NATIONAL FACILITY FOR ANIMAL TISSUE AND CELL CULTURE

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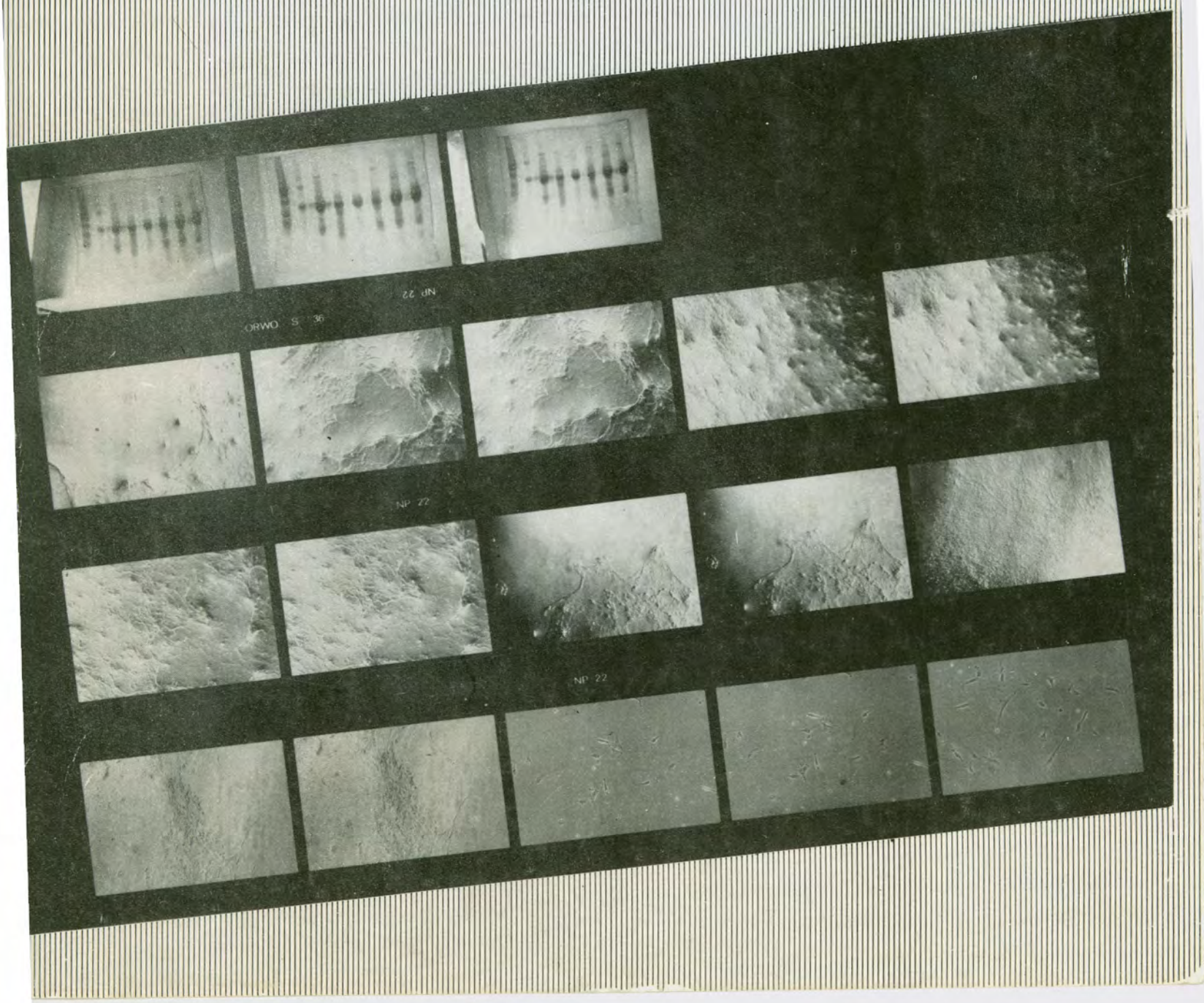
1989-90



NATIONAL FACILITY FOR ANIMAL TISSUE AND CELL CULTURE

DEPARTMENT OF BIOTECHNOLOGY
GOVERNMENT OF INDIA

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CONTENTS

TERMS OF REFERENCE

A. To receive, identify, maintain, store, grow and supply :
   i) Animal and human cell lines of both existing (typed) and newly developed
   ii) Tissues, organs and fertilised eggs and embryos.
   iii) Hybrid cells including hybridomas
   iv) Unicellular obligate pathogens, parasites and vectors.
   v) Plasmids, genes and genomic libraries.

B. Research and Development in the above cell culture-related materials and products.

C. Develop, prepare, quality-control and supply cultures and other reagents and materials independently/in collaboration with industry.

D. To establish and conduct Training courses in Animal Cell/Tissue/Organ/Embryo culture and related techniques.

2. INTRODUCTION

Since the establishment of NFATCC as a society in August 1988 the planned activities could be taken up more vigorously. The facility purchased a ready constructed building to ease the space constraints which was one of the main hurdles in the progress of NFATCC. The necessary modifications, alterations were undertaken and administrative set up was shifted to this new premises of "Jopasana" Building at Kothrud. Augmentation of essential requirements for water storage and supply, electric supply, stand-by generator facility, air conditioning, partitioning, telecommunication facility have been organised and established.

Simultaneously the process of recruitment of staff and purchase of equipment was initiated and completed.

The cell repository, one of the major function has been enriched to contain 688 cell cultures and has supplied 320 consignments comprising 72 different cell lines to the research teams and public health laboratories.

Research activities in the field of tissue banking and other R & D areas have been organised establishing very good collaborative programmes with scientists and clinicians from different organisations in the country.

Such type of functionally oriented working pattern is destined to serve more efficiently as a National Facility in the true sense.

For example, team work on Cornea bank has made further advancement by establishing a method for storage of trephined cornea for 35 days. Thus, some of the major hurdles of eye banks such as short period of 72 hours available for communication to the recepients particularly those in the rural areas, non-assurance of sterility of the stored cornea and the possibilities of damage to the endothelium have been removed.

Similarly, the work on skin bank is also progressing in the right direction with the coordinated team work of tissue culturists, dermatologists, plastic surgeons, microbiologists, immunologists and clinicians. Skin is now being maintained and cultivated as entire foetal skin explant, separated intact epidermis,isolated melanocytes and keratinocytes. Method for transplantation of cultured foetal skin to white patches of vitiligo patients has been standardised. The main objective is however to use this technology for burn cases.

Some of the new technologies which are planned to be worked out cover two important areas, namely maintenance of cells in functional state for production of cell products and use of hollow fibre technology for mass cultivation of functionally active cells. A beginning has already been made. As a first step towards development of bioartificial pancreas Islets of Langerhans isolated from rat pancreas have been maintained in a functional state on biocompatible membranes prepared by IIT group Powai, Bombay. Hybridoma cells have been maintained for more than 5 weeks in Endotronic bioreactor system and large amount of antibody has been collected. It is heartening to note that various aspects of these projects are being executed by expert scientists from different organisations working as a cohesive team.

3. SCIENTIFIC ACTIVITIES DURING APRIL 1989 TO MARCH 1990

I. SERVICE:

A. CULTURE REPOSITORY AND SUPPLY SERVICE

A-1 Cell Lines & Hybridomas :

A total of 628 cell cultures including hybridomas and primary cultures were maintained in liquid nitrogen as cryopreserved stock. These are being revived and expanded for supply service as per the requirements.

Sixty cell lines (Table I) were obtained from A.T.C.C., U.S.A. and were added to the stock. Thus the total number of cell lines in the repository is 688.
In response to the requests for the supply of cell lines, three hundred and twenty consignments comprising of 72 different cell cultures were sent to 94 research teams from 57 different laboratories.
(TABLE II & TABLE III).

A-2 Other Culture Strains :

A stock of 33 malaria culture strains, 4 obligate parasite cultures and 9 vectors are being maintained in the repository. A culture strain of Leishmania donovani was supplied to one laboratory.

TABLE I. NEW CELL LINES ADDED TO THE CELL REPOSITORY

Sr. No.	ATCC Catalog No.	Name	Origin
1.	CRL 1424	G-361	Malignant melanoma, caucasian male
2.	HTB 64	Malme-3M	Malignant melanoma, metastasis to lung, Human
3.	HTB 66	RPMI-7951	Malignant melanoma, metastasis to lymph node, Human
4.	HTB 67	SK-MEL-1	Malignant melanoma, metastasis to lymphatic system, human
5.	HTB 68	SK-MEL-2	Malignant melanoma, metastasis to skin of thigh, Human
6.	HTB 69	SK-MEL-3	Malignant melanoma, metastasis to lymph node, Human
7.	HTB 72	SK-MEL-28	Malignant melanoma, Human
8.	CL-177	XB-2	Mouse teratomal keratinocytes
9.	CRL 1623	SSC-15	Human tongue, squamous cell carcinoma
10.	CRL 1628	SSC-25	Human tongue, squamous cell carcinoma
11.	CRL 1629	SSC-9	Human tongue, squamous cell carcinoma
12.	CRL 1777	HIT-T15	Syrian hamster beta cell
13.	CRL 1615	CL-S1	Mouse mammary alveolar nodules, preneoplastic, BALB/c.
14.	CRL 1720	F-9	Mouse embryonal carcinoma

15.	TIB 223	GCT	Human fibrous histiocytoma, metastasis to lung
16.	CL 101	LLC-PK1	Pig kidney
17.	CL 101.1	LLC-PK1A	Pig kidney
18.	CRL 1743	NMU	Rat mammary adenocarcinoma
19.	CRL 1715	TM-4	BALB/c mouse testis, Sertoli cells
20.	HB 170	R4-6A2	Antimurine gamma interferon
21.	HB 8291	IFGCP-F1BA10	Antihuman gamma interferon
22.	CRL 8031	KG-1	Human acutemyelogenous leukemia
23.	CRL 1427	MG-63	Male, osteosarcoma
24.	CRL 8066	Mo	Human T cell leukemia
25.	CRL 1566	NULLI-SCC1	Mouse teratocarcinoma
26.	CRL 1572	PA-1	Human ascitic fluid cells, ovarian teratocarcinoma
27.	CRL 1535	SCC-PSA1	Mouse pleuripotent terato carcinoma
28.	HB 123	CC9C10	Anti insulin
29.	HB 127	CE9H9	Anti insulin
30.	HB 126	CG 7C7	Anti insulin
31.	HB 124	DB9G8	Anti insulin
32.	CRL 1646	FO	Nonsecreting mouse myeloma
33.	CRL 1580	P3X63-Ag8.653	Nonsecreting mouse myeloma
34.	HB 8064	Hep-3B	Human hepatocellular carcinoma
35.	CRL 8024	PLC/PRF/5	Alexander cells, human hepatoma
36.	HB 172	10 B 9	Antihuman endothelium
37.	HB 174	14 E 5	Antihuman endothelium
38.	HB 175	IR-1	Anti insulin receptor
39.	HB 180	9.3 F 10	Anti HLA DR,DQ
40.	HB 125	AE 9 D 6	Anti Insulin, residues A 8-10
41.	HB 133	BE 3 F 9	Anti insulin
42.	CRL 8163	H33HJ-JA1	Jurkat derivative, human lymphoma
43.	CCL 5	L-132	Embryonic lung, human, HeLa markers
44.	CCL 136	RD	Human rhabdomyosarcoma, embryonal
45.	CRL 8543	H9/HTLV-IIIB	Human T cell, HIV+
46.	HTB 14	U-87	MG Glioblastoma, astrocytoma, grade III, Human
47.	CRL 1721	PC-12	Rat adrenal pheochromocytoma
48.	CCL 144	MH1C1	Hepatoma, rat
49.	TIB 192	M1	Mouse myeloblast
50.	CRL 1619	A-375	Human malignant melanoma
51.	CCL 2.1	HeLa 229	Epitheloid carcinoma, cervix, human
52.	CRL 1494	Bge	Biomphalaria glaberta embryo
53.	CRL 6563	TN1.LU	Armadillo lung
54.	CRL 6005	DNI.K	Armadillo kidney
55.	CRL 6006	DNI.LU	Armadillo lung
56.	CRL 6003	DNI.F	Armadillo foreskin
57.	CRL 6607	DNI.SK	Armadillo skin
58.	CRL 6008	DNI.SP	Armadillo spleen
59.	CRL 6009	DNI.Tr	Armadillo trachea
60.	CRL 6323	B16-F1	Mouse melanoma

TABLE II. LIST OF CELL LINES SUPPLIED .

3T3 Swiss albin

A 431
ATC-15
ATC-448

BGM
BHK-21
BS-C-1
BW-5147-S

C-6/36
C-6 glial tumor
CH
CHO
COS-1
COS-7
CTLL-2
CV-1
Daudi

EL-4

FRHK-4

GM-1
GpBr-5

HDCS
HeLa
HeLa-S3
HEp-2
HepG-2
HFL-1
HL-60
HUT-78

IMR-32
Indian Muntjac

K-562
KB
L-929
LBRM-33-A-5
LLC-MK-2
L-M

MA-104
McCOY
MCF-7
MDBK
MDCK
MKTC
MRC-5

Neuro-2A
NIH 3T3
NRK-49 F
NS-1

OKM-1
OKT-3
OKT-4
OKT-5
OKT-8
OKT-11
P-388-Di
P-3x63xAg8.653
P-815
PS

RAJI
Rat-2
RAW-264.7
RK-13

Sarcoma-180
SF-9
SIHA
Sp2/0

TC-7

UC-729-6

V-79
Vero

WISH
YAC-1

CULTURE SUPPLY SERVICE

● NATIONAL FACILITY FOR ANIMAL TISSUE & CELL CULTURE

'JOPASANA'
VANAZ ENGINEERS COMPOUND,
PAUD ROAD, KOTHRUD,
PUNE - 411 029 (INDIA)

TABLE III

Record of Culture Supply Service

Total number of Cell Cultures supplied	: 320
Covering -	
Number of Cell Lines	: 72
Number of Research Teames	: 94
Number of Institutes	: 57

B. TISSUE BANK ACTIVITY :

The activities of Multiple Tissue Bank were continued. A meeting of representatives from the Dehdan Mandal, Pune and different departments of hospitals, medical colleges and consultants was convened and the issues of collection and use of cadaveric tissues were discussed. Arrangements have been made for collection and processing such tissues at the Multiple Tissue Bank.

B-1 Cornea:

(Collaborators Dr. R.D. Kolte, Naimpally Eye Bank, B.J. Medical College, Pune)

Studies on long term maintenance and preservation of cornea as organ culture were continued. Fifty eight eye balls were obtained from different eye banks in Pune. These were maintained as closed organ culture by suspending the entire corneoscleral button in nutrient medium and stored at +4°C. Transparency and viability of these cultures were checked. The results indicate that these cultures can retain transparency and viability upto 25 days. However, it cannot be used directly for keratoplasty as it needs to be trephined to specific diameter to suit recepient's requirement.

Trephination has to be carried on these corneoscleral button from endothelial side which may risk damage of endothelium. In order to overcome this practical problem following studies were conducted. Cornea trephined from epithelial side of specific diameter was cultured in media containing dextran and these were maintained at different temperatures. The results of these experiments revealed that this technique permits maintenance of trephined cornea upto 35 days.

This is an important step which would permit the use of trephined cornea directly and the further period of storage would help to communicate even to the recepients from the remote corners of villages. The benefit of corneal transplant thus could be made available to the rural masses.

B-2 Skin:

(Collaborators - Dr. B.B. Gokhale, Dr. Y.V. Tawade, K.E.M. Hospital, Pune)

Studies on cultivation of skin "in vitro" were continued. In order to test the suitability of human foetal skin for allografting, organ explant cultures were set. The skin cultures could be maintained upto 28 days. During this period the cultures exhibited melanin synthesis, development and growth of hair follicles. Such explants of cultured skin have been used for grafting to vitiligo patients. It was found that out of the four cases of allografting three exhibited successful take of the grafts. The studies revealed the potential of foetal skin for allografting in vitiligo.

B-3 Heart Valves:

(Collaborators Dr. A.P. Chaukar, L.T.M. College, Sion Hospital, Bombay)

A good number of human heart valves are available for transplants. However, by conventional method of storage at +4 °C, these can be stored up to 3 weeks which is too short a period for checking sterility and preparing a receipient. With a view to extend the storage period, methods for storage at ultralow temperature are being standardized. Four aortic valves have been obtained from the Sion Hospital, Bombay.

These have been cryopreserved at -70 °C.employing a programmable biofreezer. The cooling rate was set to 1 °C/minute up to -30 °C; then it was increased to 10 °C/minute. The viability and the mechanical strength of these cryopreserved valves are being tested after specific period of storage.

II. RESEARCH & DEVELOPMENT:

A. Keratinocyte cultures

(Collaborators Dr.B.B. Gokhale and Dr. Y. V. Tawade, K.E.M. Hospital, Pune) :

Studies on maintenance and cultivation of skin "in vitro" were continued,Keratinocytes from human foreskins and newborn mouse skins were successfully cultured in calcium free media (Figure 1). So far they have been passaged up to three tyrpsin schedules. The keratinocytes are now being cultured as three-dimensional epithelia for transplantation in burns and other cases .

B. Melanocyte cultures

(Collaborators Dr. B. B. Gokhale and Dr. Y. V. Tawade K.E.M. Hospital, Pune):

Melanocytes from the skins of normal and vitiligo subjects and from keratinocyte cultures have been cultured (Figure 2). Melanocyte growth factors are now being extracted from human foetal lung fibroblast cultures (passage 3) to enhance the melanocyte population in culture for transplantation in vitiligo cases.

C. Hepatocyte cultures:

(Collaborator Dr. P.D. Gupta, CCMB, Hyderabad)

In order to study keratin expression in hepatocytes during different developmental stages, studies were undertaken to isolate and culture hepatocytes from embryonic and adult rat liver.

Methods were standardized employing explant cultures of liver, and by "in situ" perfusion and collagenase digestion from adult mice and rats.

Cultured hepatocytes were assayed for alfa-fetoproteins, keratin, protein p150 and albumin by immunofluorescence. Presence of keratin a marker for epithelial cells as revealed by positive immunofluorescence was found in cultured hepatocytes of newborn and adult mice. However, keratin was not found in mouse embryonic and human foetal hepatocyte cultures. Presence of alfa-fetoprotein—a marker for embryonic liver __ was detected in human foetal liver cultures but it was not detected in embryonic and newborn mouse liver cultures. Presence of albumin __ a marker for adult liver was detected in liver cultures of newborn mice but not in embryonic mouse liver.

Results of these experiments suggest that hepatocyte cultures derived from different age groups of donors express different markers exhibiting change in a state of differentiation.

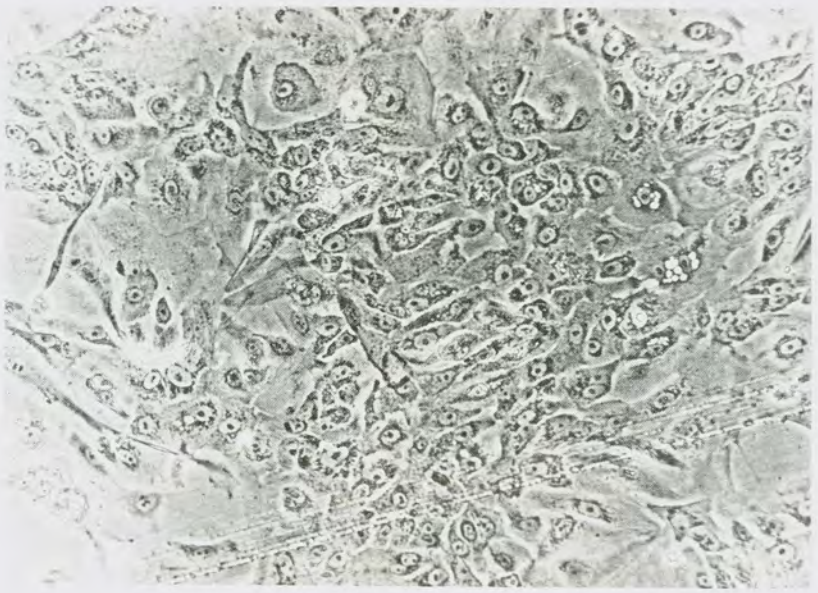


FIGURE 1 : Human skin keratinocytes growing in calcium-free medium. Note their polygonal morphology and tendency to grow as sheets. (Live, phase contrast,300 x).

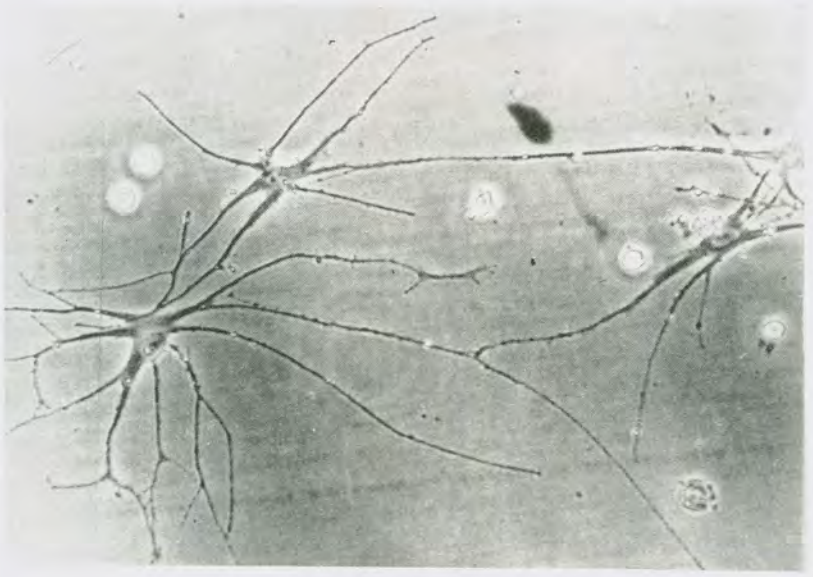


FIGURE 2 : Human melanocytes growing in media containing phorbol esters and cholera toxin. Note highly dendtritic nature of these cells. Melanin granules can also be noticed. (Live, phase contrast, 300 x).

D. Studies on organ explant cultures of pancreas:

Studies were initated to test retention of functionality of pancreatic explants "in vitro". Amylase secretion and total protein contents of culture medium were assayed as functionality markers. Organ cultures of rat (Sprague-Dawely) pancreas were prepared employing raft technique and medium RPMI 1640 supplemented with 10 % FCS. Culture fluid was collected and stored every day up to 7 days and then assessed for its amylase activity and total protein contents. Maximum amylase activity was obtained at 24 hours post culture (PC) period which decreased slowly up to 4th PC day and declined completely on 7th day.

However, addition of pancreatic extracts (10%) to culture medium on 4th day stimulated amylase secretion which was comparable to a value of amylase secretion obtained at 24 hours. This was continued for 4 more days. These studies reveal that organ culture of pancreas could retain their functionality up to 8 days "in vitro" if supplemented with appropriate nutrients.

E. 'In vitro' cultivation of islets of Langerhans on synthetic membranes to develop bio-artificial pancreas :

(Collaborator __ Prof. S. Basu, I.I.T., Powai, Bombay)

Development of bio-artificial pancreas is an exciting area of interest in the field of diabetic research. The basic object of bio-artificial pancreas is to develop a prototype for an implantable artificial endocrine pancreas. For this purpose it is necessary to grow pancreatic beta cells or islets on biocompatible synthetic membrane. Since cells cultivated in this way are protected by a membrane barrier they are not vulnerable to immune rejection.

Prof. S. Basu, has developed synthetic membranes which are required to be tested for their ability to support growth of isolated islets.

With a view to assess the use of such synthetic membranes to support growth of pancreatic islet cells,cultures of islets of Langerhans from rat pancreas were prepared following collagenase digestion. These cultures were seeded in 24 well plates (Nunc) as controls and synthetic cellulose membrane. Cultures were exposed to 16 mM glucose to stimulate insulin release in the medium. Culture medium was collected at fixed time points and stored at -20 °C for insulin assay.

Results of these experiments are presented in Tables IV & V which indicate that islet cultures could be grown on synthetic cellulose membrane without apparent cytotoxicity and the cultures respond to glucose stimulation by release of insulin in the culture medium which corresponds with the duration of exposure. The studies reveal that the synthetic cellulose membrane permits transport of glucose and insulin across the membrane without any apparent cytotoxic effects, indicating the possibilities to use the synthetic membrane for preparation of bio-artificial pancreas.

RESULTS :

TABLE IV. : Insulin release by islets at different post culture period without glucose stimulation:

Time in hours	Insulin level (mIU/ml)*	
	Explants immersed in the medium	Explants on membrane over the medium
24	5.8	6.0
48	7.9	7.5
72	8.8	8.5

* Average of 4 replicate samples.

Table V. : Insulin release by islets cultured on membrane at different time intervals following 16 mM glucose stimulation.

Time in minutes	Insulin level (mIU/ml)*			
	0	10	30	60
Control	5.8	5.8	6.0	6.0
Glucose	—	15.8	36.7	65.0

* Average of 4 replicate samples.

F. Development of culture methods for conducting Studies on allergic rhinitis: (Collaborators Dr. Avinash Bhide, Ruby Hall Clinic, Pune and Dr. Ruby Pawankar, Poona Hospital, Pune)

There is a significant increase in the incidence of allergic rhinitis due to pollutants. Viral and bacterial agents have been identified as causative agents of the disease. With a view to study the co-relationship of causative agents and their mechanism of action experimental studies were planned.

Method for establishment of explant cultures of human nasal mucosa were standardized. It is possible to maintain these cultures in a viable and functional state upto 28 days employing DMEM with 10% FCS Further studies are in progress.

G. ESTABLISHMENT OF HOLLOW FIBRE SYSTEM FOR OBTAINING CELL PRODUCTS

(Collaborator — Dr. S.N. Ghosh, N.I.V., Pune)

The potential for obtaining various secretary products from animal cell cultures need not be emphasised. The technology required for this purpose should provide suitable growth conditions, so as to obtain cells in high numbers, while maintaining their secretory function. The Hollow fibre technology with its multiple parallel capillaries provides an environment to support the growth of cells to high numbers.

Hollow fibre bioreactor, ACUSYST-R (Endotronics, Inc., USA) has been installed. Mouse hybridoma cells against Japanese Encephalitis virus (JE) were cultivated in the bioreactor for seven days and then maintained for a period of 30 days. The cell products harvested at different time points showed increase in titre of anti-JE antibodies progressively. This method permits maintenance of 2×10^8 cells and collection of several litres of the medium containing antibodies.

A collaborative project with Dr. R.A. Mashelkar and Dr. Jayaraman from N.C.L. Pune was undertaken to improve the growth conditions and to optimize the product yield. For the purpose it was essential to characterize the bioreactor with respect to mixing behaviour and retention time of molecules. Attempts have been made in this direction by injecting a pulse of high molecular weight dye and observing the elution pattern. Preliminary data show that the flow in extracapillary lumen is a plug type flow. At different flow rates and direction of the flow within the capillary lumen, it was observed that there was negligible change in the flow pattern.

H. Studies on mosquito cell fusion and premature chromosome condensation:

Studies on fusion of mosquito cells were continued. Experiments were undertaken to optimize the fusion parameters required for cell fusion. These included pretreatment and preparation of the cells, composition of fusion medium, and electrical parameters.

Varied protocols for dissociating cells from monolayers, and for holding them in suspension up to fusion procedure were evaluated in a subjective manner by assessing the fusion frequency under a microscope.

Cells dissociated using hypotonic fusion medium gave better results as compared to cells dissociated with trypsin.

Based on many trials, a medium containing glucose, calcium and magnesium, and tris was formulated, which appeared to give consistent results.

Various fusion field strengths ranging from 0.5 to 15.0 kV/cm and fusion pulse widths from 5 to 50 microseconds were evaluated for this purpose. The response was estimated in terms of percent polynucleation index, as depicted in figure 3. It appears from these results that fusion strength of 5 to 6 kV/cm and fusion pulse width of 20 microseconds yield good fusion. Higher field strengths and longer pulse durations enhanced the polynucleation index even further; however, cell viability decreased considerably. Figure 4 depicts various stages during the course of electrofusion of mosquito-mosquito cells.

The results indicate that it is possible to obtain premature chromosome condensation following electro cell fusion.

Experiments have also been initiated to standardize techniques for obtaining synchronized cell populations at specific points in the cell cycle.

I. Studies on the effect of enhancing factor (EF) on the skin and intestine "in vitro":
(Collaborators Dr. M. G. Deo, Dr. R. Mulherkar, Dr. A. Wagle, CRI, Bombay)

Enhancing Factor (EF) is a 14 kd heat and acid stable polypeptide which was isolated from small intestine of mouse. Dr. Deo's group has shown that EF enhances cellular binding of EGF. Hence, experiments were planned (i) to know whether EF is secreted by skin and intestine "in vitro"; and (ii) to localize the site of secretion of EF and (iii) to know the effect of EF on development and differentiation of mouse skin and intestine "in vitro". For this purpose, initially organ explant cultures of newborn mouse skin were employed. These explant cultures were treated with EGF, EF or a combination of these, for a period varying from 2 to 7 days. Skin samples were fixed for histopathology at specific time intervals for the assessment of growth, development of hair follicles, etc. Initial experimental results indicated that skin explants grew and differentiated "in vitro" as revealed by marked difference in histological structure of 0 day and 8 day fixed skin samples. However, presence of EF in developing skin"in vitro"was not detected as expected.

In order to assess the effect of enhancing factor (EF) on growth and differentiation, organ cultures of newborn mouse were employed. These cultures maintained in Ham F-12 and DMEM (1:1) supplemented with 10 % FCS showed necrotic changes within 48 hours. Addition of insulin, hydrocortisone and EGF to culture medium improved viability of organ explants and extended their survival up to 7 days "in vitro" without necrotic lesions.

Studies are underway to measure uptake of ^{3}H-thymidine in skin organ cultures of new born mice grown in different nutrient media with or without growth factors and EF.

J. Study of stress induced genes
(Collaborator— Prof D.N. Deobagkar, Dept. of Zoology, University of Poona.)

Induction of specific proteins was observed in *A.Stephensi* cells shifted to 35°C (normal growth temperature is 28°C); however, significant heat shock effect was seen at 37°C. Five proteins (hsp83, hsp74, hsp70, hsp63 and hsp55) were induced after heat shock.

To study whether *Drosophila* hsp genes have any homology with *A. stephensi* DNA. Total nuclear DNA was isolated from *A. stephensi* cells, digested with restriction enzymes blotted on nylon membrane and probed with 32p-labeled heat shock protein clone of Drosophila.

PstI digested DNA showed homology in the form of bands but there were many bands and a continuous smear in the background. For other enzymes total smear was lighting up.

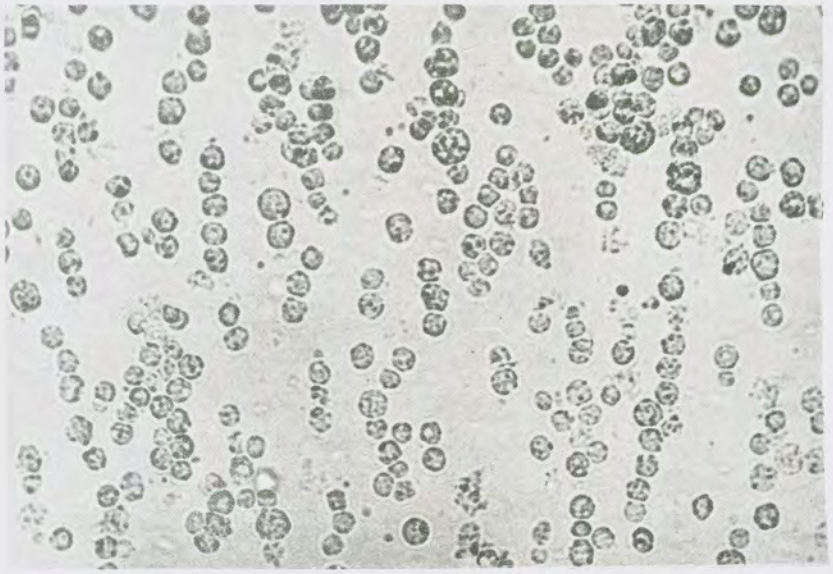
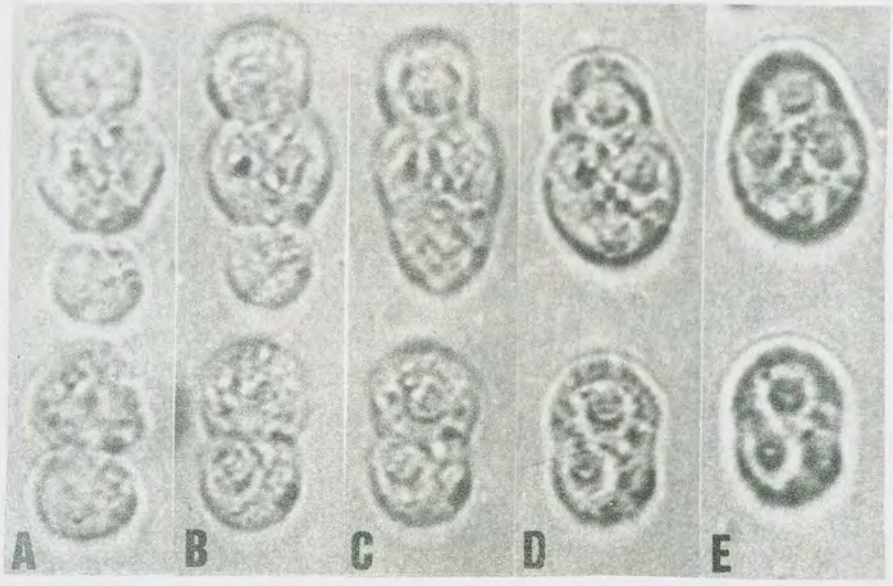


FIGURE 3 :
(a) Alignment of C6/36 cells in pearl chains following application of 1 MHz AC field, 0.21 KV/cm for 30 seconds.



(b) Selected frames from a 'time-lapse' recording, depicting post-fusion rounding off of the cells, The sequence spanned over 3 minutes.

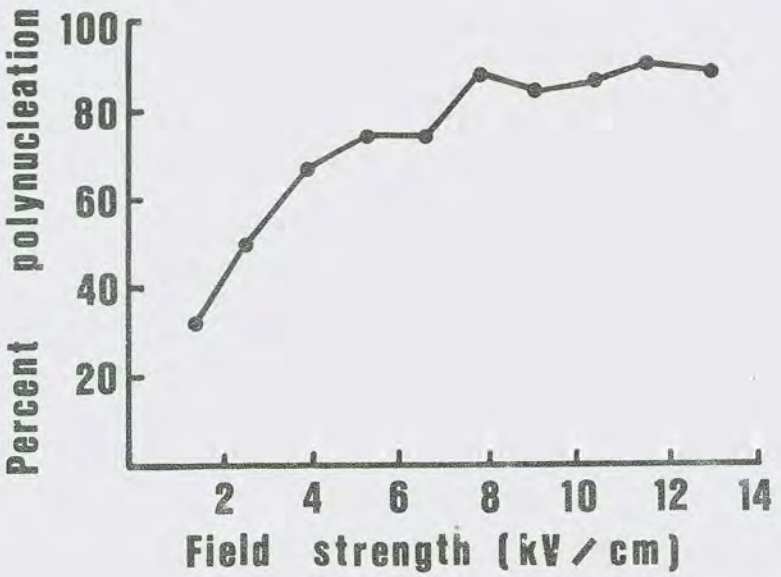
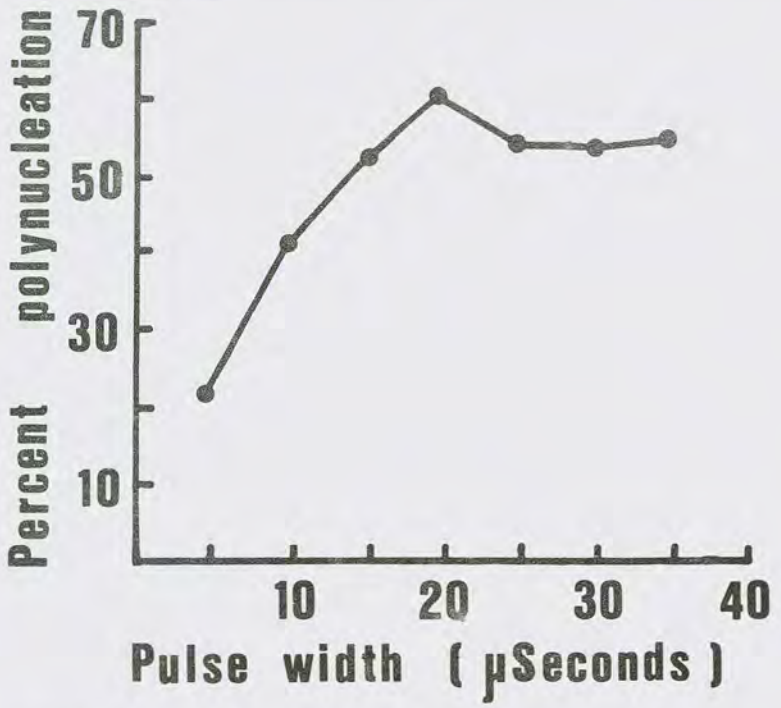


FIGURE 4 :
(a) Effect of fusion field strength on fusion eficiency. five pulses of 20 microseconds pulse width were used for fusion.



(b) Effect of fusion pulse width on fusion efficiency. Five pulses of 5.36 KV/cm were used for fusion.

To check whether heat shock induces any specific puffs in the poytene chromosomes of salivary glands of *A. stephensi,* 4th instar larvae were given heat shock at 37°C for 30 minutes and were fixed, stained with acetocarmine and squashes made to get polytene chromosome spreads.

K. **Regulation in brain hexokinase: biochemistry and molecular biology** (CSIR Project, Prof. U. W. Kenkare):

The objective of the project is to study the genetic relationship between various hexokinase isoenzymes. The question that is now being addressed is whether these isoenzymes are coded for by a single gene differentially processed in different tissues or by different genes. Three cell lines (Sarcoma 180, HepG-2 and Guinea pig brain) were chosen for investigation. The presence of hexokinase was established by spectrophotometric analysis and by activity staining on starch and polyacrylamide gel electrophoresis. It was also confirmed by Western blot technique. Using crude extracts of HepG-2 and S-180 and checking them for the presence of hexokinase using (i) activity staining, (ii) cross reactivity to the brain specific isoenzyme of hexokinase. Thus the presence of hexokinase isoenzymes and its cross reactivity to brain specific isoenzymes (bovine) was established.

To study the molecular biology of these isoenzymes a beginning was made by isolating DNA from the cell lines Sarcoma 180 and HepG-2. A dot blot was set up using a 41-mer single stranded synthetic DNA (concensus sequence for rat brain and human brain hexokinase) as a probe. Hybridization was carried out at 42°C overnight in 25 % formamide. Both S-180 and HepG-2 showed positive signals on the autoradiograph. Experiments with Southern blots are in progress.

In a parallel study, attempts are also being made to isolate and characterise the bovine brain hexokinase gene. For this purpose, a bovine brain cDNA library was screened using (i) oligonucleotide probe referred to above, and (ii) a bovine brain hexokinase specific antibody.

These plaques were picked up and subjected to a second round of screening. However, the experiments so far have not yielded satisfactory results and further efforts in this direction are in progress.

Since the library is in the expression vector gtll, the protein product produced are being screened using hexokinase antibody.

L. Screening of anti malarials on erythrocytic stages of p. falciparam culture strains in vitro. (Collaborators : Dr. B. N. Nagsampagy N.C.L. Pune)

Studies on screening antimalerial compounds. on P. falciparam strains were continued. Xanthium strumarium is a weed found throughout hotter parts of India. It is known to possess powerful anti microbial activity.

Acetone comprising of sesquiterpene lactones extract of aeriai parts of this plant were separated in 4 fractions A, B, C, & D and were tested for their antimalarial activity. Of these fractions 'C' was most active. Four xanthanolides 1, 2, 3, 4 were isolated by column chromatography and preparative thin layer chromatography (PLC) from fraction 'C' and screened.

Results obtained were as follows :

1) Fractions 1 & 2 brought about complete clearance of parasites (erythrocytic stage) in 72 hrs. at a concentration of 200 μg/ml.

2) Fraction 3 however was not effective upto the highest concentration used in the experiment viz. 200 μg/ml.

3) Fraction 4 was found to be the most effective of all the four fractions screened. A dose of 100 μg/ml brought about complete clearance of the parasites in 48 hrs.

Studies on screening of other fractions of X. Strumarium against P. falciparum strains are being continued.

M. COLLABORATING INSTITUTES :

NFATCC has established collaborative programmes with active scientists from the following institutes.

1. National Chemical Laboratory,
   PUNE.
2. Department of Zoology, University of Poona,
   PUNE.
3. Distributed Information Centre, Bioinformatics,
   Department of Zoology, University of Poona,
   PUNE.
4. B.J. Medical College & Sassoon, General Hospital,
   K.E.M.Hospital,
   Ruby Nursing Home,
   Sancheti Orthopaedic Hospital,
   Armed Forces Medical College Aundh Chest Hospital.
   PUNE.
5. Cancer Research Institute,
   BOMBAY
6. Indian Institute of Sciences,
   BANGALORE
7. Centre for Cellular & Molecular Biology,
   HYDERABAD
8. Hindustan Lever Ltd.,
   BOMBAY
9. Jankalyan Eye Bank
   PUNE

III. Teaching and Training:

NFATCC staff actively participated in the teaching programme of M. Sc. (Biotechnology) and M. Sc. (Zoology). Three students have completed the following projects under the supervision of NFATCC staff:

1. Cultivation of keratinocytes in vitro;
2. Studies on differentiation of K 562 cell line; and
3. Induction, isolation and characterization of TK- cells from Chinese hamster ovary cell line.

Following persons deputed from different laboratories in the country have successfully completed their respective specialized training programmes in tissue culture:

1.	Dr. V. THIAGARAJAN Madras	29-3-89 TO 6-9-89
2.	Shri. P. A. WADEGAONKAR New Delhi	3-10-89 TO 17-4-90
3.	DR. M. V. SAMSON Mysore	13-10-89 TO 21-10-89
4.	Mr. MURTHUZA BAIG Mysore	13-10-89 TO 21-10-89
5.	Mr. B. NATARAJAN Mysore	13-10-89 to 21-10-89
6.	Mr. R. K. SHUKLA New Delhi	16-11-89 TO 17-2-90
7.	DR. MARY MOHANKUMAR Kalpakkam	26-2-90 TO MARCH 90
8.	MS. R. L. SARADA Hyderabad	6-11-89 TO 20-11-89
9.	MR. VEERANNA Bangalore	16-11-89 TO 28-11-89
10.	DR. S. K. PANDEY Varanasi	5-2-90 TO 31-3-90

4 . SEMINARS/CONFERENCES/WORKSHOPS/SPECIALIZED TRAINING PROGRAMMES ATTENDED BY NFATCC STAFF:

1.	DR. M. MOJAMDAR	Delivered a talk on MELANOCYTE CULTURES — A NEW APPROACH TO INVESTIGATE ETIOPATHOGENESIS OF VITILIGO/ LEUKODERMA'' at the Zoology Association, Department of Zoology, University of Poona, Pune. January 20, 1990.
2.	DR. P. PARAB	INTERNATIONAL SYMPOSIUM ON MOLECULAR BASIS OF IMMUNOLOGY held at All India Institute of Medical Sciences, DBT, New Delhi. January 22-25, 1990.
3.	DR. J. M. CHIPLONKAR	NATIONAL SEMINAR ON GENETICS, Department of Zoology, University of Mysore, Mysore. February 19-21, 1990. Presented a paper entitled Electrofusion of mosquito cells.''

4.	DR. Y. SOUCHE	INDO-USSR WORKSHOP ON IN VIVO CROSSLINKING OF PROTEIN-DNA AND PROTEIN-DNA INTERACTION Held at Molecular Biophysics Unit, Indian Institute of Science, Bangalore. February 21-25, 1990.
5.	DR. M. R. WANI	HEALTH MONITORING OF LABORATORY ANIMALS held at National Laboratory Animal Centre, CDRI, Lucknow, February 27-28, 1990; and Training in LABORATORY ANIMAL HOUSE TECHNOLOGY, March 1-15, 1990.
6.	DR. J. M. CHIPLONKAR	Delivered a talk on "CELL CYCLE" for undergraduate students (Zoology), March 8, 1990.
7.	DR. M. MOJAMDAR	Delivered a talk on 3-DIMENSIONAL CULTURES OF SKIN AND ITS TRANSPLANTATION TO VITILIGO AND NONHEALING ULCER CASES" at S. K. University, Anantpur, March 19, 1990.
8.	DR. R. R. BHONDE	Delivered two lectures on TISSUE BANKING FOR TRSANSPLANTATION" for Orientation course for College Teachers at University of Poona, Pune. Feb-March 1990.
9.	DR. R. R. BHONDE	NATIONAL SEMINAR ON LIVER DISORDERS, Department of Microbiology, University of Poona, Pune. March 30-31, 1990.
10.	DR. R.R. BHONDE	XIII All India Cell Biology conference and Cell Biology Symposia. CCMB, Hyderabad Dec. 27-29, 1989. Paper presented entitled "In Vitro Cultivation if Islets of langerhans on Synthetic membranes to develop bioartificial pancreas.

. RESEARCH PUBLICATIONS BY NFATCC STAFF:

1. Y. S. SOUCHE, N. RAMESH AND S. K. BRAHMACHARI (1990).
   Probing of unusual DNA structures in topologically constrained form V DNA: Use of Restriction enzymes as structural probe.
   Nucleic Acid Research 18(2): 267-275.
2. P. B. PARAB, G. R. RAJASEKARIAH, P. A. CARVALHO AND D. SUBRAHMANYAM (1990).
   Differential recognition of Brugia malayi antigens by bancroftian filariasis sera.
   IJMR J Med Res A 91: 138-143.
3. PADMA SHASTRY, S. V. IYER, SWATI JAMBOTKAR, PRAVINA KANDOTH AND SUMAN G. KINARE. (1990).
   Antibodies to N-acetyl-glucoseamine and heparin in acute and remissioned phases of rheumatic fever.
   J. Clin. & Lab. Immunol. (In Press).
4. D. SUBRAHMANYAM, P. B. PARAB, G. R. RAJASEKARIAH, W. RUDIN, B. BETSCHART AND N. WEISS. (1990).
   Interaction of monoclonal antibodies with cuticular antigens of filarial parasites, Brugia malayi and Wuchereria bancrofti.
   Acta Tropica 47: 381-390.

6. LOGISTICS

Laboratories were fully established and became functional at the ready constructed building purchased at Kothrud. Major part of the interim facility established in the part of Department of Zoology was shifted at this new premises.

The Memorandum of Understanding between University of Poona and NFATCC was signed by the Director, NFATCC and Vice Chancellor, University of Poona at the Governing Body Meeting held on 22nd January 1990.

The process of selection of Architect for construction of Laboratory and Housing complex on Poona University Campus was completed and M/s. Beri Architects and Engineers Pvt. Ltd., were selected as Architects of NFATCC. The agreement to be signed with the Architect was finalised. The Architect has started working on master plan and preliminary sketches of the Laboratory and Housing complex to be constructed on Poona University Campus.

EFC documents for the 8th Five Year Plan were prepared with the total outlay of Rs. 1788 lakhs. The outlay was duly approved by the Governing Body in its meeting held on 22nd January 1990.

Out of 81 posts sanctioned in the 7th Five Year Plan, 41 posts have been filled. Remaining posts have been advertised and recruitment process is in progress. The staff details are as follows :

1)	Scientific staff		11
2)	Technical Staff		
	a) Laboratory		8
	b) Instrumentation & maintenance		2
3)	Administrative Staff		7
4)	Auxiliary Staff		9
5)	Trainees		
	a) Senior Research Fellows		2
	b) Junior Research Fellows		2
		Total	41
6)	Contractual Services		10

Laboratory equipments worth Rs. 46.98 lakhs were purchased during the period of this report. The equipments have been installed and are used regularly.

The meeting of the Governing Body was held on 22nd January 1990 at the Department of Biotechnology, New Delhi.

The By-laws of NFATCC were approved by the Governing Body. Draft staff rules have been prepared and are awaiting clearance from the Expert Committee constituted for the purpose.

7). COMMITTEES OF INSTITUTION

SOCIETY AND GOVERNING BODY

1. Prof. M.G.K. Menon (President of NFATCC Society)
   Minister of State for Science and Technology
   NEW DELHI

2. Dr. S. Ramchandran (Chairman, Governing Body)
   Secretary
   Dept. of Biotechnology
   Ministry of Science and Technology
   NEW DELHI

3. Prof. S.C. Gupte
   Vice Chancellor
   University of Poona
   PUNE

4. Dr. Smt. Manju Sharma
   Adviser
   Dept. of Biotechnology
   Ministry of Science and Technology
   NEW DELHI

5. Mr. B.K. Chaturvedi
   Financial Advicer
   Dept. of Biotechnology
   Ministry of Science and Technology
   NEW DELHI

6. Dr. Prema Ramchandran
   Dy. Director General
   Indian Council of Medical Research
   NEW DELHI

7. Dr. B.B. Mallick
   Director
   Indian Veterinary Research Institute
   IZATNAGAR

8. Prof. D.N. Deobagkar
   Head, Dept. of Zoology
   University of Poona
   PUNE

9. Dr. K. Banerjee
   Director
   National Institute of Virology
   PUNE

10. Prof. H. Sharat Chandra
Director
Centre For Cellular and Molecular Biology
HYDERABAD

11. Senior Scientist
National Facility For Animal
Tissue and Cell Culture
PUNE

12. Dr. U.V. Wagh (Member Secretary)
Director Incharge
National Facility For Animal
Tissue and Cell Culture
PUNE

SCIENTIFIC ADVISORY COMMITTEE

1. Prof. John Barnabas (Chairman)
Head, Biochemical Sciences Div.
National Chemical Laboratory
PUNE

2. Dr. A.N. Bhisey
Head, Cell Biology Div.
Cancer Research Institute
BOMBAY

3. Dr. B.D. Survashe
General Manager
Ventri Biological Laboratory
PUNE

4. Dr. B.N. Dhawan
Director
Central Drug Research Institute
LUCKNOW

5. Dr. S.N. Ghosh
Ex. Director Grade Scientist
National Institute of Virology
PUNE

6. Dr. B.U. Rao
Ex. Joint Director
Indian Veterinary Research Institute
BANGALORE

7. Commandant
Armed Forces Medical College
PUNE

8. Prof. A.S. Kolaskar
Officer In Charge
Bio-Informatics Centre
DIC, University of Poona
PUNE

9. Prof. B.K. Bachawat
Head, Dept. of Biochemistry
Delhi University
South Campus
NEW DELHI

10. Prof. D.N. Deobagkar
Head, Dept. of Zoology
University of Poona
PUNE

11. Prof. G.P. Talwar
Director
National Institute of Immunology
NEW DELHI

12. Prof. G. Padmanabhan
Prof. of Biochemistry
Indian Institute of Sciences
BANGALORE

13. Prof. P.N. Tandon
Dept. of Neurosurgery
All India Institute of Medical Sciences
NEW DELHI

14. Dr. U.V. Wagh
Director Incharge
National Facility
For Animal Tissue and Cell Culture
PUNE

FINANCE COMMITTEE

1. Mr. B.K. Chaturvedi (Chairman)
   Financial Adviser
   Dept. of Biotechnology
   Ministry of Science and Technology
   NEW DELHI

2. Prof. H Sharat Chandra
   Director
   Centre For Celluar and Molecular Biology
   HYDERABAD

3. Prof. Mahendra Singhvi
   Tata Management Training Centre
   PUNE

4. Dr. S. Iqbal
   Scientist, Planning and Co-ordinating Officer
   National Chemical Laboratory
   PUNE

5. Dr. U.V. Wagh
   Director Incharge
   National Facility For Animal Tissue and Cell Culture
   PUNE

BUILDING COMMITTEE

1. Dr. U.V. Wagh (Chairman)
   Director Incharge
   National Facility For Animal Tissue Cell Culture
   PUNE

2. Mr. S.R. Sapra
   Director (Finance)
   Dept. of Biotechnology
   Ministry of Science and Technology
   NEW DELHI

3. Dr. M.K. Goverdhan
   Dy. Director
   National Institute of Virology
   PUNE

4. Prof. V.R. Sardesai
Principal
BKPS College of Architecture
PUNE

5. Cheif Engineer
PWD, Pune Div.
PUNE

6. Mr. B. Bose
Sr. Manager (Admn)
National Institute of Immunology
NEW DELHI

7. Prof. D.N. Deobagkar
Head, Dept. of Zoology
University of Poona
PUNE

PURCHASE COMMITTEE

1. Dr. S.N. Ghosh (Chairman)
Ex. Director Grade Scientist
National Institute of Virology
PUNE

2. Prof. John Barnabas
Head, Biochemical Sciences Div.
National Chemical Laboratory
PUNE

3. Prof. D.N. Deobagkar
Head, Dept. of Zoology
University of Poona
PUNE

4. Major. P.K. Bapat
Administrative Officer
National Facility For Animal
Tissue and Cell Culture
PUNE

5. Mr. T.G.R. Pillai
Accounts Officer
National Facility For Animal
Tissue and Cell Culture
PUNE

6. Senior Scientist
National Facility For Animal
Tissue and Cell Culture
PUNE

7. Dr. U.V. Wagh
Director Incharge
National Facility For Animal
Tissue and Cell Culture
PUNE

INSTITUTIONAL CO-ORDINATION COMMITTEE

1. Prof. S.C. Gupte (Chairman)
Vice Chancellor
University of Poona
PUNE

2. Adviser (B)
Dept. of Biotechnology
Ministry of Science and Technology
NEW DELHI (Nominee of DBT)

3. Joint Secretary, Finance
OR
His representative, Department of Biotechnology
Ministry of Science and Technology
NEW DELHI

4. Nominee of Director General
Indian Council of Medical Research
NEW DELHI

5. Head, Dept. of Zoology
University of Poona
OR
Co-ordinator/Head Biotechnology Training Programme
University of Poona
PUNE

6. Dr. U.V. Wagh
Director Incharge
National Facility For Animal Tissue and Cell Culture
PUNE