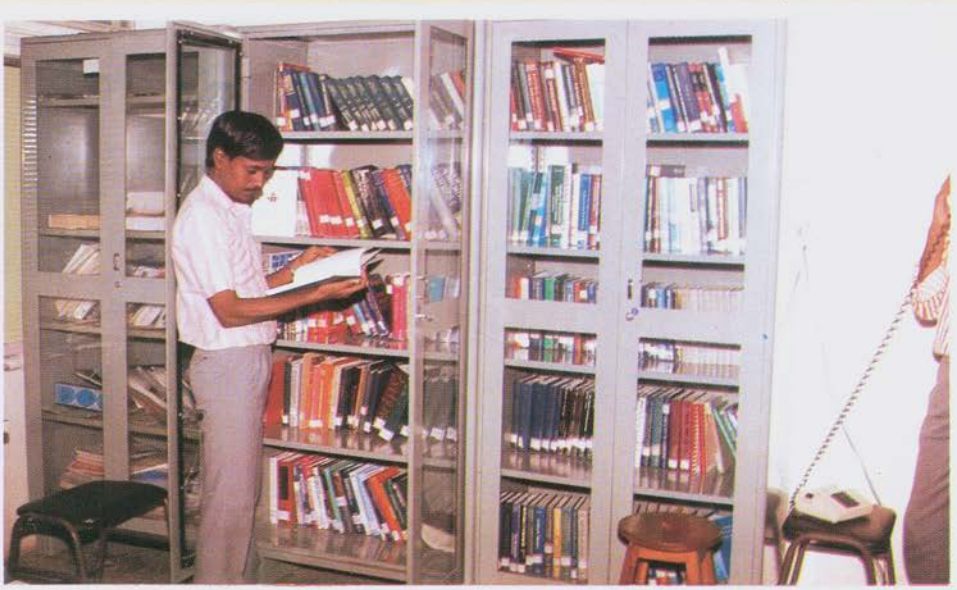
TRAINEE AT WORK

PREPARATION OF MEDIA

LYOPHILISATION

DNA SUBMARINE GEL ELECTROPHORESIS

COMPUTER SYSTEM



DOCUMENTATION & LIBRARY

DR. HAY HEAD, CELL CULTURE DEPT. ATCC, USA, OBSERVING CULTURES

MEMBERS OF THE MANGOLIAN DELEGATION "SCIENTIFIC CO-OPERATION BETWEEN INDIA & MANGOLIA" DISCUSSING WITH SCIENTISTS.

PARTICIPANTS OF THE MEETING ON ''STORAGE OF CORNEA FOR EYE BANK ACTIVITY'' DISCUSSING WITH SCIENTISTS.

PARTICIPANTS OF THE MEETING ON ''STORAGE OF CORNEA FOR EYE BANK ACTIVITY''.

COMMITTEES OF INSTITUTION

SOCIETY AND GOVERNING BODY :

- Minister of State for Science & Technology (President of NFATCC Society) New Delhi
- Dr.S.Ramachandran (Chairman, Governing Body) Secretary, Dept. of Biotechnology, Ministry of Science & Technology, New Delhi
- Prof.S.C.Gupte Vice Chancellor, University of Poona, Pune
- Dr.Smt.Manju Sharma Adviser, Dept. of Biotechnology, Ministry of Science & Technology, New Delhi
- Shri B.K.Chaturvedi, Financial Adviser, Dept. of Biotechnology, Ministry of Science & Technology, New Delhi
- Dr. Prema Ramachandran Dy.Director General, Indian Council of Medical Research New Delhi
- Dr.B.B.Mallick Director, Central Institute for Research on Goats, Mathura
- Prof.D.N.Deobagkar Head, Dept. of Zoology, University of Poona, Pune
- Dr.K.Banerjee Director, National Institute of virology, Pune
- Prof.H.Sharat Chandra Director, Centre For Cellular and Molecular Biology, Hyderabad
- Senior Scientist National Facility For Animal Tissue and Cell Culture, Pune
- Dr.U.V.Wagh Director Incharge, National Facility For Animal Tissue and Cell Culture, Pune

SCIENTIFIC ADVISORY COMMITTEE :

- Prof.John Barnabas (Chairman) Head, Biochemical Sciences Div. National Chemical Laboratory, Pune
- Dr.A.N.Bhisey Head, Cell Biology Div. Cancer Research Institute, Bombay
- Dr.B.D.Survashe General Manager, Ventri Biological Laboratory, 1376, Mile Stone, Panshet Road, Giri Nagar, Pune : 411 025

- Dr.B.N.Dhawan
  Director, Central Drug Research Institute,
  Chattar Manzil,
  P.O.B.No. 173,
  Lucknow
- Dr.S.N.Ghosh
  Ex-Director Gr. Scientist
  National Institute of Virology,
  Pune
- Dr.B.U.Rao
  Ex-Joint Director,
  Indian Veterinary Research Institute,
  Bangalore
- Commandant
  Armed Forces Medical College,
  Pune
- Prof.A.S.Kolaskar
  Officer In Charge,
  Bio-Informatics Centre,
  DIC, University of Poona,
  Pune
- Prof.B.K.Bachawat
  Head, Dept. of Biochemistry,
  Delhi University,
  South Campus,
  Pune
- Prof.D.N.Deobagkar
  Head, Dept. of Zoology,
  University of Poona,
  Pune
- Prof.G.P.Talwar
  Director,
  National Institute of Immunology,
  New Delhi
- Prof.G.Padmanabhan
  Prof. of Biochemistry,
  Indian Institute of Sciences,
  Bangalore
- Prof.T.N.Tandon
  Dept. of Neurosurgery,
  All India Institute
  of Medical Sciences,
  New Delhi
- Dr.U.V.Wagh
  Director Incharge
  National Facility For Animal
  Tissue and Cell Culture,
  Pune.

FINANCE COMMITTEE

- Shri B.K.Chaturvedi (Chairman)
  Financial Adviser,
  Dept. of Biotechnology,
  Ministry of Science & Technology,
  New Delhi
- Prof.H.Sharat Chandra
  Director,
  Centre For Cellular and
  Molecular Biology,
  Hyderabad
- Prof.Mahendra Singhvi
  Tata Management Training Centre
  Pune
- Dr.S.Iqbal
  Scientist, Planning and
  Co-ordinating Officer,
  National Chemical Laboratory,
  Pune
- Dr.U.V.Wagh
  Director Incharge,
  National Facility For Animal
  Tissue and Cell Culture,
  Pune

BUILDING COMMITTEE

- Dr.U.V.Wagh (Chairman)
  Director Incharge,
  National Facility For Animal
  Tissue and Cell Culture,
  Pune
- Shri S.R.Sapra
  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
- Chief Engineer
  PWD, Pune Div.,
  Pune
- Shri B.Bose
  Sr. Manager (Admn)
  National Institute of Immunology,
  New Delhi
- Prof.D.N.Deobagkar
  Head, Dept. of Zoology,
  University of Poona,
  Pune

PURCHASE COMMITTEE

- Dr.S.N.Ghosh (Chairman)
  Ex-Director Gr. Scientist,
  National Institute of Virology,
  Pune
- Prof.John Barnabas
  Head, Biochemical Sciences Div.
  National Chemical Laboratory,
  Pune
- Prof.D.N.Deobagkar
  Head, Dept of Zoology,
  University of Poona,
  Pune
- Major P.K.Bapat
  Administrative Officer,
  National Facility For Animal
  Tissue and Cell Culture,
  Pune
- Shri T.G.R.Pillai
  Accounts Officer,
  National Facility For Animal
  Tissue and Cell Culture,
  Pune
- Senior Scientist
  National Facility For Animal
  Tissue and Cell Culture,
  Pune
- Dr.U.V.Wagh
  Director Incharge,
  National Facility For Animal
  Tissue and Cell Culture,
  Pune

INSTITUTIONAL CO-ORDINATION COMMITTEE

- Prof.S.C.Gupte (Chairman)
  Vice Chancellor,
  University of Poona,
  Pune
- Adviser (B)
  Dept of Biotechnology,
  Ministry of Science & Technology,
  New Delhi (Nominee of DBT),
- Joint Secretary, Finance
  OR
  His representative,
  Department of Biotechnology,
  Ministry of Science & Technology,
  New Delhi
- Nominee of Director General
  Indian Council of Medical Research,
  New Delhi
- Head, Dept. of Zoology
  University of Poona
  OR
  Co-ordinator/Head Biotechnology
  Training Programme
  University of Poona,
  Pune
- Dr.U.V.Wagh
  Director Incharge,
  National Facility For Animal
  Tissue and Cell Culture
  Pune

JOSHI & SAHNEY
CHARTERED ACCOUNTANTS
1313, SADASHIV PETH,
NATU BAUG, PUNE 411 030.

AUDITOR'S REPORT

We have audited the Balance Sheet of National Facility for Animal Tissue and Cell Culture as at 31st March 1991, and also the Income and Expenditure Account of the Institute for the year ended on that date.

We report that to the best of our information and according to explanations given to us, in our opinion, the Balance Sheet read with the Notes on Accounts shows a true and fair state of affairs of the Institute as on date and the Income and Expenditure Account gives a true fair view of the excess of income over expenditure for the year ended on that date.

For **JOSHI & SAHNEY**
CHARTERED ACCOUNTANTS

(H.M. JOSHI)
PARTNER

Place : Pune
Date : 27 May, 1991

JOSHI & SAHNEY
CHARTERED ACCOUNTANTS
1313, SADASHIV PETH,
NATU BAUG, PUNE 411 030.

NOTES FORMING THE PART OF THE ACCOUNTS FOR THE YEAR ENDED 31.3.1991

1. No depreciation has been charged on the fixed assets for the year ended 31.3.1991.
2. The accounts of the Institute are maintained on cash basis.
3. The Institute has purchased during the year premises at Vanaz factory, Kothrud, Pune and has taken possession of the same. However, registration of Sale Deed in respect thereof is in process.
4. The Institute has received interest amounting to Rs. 2,09,425.85 on temperory investment of Grant-in-Aid which, as informed to us, shall be adjusted against future grant-in-Aid and has been reflected as such in the Annual Accounts.

CHARTERED ACCOUNTANTS

Place : Pune
Date : 27 May, 1991

AUDITED STATEMENT OF ACCOUNTS

JOSHI & SAHNEY
CHARTERED ACCOUNTANTS

THE BOMBAY PUBLIC TRUSTS ACT, 1950
SCHEDULE VIII [Vide Rule 17 (1)]

1913, Sadashiv Peth,
Natu Baug, Pune 30.

National Facility for Animal Tissue & Cell Culture
31st March 1991
Registration No. F-5282

PROPERTY & ASSETS	Schedule	As at 31.3.90 Rs. Ps.	As at 31.3.91 Rs. Ps.
Immovable Properties (At Cost)	III	61,11,745.54	**87,20,869.34**
Capital Work in Progress	IV	NIL	**1,26,50,000.00**
Investments :			
(Market Value of the above investment is Rs. _____)			
Furniture & Fixtures :	V	4,85,608.67	**9,88,637.45**
Library Books	VI	3,18,887.54	**8,30,390,49**
Equipments	VII	1,05,75,479.17	**1,99,26,506.86**
Vehicles	XI	1,62,234.00	**1,62,234.00**
Loans (Secured or Unsecured)			
Advances :			
To Employees	VIII	8,115.00	**11,175.00**
Deposits	IX	85,000.00	**85,000.00**
Income Outstanding :			
Cash & Bank Balance	X	77,68,748.47	**56,16,175.14**
Total Rs.		2,55,15,818.39	**4,89,90,988.28**

Date : 27 May, 1991

As per our report of even date

Sd/-
CHARTERED ACCOUNTANTS

JOSHI & SAHNEY
CHARTERED ACCOUNTANTS

THE BOMBAY PUBLIC TRUSTS ACT, 1950
SCHEDULE VIII [Vide Rule 17 (1)]

1913, Sadashiv Peth,
Natu Baug, Pune 30.

Name of the Public Trust
Balance Sheet as on

FUNDS & LIABILITIES	Schedule	As at 31.3.90 Rs. Ps.	As at 31.3.91 Rs. Ps.
Trust Funds or Corpus :			
Other Earmarked Funds : (Created under the provisions of the Trust Deed or Schedule or out of the income)	II.	2,46,50,000.00	**4,78,84,425.85**
Loans (Secured or Unsecured)			
Liabilities :			
Income and Expenditure Account :			
As per last Balance Sheet		8,20,348.58	**8,65,818.39**
Surplus as per Income & Expenditure Account		45,469.81	**2,40,744.04**
		8,65,818.39	**11,06,562.43**
Total Rs.		2,55,15,818.39	**4,89,90,988.28**

The above Balance Sheet to the best of my/our belief contains a true account of the Funds and Liabilities and of the Property and Assets of the Trust.

* **Income Outstanding :** (If accounts are kept on cash basis Rent, Interest, Other Income Total Rs.

Sd/-
DR. U.V. WAGH
Director
NFATCC PUNE