NATIONAL FACILITY FOR ANIMAL TISSUE AND CELL CULTURE

ANNUAL REPORT
1991-1992

NATIONAL FACILITY FOR ANIMAL TISSUE AND CELL CULTURE

DEPARTMENT OF BIOTECHNOLOGY
GOVERNMENT OF INDIA

JOPASANA, 85/1 PAUD ROAD, KOTHRUD, PUNE 411 029
TEL. 335928/335954 TELEX. 145 7576 NFTC IN
FAX. 91 212 369501 GRAM. ATCELL

CONTENTS

PREFACE

It has been two years since the establishment of repository activity at 'Jopasana', Kothrud. The stock in the cell repository has reached to 817 cell lines with the addition of 129 cell lines this year. Four hundred and twenty eight cell cultures were supplied to 178 research teams from 109 laboratories. The steady increase in the stock, repository and supply service indicates the healthy growth and utility of this activity.

The repository for plasmids and vectors is now set to initiate supply service from its small collection.

NFATCC has been actively involved in studies on preservation of cornea for extended period for grafting. These efforts coupled with the excellent cooperation and prompt actions of different Eye Bank Associations in the country, the Indian Council of Medical Research and the Drug Controller of Maharashtra, paved the way towards implementation of the use of cornea stored for extended period in MK medium.

The technology for cultivation of skin as 3-D epithelia for allo and auto grafting and organ culture of foetal skin for grafting to vitiligo cases is being developed, standardised and tested. The preliminary results are extremely encouraging as evidenced by the success of the grafts.

Studies on filariasis were continued with added vigour with the newly established collaboration of Vector Control Research Centre, Pondicherry and Filaria Unit of Govt. of Maharashtra. Attempts towards cultivation of infective larvae 'in vitro' yielded good results. The larvae could be maintained upto 28 days in a viable state showed differentiation. Studies on development of anti-idiotypic antibodies to F46 monoclonal antibody have been continued.

Method for Dot Blot Assay employing nitrocellulose strips for detection of Dracunculiasis (Guinea Worm) infection has been developed and standardised. This simple diagnostic test is being evaluated under field conditions in an endemic area.

Efforts have been fortified towards culturing P. vivax in vitro. The positive results in the early stages of P. vivax cultivation are heartening. But the experience forewarns that independent separate facility for handling P. vivax is most essential as handling P. falciparum in the same laboratory may lead to cross contamination.

A in vitro method for quantitation of functional antibodies to tetanus toxoid employing mouse neuro 2a cells has been developed. The method will be validated in comparison with neutralisation assay.

SP2/0 cells were adapted to goat serum and efficiency of these cells for the preparation of hybridoma is being evaluated.

Induction of advanced technology in molecular biology is of immense utility towards characterisation of cells enhancing specificity and sensitivity. Studies have been undertaken employing indigenously available technology for development, and standardisation of methods

OBJECTIVES

- To receive, identify, maintain, store, grow and supply :
  - Animal and Human Cells/Cell cultures, cell lines of both exisitng (typed) and newly developed.
  - Tissues, organs, eggs (including fertilized), and embryos.
  - Hybrid cells including hybridomas.
  - 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.

REPOSITORY

- Cell Cultures
- Obligate Parasites
- Vectors, Plasmids and Genomic Libraries

DEVELOPMENT OF TECHNOLOGY FOR TISSUE BANKING

- Cornea
- Skin
- Bonemarrow
- Heart Valves

INFRASTRUCTURAL ACTIVITY

REPOSITORY

Efforts are underway to enrich the repository by procurement of additional cultures.

CELL CULTURES

- Nuclear stock stored in liquid Nitrogen : The cell repository now has a stock of 817 cell lines, which includes primary cultures, established cell lines and hybridomas. One hundred twenty nine new cell lines were added to the existing stock of 688 cell lines.
- Stock for quality control and redistribution : Seventy cell cultures have been expanded on small scale for quality control tests and for redistribution.
- Supply services : Cell cultures are supplied to research teams and public health laboratories in various parts of the country (Fig.1). Four hundred twenty eight cell cultures in 253 consignments were provided to 178 research teams from 109 laboratories during the period April 1991 to March 1992 (Fig.2).

CELL LINE SUPPLY GROWTH

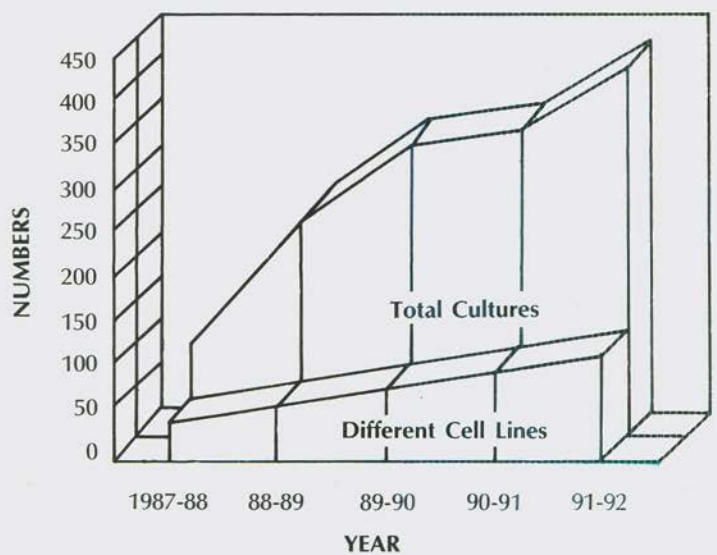


Fig. 2

CULTURE SUPPLY SERVICE

Fig. 1

OBLIGATE PARASITES

Stock Position : The obligate parasites available in the repository are *Plasmodium falciparum, Leishmania donovani, Trypanosoma congolensis, Theileria annulata* and *Toxoplasma gonidi.*

VECTORS, PLASMIDS AND GENOMIC LIBRARIES

Stock position : The repository maintains a stock of vectors, plasmids and genomic libraries. Twelve cultures were expanded from the stock and preserved as glycerol cultures. Plasmid DNA was purified from 4 strains. Four phage stocks were prepared and amplified.

The stock containing 15 host strains, 5 plasmid bearing host strains and 4 phage vectors are now available with the repository for distribution.

HOST STRAINS	PLASMID BEARING HOST STRAINS
1. *E.coli* DH10B	1. PBR 322
2. *E.coli* C600	2. PUC 18
3. *E.coli* HB101	3. PUC 19
4. *E.coli* DH 5x	4. pSV2 CAT
5. *E.coli* △ RR1	5. p Blue script
6. *E.coli* JM 101	
7. *E.coli* JM 103	**PHAGE VECTORS**
8. *E.coli* JM 105	
9. *E.coli* JM 107	1. λt 11
10. *E.coli* JM 109	2. M13 mp 18
11. *E.coli* K 802	3. M13 mp 19
12. *E.coli* Y 1090	4. Charon 4A
13. *E.coli* NM 522	
14. *E.coli* J109	
15. *E.coli* JM83	

DEVELOPMENT OF TECHNOLOGY FOR TISSUE BANKING

HIV SCREENING.

Blood samples from individuals whose tissues/cells are obtained for experimental work at the facility are screened for HIV antibody. A rapid two hour microagglutination test kit (serodia) is used for the assay.

CORNEA

Development of methodology for long-term preservation of human cornea is one of the important aspects of tissue bank activity for cornea transplantation. Moist chamber method which is currently used by the eye banks in our country permits the storage of cornea upto 48 hours only. This period, is however too short for communication with recipients in suburbs and/or rural areas. Therefore, attempts were made to modify and standardise techniques for extending the period of storage of cornea.

Organ culture of cornea-scleral button from human donor cornea at 34°C in Eagles' MEM supplemented with 10 % fetal calf serum permitted retention of endothelial cell viability, integrity and transparency of cornea upto 35 days. In order to make this technique available to the end users (eye banks), a meeting of eminent opthalmologists and members of the Eye Bank Association of India from different parts of the country was organised by the NFATCC on 5th July 1991. The members were of the opinion that as the eye banks do not have the facilities for setting up organ cultures, though the technique is efficient it is not practical for implementation. Secondly as MK medium is being used successfully for intermediate storage of cornea the members suggested the use of MK medium stored corneas as a viable alternative.

The deliberations of this meeting led to the following recommendations:

i. Instead of moist chamber system, MK medium should be used for storage of cornea in some major eye bank centres.

ii. Availability of MK medium is the limiting factor to introduce this technology in eye banks therefore preparation, quality control, and supply of MK medium should be undertaken by any research institution or commercial agency. Until such time NFATCC should supply MK medium.

iii. Ethical clearance be sought from the appropriate authority for this work. Similarly approval be obtained if necessary from the Drug Controller for the preparation and supply of MK medium.

The ICMR was approached for seeking ethical clearance for utilisation of MK medium stored cornea for grafting. In response, it was conveyed that as this method is being practised in developed countries approval is not required for utilisation of MK medium stored cornea. The NFATCC therefore has undertaken preparation of MK medium on a small scale and has supplied to three different laboratories .

SKIN

There is a dire need for development of technology for skin banking for transplantation. The major emphasis in developed countries is on banking of cadaver donor skin which is used as biological dressings. A few active research groups abroad have been involved in culturing and maintaining adult and newborn skin. Considering the availability of material and our special requirement, the following strategy was planned:

i. Organ culture of foetal skin

ii. 3-D Epithelia from donor skin for allo - and auto - grafting

iii. 3-D Intermediate thickness dermis and epidermis cultures from donor skin for allo- and auto-grafting.

As reported earlier, methods have been standardized to maintain full thickness skin without undergoing differentiation for a period of 8 days, which could be successfully transplanted to vitiligo patches (Table 1). Histopathological studies of samples obtained after 8-25 days revealed that cultured skin undergoes differentiation and subsequently leads to degeneration.

Table – 1

Follow up of Vitiligo cases grafted with organ cultured Foetal skin

Case No. Sex/Age	Repigmentation		Follow up for (months)
	Grafted area	Control area	
1. M/60	Retained	No	23
2. F/21	Sepsis	No	
3. M/25	Transient	No	21
4. F/19	Retained	No	20
5. F/19	Retained	No	14
6. M/54	Retained	No	12
7. M/16	Transient	Retained	12
8. M/20	Retained	No	6

Studies were initiated to optimise conditions for long-term maintenance of foetal skin *in vitro* without undergoing differentiation and degenerative changes.

Intermediate level split thickness skin was harvested and mounted on a raised platform. Eagle's MEM supplemented with 20% FCS, 10 ng/ml EGF and 0.4 μg/ml hydrocortisone was found to retain the structural integrity and tissue architecture of the foetal skin for upto 35 days.

Skin obtained from 7 foetuses maintained in this manner were used for histological studies. Skin organ culture from 4 foetuses were used for grafting to stable localized treatment resistant vitiligo cases. The results are shown in Table 1 which depicts 4 cases (1 to 4) of earlier work and 4 cases (5 to 8) maintained as intermediate thickness skin organ cultures.

3-D EPITHELIA FROM DONOR SKIN FOR GRAFTING ON TO BURNS AND NON-HEALING ULCER CASES

The keratinocytes being fastidious cells require a hormone and growth factors supplemented media for growth and proliferation. A media composed of DMEM + HAM'S F12 supplemented with insulin, transferrin, adenine, hydrocortisone, cholera toxin, tri-iodo-thyronine and epidermal growth factor are the defined components. In addition to these, undefined factors from mitomycin-C treated mitotically arrested feeder layers of NIH-3T3 cells and conditioned media of human fetal lung fibroblast cells have been found to be necessary for the optimum growth of these cells. 3-D epithelia sheets formed from the monolayers of these cells have been used as grafts in one burn case. The result of these grafts are awaited. A special type of cultured epidermis called as "Melanocyte Bearing Epithelia" is being prepared for transplantation in vitiligo cases.

Normally one does not ensure the presence of melanocytes in cultured epithelia used for allo and auto grafts for burn cases. In case of vitiligo however, it is necessary to ensure that melanocytes are present in the cultured epithelia and only autografts be used, as melanocytes, unlike keratinocytes are known to be antigenic and make antimelanocyte antibodies. One such melanocyte bearing epithelia has also been cultured and used as autologous graft (Fig. 3, 4 and 5). Techniques to cryopreserve such cultured epithelia are being standardized.

3-D INTERMEDIATE THICKNESS DERMIS + EPIDERMIS CULTURE

Normally before transplantation the dermis equivalent prepared from

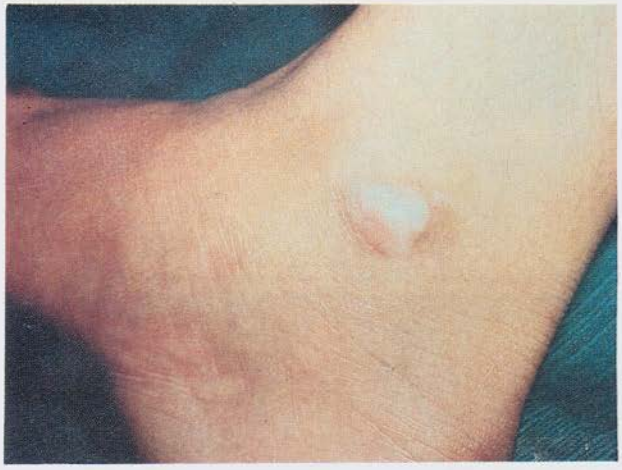


Fig. 3 :
A Case of resistant vitiligo patch.

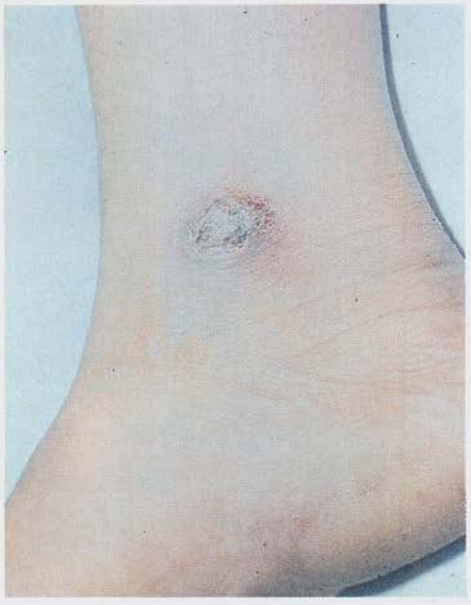


Fig. 5 :
One Week post grafting.
Pigmentation has started to appear.

Fig. 4 :
Cultured melanocyte bearing epithelia.

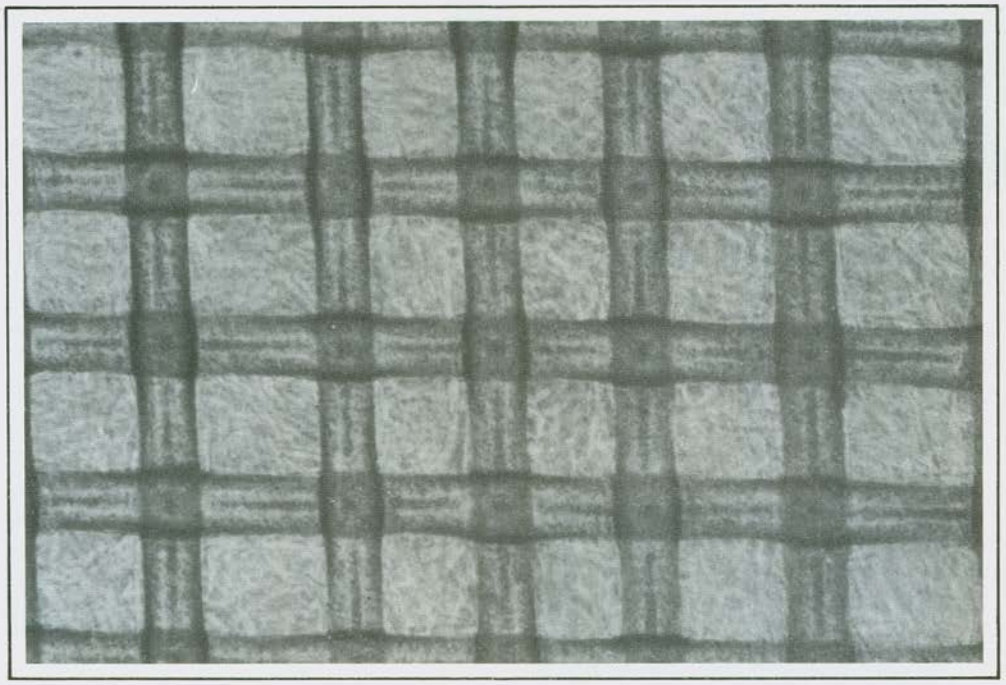


Fig. 6 : Dermal equivalent prepared from cultured fibroblast on nylobolt cloth

mitomycin-C treated NIH 3T3 cells are removed and the epithelia detached from the culture dish. The cultured epithelia being only 5-6 cell layer thick is very fragile and lacks flexibility due to the absence of dermis. To improve the graft take, artificial cultured dermis was prepared using fetal lung fibroblast cell lines and autologous dermal fibroblast. Since fibroblast grow only as a monolayer and have no tendency to form 3-D sheets, artificial 3-D surface area was provided in the form of Nylon Bolting Cloth. It was found that fibroblast do grow in a 3 dimensional manner on these Nylon Bolting Cloth as shown in figure. (Fig. 6). Keratinocytes have been successfully grown on these artificial culture dermis and will be used for grafting, hopefully with more successful takes.

MELANOCYTE CULTURE IN TUMOUR PROMOTOR FREE MEDIA

Melanocytes from vitiligo and normal human subjects grow only in the presence of potent tumour promotors such as tetradecanoyl phorbol acetate (TPA) and as such cannot be used for transplantation. Therefore, attempts were initiated to grow human melanocytes in tumour promotor-free media. Last year's studies revealed that by using fibronectin coating the concentration of TPA could be brought down from 10 ng/ml to 4 ng/ml. However, *in vitro* conditions TPA is not present to stimulate the growth of melanocytes. During wound healing for example melanocytes do multiply and the stimulus for this multiplication could be coming either from fibroblasts or keratinocytes. Therefore, melanocytes growing in the presence of TPA was trypsinized and plated on dermal equivalents prepared from HF6LF cells growing on Nylobolt cloths (see Fig. 6) Three such attempts were made and in none of the cases the melanocytes grew on these dermal equivalents. Further experiments to grow melanocytes as dermal equivalents are being tried out. To understand whether keratinocytes provide the growth stimulus for melanocytes, a co-culture of melanocytes and keratinocytes at a ratio 1:30 respectively was done. This ratio of melanocyte:keratinocytes was selected because it is known that under *in vitro* conditions one melanocyte interacts and donates pigment to sixteen to thirty two keratinocytes. The media used for co-culture was KGM (Keratinocyte Growth Medium), a media designed for the optimum growth of keratinocytes (see 3-epithelia cultures for the composition of the media). Under these conditions melanocytes under phase contrast could be identified upto 3 passages. So far only one attempt at co-culture has been made. Further such attempts to grow melanocytes in tumour promotor free media and identification of melanocytes by DOPA-reaction and by anti melanocyte antibodies are being attempted.

CHARACTERIZATION OF A FACTOR THAT PROMOTES THE GROWTH OF HUMAN SKIN KERATINOCYTES

Feeder layer made from mitomycin - C treated mitotically arrested human lung fibroblast cell line HF6LF was found to support the active proliferation of human keratinocytes. Hence, attempts to isolate and characterize the factors promoting the growth of keratinocytes secreted by HF6LF cells was initiated. HF6LF cells at 70% confluency are used to make feeder layers and therefore collection of conditioned media (CM) was done at 70% confluency. Initially CM was collected in DMEM alone without FCS. This CM did not support the growth of keratinocytes. As it is known that feeder layers secrete shortlived growth factor an attempt to stabilize the conditioned media growth factor was made by collecting them in DMEM + 10% bovine serum albumin. Conditioned media thus collected was found to improve the growth of keratinocytes as revealed by ability of adult keratinocytes to reach confluency earlier than those grown without CM. Conditioned media was, therefore, collected from HF6LF at passage 6, 7 and 8 and freeze dried and dissolved in a small quantity of DMEM. This was subjected different molecular weight cut off dialysis. The dialysate after 12-1400 m.w. cut off dialysate continued the keratinocyte growth promoting factors. SDS-PAGE of the dialysate revealed three bands. Further purification and characterisation of this factor is being done.

BONE MARROW

Studies on standardisation of technology for cryopreservation of bone marrow have been undertaken. Such technology is of great importance for patients undergoing radical treatment for various malignancies and also for people working in hazardous areas such as nuclear reactor plants.

It is planned to collect bone marrow, characterise the various cell populations, process for storage in liquid nitrogen and analyse cell populations on revival.

So far 35 normal human bone marrow samples have been collected and processed for cryopreservation employing DMSO or glycerol at 10% level as cryoprotectant. The rate of cooling was 1°C/min. 3 parameters were used to test the efficacy of cryopreservation.

Colony forming unit Granulocyte Monocyte assay (CFU-GM) :

Preliminary experiments revealed that the specimens which had shown good CFU-GM formation failed to develop colonies after revival (except sample number 1). Table 2.

Colony forming unit Granulocyte Erythroid, Monocytoid, Megakaryocyte assay (CFU-GEMM) & CD34 Ab staining :

After procurement of Erythropoietin (Boheringer Manheim) and CD34 monoclonal antibody (Beckton & Dickinson) it was proposed to check the frozen cells for CFU-GEMM which detects very early progenitors and also CD34 antigen +ve stem cells. Experiments with 2 samples showed that the cells gave rise to significant number of CFU-GEMM colonies and a +ve fluroescence in nearly 30% of the cells with anti CD34+ antibody. These results suggest that though there is a loss of more mature cells, the stem cells and early progenitors were protected. Further studies are in progress.

TABLE – 2

REVIVAL OF FROZEN BONE MARROW CELLS

Sr.No.	CFU-GM before freezing				Days after freezing	% Viability (Trypan blue)
	7d		14d			
	clust	col.	clust	col.		
1.	400	264	1320	364		
1B.*	652	141	917	341	13	80
2.	211.2	64	704	336	4	85
3.	244.02	34.86	197.54	127.82	18	80
4.	209.16	319.55	238.21	180.19	60	75
5.	220.78	168.49	226.59	273.07	165	95

* 1B – Values obtained after 13 days of freezing.

Samples 2-5 showed no CFU-GM after revival.

HEART VALVES

An estimated 1.5 million of the 15 million people with rheumatic affection require valve replacement in India. Currently either artificial valves or gluteraldehyde fixed cadaver homograft valves are being used. Both types of valves have their problems of embolism, wear and tear etc and a second valve replacement surgery needs to be done on these people. Heart valves that are viable are expected to be capable of repairing themselves in case of damage and therefore expected to function longer after replacement surgery.

Since it takes more than ten days to conduct preliminary tests on sterility etc. it is essential to ascertain that the valves are kept in viable condition till they are used. Thus this period has to be extended as much as possible. Is is possible

to achieve this objective by cryopreservation of valves in liquid nitrogen. Hence this work was undertaken. It was planned to prepare explant cultures for studying the viability and Sashmira Silk Thread method for studying the mechanical strength.

A set of four aortic and four pulmonary valves was frozen by rapid cooling in liquid nitrogen and a set of three aortic and three pulmonary valves were frozen by a slow cooling method. Before cryopreservation explant culture of fibroblast and endothelial cells were set from these valves. However, none of these established outgrowth of cells. The tensile strength of the valves after 0 and 6 months of cryopreservation was tested. The preliminary results indicate that mechanical strength remains unaffected by cryopreservation. Conditions for collection and further processing of heart valves to retain their viability are being studied.

DEVELOPMENT OF PRIMARY CULTURES & RETENTION OF FUNCTIONALITY OF CELLS *IN VITRO*

- Development of primary cultures from human fetuses
- Development of primary cultures from monkey tissues
- *In Vitro* cultivation of pancreatic cells
- Immortalization of bone-marrow derived stromal and stem cells

DEVELOPMENT OF PRIMARY CULTURES & RETENTION OF FUNCTIONALITY OF CELLS *IN VITRO*

DEVELOPMENT OF PRIMARY CULTURES FROM HUMAN FETUSES

A total of 23 human foetuses from MTP cases were procured from Sassoon and KEM Hospital. Three of them were found to be masserated and were discarded. Primary cultures from liver, lung, kidney and pancreas were set up from 19 foetuses. Of these, primary cultures from 12 foetuses, either did not grow at all or were found to be contaminated and therefore discarded. From the remaining, seven primary cultures have been established and have been stored in LN_2. These cells are being tested for their abilities to produce/ secrete growth factors, serve as feeder layers and form dermal equivalents.

DEVELOPMENT OF PRIMARY CULTURES FROM MONKEY TISSUES

Attempts were made to prepare primary cultures from monkey kidney and liver tissues. CDRI (Lucknow) kindly provided the animals and extended the facilities to carry out the laboratory work for initiation of primary cultures . Monkey kidney primary cultures were prepared at CDRI and brought to NFATCC . Primary kidney cultures were grown and stored frozen in liquid nitrogen. Attempts to grow primary cultures from liver did not meet with success as some of the cultures got contaminated. Organ cultures were prepared from bronchi, these bronchi exhibited ciliary activity indicating that they were functional. The cultures are preserved in liquid nitrogen.

IN VITRO CULTIVATION OF PANCREATIC CELLS

Development and maintenance of different cells of pancreas is of great significance for understanding of diabetics. Retention of functionality - synthesis and secretion of insulin from these cells *in vitro* is important. Hence studies have been undertaken to develop culture of these cells with retention of their functionality.

DEVELOPMENT OF PRIMARY CULTURES & RETENTION OF FUNCTIONALITY OF CELLS *IN VITRO*

DEVELOPMENT OF PRIMARY CULTURES FROM HUMAN FETUSES

A total of 23 human foetuses from MTP cases were procured from Sassoon and KEM Hospital. Three of them were found to be masserated and were discarded. Primary cultures from liver, lung, kidney and pancreas were set up from 19 foetuses. Of these, primary cultures from 12 foetuses, either did not grow at all or were found to be contaminated and therefore discarded. From the remaining, seven primary cultures have been established and have been stored in LN_2. These cells are being tested for their abilities to produce/ secrete growth factors, serve as feeder layers and form dermal equivalents.

DEVELOPMENT OF PRIMARY CULTURES FROM MONKEY TISSUES

Attempts were made to prepare primary cultures from monkey kidney and liver tissues. CDRI (Lucknow) kindly provided the animals and extended the facilities to carry out the laboratory work for initiation of primary cultures . Monkey kidney primary cultures were prepared at CDRI and brought to NFATCC . Primary kidney cultures were grown and stored frozen in liquid nitrogen. Attempts to grow primary cultures from liver did not meet with success as some of the cultures got contaminated. Organ cultures were prepared from bronchi, these bronchi exhibited ciliary activity indicating that they were functional. The cultures are preserved in liquid nitrogen.

IN VITRO CULTIVATION OF PANCREATIC CELLS

Development and maintenance of different cells of pancreas is of great significance for understanding of diabetics. Retention of functionality - synthesis and secretion of insulin from these cells *in vitro* is important. Hence studies have been undertaken to develop culture of these cells with retention of their functionality.

EXOCRINE PANCREAS

Experimental studies were undertaken to optimize conditions for retention of secretory activity of pancreatic acinar cells *in vitro*. Pancreatic organ cultures were prepared from adult rat (Sprague-Dawley) pancreas employing raft technique and explants were seeded in 24 well plastic plates (Nunc). Different experimental media with / without serum were used and their effect on amylase secretion was assessed. The other culture conditions included in the protocol were presence of specific additives such as insulin, hydrocortisone, carbamylcholine (secretagogue for amylase) and soyabean trypsin inhibitor (STI). The effect of these additives on amylase secretion was studied by supplementing the media with these substances. Samples were collected at definite time points from control and experimental cultures for amylase assay. It was found that different experimental media (DMEM, L-15, Ham F12) used for culturing pancreatic explants without any additive permitted secretion of amylase by pancreatic explants upto 8 days . Amylase activity was highest in supernatants collected on day 2 and decreased gradually till day 8. There was no significant difference in the amylase secretion when cells were cultured in different media used (Table 3). When the pancreatic explants were cultured in media supplemented with STI (0.1 mg/ml) there was an increase in amylase secretion from initial level of 200 to 300 U/ml, in all the media (Table 4). Addition of 5% serum to culture medium with/without STI increased amylase secretion. Further experiments were concentrated on using serum free medium with addition of hormones and secretagogues. It was found that 5 μg insulin/ml, 0.1 mg/ml STI and 0.1 mg/ml carbamylcholine to Ham's F12 culture medium without serum permitted retention of amylase activity by the explants upto 10 days. The viability and cellular architecture of the pancreatic explants were examined by histopathological studies which revealed retention of tissue architecture upto 10 days. These results indicate that it is possible to maintain secretory activity (with reference to amylase) of pancreatic explants in organ culture upto 10 days in serum free medium supplemented with insulin and STI.

TABLE – 3

EFFECT OF DIFFERENT CULTURE MEDIA ON AMYLASE SECRETION IN PANCREATIC ORGAN CULTURE

Amylase secretion in IU/ml in different media			
Days	**DMEM**	**Ham's F12**	**L-15**
2	360	350	280
4	300	290	260
6	200	230	210
8	130	145	110

TABLE – 4

EFFECT OF STI SUPPLEMENTATION TO CULTURE MEDIA ON AMYLASE SECRETION IN PANCREATIC ORGAN CULTURE

Amylase secretion in IU/ml in different media			
Days	**DMEM + STI**	**Ham's F12 + STI**	**L-15 + STI**
2	560	500	400
4	430	440	380
6	270	280	240
8	180	160	120

STI - Soyabean trypsin inhibitor

CULTURE OF ACINAR CELLS

Pancreatic organ culture system permitted maintenance of functional pancreas upto 8 days only. Therefore further studies were carried out using pancreatic acinar cells obtained by collagenase digestion. It was found that pancreatic acinar cells retained their activity upto 23 days *in vitro* in Ham's F12 medium supplemented with insulin and STI.

ENDOCRINE PANCREAS

In an attempt to optimize conditions for retention of secretory activity of endocrine pancreas, organ cultures of adult rat pancreas were exposed to different culture conditions with respect to nutrient medium with/without serum and secretagogues. Functionality of endocrine pancreas in organ culture was assessed by insulin production. It was observed that pancreatic explants cultured in nutrient medium with/without serum but in presence of STI yielded similar levels of insulin secretion for a period of 8 days. Thus indicating that the secretory activity of islet cells is not affected in the absence of serum. A 16 mM glucose pulse on day 3 culture for 60 min enhanced insulin production confirming the retention of responsiveness of pancreatic explants to secretagogue.

ENCAPSULATION OF ISLETS IN CHITOSAN CAPSULES

For mass cultivation of islets it is necessary to maintain the islets as isolated units. So also for according protection from the blood system to the islet explants, when implanted in the body encapsulation of these islets in a biocompatible membrane is essential. Experiments were designed to achieve this objective. Islets were isolated from adult Sprague-Dawley rats (fasted overnight) employing collagenase digestion. Islets were purified on percoll discontinuous gradient. The purified islets were encapsulated in calcium alginate and chitosan membranes. The encapsulated and unencapsulated islets were cultured in 96 well plate in Ham's F12 medium supplemented with 10% FCS. The islets were stimulated with 16 mM glucose and insulin secretion was estimated from these samples collected at various time points between 10 minutes to 6 hours (Figs. 7 & 8). The results reveal that both encapsulated and unencapsulated islets respond to glucose stimulation. The insulin release is directly proportional to the period of glucose stimulation. Initially there is a steep rise in insulin, followed by steady level of insulin release.

Insulin released by islet culture

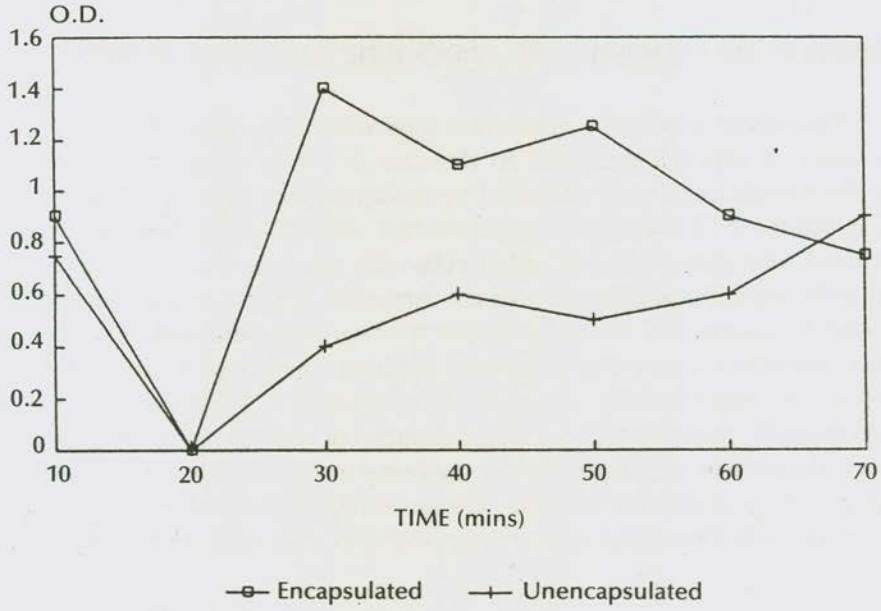


Fig. 7

Insulin released by islet culture

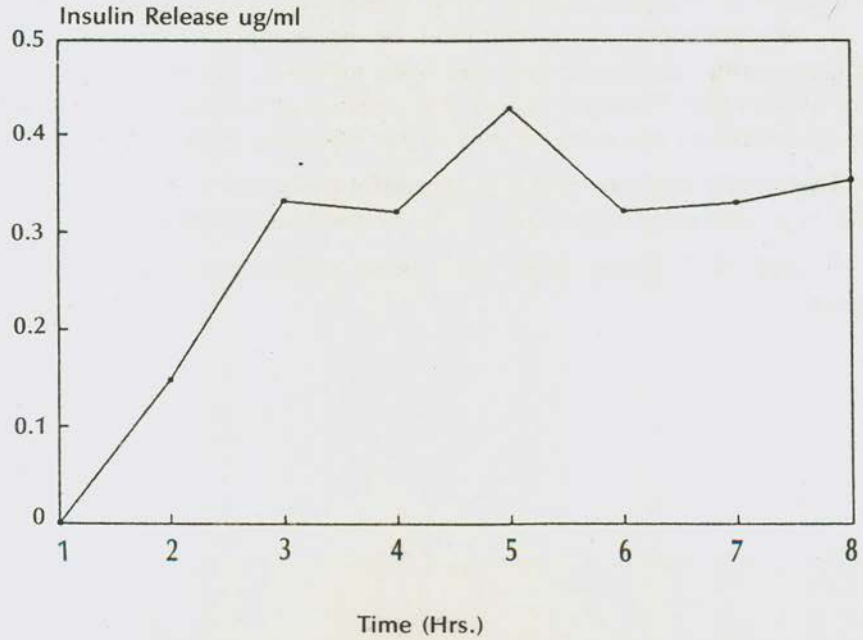


Fig. 8

INTERACTION OF FXOCRINE AND ENDOCRINE PANCREAS *IN VITRO*

Pancreatic exocrine - endocrine interactions *in vitro* have been known for many years. It was felt desirable to develop an *in vitro* model for such kind of study. Pancreatic cells were obtained by collagenease digestion and incubated at 37 °C in Ham's F12 medium supplemented with 5% FCS. Selective dysfunction of the islets was achieved by treating cells with glucose free medium, and that of acinar cells with chick embryo extract. Amylase activity and insulin levels were estimated to assess the acinar and islet functions respectively. It was found that amylase secretion was lower in treated cultures compared to untreated cultures indicating non-functionality of acini after treatment.. Similarly chick embryo extract treated cultures led to degeneration of acinar cells and decrease in amylase production accompanied by decrease in insulin levels of culture medium as compared to untreated cultures. These results indicate that interaction occurs in exocrine-endocrine pancreatic areas under *in vitro* culture conditions.

IMMORTALIZATION OF BONE-MARROW DERIVED STROMAL AND STEM CELLS

It is proposed to develop clonal lines from bone-marrow to facilitate the studies related to leukemogenesis. Such clonal lines are especially useful for the study of differentiation. Immortalization of bone-marrow stromal cells using cloned oncogenes via transfection has been initiated. The first set of oncogenes tried are SV-40 large T antigen (pSV3.dhfr) and polyoma middle T ag (pSV5.dhfr). The results obtained are summarized in the following table. (Table 5).

The results indicate that for successful transfection it is necessary to use the cells in proliferative stage (fresh cells or stimulated cultured cells).

A total of 7 clonal lines have been obtained and are under regular passaging.

TABLE – 5

No. of transfections	Status of cells when transfected	Method of transfection	Plasmid	Comment
2.	Stromal cells from 4 wk. old LTBMC	Lipofectin mediated	pSV3.dhfr	Cells selescent
5.	Stromal cells from 4 wk. old LTBMC*	Calcium phosphate Co-ppt	pSV3.dhfr	cells selescent
1.	Erythropoietin induced stromal cells from 4 wk. LTBMC	Calcium phosphate Co-ppt	pSV3.dhfr	Cells selescent
4.	Stromal cells from 4 sk.old LTBMC	Microinjection	pSV3.dhfr	1 cell line showing foci formation
5.	Erythropoietin induced stromal cells from 4 wk. old LTBMC	Calcium phosphate Co-ppt	pSV5.dhfr	4 lines in passage
2.	Mononuclear cell fraction from fresh bone- marrow cells	Calcium phosphate Co-ppt in suspension	pSV5.dhfr	2 lines in passage

* LTBMC - Long term bone marrow culture

CHARACTERISATION OF CULTURES

- Characterisation of cell lines by DNA fingerprinting
- Characterisation of parasite DNA by megabase DNA Electrophoresis
- Characterisation of cell lines using mitochondrial DNA as a parameter

CHARACTERISATION OF CULTURES

CHARACTERISATION OF CELL LINES BY DNA FINGERPRINTING

Analysis of DNA in terms of its sequence and organisation can be useful for the characterisation. In eukaryotes DNA is present in organelle like mitochondria, in addition to nucleus. Studies on the DNA from both the sources is underway for the purpose.

Cross contamination among cultured cell lines has been reported to occur at frequencies as high as 16-35% . Detection is particularly difficult if cells express similar phenotypes. Conventionally available methods for detecting cell contamination are isoenzymology and karyotyping. Recently Alec Jeffreys and others demonstrated that some sequences in human DNA are hypervariable. These give a complex pattern of hybridisation (fingerprint) that is specific for a individual. This approach has been used for characterisation of cell lines which would also detect cross contamination.

It was proposed that all the cell lines present in the repository will be characterised by their specific fingerprint. Dr. Lalji Singh from CCMB (Hyderabad) has developed a probe 2 (8) which is being used for this purpose.

High molecular weight DNA was prepared from 5 human, 5 mouse, 2 monkey and Indian Muntjac and Culex cell lines. It was cleaved with restriction enzyme Hinf I. The digest was then size fractionated on .8% agarose gel, transferred to nitrocellulose filter and hybridised with radiolabelled probe 2(8). Autoradiogram was developed after 48 hrs and showed a very good pattern with all the cell lines tested.

M13 DNA contains a sequence in the gene 3 that detects hypervariable loci in human and bovine DNA. Attempts were made independently to use this as fingerprinting probe. In this procedure, single stranded M13 DNA was radiolabelled and used to probe Hae III digest of genomic DNA with milk powder as a blocking agent for the hybridisation. Conditions for depurination and electrotransfer of the DNA were standardised but problems were encountered at the stage of hybridisation wherein no radioactivity was retained on the filters after the wash. Efforts are being made to overcome this difficulty.

CHARACTERISATION OF PARASITE DNA BY MEGABASE DNA ELECTROPHORESIS

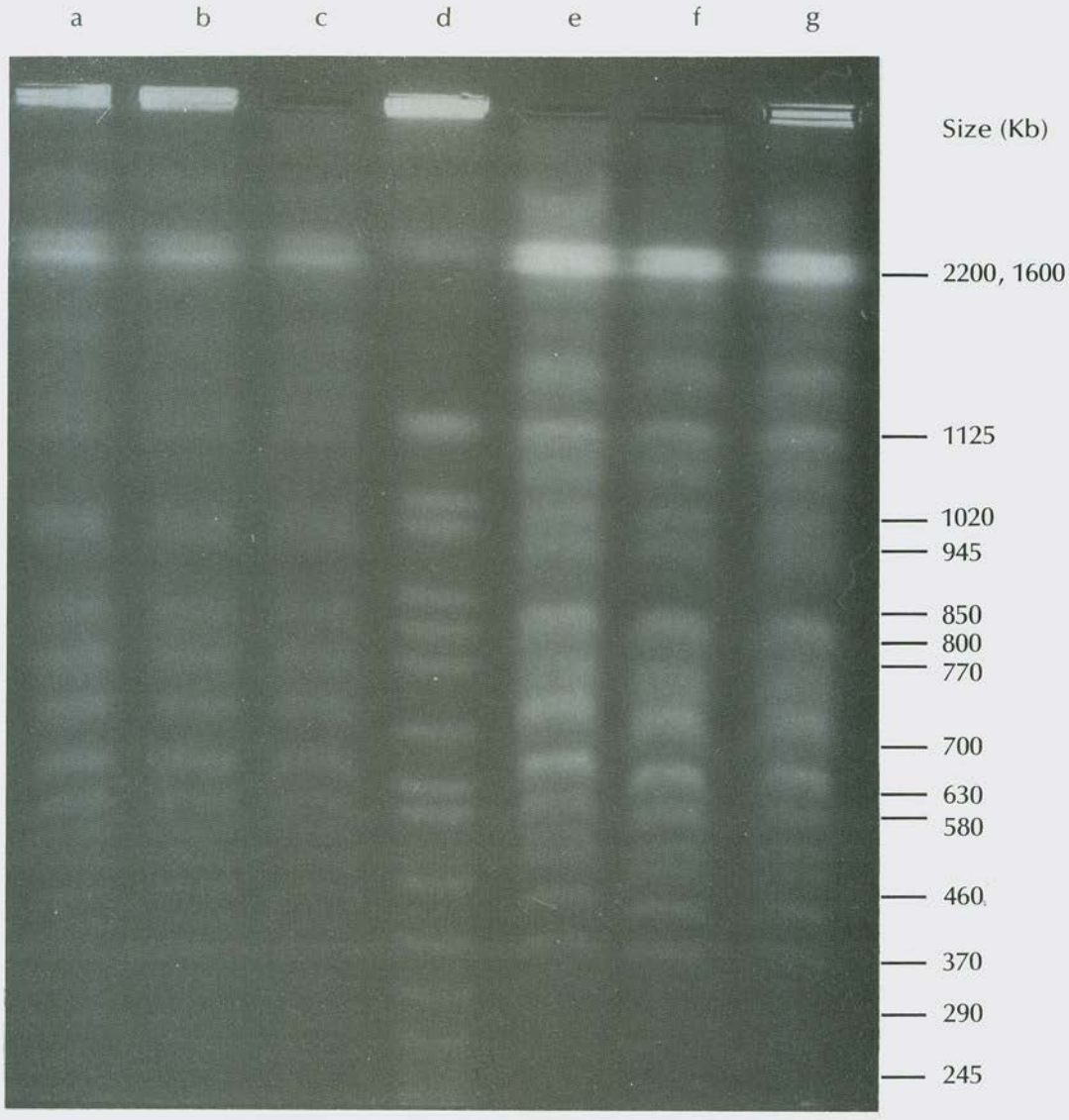
The repository has in the stock *Leishmania donovani* strains from two sources- One from Germany and the other Indian strain UR 6. Thr morphological features and growth characters of both these appear to be comparable. With a view to assess if these two cultures are similar or have variation amongst them, chromosome organisation in these parasites was studied.

Most unicellular parasites are not amenable to conventional methods of genetic analysis by recombination. This problem can be circumvented by use of a technique that can resolve DNA in the megabase range. Using the conditions for in situ lysis of the cells in agarose blocks and gel running, chromosomes of two different strains of *Leishmania donovani* were analysed. In case of *Leishmania donovani* german strain 16 chromosomes were resolved whereas strain UR 6 showed 17 chromosomes. (Fig. 9)

CHARACTERISATION OF CELL LINES USING MITOCHONDRIAL DNA AS A PARAMETER

Mitochondrial DNA is circular and a small molecular weight DNA having length of 16-20 kb in vertebrates. One molecule is highly conserved with respect to the proteins, rRNA and tRNA it codes for. But due to high frequency of mutations at the 3rd base of codon , the sequence differences are observed in various species and conspecific. These sequence differences can be easily detected by utilising restriction fragment length polymorphism (RFLP).

RFLP of micochondrial DNA (mt DNA) is also one of the criteria to characterise the animal cell lines. Reports on studies using vertebrate cell lines have shown that mt DNA analysis can be effectively used for identification and to detect cross contamination of these cell lines. Most of the mosquito cell lines cannot be differentiated on the basis of morphology, karyotype and isoenzyme analysis. So the studies were undertaken to see whether these cell lines can be distinguished by analysing mt DNA. Low passage mosquito cells were grown *in vitro* and mitochondria and mt DNA were purified by differential centrifugation and isophycnic density gradient centrifugation respectively. Mt DNA was cleaved by restiction enzymes and the digestions were analysed after end labelling of fragments generated by clevage. The restriction patterns for mt DNA of *Anopheles stephensi* and *Aedes albopictus* cell lines were obtained and analysed. The results indicate that the patterns are different for the two cell lines studied.



Lanes a-c, German strain
lanes e-g, strain UR 6
lane,d, yeast molecular weight markers.

Fig. 9

DEVELOPMENT OF INDIGENOUS TECHNOLOGY & SCREENING OF DRUGS etc.

- Adaptation of SP2/0 cell line to goat serum
- Studies on Lymphatic filariasis
- Development of antiidiotypic antibodies to F 46
- Development of dot-blot assay for detection of Dracunculusis (Guinea worm) infection in endemic areas
- Development of an assay system for quantitation of functional antibodies to tetanus toxoid (TT) in an *in vitro* model
- Screening of MAB to insulin to assess their utility for estimation of immmunoreactive insulin by ELISA.
- Screening of antimalarials against erythrocytic stages of *plasmodium falciparum*
- Toxicity testing of Aureofungin

DEVELOPMENT OF INDIGENOUS TECHNOLOGY & SCREENING OF DRUGS etc.

ADAPTATION OF SP2/0 CELL LINE TO GOAT SERUM

Efforts to adapt myeloma cell line SP2/0 to goat serum were continued and these cells have been successfully adapted to 6% goat serum. Supplementation of soybean lipids (20 μg/ml) in the culture medium i.e. DMEM with 6% goat serum was found to stabilise the cells. The cells were revived after 1-6 months following storage in liquid nitrogen. The functionality of the revived cells was assessed for HGPRTase (-ve) locus on the adapted cells by maintaining these cells in 8 azaguanine (20 μg/ml) medium. Balb/c mice were immunised with *Leishmania donovani* promastigate antigen. Splenocytes from these immunised mice were fused with goat serum adapted SP2/0 cells. Three fusions were carried out of which one fusion was successful yielding 44 hybrids. Supernatants from 4 hybrids were positive for antibody activity when screened by ELISA. Cloning and subcloning of these 4 hybrids is in progress.

STUDIES ON LYMPHATIC FILARIASIS

Over 90 million people inhabiting tropical and subtropical areas of the world are infected with lymphatic filarial parasites e.g. *W. bancrofti, B. malayi* and *B. Timori* (WHO Report). The infection is initiated with cutaneous penetration of mosquito borne infective larvae. The larvae then migrate to the lymphatics and mature into adult worms. There are hardly any reports about cultivation of infective larvae *in vitro* Cultivation of the infective larvae in artificial medium would facilitate the prophylatic and immunological studies in filarial infection.

- Cultivation of *W. bancrofti* infective larvae

In vitro cultivation of infective larvae of *W. bancrofti* were undertaken in collaboration with Vector Control Research Centre, Pondicherry. Infected mosquitoes supplied by VCRC were dissected and infective larvae isolated. Larvae were maintained in Franke's medium (NCTC-135 with IMDM 1:1 ratio) for 28 days. The morphological and developmental changes were monitored. First molting of the worms was observed after 10 days (Fig 10). Thickening of cuticle of the anterior part of the worm and differentiation of excretory pore was noted after 28 days (Fig 11). Studies are in progress by using various media combinations for maintenance of infective larvae for a longer period of time.

DEVELOPMENT OF ANTIIDIOTYPIC ANTIBODIES TO F. 46

F.46 monoclonal antibody raised against infective larvae of *B. malayi* found to be cytotoxic in various *in vivo* and *in vitro* assays. Development of antiidiotypic antibody (internal image antigen) to F. 46 could be use in active immunization where adequate quantity of immunogenic material is a problem. Hence in view of developing antiidiotypic antibody to F.46, Balb/c mice were injected with F. 46 antibody. Splenocytes from the immunised mice were fused with SP2/0 myeloma cells. Two experiments were carried out but did not yield any secretory hybrids.

DEVELOPMENT OF DOT-BLOT ASSAY FOR DETECTION OF DRACUNCULIASIS (GUINEA WORM) INFECTION IN ENDEMIC AREAS

Dracunculiasis is an incapacitating disease and is fairly wide spread in South-East Asia as well as in North Africa where atleast 12-50 million people are affected annually. There have been few attempts to develop diagnostic test for the detection of this disease. Conventional ELISA used for detection of Guinea worm infection is time consuming and requires adequate laboratory facilities such as ELISA plate reader, plasticware etc., therefore it was of interest to develop a simple diagnostic assay which could be useful in field conditions. The adult worms were collected from endemic areas of Rajasthan from infected individuals. The soluble antigen was prepared by homogenizing adult guinea worms. The nitrocellulose strips were coated by soluble adult worm antigen of D. medinensis in dots (2 μl/dot). Antigen dots were blocked by incubating the strips in 1% BSA/TTBS for 1 hour at 37°C. Strips were then washed 3 times with TTBS and the antigen dots were exposed to various infected sera. (1:50 dil. in TTBS 2 μl/dot) and incubated for 3 hours at 37°C. The strips were washed 3 times with TTBS and exposed to antimouse IgG peroxidase (1:750) for 1 hour at 37°C. Strips were again washed 3 times with TTBS and developed with 4 chloro-napthol. Development of dark spots corresponded to positive reaction of infected sera whereas normal sera showed faint grey background. Forty three infected sera, ten normal sera and ten endemic sera were tested in ELISA and dot-blot ELISA. Forty one out of forty three infected sera were positive in dot-blot ELISA. All the normal and endemic sera tested were negative. There was a good correlation between the two assays used. Dot-blot ELISA thus found to be less time consuming, easy to perform and large number of population can be studied in any field laboratories having limited laboratory facilities.

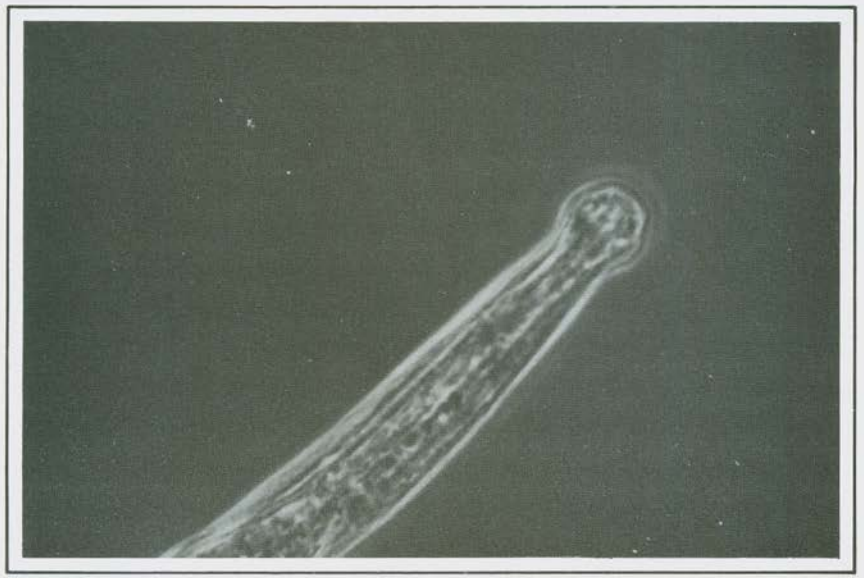


Fig. 10 : Anterior end of infective larvae after 28 days.
Round thickened cuticle seen at the head

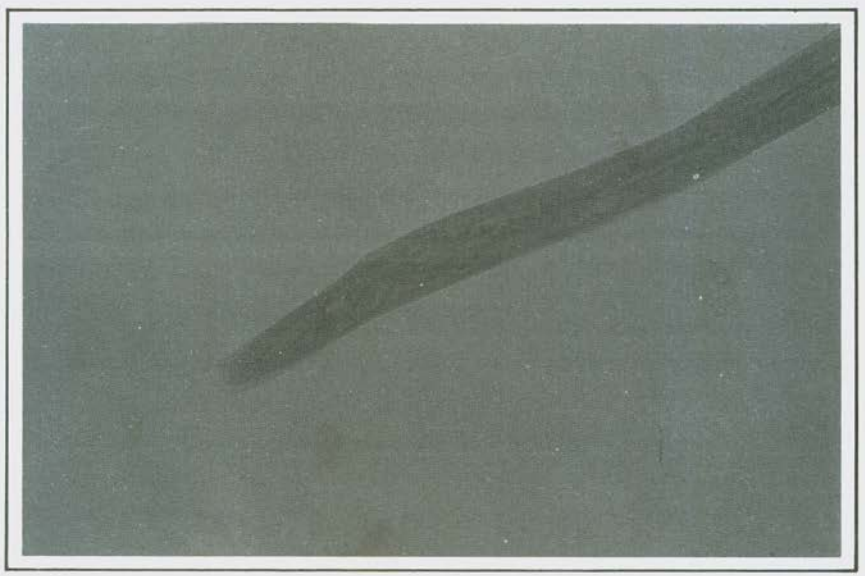


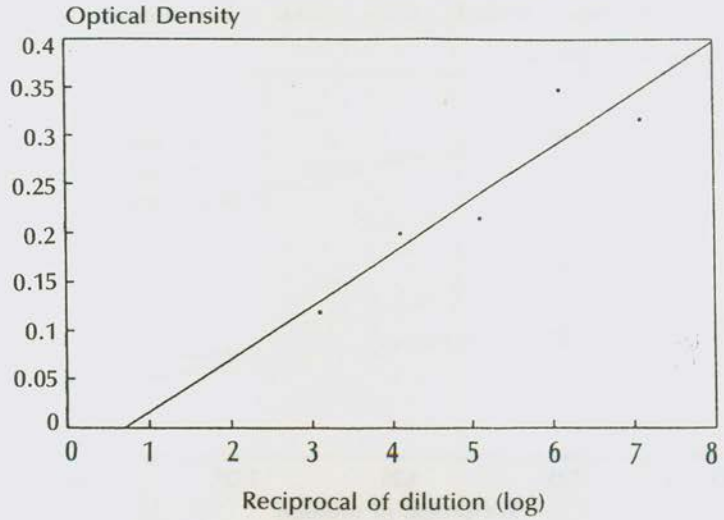
Fig. 11 : Posterior end of infected larve after 28 days.
Formation of excretory pore.

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

Various techniques are available for measuring anti-TT antibodies. Each of these techniques has some merits and demerits. The toxin neutralisation assay has the advantage of measuring exclusively neutralising antibodies but is not very sensitive. The ELISA on the other hand is sensitive but the antibodies estimated by it are total antibodies and includes non-neutralising antibodies which are not biologically important.

The aim of the project is to develop an *in vitro* assay for quantitation of antibodies to TT. Mouse neuro 2a cells were used as a source for toxin receptors. The presence of the T. toxin receptors was demonstrated by direct immunofluorescent technique, using FITC labelled toxin. A direct cellular ELISA with neuro 2a cells was standardised with alkaline phosphatase labelled toxin. Neuro 2a cells were seeded in 96 well (NUNC) plates and after 5 days in culture, the cells were fixed and used for the assay. The cells were treated with serial dilutions of labeled toxin. Titration curve was drawn with OD Vs log of dilution of labelled toxin. There was a good correlation between OD and the dilution of the labelled toxin as estimated by regression analysis. A commercial antibody tetanus

Standard graph for * toxin binding
Standard-Tetanus Immunoglobulin (TIG).



Antibody conc.range : 0.0001- 1 IU/ml. **Fig. 12**

immunoglobulin (TIG) was used for standardisation for estimation of TT antibodies. A two step, modified inhibition assay was developed for this purpose. In the first step, labelled toxin was incubated with varying concentrations of TIG (0.000016-2.5 IU/ml). In the second step the mixture was added to cells in 96 well plates. Paranitrophenyl phosphate (PNPP) was used as the substrate. The % binding was calculated and a graph was plotted with % binding Vs log of concentration of TIG (Fig 12). The The least square regression analysis was used for calculating the r value. The sensitivity achieved by the assay was 0.002 IU/ml. and the r value was between 0.85- 0.95 in the 6 experiments carried out.

SCREENING MONOCLONAL ANTIBODIES TO INSULIN TO ASSESS THEIR UTILITY IN ELISA FOR ESTIMATING IMMUNOREACTIVE INSULIN.

Four monoclonal antibodies viz CC9C10, AE9D6, CE9H9 and DB9G8 were screened for antibody activity against human insulin. These MABs were produced against insulin from different species and crossreacted to varying degrees with insulin from other species. An indirect ELISA using a commercial human insulin preparation was standardised. The CC9C10 and AE9D6 MABs showed significantly higher reactivity and titres in comparison with DB9G8 and CE9H9 (Fig. 13). Therefore, these two antibodies were used for further experiments in capture ELISA. The plates were coated with CC9C10 antibodies (0.2 μg/well) and treated with varying concentrations of human insulin ((0.1 μIU-10 IU/ml). The AE9D6 antibody labelled with alkaline phosphatase was used as a

Comparision of antibody activity of MABs to human insulin

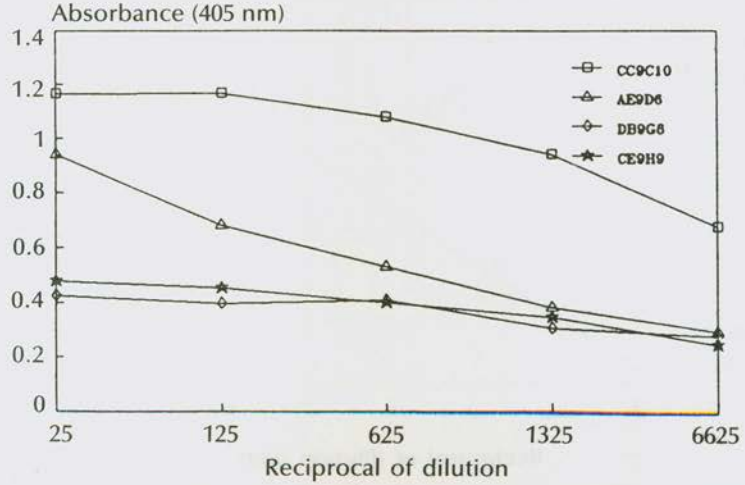


Fig. 13

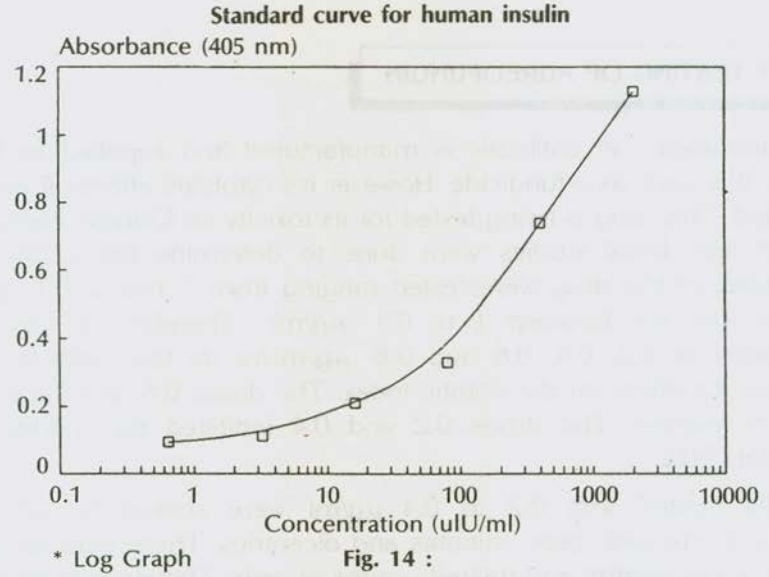


Fig. 14 :

secondary antibody. Graph was plotted with OD vs IU/ml of human insulin (Fig 14). The correlation was significant ($r > 0.9$) using the least square regression analysis.

SCREENING OF ANTIMALARIALS AGAINST ERYTHROCYTIC STAGES OF *PLASMODIUM FALCIPARUM*

For *in vitro* screening of antimalarials tritiated Hypoxanthine uptake method is being standardized. Results of a few preliminary experiments are incorporated in Table 6.

TABLE – 6

^{3}H HYPOXANTHINE UPTAKE BY PLASMODIUM FALCIPARUM CHLOROQUINE RESISTANT STRAIN SO-HS

Expt. No.	Control	Parasites	Parasites with 1.25 nM	Chloroquine 2500 nM
1.	612.75 (0)	7354.75 (1.12)	N.D.	N.D.
2.	555.64 (0)	13,119.74 (2.5)	10,879.49 (2.12)	477.11 (0)

Values in parantheses indicate % parasitemia by microscopy.
Initial % parasitemia was adjusted to 0.5.

(Values in disintegrations/min. DPM)

TOXICITY TESTING OF AUREOFUNGIN

Aureofungin, an antibiotic is manufactured and supplied by Hindustan Antibiotics. It is used as a fungicide. However it's cytotoxic effects, if any requires to be studied. This drug is being tested for its toxicity on Chinese Hamster Ovary (CHO) cell line. Initial studies were done to determine the LD50 . Several concentrations of the drug were tested ranging from 1 mg to 0.1 μg/ml. The LD50 after 24h lies between 1 to 0.1 μg/ml . Therefore, it was tested at concentrations of 0.2, 0.4, 0.6 and 0.8 μg/ml for its toxic effects. This was evaluated by it's effect on the mitotic index. The doses 0.6 and 0.8 μg/ml were inhibitory to mitosis. The doses 0.2 and 0.4 inhibited the mitotic index to approximately 50%.

Cells treated with 0.2 to 0.4 μg/ml were scored for chromosome abberations like breaks, gaps, minutes and dicentrics. There were no significant differences in the control and treated groups of cells. Therefore, sister chromatid exchanges will be scored as well in future.

OTHER PROJECTS

- *In vitro* cultivation of erythorcytic stages of Plasmodium vivax
- Indentification and study of oncogenes/s involved in melanoma
- Mass cultivation of animal cells
- Regulation of polytenization in salivary gland cells
- Studies on stress proteins in *Ano. stephensi* culture.

OTHER PROJECTS

IN VITRO CULTIVATION OF ERYTROCYTIC STAGES OF PLASMODIUM VIVAX

Malaria is one of the major health problems in our country. *Plasmodium vivax* infections are highly prevalent in India. Yet unlike *P. falciparum* there is no culture system available for experimental study. Hence studies were undertaken to culture the erythrocytic stages of *Plasmodium vivax*. Since *P. vivax* preferentially invades young erythrocytes, one prerequisite for its culture is to generate young erythrocytes. Young erythrocytes were obtained from bone-marrow and foetal liver.

Bone-marrow :

Normal human bone-marrow cells separated from rib specimens or iliac graft specimens were grown as long term cultures in Dexter system. (IMDM + 12.5% FCS + 12.5% horse serum and 10^{-7} M hydrocortisone). The non-adherent cells containing erythroblasts were washed, suspended in IMDM with 20% FCS and differentiated with erythropoietin. This induced erythroid cell generation. These cells were used for weekly feeding the vivax cultures.

Foetal liver cells :

Foetal liver is a rich source of erythroid progenitors. Foetuses of MTP cases in second trimester were obtained from Sassoon General Hospital and immediately dissected. The liver was washed several times with plain medium and then finely chopped. The pieces were treated with 0.01% collagenase for 15 minutes. The cells were collected and separated on ficoll hypaque. The mononuclear cells at interphase were washed and cultured in IMDM supplemented with 20% FCS and 10^{-7} M hydrocortisone. Every week the cultures were demidefoliated. The non-adherent cells taken out during demidefoliation were washed and suspended in IMDM + 10% FCS and differentiated with erythropoietin. These cells were used for feeding of vivax cultures. Blood samples from untreated *P. vivax* cases were used for *in vitro* cultivation of erythrocytic stages of *Plasmodium vivax*.

Blood samples were collected from village Wagholi (District - Pune) as well as from Sassoon General Hospital, Pune. The samples were cultured by using four different cell types viz foetal liver haematopoietic cells, normal Human Bone-marrow cells, (induced to differentiate with erythropoietin), HEL human erythroid leukemia (treated with Hemin) and K 562 human erythroid leukemia. (treated with sodium butyrate). Human ORh +ve RBCs (using conventional dessicator method) were also inoculated simultaneously.

Infected RBCs were inoculated in differentiated cells with media containing FCS. The cultures were incubated at 37°C in 5% CO_2 for a week and then cultured by conventional desicator method for maintaining the cultures.

Out of the 5 samples inoculated one could be successfully cultured in Foetal liver haematopoietic cells. The sample is now in passage 24 (11 weeks old) shows 8-10% parasitemia. It has been cryopreserved using 15% glycerol and could be successfully revived after one month of cryopreservation. Though initially the culture exhibited erythrocytic stage of *P. vivax*, after certain passages the culture showed close similarity to *Plasmodium falciparum* indicating multiplication of *P. falciparum*. It is possible that the original sample might have mixed infestation of *Plasmodium vivax* and *Plasmodium falciparum* with higher population of *P. vivax* in initial stages. In continuous culture *Plasmodium falciparum* might have gradually taken over or the cultures might have got cross contaminated with *P. falciparum* cultures. It is proposed now to carry out this work in isolated chamber reserved for *P. vivax* only. Facilities for these are being established.

IDENTIFICATION AND STUDY OF ONCOGENES/S INVOLVED IN MELANOMA

Melanomas are highly variable with respect to aberrant gene expression and chromosomal lesions but share a common characteristic of an acquired independence from growth factors that are required for proliferation of melanocytes.

An interesting feature of melanoma is that there is a simultaneous expression of differentiation genes and oncogene/s. The differentiation pathway leads to melanogenesis with expression of genes for tyrosinase, melanosomal proteins and genes responsible for dendrite formation, whereas the expression of oncogene/s leads to tumorigenesis.

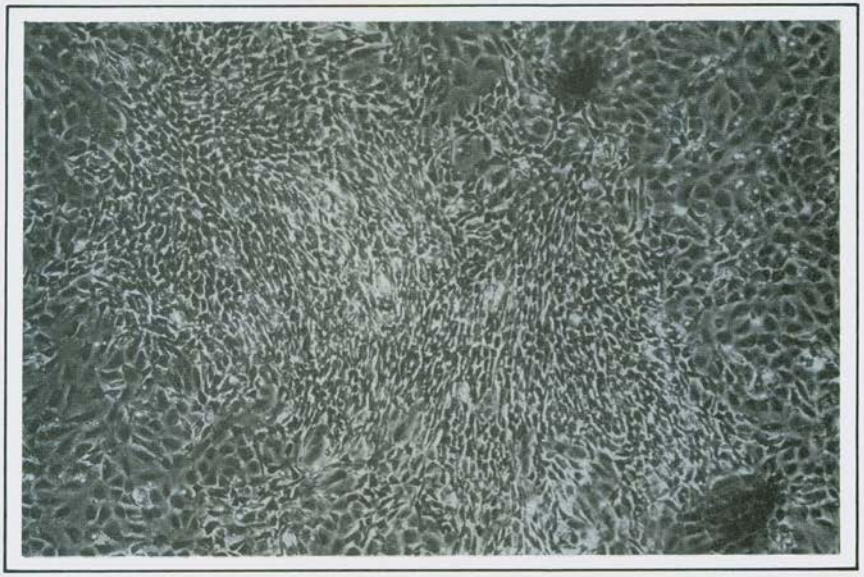


Fig. 15 : Foci of transformation after primary transfection of M3 melanoma DNA into NIH3T3.

Fig. 16 : Foci after secondary transfection as revealed by light modulation.

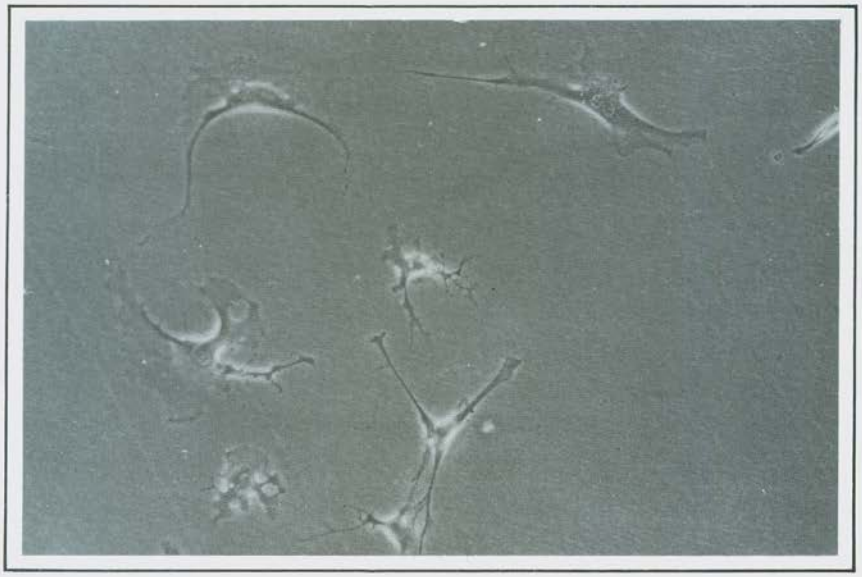


Fig. 17 : Branched dendrites typical of melanocytes in some clones after secondary transfection

Fig. 18 : Melanin pigmented cells observed in secondary transfectant

The aims of present study are :–

i. Identification of one or different classes of oncogene/s responsible for maintaining the tumorigenic and metastatic potential.

ii. Investigation of the relationship between oncogenes is and differentiation events with respect to tumorigencity and metastatic ability in melanoma.

Cell lines chosen for the study have been mouse melanoma (B16F1 & B16F10) and Clone M3 mouse melanoma (derived from cloudman melanoma). NIH3T3 cell line was used as recipient in DNA transfection assay.

Standard method of DNA transfection into NIH3T3 using calcium phosphate reagent and lipofectin reagent was performed. Foci of transformation were observed in transfected plates in 2 to 3 weeks time. (Fig. 15). Five such foci, with cells piling on each other, were picked up, grown and expanded in culture. DNA was isolated from these clones and re-transfected into NIH3T3 cell line. Secondary transfection was done to confirm the foci forming ability of the melanoma DNA (Fig. 16). These secondary foci were expanded in culture. For ease of handling, ten clones were frozen in liquid nitrogen. Eight clones were continued for further study.

The clones were characterized by the peculiar morphology of cells. Two clones showed preponderance of cells with branched dendrites, typical of melanocyte morphology (Fig. 17). One of the clones showed presence of pigmented cells containing melanin (Fig. 18). Few clones expressed tyrosinase as demonstrated by histochemical studies using DOPA (L-ß,3,4 dihydroxy phenylalanine) reaction.

The next set of experiments involve identification of the oncogene fragment by restriction enzyme digestion of DNA from the transformed foci. Further, it is also planned to develop a highly efficient expression cDNA cloning system for isolation of the oncogene/s.

MASS CULTIVATION OF ANIMAL CELLS

Fetal bovine serum is one of the important ingredients in tissue culture area. Several reports describe effect of serum concentration on growth and productivity of animal cells. A project was undertaken to study the effect of different serum concentrations on growth and metabolism of mouse hybridoma

cells (CC9C10). There was no effect of serum concentrations (10%, 6%, 2% and 0%) on the specific growth rate of these cells. However during the second subcultivation the cells in 10% had better growth rate in comparision with the lower concentrations of the serum. When the cells were in low density in logarithmic phase then concentration dependent growth of hybridoma was not observed. In fed batch experiments specific growth rate of the cells and extent of growth was found to be independent of serum concentrations. The cells in serum free medium showed very little growth. The metabolic activity of the cells as assessed by glucose consumption and antibody production, did not differ in cultures with 2% to 10% of the serum concentration. The other requirements of the cell such as effect of glucose and glutamine concentration were also standardised. By using this data cells are now being cultivated in hollow fiber reactor.

REGULATION OF POLYTENIZATION IN SALIVARY GLAND CELLS

The Drosophila cell line H33 was used to standardize the parameters for optimal conditions for electroporation. This was done with the aim of introducing certain putative factors from salivary gland cells of III instar larvae into this cell line (H33). The physical parameters which determine electroporation are the field strength , pulse width, number of pulses and the the fusion medium. The biological variables are the radii of the cells, initial viability of the culture and the days after subculture. Experiments were done on the second day after subculture and the initial and final viability was determined at different time points to arrive at an optimum field strength and pulse width. The viability and poration was checked by the permeability of pulsed and control (not pulsed) cells to propidium iodide. The fluorescence was observed and cells were counted on a hemocytometer under a fluorescence microscope using filter combination BP 546, FT 580 and LP 590.

From a series of five experiments, in which cells were monitored before the pulse, immediately afterwards, and every 24 hours thereafter, it was found that a field strength of 2.3-2.4 kV/cm for 5 pulses of 10 usec was insufficient for poration. However 5 pulses of 99 usec at a field strength of 2.0- 2.5 kV/cm was satisfactory. Field strength of 3.75 kV/cm was deleterious. The project has been discontinued.

STUDIES ON STRESS PROTEINS IN *ANO. STEPHENSI CULTURE*

Cloning of heat shock specific transcripts of *Ano. Stephensi.*

Total RNA was isolated from normal cells (grown at 28° C and heat shocked cells (incubated at 37° C 2 hrs. after 80% confluent growth at normal temperature). Subtractive hybridization of single stranded cDNA from induced cells and poly(A)+ RNA from normal cells was done. Single stranded and double stranded nucelic acids were separated on hydroxyapatite column. Selected cDNA was cloned in EcoRI site of vector -λt10. This cDNA library was screened with Drosophila HSP70 clone (PPW 229) DNA. Library screening was done in three steps and eight strongly hybridizing clones were picked. DNA of these clones was isolated by mini lysate method. It is planned to carry out further studies for characterization of these clones by mapping, sequencing and finally comparing with known HSP70 sequences from various organisms.

COLLABORATORS

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

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
Bharati Vidyapeeth Ayurved College Pune	Dr. B.R. Mardikar Mrs. Gudrun Ulrich - Merzenich
Janakalyan Eye Bank Pune	Dr. R.D. Kolte
King Edward Memorial Hospital Pune	Dr. B.B. Gokhale Dr. Y.V. Tawade Dr. S.B. Kelkar Dr. C.S. Yajnik Dr. U.D. Sutaria Dr. D. Bhattacharya
Mahatma Gandhi Eye Bank Pune	Dr. N. Shah
Malaria Corporation Unit Pune	Mr. J.K. More
Marathe Clinic Pune	Dr.R.L. Marathe
Naik Hospital Pune	Dr.S.N. Naik Dr.(Mrs.) S.S. Puntambekar
National Chemical Laboratory Pune	Dr. R.A. Mashalkar Prof. John Barnabas Dr. M.S. Srinivasan Dr. Prakash Deshpande
Ruby Nursing Home	Dr. C.A.Apte

University of Poona Pune Indian Red Cross Society Ahmedabad	Prof.D.N. Deobagkar Dr. N.N. Godbole Mr. G. Mazmudar
Lokmanya Tilak Muncipal Hospital Bombay	Prof. A.P.Chaukar Dr. Alka Gogate
Cancer Research Institute Bombay	Dr. A.N. Bhisey Dr. S.G.A. Rao
Indian Institute of Technology Bombay	Prof. S.Basu Prof.(Mrs.) Bhat
Hindustan Lever Research Centre Bombay	Dr. V. Sheorain
King Edward Memorial Hospital Bombay	Dr. S.R. Tambwekar
Tata Institute for Fundamental Research, Bombay	Dr. L.C. Padhy
Centre for Cellular & Molecular Biology, Hyderabad	Dr. P.D. Gupta Dr. Lalji Singh
Central Drug Research Institute, Lucknow	Dr. A. Bhaduri
Vector Control Research Centre Pondicherry	Dr. V. Dhanda Dr. K. Balaraman
National Filaria Control Unit Thane	Dr. S.B. Dongre

* Bhonde,R.R., Kolte,R.D., Kelkar,S.B.(1991)
Long-term preservation of human cornea for eye banking.
In: Ocular pharmacology Recent advances (Gupta,S.K.Ed.)
pp. 193-196.

* Chiplonkar,J.M. (1992)
Electrofusion of mosquito cells.
Invitro, **28A** : 230-232.

Chiplonkar,J.M., D.D. Vandre and J.M. Robinson (1992)
Stimulus-dependent relocation of microtubule organizing center in human polymorphonuclear leucocytes.
J. Cell Science (In Press).

* Gokhale B.B., Tawade Y.V., Bharatiya P.R., Parekh A.P., Mojamdar M., Bhonde R.R. and Wani M.R. (1991)
Use of Organ Cultured Fetal Skin as Allografts in the Treatment of resistant Vitiligo. Ind. J. Dermatol Venereol & Leprol, **57** : 272-275.

Guha Roy, S. and Bhisey, A.N. (1991)
Regulation of cytoplasmic microtubule complex organisation in somatic cell hybrids.
Cell Biol. Int. Reports, **15**:377-387.

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

* Gupta P.D & Bhonde, R.R.
Increased expression of keratin polypeptides in long term cultures of adult rat hepatocytes.
Cytobios (In press).

Kale,R.K. and Sitasawad,S.L. (1991)
Non linear pattern of radiation induced lipid peroxidation is not affected by vitamin E, Fe^{2+} ions and molecular oxygen.
Ind. J. Exptl. Biol., **29**: 778-781.

Parab,P.B. and Subramaniam D.(1991)
Role of monoclonal antibodies in diagnosis and protection in lymphatic filariasis. Illustrated Biodigest(Biotechnology Special), **5 & 6**:53-58.

Rajasekariah,G.R., Parab,P.B., Subramaniam D.(1991)
Detection of Wuchereria bancrofti specific antigens in the serum of endemic residents.
Trop. Med. Parasitol., **42**: 103-105.

Rajasekariah,G.R., Parab,P.B., Chandrashekhar, R.; Deshpande, L. Subramaniam D.(1991)
Pattern of Wuchereria bancrofti microfilaraemia in young and adolescent school children in Bassein, India, an endemic area for lymphatic filariasis.
Annals of Trop. Med. & Parasitology, **85**:

Ulemale A.H., Wani M.R. and Kulkarni P.E.
Effect of Electromagnetic Stimulation on osteogenesis in Long Bone Fracture of Calves (An experimental study) .
Ind. J. Vet. Surg. (Accepted for publication).

Ulemale A.H., Wani M.R. and Kulkarni P.E.
Treatment of severely Damaged Diphseal Fracture of Long Bones using Entire Segmental Allografts (An experimental study).
Ind. J. Vet. Surg. (Accepted for publication).

Wani M.R. , Ulemale A.H., Narkhede M.D. and Kulkarni P.E.
Pinch Skin Grating in the Granulating Wounds of Canines
Ind. Vet. J. (Accepted for publication)

PUBLICATIONS IN PROCEEDINGS

* Mojamdar, M.
Cultured epithelia. A new model for investigating carcinogenesis.
Proc. Brain storming Session on Cancer Research. Cancer Research Institute, Bombay (In press).

Note : * Work carried out at NFATCC.

MOJAMDAR M.V

- "TISSUE CULTURE OF MELANOCYTES FROM NORMAL AND VITILIGO SUBJECTS AND ITS CHEMICAL APPLICATIONS"
  IAPM meeting March 22, 1991.

- Invited lecture on culture of keratinocytes and melanocytes
  Biotechnological and Biomedical applications at Hindustan Lever Research Center, Sept. 5, 1991.

- Brain storming sessions on Cancer Research. Participated in the sessions on "MECHANISM OF CARCINOGENESIS", at Cancer Research Institute Bombay. Nov. 25-27, 1991.

GUHA ROY S

- "CULTIVATION OF FASTIDIOUS CELLS AND MODERN METHODS OF CELLULAR MANIPULATIONS" A training course conducted at NFATCC from Dec. 1-15, 1991.

- "FLOWCYTOMETRY OF EUKARYOTIC CELLS" Workshop conducted at BARC from Feb. 24-March 6, 1992.

OVERSEAS ASSOCIATESHIP

Dr. J.M. Chiplonkar was awarded the Department of Biotechnology Overseas Associateship (long term) for 1990-1991. He worked at the department of Cell Biology, Neurobiology & Anatomy, Ohio State University, Columbus, Ohio, USA during the period May 19 1991 to May 18 1992.

i. Organised and conducted the "Popular Lecture Series" by Eminent Scientists sponsored by DBT.

ii. Conducted the following workshops :-

   a. "Introduction to Animal tissue culture and its applications" sponsored by NFATCC (for college & University teachers).

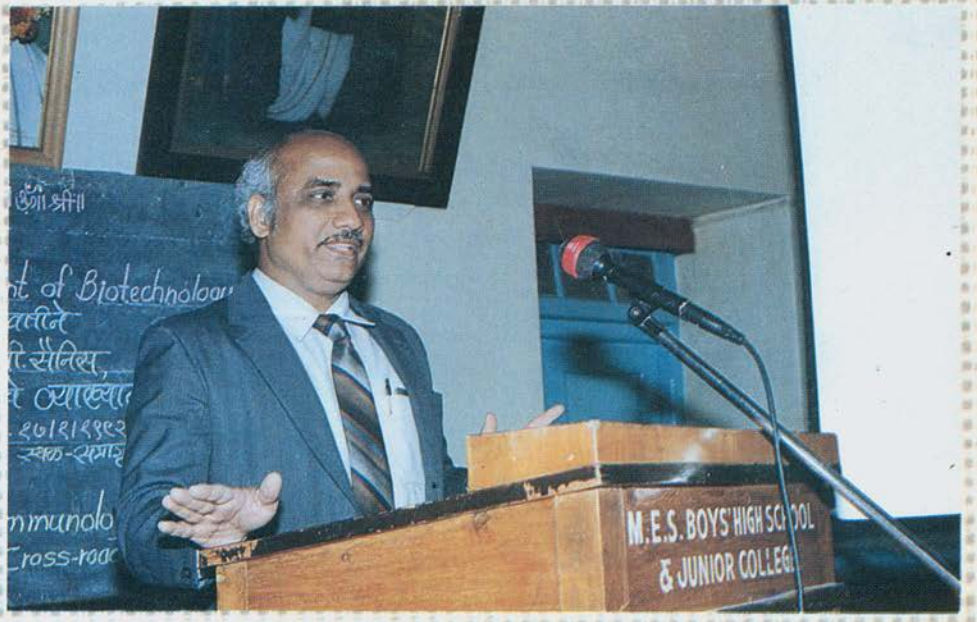
   b. "Culture of Fastidious cells & modern techniques of Cell manipulation" sponsored by DBT.

iii. Participated in University of Poona teaching and training.

iv. Individual training for deputed candidates.

NAME	INSTITUTE	TRAINING From	PERIOD To
Dr. S.V. Kulkarni	Disease Investigation Section, Aundh, Pune	01.09.91	15.09.91
Ms. Supriya Patil	Center for Genetic Engineering, Indian Inst. of Sci. Bangalore	15.11.91	30.11.91
Mr. V.K. Srinivas Mr. K. Khaleel-Ur - Rehman	Dept. of Gastroenterol Osmania General Hosp. Hyderabad	17.02.92	29.02.92
Ms. Asmita Nikalje	Dept. of Zoology University of Poona Pune	17.05.91	04.07.91
Mr. Ramesh Sharma Ms. Manisha Fousdar	Divn. of lab. animals C.D.R.I., Lucknow	03.03.92	17.03.92

• DR. A.N. BHISEY UPDATING THE AUDIENCE WITH "CURRENT TRENDS IN CANCER RESEARCH. •



• DR. K.B. SAINIS EXPLAINING "IMMUNOLOGY AT CROSS ROADS". •

• DR. BAL PHONDKE HIGHLIGHTING THE "FRONTIERS IN VACCINE PRODUCTION". •

• YOUNG ENTHUSIASTIC AUDIENCE LISTENING ATTENTIVELY TO THE LECTURE •

• FACULTY MEMBERS AND PARTICIPANTS OF THE WORKSHOP "CULTURE OF FASTIDIOUS CELLS AND MODERN TECHNIQUES OF CELL MANIPULATION", SPONSORED BY DBT. •

COMPUTERS

The computer unit has been equipped with a 386 based machine (1 no.) and 286 based machines (2 nos.). The 386 based machine is used as file server for network with PCs. One 286 based machine is dedicated for library and other for scientific analysis, data storage and report generation. PCs and XTs have been installed in various sections for different activities.

IMPLEMENTATION OF SOFTWARE DEVELOPED IN HOUSE

Software packages developed for scientific, administration and library activities identified during the previous year have been successfully implemented.

Areas computerised so far :–

- Personnel Management
- Financial Management
- Library Management
- Office Automation
- Stores and purchase
- Cell line cataloguing and supply
- Statistical Analysis (modules to suit the requirement)
- Educational Aids

USER AWARENESS AND GUIDANCE

The computer users are guided for updating with new versions of software packages and developments in the field of computer technology.

A Desk Top Publishing (DTP) facility has been set for generation of reports, preparation of catalogues and for presentation & publications.

Communication Aids :

- Compact Disk Read Only Memory (CD-ROM) drive has been setup for literature search.
- Fascimile was installed for efficient and fast communication.

LIBRARY AND DOCUMENTATION

The main activities of the Library & Documentation have been focused on collection and dissemination of Science and Technology information. The main areas covered for this purpose are Animal Tissue & Cell Culture and its allied subjects.

So far the library has acquired approximately 1500 documents which include books, reports, conference proceedings, journals and periodicals. The library subscribes to 31 scientific journals and about 15 journals are received on Gratis basis.

The library has continued to offer CAS/SDI services from a bibliographic database of research articles relevant to NFATCC activities from various periodicals.. The database is designed and developed by augmenting CDS/ISIS software received from NISSAT, (Govt. of India). The library has contributed in compiling a bibliography on "Fastidious Cells"

The Library has received Health Foundation Phenytoin Database from USA on Gratis which covers literature from 1937 onwards.

The Library has installed high density optical storage media CD-ROM to provide pin-pointed exhaustive and expeditious information to its users and has subscribed for DIALOG-ON-DISC (MEDLINE) from 1984 and onwards. The Library has carried out 70 searches for the scientists at NFATCC. Searches have been carried out from Medline for other institutes also.

In order to satisfy the concept and add value to "Resource Sharing" through shared library documents the library continues to share its resources with other libraries. Inter-library loan and bibliographic services are rendered on requests.

INSTRUMENTATION AND MAINTENANCE

Instrumentation & Maintenance unit provides the following technical and maintenance services :–

i. Electrical
ii. Civil and Mechanical
iii. Airconditioning
iv. Instrumentation
v. Other technical assistance and guidance to users.

Regular and preventive maintenance of all equipments and instruments are carried out. Updation of documentation and records of equipments is executed. During the year 1991-92, the unit has successfully completed the following jobs :–

Interaction and coordination with C & S department, DAE, technical staff of the University of Poona, Architects and Poona Municipal Corporation towards approval of NFATCC plans on the University campus.

. Tree plantation at university site and providing water supply system for irrigation and construction work.

. Laying of chain link fencing at University site.

. Supervision and coordination of Jidnyasa building.

. The interior jobs which included partitioning, air conditioning, electrical connections have been planned and executed at Jidnyasa.

construction of laboratory and residential complex of NFATCC on Poona University campus gathered good momentum during the period of this Annual Report. The salient points in this regard are as follows :

a) Approval of the building plans by Pune Municipal Corporation.

b) Erection of chain link fencing all along the boundaries of the plot allocated by Poona University to NFATCC for the construction of laboratory and residential complex.

c) Memorandum of Understanding between NFATCC and Department of Atomic Energy (C & S) group, Bombay was signed.

d) Department of Atomic Energy (C & S) group, Bombay floated technical and financial tenders regarding construction of laboratories of NFATCC on Poona University campus.

The budget estimates for the construction of laboratories have been revised from Rs.638 lacs to Rs.737.27 lacs and have been approved in principal by Department of Biotechnology. It has now been planned to contruct laboratories admeasuring 56800 sq.ft. initially. Regular meetings between the concerned staff of DAE (C & S) group, Bombay, the Architects M/s.Beri Architects & Engineers Pvt.Ltd., and Director, NFATCC were held to co-ordinate the construction activity.

Completion of remaining construction of an unfinished RCC structure purchased from M/s.Vanaz Engineers Ltd., is nearing completion. The budget estimates for the completion of remaining construction were revised to Rs.40.99 lacs and were approved in principal by DBT. Infrastructural staff has already started functioning from this new premises named 'Jidnyasa'. Office accommodation leased out from Red Cross for infrastructural staff has since been vacated.

The meetings of the important statutory committees were held as follows :

a)	Fourth meeting of the Governing Body	8th July, 1991
b)	Fifth meeting of the Governing Body	17th Jan.1992
c)	Second meeting of the Society	8th July, 1992
d)	Third meeting of the Scientific Advisory Committee	27th Aug.1991
e)	Sixth meeting of the Finance Committee	9th Dec.1991

Construction of laboratory and residential complex of NFATCC on Poona University campus gathered good momentum during the period of this Annual Report. The salient points in this regard are as follows :

a) Approval of the building plans by Pune Municipal Corporation.

b) Erection of chain link fencing all along the boundaries of the plot allocated by Poona University to NFATCC for the construction of laboratory and residential complex.

c) Memorandum of Understanding between NFATCC and Department of Atomic Energy (C & S) group, Bombay was signed.

d) Department of Atomic Energy (C & S) group, Bombay floated technical and financial tenders regarding construction of laboratories of NFATCC on Poona University campus.

The budget estimates for the construction of laboratories have been revised from Rs.638 lacs to Rs.737.27 lacs and have been approved in principal by Department of Biotechnology. It has now been planned to contruct laboratories admeasuring 56800 sq.ft. initially. Regular meetings between the concerned staff of DAE (C & S) group, Bombay, the Architects M/s.Beri Architects & Engineers Pvt.Ltd., and Director, NFATCC were held to co-ordinate the construction activity.

Completion of remaining construction of an unfinished RCC structure purchased from M/s.Vanaz Engineers Ltd., is nearing completion. The budget estimates for the completion of remaining construction were revised to Rs.40.99 lacs and were approved in principal by DBT. Infrastructural staff has already started functioning from this new premises named 'Jidnyasa'. Office accommodation leased out from Red Cross for infrastructural staff has since been vacated.

The meetings of the important statutory committees were held as follows :

a) Fourth meeting of the Governing Body — 8th July, 1991

b) Fifth meeting of the Governing Body — 17th Jan.1992

c) Second meeting of the Society — 8th July, 1992

d) Third meeting of the Scientific Advisory Committee — 27th Aug.1991

e) Sixth meeting of the Finance Committee — 9th Dec.1991

Construction of laboratory and residential complex of NFATCC on Poona University campus gathered good momentum during the period of this Annual Report. The salient points in this regard are as follows :

a) Approval of the building plans by Pune Municipal Corporation.

b) Erection of chain link fencing all along the boundaries of the plot allocated by Poona University to NFATCC for the construction of laboratory and residential complex.

c) Memorandum of Understanding between NFATCC and Department of Atomic Energy (C & S) group, Bombay was signed.

d) Department of Atomic Energy (C & S) group, Bombay floated technical and financial tenders regarding construction of laboratories of NFATCC on Poona University campus.

The budget estimates for the construction of laboratories have been revised from Rs.638 lacs to Rs.737.27 lacs and have been approved in principal by Department of Biotechnology. It has now been planned to contruct laboratories admeasuring 56800 sq.ft. initially. Regular meetings between the concerned staff of DAE (C & S) group, Bombay, the Architects M/s.Beri Architects & Engineers Pvt.Ltd., and Director, NFATCC were held to co-ordinate the construction activity.

Completion of remaining construction of an unfinished RCC structure purchased from M/s.Vanaz Engineers Ltd., is nearing completion. The budget estimates for the completion of remaining construction were revised to Rs.40.99 lacs and were approved in principal by DBT. Infrastructural staff has already started functioning from this new premises named 'Jidnyasa'. Office accommodation leased out from Red Cross for infrastructural staff has since been vacated.

The meetings of the important statutory committees were held as follows :

a)	Fourth meeting of the Governing Body	8th July, 1991
b)	Fifth meeting of the Governing Body	17th Jan.1992
c)	Second meeting of the Society	8th July, 1992
d)	Third meeting of the Scientific Advisory Committee	27th Aug.1991
e)	Sixth meeting of the Finance Committee	9th Dec.1991

Annual Report both in English and Hindi was prepared for the year 1990-91 and was furnished to DBT for onward presentation to the Parliament.

As the new building 'Jidnyasa' is nearing completion, additional working space has become available. In view, 37 posts as per following details have been advertised.

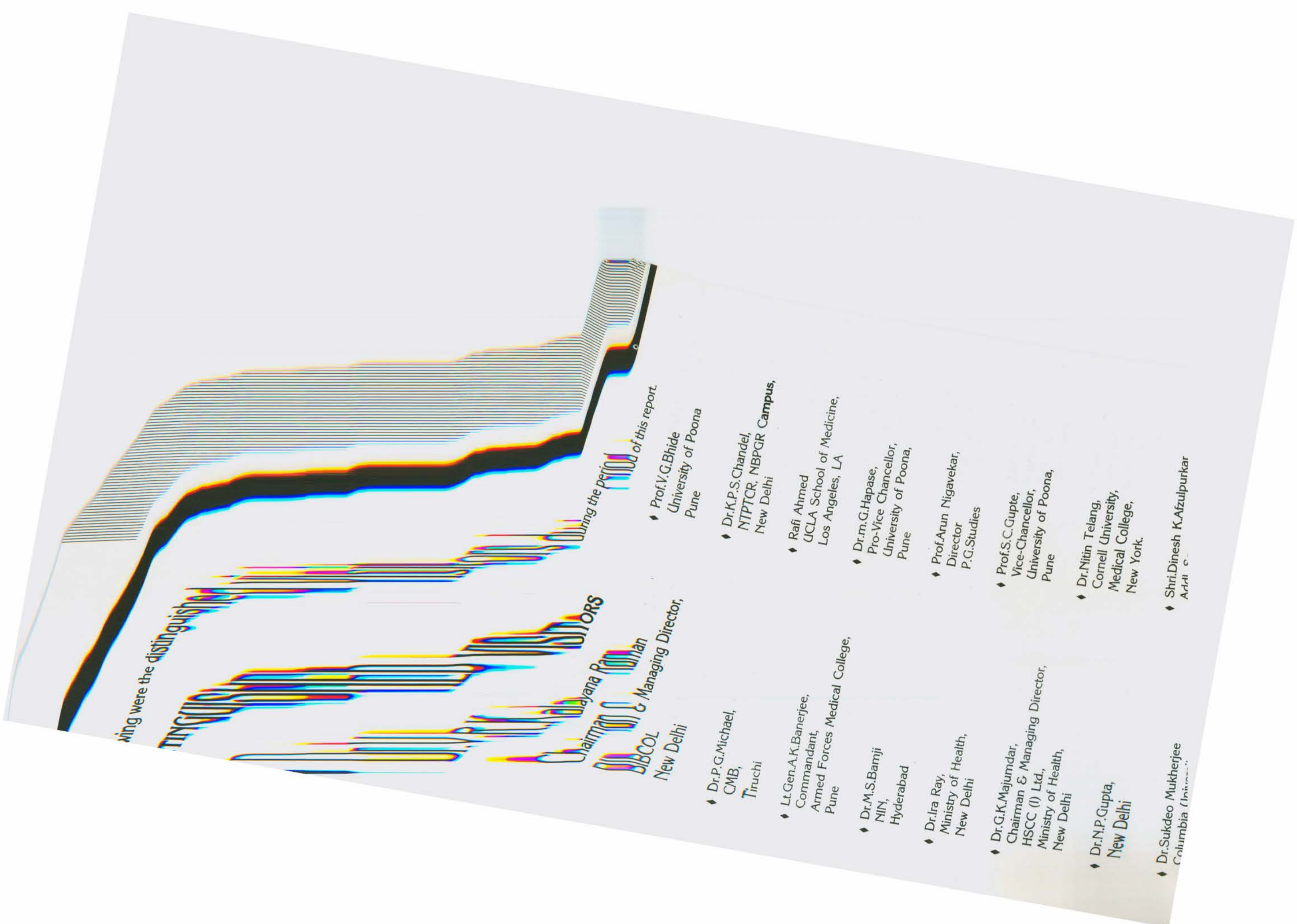
a) Scientists	8
b) Technical Staff	13
c) Administrative Staff	3
d) Auxiliary Staff	4
e) Trainees	9

Selection process is in progress. Special recruitment drive for SC/ST candidates have been undertaken.

The staff complement of NFATCC as on 31.3.92 is as follows:

a) Scientific Staff	13
b) Technical Staff	
i) Laboratory	11
ii) Instrumentation & Maintenance	5
c) Administration Staff	9
d) Auxiliary Staff	16
	54
e) Trainees	3
f) Contractual services	8

Laboratory equipments worth Rs.43 lacs were purchased during the year. The equipments have been installed and are used regularly.


wing were the distinguished
TINGUISHED VISITORS
uring the period of this report.
Chairman & Managing Director,
BBCOL
New Delhi
Prof.V.G.Bhide
University of Poona
Pune
Dr.K.P.S.Chandel,
NTPTCR, NBPGR Campus,
New Delhi
Rafi Ahmed
UCLA School of Medicine,
Los Angeles, LA
Dr.m.G.Hapase,
Pro-Vice Chancellor,
University of Poona,
Pune
Prof.Arun Nigavekar,
Director
P.G.Studies
Prof.S.C.Gupte,
Vice-Chancellor,
University of Poona,
Pune
Dr.Nitin Telang,
Cornell University,
Medical College,
New York.
Shri.Dinesh K.Afzulpurkar
Dr.P.G.Michael,
CMB,
Tiruchi
Lt.Gen.A.K.Banerjee
Commandant,
Armed Forces Medical College,
Pune
Dr.M.S.Bamji
NIN,
Hyderabad
Dr.Ira Ray,
Ministry of Health,
New Delhi
Dr.G.K.Majumdar,
Chairman & Managing Director,
HSCC (I) Ltd.,
Ministry of Health,
New Delhi
Dr.N.P.Gupta,
New Delhi
Dr.Sukdeo Mukherjee

- Prof.H.Y.Mohan Ram
  Dept. of Botany,
  University of Delhi,
  Delhi

- Dr.P.Richard Masillamony,
  Vice Chancellor,
  TN Veterinary and Animal
  Science University,
  Madras

- Dr. B.K. Soni
  Former Regional Animal
  Foundation & Health Officer,
  FAO, Bangkok

- Dr.Y.K.Kauna,
  Prof. & NF,
  Vet.Micribiology,
  HAU, Hisar

- Dr.D.N.Garg,
  Mycoplasms Lab,
  Dept. VPHE,
  HAU, Hisar

- Dr.Minakshi,
  Asstt.Scientist,
  Vet. Microbiology,
  HAU,
  Hisar

- H.M.Pandit
  Prof.Emeritus,
  54, Danebrock,
  New York

- Dr.Smt. Kamal J.Ranadive,
  Emeritus Scientist,
  1/27, Nishgandh,
  Roopnagar,
  Bandra, Bombay

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 S.B.Krishnan,
  Joint Secretary & 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
  Joint Director (Research),
  Indian Veterinary Research Inst.
  Izatnagar : 243 122 (UP)

- Prof.A.S.Kolaskar,
  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

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

SCIENTIFIC ADVISORY COMMITTEE :

- Prof.H.Sharat Chandra, (Chairman)
  Director,
  Centre For Cellular &
  Molecular Biology,
  Uppal Road,
  Hyderabad : 500 007

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

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

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

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

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

- Dr.Rama Mukherjee,
  Staff Scientist V,
  Microbiology Div.,
  National Institute of Immunology,
  Shaheed Jeet Singh Marg,
  New Delhi : 110 067

- Dr.S.Sriramachari,
  Ex-Additional Director General
  ICMR
  P.B.No.4909
  Safdarjang Hospital campus,
  New Delhi : 110 029

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

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

- Prof.U.W.Kenkare,
  Emeritus Scientist, CSIR
  National Facility For
  Animal Tissue & Cell Culture,
  Pune

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

FINANCE COMMITTEE

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

- Prof.H.Sharat Chandra
  Director,
  Centre For Cellular and
  Molecular Biology,
  Hyderabad

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

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

BUILDING COMMITTEE

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

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

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

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

- Shri M.V. Patil
  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. C. Sivaraman (Chairman),
  Ex-Director Gr. Scientist,
  National Institute of Virology,
  Pune

- Prof.U.W.Kenkare,
  Emeritus Scientist, CSIR
  National Facility For
  Animal Tissue and Cell Culture,
  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
  National Facility For Animal
  Tissue and Cell Culture,
  Pune

INSTITUTIONAL CO-ORDINATION COMMITTEE

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

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

- Shri S.B. Krishnan
  Joint Secretary & Financial Adviser,
  OR
  His representative,
  Department of Biotechnology,
  Ministry of Science & Technology,
  New Delhi

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

- Prof. D.N. Deobagkar
  Head, Dept. of Zoology
  University of Poona
  OR
  Co-ordinator/Head Biotechnology
  Training Programme
  University of Poona,
  Pune

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