ANNUAL REPORT
1993 - 94

REGIONAL MEDICAL RESEARCH CENTRE
(INDIAN COUNCIL OF MEDICAL RESEARCH)
BHUBANESWAR, ORISSA

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PREFACE

This Centre, located in the upper part of Eastern Coastal Region has been traditionally having filariasis, as its primary focus of research for several years. Based on this tradition, majority of projects continued to have their thrust on various basic and applied aspects of human filariasis. Studies on different components of prevention and treatment of human filariasis are in progress with the assistance of international agencies. Different animal experimental models have been attempted with varying degrees of success to understand the immunological profile at various stages of filarial parasite. A programme is being developed to move out from the laboratories to the communities to document the health and nutritional status of different areas of the region. Field Unit at Jeypore has been strengthened. Attempts are being made to study haemoglobinopathies at community level, rather than continuous monitoring in a hospital setting. Epidemic of diarrhoeal disorders was investigated in the undivided district of Koraput. Interaction with the sister institutions of the Council and other agencies and participation in scientific deliberations continues to be a major activity of the Centre. I take this opportunity to thank all our SAC members and staff of this Centre for helping me to steer this Centre with greater confidence and commitment. We are grateful to Dr.S.P.Tripathy, Ex-Director General and founder Director of this Centre for helping us to overcome our problems and for providing guidelines for our activities. We extend a warm welcome to Dr.G.V.Satyavati, our new Director General and we look forward to work under her leadership.

K. Satyanarayana

Place: Bhubaneswar
Date: 15.05.94

(DR.K.SATYANARAYANA)

Director

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R.N.Nayak	B.A.	Census Taker

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Field Research Stations

RMRC Field Unit,Sambalpur(Burla)

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C.Nayak		Sweeper-cum-Attnt.

RMRC Field Unit, (Jeypore)

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S.Nayak		L.D.C.
S.K.Das	B.Com	L.D.C.

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B.S.Rao		U.D.C.

Supporting Staff

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R.K.Patra	Insect Collector
B.Pradhan	Insect Collector
C.S.Tripathy	Insect Collector
S.S.Beuria	Insect Collector
G.Simhachalam	Insect Collector
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K.C.Dalai	Generator Operator
J.Behera	Pump House Operator
B.K.Moharana	Plumber-Cum-Carpenter
Md.Daulat Khan	Driver
Sibaram Patra	Driver
R.Pradhan	Driver
Anakar Nayak	Driver
A.R.Khan	Driver
P.K.Behera	Driver
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B.K.Kanhar	Laboratory Attnt.
C.R.Samantaray	Laboratory Attnt.
H.K.Jena	Field Attnt.
R.K.Hembram	Field Attnt.
S.K.Das	Animal House Attendant
S.K.Mallick	Animal House Attnt.
A.Senapati	Animal House Attnt.
S.C.Das	Animal House Attnt.
K.C.Jena	Office Attnt.
R.C.Dash	Office Attnt.
J.Naik	Sweeper-c-Attnt.
Banamali Naik	Sweeper
K.G.Samal	Sweeper
Banamali Sahoo	Gardener
Sankar Pr.Sharma	Watchman
M.B.Thappa	Watchman
R.S.Rai	Watchman
Som Pr.Sharma	Watchman
T.Bahadur	Watchman
R.S.Bahadur	Watchman

CONTENTS

Page No.

I. PARASITE IMMUNOLOGY DIVISION

1.1 Immunological studies of filariasis:

Scientists: Dr. Manoj K. Das
Dr.M.K.Beuria
Technical Staff: Mr.H.S.Naik

This Centre has earlier described the levels of IgE antibodies to infective- larvae (L_3) antigen of *Wuchereria bancrofti* and to a purified allergenic fraction (Fr.III) isolated from bovine parasite *Setaria digitata* (which is available relatively in higher quantity) in people living in filaria endemic regions of Orissa. Immunologic changes seen in asymptomatic microfilaraemic patients (AS) after treatment with diethylcarbamazine (DEC) have been documented previously by other workers. IgE antibodies have not received adequate attention following drug therapy. IgE responses to purified allergen (Sd30) and to infective-larvae(L_3) antigen for comparison were followed after DEC treatment in asymptomatic microfilaraemic patients (n=18). A transient increase, though significant was noticed by day 15 for IgE. This was followed by a sharp decline leading to values considerably lower than the pre-treatment IgE values. The reduction observed at about 1 month reached nadir, around 3 months after treatment with DEC. It is to be noted that IgE levels to Fr.III underwent greater changes than that to W.bancrofti L_3 antigen. At 3 months, fifteen patients exhibited reduction in IgE to Sd30 antigen ranging from 72 to 100%, two patients however did not exhibit any change during the therapy. The reduction continued till 8 months after the treatment, during which all the patients remained amicrofilaraemic.

In a separate field study an attempt has been made to compare serum IgE and IgG subclasses with skin test reactivity to Fr.III antigen. Immediate type hypersensitivity reaction (ITH) was evaluated in 103 individuals living in a filarial region (Bajapur village, Khurda district) of Orissa. The extent of ITH positivity to Fr.III in infected individuals ranged from 20% in chronic filarial patients (n=20) to 56% in asymptomatic microfilaraemic (AS) carriers (n=41). About 62% of endemic normals (n=42) were also ITH positive. IgE and IgG_4 levels were found to be inversely related only in the AS group with respect to ITH reaction. Asymptomatic patients (AS) with positive ITH reaction had higher IgE but lower IgG_4 than ITH negative individuals from the same group. EN (endemic normals) and CP (chronic filariasis) groups did not exhibit such reciprocal relationship in IgE/IgG_4 levels. The role of IgG_4 as blocking antibodies is to be noted.

1.2. Characterization of anti-filarial activity in the plant *Streblus asper* (Project completed).

Scientists Dr.Manoj K.Das
Dr.D.Das

Earlier this centre had detected an agent with potent *in vitro* anti- filarial activity in aqueous bark extract of *Streblus asper*. About 100 gm of stem bark after extraction yielded an average of 9 gm of lyophilized material. The agent which was of high molecular weight was purified by gel-filtration (Sephadex G-100). Purified active component constituted 2% of lyophilized extract. The agent contains carbohydrate residues (5%) which were crucial for filaricidal activity as periodate oxidation abolished the activity. The dose required for single worm toxicity *in vitro* was determined. An amount of 70 ug of purified agent could kill an adult (female) *S.digitata* as checked by MTT-formazan assay.

1.3. Antigenicity of filarial enzymes in endemic population.

Scientists Dr.Manoj K. Das
Ms.M.S.Bal
Technical Staff Mr.H.S.Naik

The following enzymes of filariae (human and cattle) parasites were investigated.

1. Superoxide dismutase (SOD) : This enzyme was purified to 29 fold from adult *S.digitata*. It constituted 1.6% of total soluble proteins. The distribution pattern of enzyme activity showed that the majority of SOD activity is in the surface components of parasites, indicating its importance in parasite defence. SOD activity was detected in excretory-secretory products of adult *Setaria*. The amount of SOD released peaked at 24 hr and then declined rapidly. Filarial IgG (from chronic patients of filariasis) could inhibit the SOD activity in *B.malayi* L_3, *S.digitata* adult and ES. IgG from non-endemic normal individuals did not neutralize the activity. Higher extent of seropositivity to SOD was noticed in chronic filariasis patients (100%) and asymptomatic microfilariae carriers (76.6%) compared to endemic normals (33%, n= 30 in each group). Individuals of non-filarial regions (Jeypore, Koraput) of Orissa were seronegative to SOD.

Proteases: Proteolytic activities were detected as "somatic" and "secreted" (ES products) forms in adult *Setaria*. It is also detected in infective-larval (L3) and microfilarial (mf) stages of human parasite *B.malayi* and *W.bancrofti*. Two proteases with high activities (SdPI and SdPII)

were purified from somatic extracts of *S.digitata*. High antibody levels to SdPI were detected only in filariae infected cases (asymptomatic mf carriers (AS) and chronic patients (CP)) compared to endemic normals (EN). For example, 42 out of 44 in CP group (95.4%), 62 out of 71 in AS (87.3%), 7 out of 45 in EN (15.5%) and none out of 20 in control group (non-filarial region) were seropositive. The two individuals who were seronegative in CP group are found to be suffering from hydrocele. However, EN and AS individuals could not be differentiated by antibody levels using whole extract of adult worm as antigen. These results would indicate a good potential for SdPI as a marker of infection. Antibody response to SdPII and the proteases in ES are being looked into.

Glutathione-S-transferase (GST):

The enzyme was affinity purified on a Glutathione-agarose column and an average yield of 0.5% from *S.digitata* adult was obtained. An enhanced enzymatic activity was noticed in affinity purified preparation over the crude parasitic extract. Immunological response to purified GST in filarial sera is currently under investigation.

1.4. Immunological investigations in human filariasis using purified antigens.

Scientists	Dr.Manoj K.Das
	Dr.M.K.Beuria
	Ms.M.S.Bal
Technical Staff :	Mr.H.S.Naik
Starting date	October 1993

Characterization of filarial allergen Fr.III

The molecular wt. of Fr.III of cattle filarial parasite *Setaria digitata* was determined to be 30 KDa by SDS-PAGE, which is also supported by sephadex (G- 200)column chromatography.

Proteolytic activity: In the initial experiments it was observed that Fr.III (2ug) was able to clear a zone of hydrolysis in casein-agar plate. The specific proteolytic activity of Fr.III against azocoll, a general protease substrate, was 993.4±60.3 as compared to 103.2±32.21 of an adult *S.digitata* homogenate. The activity peaked at pH 7.0 and then declined in alkaline pH. Protease activity was completely absent at pH lower than 5.0. The effects of various protease inhibitors on the hydrolysis of azocoll by Fr.III were evaluated. Activity was completely inhibited by EDTA, Iodoacetamide and mercuric chloride. Phenylmethyl sulfonyl fluoride (PMSF), a serine proteinase inhibitor, has no inhibitory effect. These data indicate that Fr.III could be a metal dependent cysteine protease. This is the first report in filariasis demonstrat-

ing an allergen to be a proteolytic enzyme (cysteine proteinases are widely distributed and are often allergenic: Papain, for example).

Detergent soluble fraction of *S.digitata* (DSSd):

Work is continuing on DSSd antigen, antibody (IgG) response to which was particularly suppressed in asymptomatic microfilaraemic (AS) carriers. Such AS individuals on DEC treatment exhibited 10 fold enhancement in IgG titer, similar to the levels in endemic normals. Chronic filarial patients had the highest level. Preliminary studies indicate that IgM levels similar to IgG are also depressed in AS individuals. Antibody response to this antigen is negligible in the sera from non-filarial (Koraput, Jeypore) regions of Orissa.

Training Programme:

A student from the department of Biotechnology (B.H.U.) received laboratory training for one week (First week of November, 1993) in immunological methodologies.

Other important events:

1. Dr.M.K.Das participated as a faculty in ICMR-WHO Workshop on Biomedical communication September 28 - October 1, 1993 held at RMRC, Bhubaneswar.

2. Dr.M.K.Das was appointed as Ph.D. examiner to review a Ph.D. thesis submitted in the Dep. of Biochemistry and Biophysics, University of Delhi.

Publications:

A.K. Praharaj and M.K. Das Enhanced antibody response to a detergent soluble antigen in human filariasis after treatment with diethylcarbamazine. J. Bioscience (In Press)

II. APPLIED IMMUNOLOGY

2.1 Immunological studies on antibodies to Diethylcarbamazine- an approach for developing an immunoprophylactic agent against filarial parasite.

Scientists	Dr. B. Ravindran
	Dr. A.K.Satapathy
	Ms. S. Mukhopadhyay
Technical Staff :	Mr. P.K. Sahoo
Starting date :	November, 1990

Previous investigations from our laboratory had revealed the existence of antigenic mimicry between sheath antigens of microfilariae and the antifilarial drug, Diethycarbamazine (DEC). Polyclonal antibodies to DEC raised in Rabbits and *Mastomys* sp. were found to cross-react with mf sheath antigens of *W.bancrofti, B.malayi* and also *Setaria digitata*. During the last one year, the laboratory addressed itself to two major aspects of this work:

(i) Identification and immunochemical characterization of the filarial antigen that cross-reacts with anti-DEC. This was considered essential for evaluating the role played by this antigen during the natural course of the disease in human communities.

(ii) Studying the *in vivo* significance of anti-DEC in animal models- to monitor the fate of circulating microfilariae in the presence of anti-DEC, after active and passive immunization procedures.

Solubilised somatic extracts of *S.digitata* were found to contain an antigen that could precipitate in agarose with Wheat germ agglutinin (WGA). Since WGA reacts very strongly to mf sheath of filarial parasites, attempts were made to affinity purify this antigen through a column of WGA-Sepharose. The affinity purified antigen was found to react with anti-DEC as revealed by ELISA. A lectin- ELISA involving a double sandwich method using WGA and WGA-peroxidase was developed to detect and quantify the WGA-binding filarial antigen. The WGA binding antigen was found to be heat stable (100° C for 5 min) and was extractable using any of the non-icnic detergents. It was also found to be metabolically released as 'ES products' in in vitro culture systems. The WGA- binding antigen was found to have a very large molecular

weight (about $2x10^5$) getting eluted at the void volume in Sephadex G-200 column and has a slow ß- electrophoretic mobility. Further work is in progress to study the immune response to the WGA binding antigen in filariasis patients(with different clinical manifestations) and in experimental filariasis in animal models.

Attempts have been made to study the *in vivo* effect of anti-DEC on circulating microfilariae in two animal models.

a) *B.malayi* - L_3 infected *Mastomys* sp and
b) *S.digitata* adult worms implanted *Mastomys* sp.

The former (a) is a system wherein there is continuous production of microfilariae by adult worms and in the latter (b) mf are released into the peritoneal cavity by gravid implanted adults (introduced by surgical procedures) and are present in the circulation for about 3 months post implantation. Such *in vivo* investigations can not be undertaken for W.bancrofti, since no laboratory animal model exists for this parasite. Since anti-DEC antibodies react with the surface of mf, their effect on circulating Setaria mf was studied by immunization of microfilaraemic animals with MPCA-BSA. Two to three doses were injected intraperitoneally without adjuvants at fortnightly intervals. Results so far have indicated significant lowering of microfilaraemia of *S.digitata* mf in *Mastomys* sp. However anti-DEC antibodies did not mediate mf clearance in *B.malayi* infected animals but subsequent administration of very small doses (10 mg/kg) of DEC for 5 days had shown complete clearance of microfilaremia in 7 days in such pretreated animals [the standard dose of 250 mg/kg for 5 days does not lead to total clearance of mf in *B.malayi* infected *Mastomys* sp.]. These observations, although preliminary indicate the potentiation of microfilaricidal activity of DEC once the animal has been made to produce anti-DEC antibodies by experimental (active) immunological procedures. This is in conformity to the known mode of action of DEC. Investigations are now under progress to study the relationship between anti-DEC titres in asymptomatic mf carriers and their ability to clear microfilariae with the administration of DEC at much reduced dose levels.

2.2 Studies on anti-sheath antibodies in Bancroftian Filariasis.

Scientists	:	Dr.B.Ravindran
		Dr.A.K.Satapathy
Technical Staff	:	Mr.P.K.Sahoo
Starting date	:	June'86

Antibodies reacting to the sheath of microfilariae have been incriminated in the elimination of circulating mf in bancroftian filariasis. Previous investigations from our laboratory had established an inverse relationship between the presence of mf in circulation and the presence of anti-sheathantibodies. However, it was not possible to explain the presence of anti-sheath antibodies in a small proportion (about 20%) of asymptomatic mf carriers who had shown antisheath antibodies in circulation, but had mf in circulation. Conventionally anti-sheath antibodies are quantified by immuno-fluorescence assay using a batch of mf purified from one or two mf carriers. It is generally assumed that the mf sheath is antigenically homogenous and non-polymorphic. Assumption of a possible existence of polymorphism of sheath antigens should explain the presence of anti-sheath antibodies in mf carriers since generally autologous mf are not used for the immunofluorescence assay.

Microfilariae of *W.bancrofti* purified from five different mf carriers were used separately as antigen to identify anti-sheath antibodies. Sera from these individuals did not react with mf prepared from their own blood (autologous). These five mf preparations were tested with (heterologous) sera from 15 asymptomatic mf carriers (AS cases) and sera from 7 patients with chronic filarial manifestations (CP). All the 7 chronic patients sera reacted with all the 5 mf preparations. In the case of AS individuals, sera from 4 persons (27%) reacted with one mf preparation and for two more mf preparations 2 individuals showed reaction (13% each; Figure-1). For the remaining 2 mf preparations none of the 15 AS individuals showed any reaction. Such a differential reactivity to the sheath was found to be a feature of antibodies in AS cases only. Sera of seven amicrofilaraemic patients with elephantiasis (chronic filariasis) reacted uniformly with all mf preparations. The intensity of the reaction (immuno-fluorescence) was graded from 1 + to 4 +. Mild grade reaction (1 +) was seen in 47% of AS individuals (Figure-2). Higher grades of reactivity (3 + and 4 +) was seen in 43% and 29% of chronic cases and none of the AS individuals had shown reactivity with this intensity.

Chronic filariasis patients (CP) not only reacted with all the 5 mf preparations made, but also showed intense reaction in most instances. These findings appear to indicate the possible existence of polymorphic and conserved antigens on the mf sheath in *W.bancrofti*. Asymptomatic mf carriers (AS) appear to respond only to the variable antigen(s) while amicrofilaraemic chronic patients (CP) possess antibodies to the conserved antigens that could in turn facilitate the removal of all "strains" of circulating mf. Extending the investigations of the present study to a larger panel of mf from different geographical regions may lead to additional serological evidence for establishing the existence of various "strains" of *W.bancrofti*.

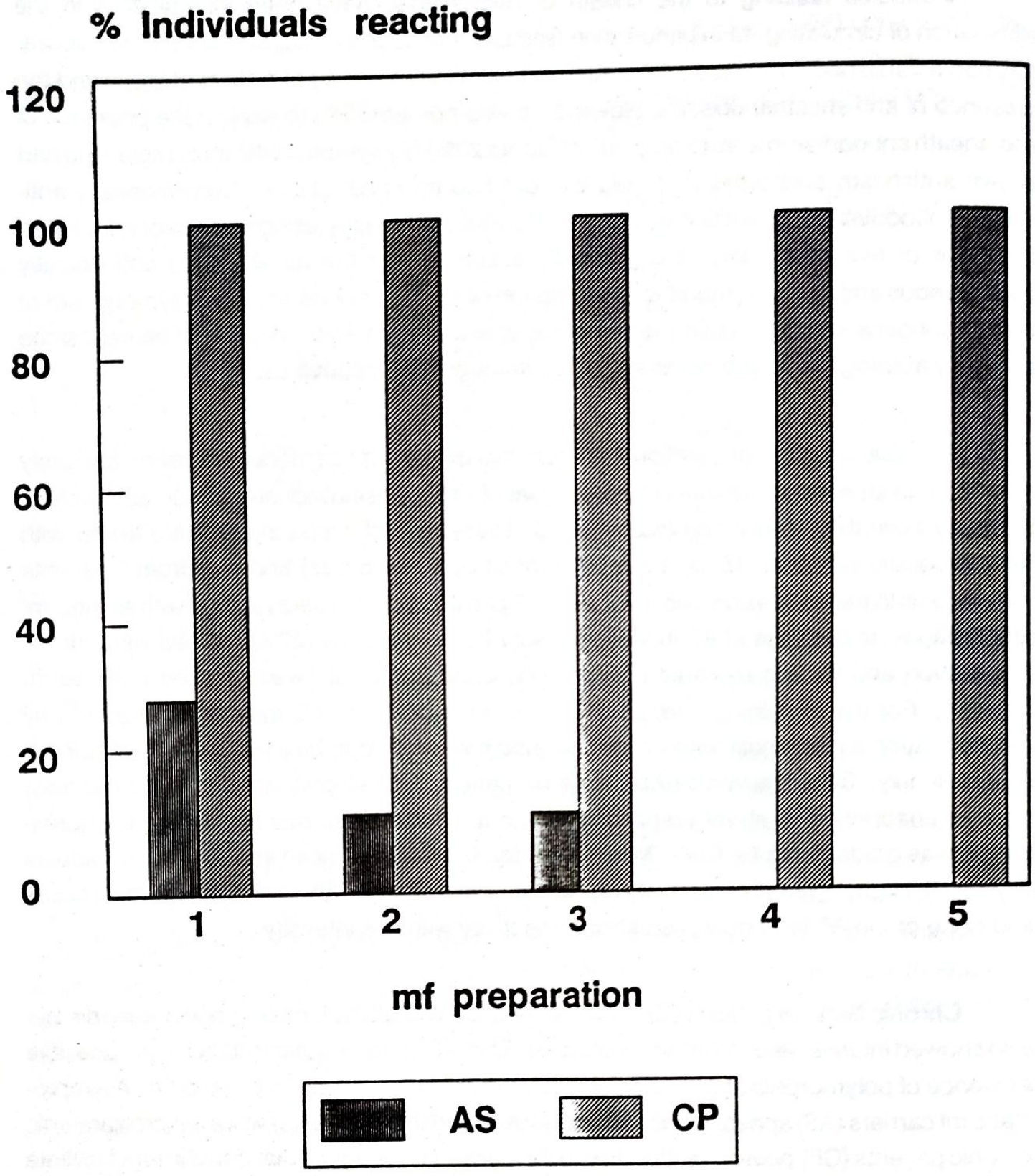

Fig - 1 Percentage of AS and CP cases showing reactivity to the 5 mf preparations from different persons

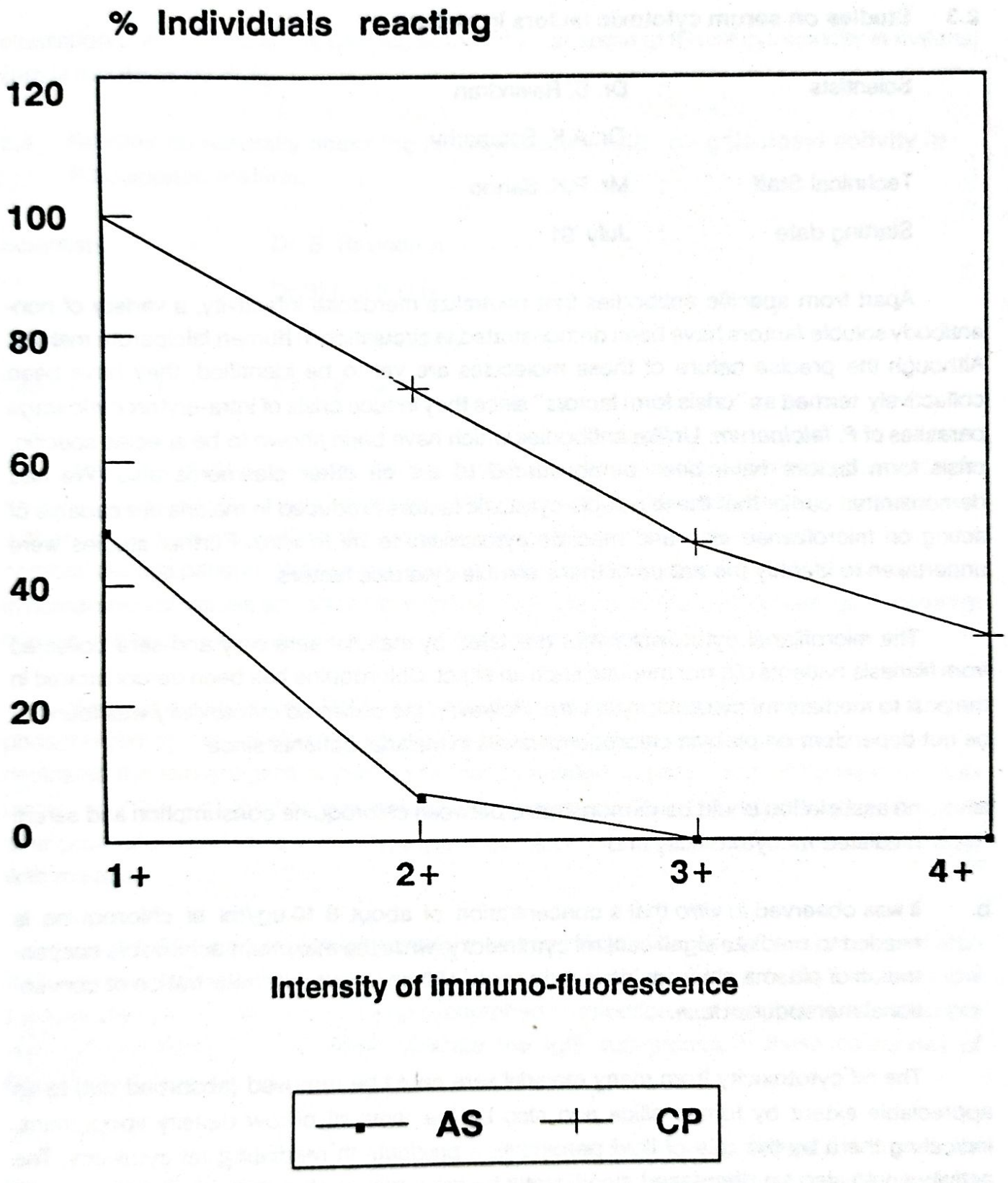

Fig - 2 Intensity of reaction among groups for anti-sheath anti bodies

2.3 Studies on serum cytotoxic factors in malaria

Scientists	:	Dr. B. Ravindran
		Dr. A.K. Satapathy
Technical Staff	:	Mr. P.K. Sahoo
Starting date	:	July '91

Apart from specific antibodies that neutralize merozoite infectivity, a variety of non-antibody soluble factors have been demonstrated in circulation in human falciparum malaria. Although the precise nature of these molecules are yet to be identified, they have been collectively termed as "crisis form factors" since they induce crisis of intra-erythrocytic stage parasites of *P. falciparum.* Unlike antibodies which have been shown to be species specific, crisis form factors have been demonstrated to act on other plasmodia also. We had demonstrated earlier that these soluble cytotoxic factors produced in malaria are capable of acting on microfilariae also and mediate cytotoxicity to mf *in vitro.* Further studies were undertaken to identify the nature of these soluble cytotoxic factors.

The microfilarial cytotoxicity was mediated by malarial sera only and sera collected from filariasis patients did not mediate such an effect. Chloroquine has been demonstrated in the past to mediate mf cytotoxicity *in vitro.* However, the observed cytotoxicity was found to be not dependent on plasma chloroquine levels in malarial patients since

a. no association could be demonstrated between chloroquine consumption and serum mediated mf cytotoxicity and

b. it was observed *in vitro* that a concentration of about 8-10 ug/ml of chloroquine is needed to mediate significant mf cytotoxicity, while the maximum achievable concentration of plasma chloroquine is only about 150 ng/ml after administration of conventional therapeutic dose.

The mf cytotoxicity from many malarial sera could be removed (absorbed out) to an appreciable extent by fumed silica and also by the removal of low density lipoproteins, indicating there by the role of lipid peroxidation products in mediating mf cytoxicity. The activity could also be decreased significantly by the addition of nordihydroguaiaretic acid (NDGA), a phospholipase A_2 (PLA_2) inhibitor. Experiments performed with porcine pancreatic PLA_2 indicated that mf as well as adult parasites are susceptible to very low concentrations (< 2 ug/ml) of PLA_2. Since increased levels of extracellular PLA_2 has been demonstrated in

circulation during malaria, it is concluded that at least some of the mf cytotoxicity in malarial sera is mediated by PLA_2.

2.4 Studies on naturally occurring autoantibodies with α - galactosyl activity in *P.falciparum* malaria.

Scientists: Dr. B. Ravindran
Dr.Bidyut K.Das
Dr. A.K. Satapathy

Technical Staff: Mr. P.K. Sahoo

Naturally occurring autoantibodies have been shown in recent times to play a role in immune response to various parasites. We had earlier demonstrated the presence of autoantibodies with specificity to alpha linked galactose in cerebrospinal fluid (CSF) of cerebral malaria patients. We had also demonstrated the presence of α - galactose residues in human neural tissues and attempts were made to correlate the levels of anti-gal in cerebral malaria cases as well as uncomplicated *P.falciparum* malaria.

Another autoantibody that has attracted the attention in malarial immunology is anti-phospholipid(anti-PL) antibodies, since they have been demonstrated (in animal models) to neutralise the exo-antigens of plasmodia that stimulate the production of Tumour necrosis factor - α (TNF - α) Studies in murine malaria have shown that anti-phospholipid antibodies offer protection (anti-toxic immunity) against some of the clinical manifestations associated with malaria.

Anti-gal levels were found to be elevated in uncomplicated cases of *P.falciparum* and also in cerebral malaria cases who responded to treatment with quinine, while the titres were low in those cases of cerebral malaria who succumbed to infection and lost their lives. Attempts are now underway to qualitatively analyze the IgG sub-groups in these categories of *P.falciparum* malaria.

IgG anti-phospholipid (anti-PL) antibodies were found to be significantly high in those cases of cerebral malaria where the prognosis was good, in comparison to cerebral malaria patients who died despite administration of intravenous quinine. Interestingly such an association was not seen when IgM antibodies to phosphatidyl-choline were measured. More significantly there was a significant inverse relationship between IgG_1 anti-phospholipid

antibodies and levels of circulating Tumour Necrosis factor- α . These observations clearly indicate the possibility of anti-PL neutralising the toxic exo-antigens of *P.falciparum* that stimulate TNF - α production which has been found to be responsible for many of the clinical manifestations seen in malaria. Higher levels of anti-PL, with its inverse relationship to TNF - α may thus be expected to confer benefit to patients by reducing the risks of some of the clinical manifestations associated with malaria.

2.5 Development of a laboratory animal model for *Wuchereria bancrofti*

Scientists : Dr B.Ravindran

Dr A.P.Dash

Ms.S.Mukhopadhyay

Technical staff : Mr P.K.Sahoo

W.bancrofti is primarily responsible for more than 90% of human lymphatic filariasis cases occurring globally. However the parasite is yet to be adapted in laboratory animals. Various attempts in the past to adapt in jirds, gerbils, *Mastomys sp,* and even in nude mice have met with little success. Although the infective larvae (L_3) undergo one or two moultings, establishment of fertile adults (both male and female) and the consequent production of microfilariae in circulation has not been achieved so far. Establishment of a microfilaraemic model (without the development of adult worms) in small laboratory animals itself may find immense application for evaluation of new antifilarial drugs, in understanding the biological processes involved in the clearance of microfilariae from the circulation and more importantly, in identifying the protective surface antigens on mf sheath that play a vital role in the elimination of microfilariae. For this, breeding pairs of CBA/N strain of mice were recently procured from Jackson Laboratories, USA, with the help of NII, New Delhi. These animals are genetically deficient in immune response to T-independent antigens. Earlier studies have indicated that some of the filarial antigens, particularly those present on microfilarial sheath are carbohydrate in nature and may thus be T- independent antigens. Inability to mount an immune response to these antigens is expected to sustain the filarial parasites (mf) in circulation. Experimental studies have been initiated in this direction.

Since the scarce availability of parasite material of *W.bancrofti* from infected human subjects would not allow different experimental combinations (viz., doses, routes, immunosuppresssion with different agents etc) it was decided to use a more easily available cattle filarial parasite Setaria digitata in *Mastomys sp.* Although it was originally intended to be a model parasite for standardization of experimental methodologies, the results have indicated

a possibility for further studies and utilization of this animal model for purposes other than it was originally conceived. Intraperitoneal implantation of gravid adult female worms of *Setaria digitata* in *Mastomys sp.* resulted in microfilaraemia from 7th day and it lasted for nearly 110 days. Consistantly high microfilaraemia was found only in animals weighing > 45 gms and < 70 gms. There was a statistically significant inverse relationship between the number of mf in circulation and decrease in body weight of animals- post implantation. Animals that registered greater loss of body weight after surgical implantation of gravid worms, supported relatively greater number of mf in circulation.

Apart from anemia and leucopenia there was a prominent eosinophilia in the worm implanted animals. The nine fold increase in the total eosinophil count peaked around the 21st day and normal levels were reached only after 50 days. This is somewhat similar to eosinophilia observed in the mankind, after exposure to parasites. There was a four fold increase in spleen weight by 7th day and the splenomegaly regressed by 8-9 weeks post implantation. The immune response to three different antigenic fractions of somatic antigen (purified by gel filtration on Sephadex G-200) and ES products were studied in groups of *Mastomys* sp. implanted with live or dead (cold stunned) worms. Antibodies to Fraction I & III were high in animals implanted with live worms, while in animals implanted with dead worms the response was generally poor. Implantation of adult worms resulted in generalised immunosuppression as revealed by immune response to tetanus toxoid administered one week after implantation.

We may thus have two kinds of microfilaraemic models, the one that is already established for cattle parasites, by intraperitoneal implantation of gravid female worms and the other model [CBA/N mice] for *W.bancrofti* microfilariae by maintaining mf derived from patients, for longer periods for experimentation under *in vivo* conditions. These microfilaraemic models could be used to monitor the efficacy of DEC on elimination of circulating mf indicating the utility of this model, in the *in vivo* evaluation of new antifilarial drugs. The *Setaria* model could also be used for monitoring the immunological effect of anti-parasite antibodies (for more details please see section No.2.1. wherein the model has been used for anti-DEC antibodies). The induction of eosinophilia is another feature that merits detailed study.

Activities :

1. Dr.B.Ravindran was invited to attend a "Brain storming session on Molecular Immunology" in Delhi University (South Campus) Jan 29-31, 1993 and presented a working paper on "Antigenic mimicry between Diethylcarbamazine and filarial parasites".

2. Dr.B.Ravindran was invited by Dept. Zoology, Utkal University, Bhubaneswar to give lectures in the Refresher course for College Teachers. Two lectures on 'general immunology' were given by him on 6th and 10th Dec.1993.

3. Dr.B.Ravindran delivered a lecture on "Role of immunity and vaccines in relation to malaria" on 22nd July 1993 at Hotel Kalinga Ashok, Bhubaneswar in a Seminar/ Workshop on 'Action plan for Districts of Orissa in control of Malaria'.

4. Dr.B.Ravindran participated as a faculty member in "ICMR-WHO Workshop on Bio-medical communications" held at RMRC,Bhubaneswar; November 29 to December 1; 1993.

5. Dr.B.Ravindran was invited to participate in the "Second brainstorming sessions on molecular Immunology" held at Bhabha Atomic Research Centre, Bombay from 21st to 23rd February 1994. He presented a working paper on "Murine Malaria: Anti-erythrocytic antibodies recognize N-acetyl neuraminic acid residues" during the sessions.

6. Dr.B.Ravindran delivered a lecture on "Antigenic mimicry and filarial parasites" at the Molecular Biology Unit of the Tata Institute of Fundamental Research, Bombay on 25th February 1994.

7. Dr.B.Ravindran delivered a series of six lectures on "Immunology" to the final year M.Sc. (Zool.) students at the Dept. of Zoology,Utkal University in the month of March 1994.

Other events :

Dr.K.R.Bhardwaj, Deputy Director and Head, Div. of Laboratory Animals, CDRI, Lucknow visited this Centre on 2-3rd Aug. 1994 for inspection of the animal house at RMRC.

Publications :

1. A.K.Satapathy, M.K.Das and B.Ravindran (1993) Murine Malaria: Anti- erythrocytic antibodies recognize N-acetyl neuraminic acid residues. Immunology, 80;546.

2. B.Ravindran, A.K.Satapathy and P.K.Sahoo (1994) Bancroftian Filariasis- Differential reactivity of anti-sheath antibodies in microfilariae carriers. Parasite Immunology (In press).

3. C.A.Labo, Santosh K.Kar, B.Ravindran *et al.* (1994) Novel proteins of *P.falciparum* identified by differential immuno-screening using immune and patient sera. Infection Immunity (In press).

III CLINICAL DIVISION

3.1 Comparative study on efficacy of ivermectin and DEC in the treatment of Bancroftian filarial adenolymphangitis (ADL) (WHO/TDR supported).

Scientists	:	Dr. S.K. Kar
		Dr. J. Mania
Technical Staff	:	Mr. T. Moharana
		Mr. K. Dhal
		Mr. R.N. Nayak
Starting date	:	1991

Objective:

A double blind study was undertaken to compare the efficacy of multiple doses of Ivermectin (MK-933) or DEC-C in the treatment of adenolymphangitis (ADL) of *W.bancrofti* lymphatic filariasis in the prevention of future episodes of ADL.

This Centre had earlier carried out studies on the treatment of microfilaraemics admitted to our local hospital facility, with the new drug Ivermectin. About 60 men were administered single oral dose of Ivermectin at different dosage levels i.e. 20, 50, 100 and 200 ug/kg body weight, as in-patients. The clearance of mf and side reactions were monitored. The mf in the circulation disappeared in all categories of patients, including 20 ug/kg body weight by two weeks. However, the mf started reappearing in the peripheral circulation by 3rd month to 6th months. The mf re-appearance was 12 and 44% of pre-treatment mf values observed at 3rd and 6th month post drug respectively in the groups. The group receiving maximal dose of drug (200 ug/kg body weight) showed not only rapid clearance of mf from circulation but also delay in the re- appearance time of mf. Based on these experiences a project was initiated at this centre with WHO/TDR support to test the efficacy of Ivermectin at higher dosage level, coupled with one year treatment with doses at monthly intervals in clinical cases of filarial disease. Thus the current year WHO/TDR project envisages 12 doses of Ivermectin at 400 ug/kg body weight level, at monthly intervals, instead of single dose employed in our earlier study. Also the dosage now employed is double the maximum dose used in our earlier study. It was envisaged to administer this level of the drug on 12 occasions over 12 months. The patients will be followed for another 12 months period after therapy.

Thus the double blind chemotherapy trial with 1) Ivermectin 2) DEC and 3) placebo is being conducted in bancroftian endemic population of Puri district of Orissa, to evaluate the efficacy of Ivermectin in preventing future episodes of filarial adenolymphangitis (ADL) in 120 eligible cases.

Table I : Coverage of cases according to body weight for the double blind therapy schedule.

	Body weight (Kg)				
	No of Cases	30-39	40-49	50-60	61-65
Initial target	135	15	60	45	15
Under therapy	105 (77.8%)	14 (93.3%)	52 (86.7%)	33 (73.3%)	6 (40.0)

The study is progressing smoothly with a coverage of 105 eligible cases under chemotherapy schedule (Table-1). A target of 135 subjects for therapy programme was kept to compensate for drop outs and to retain 120 subjects on the treatment schedule for 12 months either with one of the drugs or with the placebo. The target for lower body weight groups could be reached easily, while the target for higher body weight groups was found to reach only 40% in 61-65 kg body weight group (Table-I), since patients with more than 60 kg body weight were not many in that community. Out of the 105 cases that were already registered, 54 completed the 12 months of therapy phase and entered Post-therapy one year observation phase. Another group of 30 cases completed about 6 months of therapy phase. A third group of 21 cases were recruited recently. Another group of 30 patients have to be taken into the study for completion of the target. Persons with history of at least two ADL attacks in the past and currently experiencing ADL as per the study criteria were registered into the study in smaller groups (batches) for operational convenience and follow-up in the field.

Age group and sex distribution of 105 individuals under therapy is shown in Table-II. Half of them (48.6%) belonged to younger age group (18-30 years) and 80% of this group were men. The proportion of women was the least in the younger age group and women patients constituted about 28% of the total cases registered. Before the commencement of the treatment, majority (46.7%) had two attacks of ADL per year. More than one third (37.1%) had 3-5 attacks per year and remaining one-sixth of them had more than 5 attacks per year (Table-II). The clinical symptoms/signs of ADL during intake revealed that nearly one-third of cases had genital inflammation in the form of epididymo-orchitis and only very few had severe affliction. Out of 105 cases only 48 Subjects experienced ADL attacks during the period of

Table II : Age and sex distribution of cases under therapy with number of ADL attacks in the previous year.

Age group (yrs)	No of cases			Number of Cases with different Frequency of ADL attacks in the previous year			All Categories
	Males	Females	Total	2	3-5	>5	
18-30	41	10	51	22	18	11	51 (48.6)
31-42	18	12	30	14	12	4	30 (28.6)
43+	17	7	24	13	9	2	24 (22.8)
Total	76 (72.4)	29 (27.6)	105 (100.0)	49 (46.7)	39 (37.1)	17 (16.2)	105 (100.0)

Figures in parentheses are percentage of total subjects.

therapy. Most of these attacks of ADL (n=63) occurred during the 1st to 6th months of therapy. Factors like severity of oedema and duration of disease did not seem to affect the post-treatment ADL frequency. Out of 135 ADL attacks that had occurred after the commencement of the therapy 30 ADL attacks had evidence of injury/infection. As compared to Pre-treatment ADL frequency, the Post-treatment ADL frequency was found to be significantly lower when all cases were considered as one entity. There is a reduction in the circumference of affected limbs and hydrocele diameter in cases, who have completed 12th month drug therapy. Since, it is a double blind study and drug code is not known, it is not possible to ascertain the effect of either drug or placebo on the reduction in the frequency of ADL attacks or remissions in clinical filarial lesions. The haematological and biochemical parameters of blood were within normal limits in the study population both during the pre and post treatment periods.

The drugs and the placebo were tolerated safely and no untoward side reactions were noted on the day of consumption of drug or during the subsequent period of observation. Co-

operation could be obtained from all those under the study, excepting few cases who were out of station for longer periods. The drug distribution was discontinued in 4 pregnant women as per the protocol. Drug administration will continue till all available 135 drug packages are distributed to the eligible subjects as per the protocol.

Reasons for Continuation

i) Thirty more cases will be included in the study for chemotherapy, mostly those belonging to higher weight groups as per protocol.

ii) The enroled cases (51 cases who have not yet completed one year) have to be covered for monthly drug doses assigned, to complete the 12 monthly treatment schedule with one of the drug or placebo.

iii) All the cases have to be followed up for another 12-months period (after the administration of last drug dose) to record the occurrence of Adenolymphangitis attacks among the various groups of subjects belonging to all treatment categories. This would allow the study of the influence of the drug, if any, on adult filarial worms and consequently on mf prevalence, relative load and disease course. Second year of the study (one year of therapy and followed by the second year drug free observation) is crucial for this investigation. Besides, the clinical follow up examination for ADL, blood samples would be collected from each case during pre-drug period, during follow-up visits of therapy phase and on day 14, 180 and 360 of post-therapy drug free observation period. These blood samples will be analyzed for circulating filarial antigens, specific antibodies, besides repeating other tests, carried out during pre-drug period. This study is in progress.

3.2 Evaluation of cardiopulmonary involvement in the Tropical Pulmonary Eosinophilia (TPE) and response to DEC therapy - A follow up study.

Scientists	:	Dr. S.K. Kar
		Dr. J. Mania
Technical Staff	:	Mr. T. Moharana
		Mr. K. Dhal
		Mr. R.N. Nayak
		Mr. S.C. Rout
Starting date	:	June, 1988

The ongoing clinical study is being carried out at Unit III, Government Dispensary, out patient department (OPD), Bhubaneswar. Suspected cases of TPE are referred to this OPD from other OPDs. During the year 1410 cases were examined. Based on the presence of symptoms/signs, 23 were suspected to be TPE cases on clinical grounds. Further haematological examination revealed seventeen confirmed cases of TPE with absolute eosinophil count (AEC) more than 3000 cells/cm. These were then assessed by further clinical examination, Spirometry and Electrocardiogram. Sera were collected from these patients for baseline haematological and immunological tests before administration of chemotherapy with DEC. They are being followed up as per protocol on the 1st, 4th, 12th, 18th and 24th month of treatment to assess their response to therapy. Changes in eosinophil levels, lung function tests and immune responses and recurrence pattern of illnesses were documented.

There were 17 confirmed cases of TPE recorded during the year. Their average age was 25 $\pm$ 11 years. The AEC count averaged to 4674 $\pm$ 3066 cells per cm. The average eosinophil count as percentage of total leucocyte count was 39.9 $\pm$ 18.4. All TPE cases were positive for circulating filaria specific IgG and total IgE, respectively. The mean circulating immune complex levels were very high. Fifteen cases showed improvement in their lung functions, following 1st course of therapy with DEC. Twenty five cases registered last year (during 1992-93) also attended the OPD for periodic follow-up examinations.

This follow up study will highlight the clinical pattern of manifestations in TPE, response of TPE patients to DEC therapy, concomitant alterations in eosinophilic and immune responses and recurrence pattern of TPE.

3.3 Filarial hydrocele and its treatment with DEC.

Hydrocele formation is the most frequent chronic clinical manifestation reported in men, in many bancroftian endemic areas. However, the clear relationship between the hydrocele formation, microfilaraemic status and immunological status could not be elucidated in earlier studies. The present study investigated the most common chronic filarial manifestation i.e., hydrocele, its association with microfilaraemia and immune response in bancroftian endemic areas, with in a population of 4680, in the state of Orissa. Besides the data collection on hydrocele a pilot study was undertaken to evaluate the efficacy of long term high dose therapy with Diethylcarbamazine-C (DEC) in the reduction of size of filarial scrotal swelling.

Table III : Prevalence(%) of chronic filarial signs in the population studied

Age(Yrs)	Number examined	% with lesions*		
		1	2	3
0-9	325	0	0	0
10-19	370	10.3	0.3	0
20-29	270	43.0	3.3	2.2
30-39	176	52.8	1.1	9.7
40-49	135	57.0	1.5	13.3
50-59	130	50.0	2.3	16.9
60+	118	42.4	5.9	19.5
Total	1524	28.8	1.2	5.6

* 1: Hydrocele, 2: Limb lymphoedema, and 3: Cases with both the lesions.

Hydrocele alone was the most prevalent (28.8%) chronic clinical sign amongst men (Table-III) and in comparison to this the prevalence of lymphoedema was found to be significantly low (1.2%; $P < 0.001$). The mf rate amongst lymphoedema group (5.3%), although low, was not significantly different from that of cases with hydrocele (21.9%). Out of the 439 cases of hydrocele, 86 persons had neither detectable microfilaraemia nor had any history of ADL, the features that support filarial aetiology. These cases could therefore be termed as "idiopathic". The group of subjects with hydrocele were compared with those who has lymphodema of limbs for their immune responses. Such a comparison revealed significantly lower mean IgG titre (0.089 ± 0.084) and percent positive for antigen (19.8%) in cases with hydrocele as compared to the cases with limb involvement. There was significant reduction (<0.05) in the scrotal diameters and skin thickness in cases who were treated with DEC 10 mg/kg body weight dosage daily for 3 weeks, in each month for 6 consecutive months, than those who received two standard courses of DEC (6 mg/kg/day/12 days), during 1st and 6th months of therapy.

Present study revealed the possibility of non-filarial aetiology in one-fifth of hydrocele cases seen in endemic areas and these could be referred to as 'idiopathic' cases. This study also emphasised on longitudinal study of immunoregulatory process of host that involved in the expression of clinical pathology of hydrocele manifestation. Prolonged DEC schedule may be useful for beneficial effect in the treatment of hydrocele.

3.4.1 The sheath of the microfilaria of *Wuchereria bancrofti* has albumin and immunoglobulin on its surface.

Scientists : Dr. S.K. Kar
Dr. J. Mania

Technical Staff : Mr. T. Moharana
Mr. K. Dhal
Mr. R.N. Nayak
Mr. S.C. Rout

Using direct fluorescent antibody analysis it was shown that the sheath of live microfilariae of *Wuchereria bancrofti* had human albumin and the immunoglobulin G subclasses IgG1, and IgG4 on its surface (Table-IV).

Table-IV: The degree of fluorescence of microfilariae of *Wuchereria bancrofti* when probed with various FITC labelled anti- IgG isotypes or with anti-albumin, (Level 0 is a negative and level 3 the strongest reaction).

Conjugate	Percentage of mf with degree of fluorescene			
	0	1	2	3
MoAb anti-IgG (Fc)	100	0	0	0
Polyclonal anti-IgG (r)	40	26	21	13
MoAb anti-IgG_1	61	36	3	0
MoAb anti-IgG_2	100	0	0	0
MoAb anti-IgG_4	48	35	2	15
Polyclonal anti-human albumin	0	0	10	90

Approximately 60% of mf had IgG on their surface and the tests with anti-subclass reagents showed that this was both IgG1 and IgG4. There was no evidence of IgG2 on the sheath. Although these tests were not quantitative, the dilutions of each reagent were adjusted so that there was a range of fluorescence amongst the positive mf. The observations that there were still numerous non- fluorescing mf with all the anti-IgG reagents suggests that, as in cats infected with *B.pahangi*, mf are born without IgG on their sheaths and accumulate

it as they mature. On the other hand, this study could not detect any mf which did not have albumin on their sheath which suggest they may be coated with albumin very soon after birth.

3.4.2. Detection of albumin and immunoglobulin on surface of *Brugia malayi* mf by immunofluorescence.

Direct fluorescent analysis revealed the presence of human albumin and the immunoglobulin G on the sheath of live microfilariae of *B.malayi* (Table-V)

Blood samples were collected from four microfilaraemic individuals living in a village of Balasore district (Orissa), endemic for *B.malayi* infection. The mf were confirmed to be of *B.malayi* by microscopic examination of the specimens. Each of the samples had more than 1500 *B.malayi* mf per ml of blood. As shown in the Table V, all mf probed with anti-human albumin conjugate fluoresced (100% B.malayi) and the majority reacted at level 3(75%) of fluorescence revealing the presence of human albumin on their surface as seen on the mf of *W.bancrofti*. These observations suggest that the mf become coated with albumin very soon after birth.

Table-V: The degree of fluorescence of microfilariae of *B.malayi* when probed with FITC labelled anti-IgG or with anti- albumin (grading as in table IV).

Conjugate	Percentage with varying degree of fluorescence			
	0	1	2	3
Polyclonal anti-IgG (r)	50	6	31	13
Polyclonal anti human albumin	0	12	13	75

3. Scientific Conferences/Workshops/Seminars attended : Dr. S. K. Kar

12th Annual Conference of the Association of Physicians of India (Orissa) State Branch on 9th and 10th January 93 at Burla, Sambalpur, Orissa	i) Immuneresponses in patients with filarial fever.
48th Joint Annual Conference of the Association of Physicians of India (APICON-93) at New Delhi 20th to 24th January 93.	i) "Ivermectin treatment in bancroftian microfilaraemia in Orissa.
Joint TDR/ICMR informal consultation meeting ivermectin trials at TRC, Madras from 11 to 13 Feb. '93.	Studies on the efficacy & tolerance of ivermectin & DEC in the management of Adenolymphangitis (ADL).
3rd Asian Congress of Parasitology held at CDRI, Lucknow from 18th to 21st February, 93.	Guest lecture on "Clinical Filarial Manifestations and Chemotherapy".
"Invited as a "Resource Person" to the "Academic Staff Orientation Programme" by UGC at Andhra Uni, Visakhapatnam March, 1993 to deliver two lectures.	i) Epidemiology and clinical manifestation of Lymphatic filariasis ii) Chemotherapy and control of lymphatic filariasis.
Workshop on "Lymphatic pathology in filariasis" with demonstration of lymphatic cannulation at V.C.R.C., Pondicherry, Aug 93.	
Invited as guest speaker by OXFAM (INDIA) TRUST & Bapuji Gram Kalyan Samaj (BGKS).	"Malaria treatment & control".
14th International Congress of Lymphology. WHO/ISL Symposia on Lymphatic Filariasis, treatment with DEC" Washington, D.C., USA, 20th-26th September 93."	"Filarial Hydrocele & its treatment with DEC"
Invited to the Task force for study & investigations of methyl alcohol poisoning by ICMR, New Delhi on 19th October 93.	"Methyl alcohol poisoning"
Attended API-Conference, Orissa branch at Cuttack on 11th & 12th Dec 93.	
Attended a meeting on Ivermectin trial on ADL at TRC, Madras, 14th to 16th Dec 93.	

4. **Papers published:**

1. Kar S.K., Mania J., Baldwin CI., Denham D.A. (1993). The sheath of the microfilariae of *Wuchereria bancrofti* has albumin and immunoglobulin on its surface. Parasite Immunology 15:297-300.

2. Kar S.K., Mania J., Kar P.K. (1993). Humoral immune response during filarial fever in bancroftian filariasis. Transaction of the Royal Society of Tropical Medicine and Hygiene" 87:230-233.

3. Kar S.K., Patnaik S., Mania J., Kumaraswami V. (1993). Ivermectin in the treatment of Bancroftian filarial infection in Orissa, India. The South- East Asian Journal of Tropical Medicine and Public Health 24(1):80-86.

4. Kar S.K., Patnaik S., Kumaraswami V., Murty R.S.N. (1993). Side reactions following Ivermectin therapy in high density Bancroftian microfilaraemics. "Acta Tropica" 55:21-31.

5. Kar S.K., Mania J., Kar P.K. (1993). Prevalence of lymphatic nodule in Bancroftian endemic population. "Acta Tropica" 55:53-60.

6. Kar S.K., Mania J., Kar P. K. (1993). Clinical filarial disease in two ethnic endemic communities of Orissa, India. "Journal of Tropical Medicine and Hygiene" 96:311-316.

7. Kar S.K., Mania J., Patnaik S. (1994) The use of Ivermectin against scabies. "The National Medical Journal of India". Vol-7 No.1, 1994.

8. Mania J., Kar S.K. (1994) Detection of albumin and immunoglobulin on surface *B.malayi* mf by Immunofluorescence. "Indian Journal of Medical Research" 99 : 65-67.

9. Kar S.K., Mania J. (1994) Filarial hydrocele and its treatment with DEC. In :"Progress in Lymphology XIV, Proceedings of the 14th International Congress of Lymphology, September 20-26, 1993. Witte, MH, CL Witte (Eds.), The International Society of Lymphology, Zurich, Switzerland and Tucson, Arizona, 1994."(in press)

IV MICROBIOLOGY

4.1 HIV/AIDS

HIV Sero surveillance was initiated at this centre in 1987 as a part of the ICMR's nation-wide net work of HIV sero surveillance centres. In 1991, this centre also initiated Sentinel Surveillance activity. The surveillance activities are also supported by the NACO.

4.1.1. Sentinel Surveillance

Scientists : Dr . V. R. Subramanyam
Dr . B. B. Pal

Started : Dec 1991

Completed : March 1993

A total of about 500 sera were collected and tested during this year(January to March 1993). The total under this category reached 1211 by March 1993 when surveillance activity was closed down. All the samples were collected from the OPDs of Capital Hospital, Bhubaneswar. About 200 samples collected from the blood bank were included in the larger group discussed in the next section. None of the samples from sentinel surveillance were found to be positive for HIV antibodies.

4.1.2. Screening of blood donor samples for HIV antibodies.

Scientists : Dr. V. R. Subramanyam
Dr. B. B. Pal

Started : 1989

Ongoing

	No. tested	ELISA+ WB+	Seropositive/1000	
Jan,93-Mar.94	888*	7	6	6.8
Cumulative for 7 years	3401**	9	6	1.8

* Includes 211 sentinel surveillance samples.

** Includes 479 sentinel surveillance samples.

This year a total of 888 blood donor samples (including 211 sentinel surveillance collections) were tested and six of them were found to be sero- positive by Western Blot test. Two of these (both referred to this Centre, by the Dept. of Microbiology, SCB Medical College, Cuttack) were positive for HIV antibodies by ELISA as well as Western Blot tests. The latter test was performed by the NICED, Calcutta. It is for the first time that donor blood samples from Orissa are found positive for HIV antibodies. During the first quarter of 1994, Red Cross Blood Bank, Berhampur had referred six blood donor samples for testing. Out of these five samples were found to be repeatedly ELISA reactive and four were confirmed to be WB positive by the NICED, Calcutta. All of them were from Orissa state and some had history of migration to other cities in India for work and business. The Red Cross Blood Bank at Berhampur had so far screened about 1000 persons (estimated) and the rate of sero positivity for that city would work out to be 4 per 1000 samples.

Apart from the 6 Western Blot positive samples seen in the blood donor samples, there were four more Western Blot positive individuals in the current year's surveys. This included two sero positive persons from the TB patients discussed in the next section and two individuals belonging to high risk groups. During the year 1993-94 the seropositivity was found to be 6.16/1000 samples (10/1624). This figure registers a sharp rise from the very low rate of 0.18/1000 computed for the six years' total between 1987 to 1992 (1/5439). Including the current years figures the seven years' cumulative data (11/7063) showed a rate of 1.56/ 1000 persons screened. Overall picture registered a several fold increase in the seropositivity rate between last year and the current year (0.18/1000 against 1.56/1000).

4.1.3. Screening of blood samples from TB patients for HIV antibodies.

Scientists : Dr. V. R. Subramayam

Dr. B. B. Pal

Started : Sept 1993

Completed : Dec 1993

In Orissa, though HIV screening activity started way back in 1987, work so far has not focused on any specific group except for blood donor samples. The various at-risk groups are almost unexplored. Because of the often reported close association between TB and HIV infection and for operational reasons we chose to examine samples from TB patients undergoing treatment at the 3 TB hospitals in this state. The 3 TB Hospitals in Orissa are located at Puri, Chandpur and Uditnarayanpur. The first two are in Puri District and the other is in Bhawanipatna District. The number of TB patients were Chandpur: 200, Puri: 50 and

Uditnarayanpur: 65. Out of these in-patients we could test samples from 124, 43 and 59 adult patients respectively, from the 3 hospitals. Children were not included in the screening. Samples were coded and testing was done as per standard protocol. Test results are presented in Tables 1 & 2.

Table 1. Details of samples from hospitalized TB patients for HIV antibodies

	Chandpur	Puri	Uditnarayanpur	Total
Males	89	28	46	163
Females	36	15	13	64
Total	125	43	59	227
Age(Years)				
Range	16-70	16-78	17-65	16-78
Median	36.5	39	30	
ELISA +ve	1	3	0	4 (17.6/1000)
WB+ve	1	1	0	2 (8.8/1000)

Table 2. Details of samples from hospitalized TB patients positive for HIV antibodies

Age	Sex	ELISA	WB
20	Male	+	+
25	Male	+	–
55	Male	+	+
25	Male	+	–

Thus, out of the 227 samples tested, 4 (17.6 per 1000) were found to be repeatedly positive for HIV antibodies by ELISA. Two of these were confirmed to be positive for HIV-1 antibodies (8.8/1000) by the National HIV Reference Centre, NICED Calcutta.

The prevalence of HIV infection in patients with tuberculosis and other respiratory diseases in India has been reported earlier to be 5/356 (14 per thousand) in Pune (Pahwa *et al* ., 1992 JAPI, 40:802), 20/572 (34 per thousand) in Pondicherry (Arora *et al* ., 1993 Ind

J Chest Dis All Sci. 35:103), 57/1950 (29 per thousand) in Bombay (Mohanty *et al.*, 1993 Ind J. Tubs 40:5) and 3/392 (7 per thousand) in Madras (Anuradha *et al.*, 1993 Ind J Tubs 40:13). In our study only two out of the four repeatedly ELISA positive samples have been found to be positive by the Western Blot method (8.8 per thousand). The general rate at our centre was found to be less than 2 sero-positive per 1000 samples screened. Our study on the in-patients of TB hospitals of Orissa revealed a five fold higher seropositive rate (8.8 per 1000) in this group compared to the rate of 1.56 per 1000 samples in the entire study. These data would suggest that TB patients may be having greater biological and iatrogenic risk.

We suggest that tuberculosis patients could be a very valuable sentinel group for epidemiological investigations on HIV prevalence. In the wake of the dual infections (with tuberculosis and HIV), it becomes imperative for health professionals to be more alert to the possibility of spreading HIV and other infections, if universal safety precautions for therapeutic procedures are not strictly enforced in health institutions. This is because the prevalence of HIV infection could be many fold the general figure in specific risk groups.

4.1.4. IEC activities (HIV/AIDS)

Scientists : Dr . V. R. Subramanyam
Dr. B. B. Pal

Tech. Staff: Mr. C. C. Rath

Started : 1992

Ongoing:

A wealth of technical information is available on HIV/AIDS. But there is a paucity of informative/educational material, particularly in regional languages. Keeping this in mind, our centre has taken keen interest in IEC activities at technical as well as nontechnical levels. We have procured video cassettes and posters etc in Hindi and English on HIV/AIDS and distributed them to NGOs and representatives from high risk groups and institutions. We have also prepared posters in Oriya. An audio cassette in Oriya has also been prepared bringing out salient information about HIV/AIDS in a simple language. These audio-visual aids were repeatedly used on various occasions.

Three IEC programmes were conducted this year for the benefit of

1) Jawans, Officers & families of a battalion of Territorial Army,
2) Participants of the rally organized by Territorial Army at Bhubaneswar and
3) Inmates of Circle Jail, Choudwar.

Interactive discussions were held with the medical officers of the special jail, Bhubaneswar and the Circle Jail, Choudwar as a prelude to HIV testing of samples from prisoners. About 100 samples were screened from voluntary potential blood donors from Circle Jail, Choudwar. All the samples were found to be negative by ELISA technique. The Deputy Inspector of Jails had observed that the IEC programme on the prevention of HIV/AIDS is a valuable programme for high risk groups. The Jail authorities of Orissa requested this Centre to carry out similar IEC programmes for all the Circle Jails and District Jails. This activity will be taken up in due course after obtaining proper clearances and resources.

A document "Guide lines for HIV testing" has been prepared and submitted to the Directorate of Health Services, Govt. of Orissa.

4.2. Diarrhoeal Disorders:

Scientists	:	Dr. V. R. Subramanyam
		Dr. S. S. S. Mohapatra
		Dr. B. B. Pal
		Dr. D. Das
		Mr. P. K. Jangid
Technical Staff	:	Mr. C. C. Rath
Started	:	1986
Ongoing		

Diarrhoeal disorders pose a great threat in this region due to many factors. This Centre has been sensitive to this regional problem and has been working in close association with the state health authorities, the NICED, Calcutta and other institutions. While systematic studies on this problem have not been possible due to constraints of resources, we have been engaged in periodic studies to document the epidemiological and microbiological findings.

In the face of a possible outbreak of diarrhoeal disorder in the districts of Koraput and Nowrangpur in August'93, a study was undertaken. A request was made for the NICED, Calcutta to send a team for the investigation of 1993 epidemic. The investigating team consisted of Dr S.K.Mondal, Dr S.K.Niyogi, Dr Banerjee and Mr G.N.Dey of NICED, Calcutta and the team from RMRC, Bhubaneswar as indicated above.

A detailed report along with short term and long term recommendations have been submitted to the Director of Health Services, Govt. of Orissa for appraising the situation and follow up action.

The team visited various villages in Nowrangpur and Koraput districts. Data were collected from the affected PHCs/ZHCs (Jhorigam and Umarkote respectively), and from the CDMO'S office, pertaining to the districts of Koraput, Nowrangpur and Raygada. Malkangiri, the 4th part of undivided Koraput district seems to be unaffected. In addition, samples of rectal swab (34), water (4) and meat (2) were collected from PHCs, villages and Koraput HQs. hospital for bacteriological investigations.

Description of the epidemic:

It would be appropriate to mention certain points about Koraput District (undivided). The scheduled tribe population constitutes 16.36 lakhs (54.5%) and scheduled caste population is about 4.52 lakhs (15%) out of a total population of 30 lakhs. Literacy rate is low (18.5%). There are at least 220 identified "problem villages" for drinking water facilities. About 24% of the total geographic area is covered by forest.

The data pertaining to the current (1993) investigation are incorporated in Tables 1 and 2.

During the period of this epidemic the health authorities have recorded about 2636 cases with the history of repeated diarrhoeal attacks during the 8 months period between January '93 and August '93. Among these patients, 285 persons expired due to illness and its consequences, constituting 10.7% mortality among the total diarrhoeal attacks recorded. During the visit of ICMR teams, details could be collected on 1798 patients with regard to age, sex, period of illness including deaths that had taken place. Out of the 1798 cases where we had these details, 226 persons have been reported to have died during the course of illness (Table-1). This constituted 12.6% deaths among the persons on whom detailed data were available. This rate is slightly higher than 10.7% deaths that was recorded in the over all figures.

According to the data made available by the state health authorities, 159 diarrhoeal cases were recorded in the month of April '93, constituting about 9% of total cases (Table-1). There was a slight rise in the month of May'93, with about 16% of cases being recorded in that month. However, there was a decline in the occurrence of cases in the month of June and only 7% of cases occurred in that month. In the month of July, 352 cases were recorded constituting about 1/5th of total cases recorded and this was followed by the occurrence of 875 cases in the month of August '93 and the proportion of cases recorded in this month was about 49%.

Regarding the distribution of the 226 deaths in the 5 months of the current epidemic

Table 1: Month wise break-up of Diarrhoeal attacks/deaths 1993.

Month	Attacks		Deaths		
	No (1)	% of total	No (2)	% of total	(2) as % of (1)
April	159	8.9	12	5.3	7.5
May	288	16.0	17	7.5	6.0
June	124	6.9	14	6.2	11.3
July	352	19.6	46	20.4	13.1
August	875	48.6	137	60.6	15.7
Total	1798	100.0	226	100.0	12.6

(Table-1), 1/5th of the deaths were seen in July '93 and 3/5th were seen in August '93. During the month of August '93, the case fatality rate was found to be 157 per 1,000 (15.7%; Table-1), which was double the rate that was registered in the initial month of this epidemic (75 per 1,000, April 93 [7.5%]). The overall case fatality rate was found to be 107/1000 in this epidemic.

Table 2 shows the distribution of 1798 cases in the 7 age groups. Children up to 10 years of age constituted 1/3rd of the diarrhoeal cases recorded by the health authorities. Among the other age groups, persons aged 41-50 constituted 8.2%; 51-60 constituted 5.1% and 61 years or more constituted 3.2% of total cases. The other three groups (11-20,21-30 & 31-40 years) contributed half of the cases, each group contributing about 16-18% of total cases.

Distribution of 226 deaths into the 7 age groups is also shown in Table-2. Children with less than 10 years of age constituted more than 1/3rd of the total deaths. Each of the 4 age groups between 11-50 years constituted 10-15% of total deaths. Age groups 51-60 with 6.2% and more than 61 years with 8% had shown higher deaths than their proportional representation for the diarrhoeal attacks. For this reason 31.5% of the oldest age group were observed to succumb to the illness as against 10-15% individuals succumbing to the illness in the other 6 age groups. Older adults therefore constituted a vulnerable segment. This could be due to other factors like their earlier nutritional status before the onset of the illness.

Of the two regions that were studied in detail, initial cases occurred according to official records, on 24/7/93 (in Chikili and Banjiana villages of Jharigam PHC) and on 25/8/93 (in Upper Mangara village of Mathalput PHC).

Table 2: Age wise break of Diarrhoeal attacks/deaths 1993.

Age in years	Attacks		Deaths		
	No (1)	% of total	No (2)	% of total	(2) as % of (1)
< 10	603	33.5	80	35.4	13.3
11-20	284	15.8	29	12.8	10.2
21-30	328	18.2	34	15.1	10.4
31-40	287	16.0	29	12.8	10.1
41-50	148	8.2	22	9.7	14.9
51-60	91	5.1	14	6.2	15.4
> =61	57	3.2	18	8.0	31.5
Total	**1798**	**100.0**	**226**	**100.0**	**12.6**

Stratification of villages:

The month of August'93 has been the worst period, accounting for a large number of cases as well as deaths due to diarrhoeal disorders as described above. The villages/ hamlets could be approximately stratified as belonging to category I, II and III depending on their proximity to a health centre and on the degree of urbanization.

Category I areas are urbanized and are in close proximity to a ZHC or a PHC and have access to safe drinking water sources.

Category II areas are a little farther from the health care units, but have easy access to the health care centres. Safe drinking water sources though existing, are perceived to be inadequate in these areas.

Category III areas are very remote from the health care units and mostly consist of unauthorised settlements in reserve forest areas. Here, safe drinking water facility is non existent. Because of difficult terrain (no roads exist), providing health care in these areas is a formidable task. A majority of residents of this area are tribals or refugees from Bangladesh.

Not surprisingly, the cases and particularly deaths due to diarrhoeal disorders have occurred mostly in category III villages.

Bacteriological findings:

From the 12 rectal swab samples and one water sample collected during the first team's visit, no pathogen could be isolated. This could be attributed to the constraints and limitations of the methodology (no incubator was available) and the inferior quality of the bacteriological media used.

However, from the second lot of samples, which were investigated along with NICED, Calcutta team. 16 of the 34 rectal swabs yielded growth of *Vibrio cholerae* 01, Inaba strain (Table-3). Thus about 47% of rectal swabs collected from patients revealed the presence of the pathogen, *Vibrio cholerae*. This would indicate the need for strengthening of primary and secondary level prevention measures in remote villages in a big way. Three out of 4 water samples also yielded growth of the same organism. Detection of *Vibrio cholerae* in the stream water is a serious matter and indicated repeated contamination of this source. This would reflect absence of even rudimentary sanitary measures. One rectal swab yielded growth of *Vibrio parahaemolyticus*.

Out of the two samples of 'dried meat' both yielded growth of coliform group of organisms, indicating poor hygiene.

Table-3 Results of bacteriological investigations.

Sample	Numbers	Report
Rectal swab	34	16 positive for *Vibrio cholerae* 01 Inaba; 1 positive for *Vibrio parahaemolyticus;* 18 negative.
Water (open well)	3	2 positive for *Vibro cholerae* 01 Inaba; 1 negative.
Water (Stream)	1	1 positive for *Vibro cholerae* 01 Inaba
Dried meat	2	2 positive for coliforms

The isolation of *V.cholerae* 01 in this remote endemic foci assumes importance because the current epidemics in many part of the country, including Andhra Pradesh & West Bengal, are due to *V.cholerae* 0139 by replacing *V.cholerae* 01.

Recommendations:

Short term measures:

The immediate task was to reduce mortality in the affected persons.

1) It was observed that while adequate supplies of ORS, normal saline and tetracyclines were by and large available in the health centres, there was inadequate supply of Ringer lactate. Adequate quantities of Ringer lactate is essential to rally round moribund patients of diarrhoeal disorders. ORS, Normal saline, tetracyclines and some antiemetics must also be adequately stocked.

2) The current practice of chlorinating the wells once a week or so has to be replaced by chlorinating every night, at least as long as the outbreak lasts. Adequate quantities of bleaching powder must be used every time. The public health authorities should ensure proper and adequate chlorination, by spot checking of the waters for available residual chlorine, to take care of contamination at a later stage during storage and distribution. It is pertinent in this context to note that out of the 3 well water samples tested by us, 2 were positive for *Vibrio cholerae*. One of these 'positive' wells was 'chlorinated' 7 days prior to sample collection. This finding highlights the need to chlorinate a) adequately and b) more frequently.

3) Health workers must implore and impress upon the villagers to drink water only from the chlorinated sources. Health education measures may help in this regard.

Long term measures:

1. **Improvement of personal hygiene and general sanitation:** The people in a ffected areas live in the company of a variety of live stock (fowls, pigs, cattle etc) and the latter have free access to every part of the house and also, at times, to the food consumed by the people, leading to very poor hygienic conditions. The places used for disposal of wastes are very near to the human settlement. Defecation is usually done around the village in bushy places. Such places get washed into low lying areas in rainy season. In remote unauthorised settlements, wells were not protected by raised platforms. As a consequence, there are chances for contamination of community drinking water supplies on a regular basis. This should be prevented by encouraging people to construct protective embankments around the wells.

2. Poverty, inadequate and irregular employment and illiteracy are major socio-economic problems of affected areas. Because of poverty these tribal people are believed to

consume whatever is available, particularly in the lean seasons (monsoon months). Attempts must be made by the various Govt. departments to initiate poverty alleviation programmes.

3. There are definite "problem villages" and "peak seasons" in the context of diarrhoeal attacks. Having seen two epidemics, one in 1991 and the other in 1993, the Govt. may reorient its resources much in advance so that the cyclic epidemics can be controlled successfully. The system of 'surveillance' needs to be revamped. The services of the laboratory of the State Govt's Pathologist & Bacteriologist, Cuttack, should be optimally utilized.

4. A majority of the cases and particularly deaths occur in the reserve forest areas, among unauthorised settlements, where basic civic amenities are not available. The Govt. should a) not allow unauthorized settlements or b) recognise such settlements and provide basic civic amenities.

5. A lot of misconceptions and prejudices exist both among the villagers as well as the health care personnel regarding tribal habits and taboos. It is generally believed that tribals are the most affected because they "eat all sorts of things' such as bamboo shoots, mushrooms, various leaves (greens) and dried meat of cattle. While some of these "food" items could at times be hazardous, these alone are not responsible for the diarrhoeal disorders. In the current study, community water sources were found to be contaminated, because of the lack of primary preventive measures. The people of affected areas should be imparted basic knowledge on health and personal hygiene. They must be instructed about the importance of timely medical intervention and must be weaned away from the habit of relying on 'Gunis' (local "spiritual" person, who can ostensibly ward off the 'evil spells'). The notion that intake of fluids during attacks of diarrhoea aggravates the condition must be dispelled, with health education. Poor people must be persuaded to use protected water during peak seasons, if not in all seasons. Adequate chlorination, repeated broadcasting of health education messages, practice of personal hygiene are corner stones in the prevention of these repeated cycles of diarrhoeal disorders. Mothers (who are mostly illiterate) should be selected as target groups for special health education campaign, as one-third of deaths are constituted by young children. This would call for innovative approaches and should be able to reach tribal folk.

4.3. Laboratory studies on the growth and survival of *Vibrio cholerae* and other bacteria in millet gruel.

Scientist: Dr V.R.Subramanyam

Tech. Staff: Mr C.C.Rath

Vibrio cholerae is one of the major aetiologic agents of diarrhoeal diseases and is still a cause of great concern in areas endemic for cholera, where periodic outbreaks take a heavy toll of human life. While rapid strides have been made in our understanding of pathogenesis and hence in greatly reducing the mortality due to cholera, the role of local food habits in transmitting the disease cannot be undermined.

Orissa in Eastern India has pockets endemic for cholera. While sporadic cases go unnoticed, outbreaks occur around monsoon season, sometimes reaching epidemic proportions as happened in 1991 and again in 1993. During investigations into these outbreaks of cholera in Koraput district of Orissa, it was observed that a minor millet locally called "mandia" or "ragi" *(Eleusine coracana)* constituted the major and often the only food consumed. A thin gruel is made by adding "ragi" flour to hot water along with some salt for taste. The gruel is used both as food and also for quenching thirst, often for several hours after preparation. We therefore, investigated the potential of "ragi" gruel in supporting the growth of enteric pathogens, as the gruel is made in bulk and consumed by all family members over a period of several hours.

Table 1. Growth of *V.cholerae* and *E.coli* in 1% "ragi" gruel.

Bacteria	Inoculum*	Count after incubation+
V. cholerae	1.8×10^3	a. 1.2×10^7 b. 1.8×16^8
E. Coli	8.7×10^3	a. 2.2×10^8 b. 4.6×10^7

* Counts are averaged from triplicates, expressed as cfu/ml.

\+ Incubation at a) ambient temperature (min. 32°C, Max 36°C) or b) at 37°C for 18-24 hours.

Growth of *V. cholerae* and *E. coli* in "Ragi" gruel:

It is clear from Table 1 that "ragi" gruel is a good nutrient medium supporting the

growth of *V. cholerae* and *E. coli*, even at ambient temperatures. In fact, for an identical inoculum, 5-7 fold higher counts were obtained when incubation was at ambient temperature (32-36° C) as compared to the counts after incubation at 37° C.

Length of survival of *V. cholerae* and *E. coli* in "ragi" gruel:

Both the organisms remained viable in "ragi" gruel at either temperature, up to 24 days (the maximum period of observation).

Table 2. Growth of bacteria on "ragi" agar.

Bacterium	"Ragi" agar with DW*			"Ragi" agar with TW*		
	0.01%	0.1%	1%	0.01%	0.1%	1%
Staph. aureus	+	+	+	+	+	+
Bacillus sp.	+	+	+	+	+	+
Escherichia coli	+	+	+	+	+	+
Shigella flexneri	+	+	+	+	+	+
Salmonella typhi	+	+	+	+	+	+
Vibrio cholerae	++	++	++	+++	+++	+++

* "Ragi" agar was made by boiling "ragi" flour (0.01, 0.1 and 1% w/V) and Na Cl (0.5% w/V) in distilled water (DW) or in tap water (TW) , prior to autoclaving. Growth is graded as moderate (+), good (++) or luxuriant (+++).

Growth of various bacteria on "ragi" agar

"Ragi" agar was tested for its ability to support the growth of a variety of bacteria (Gram positive as well as Gram negative). All the six bacteria tested could grow on "ragi" agar, even at a "ragi" concentration of 0.01% (w/v). Increasing the concentration of "ragi" up to 1% did not have any effect on the quality of growth (Table 2). However, *Vibrio cholerae* consistently gave a better growth on "ragi" agar as compared to other organisms. Substituting tap water for distilled water in preparing "ragi" agar improved the growth of *Vibrio cholerae* but had no effect on other bacteria.

In areas endemic for cholera, out breaks of the disease occur primarily as a result of massive contamination of the drinking water sources with human excreta containing *V. cholerae*. Secondary spread of the disease results from person-to-person transmission, due

to poor personal hygiene (Wilson 1984). In rural India it is not unusual for the entire family to partake food from a common container carried to the place of work (agricultural fields etc.). Under conditions of poor hygiene and sanitation, such food could be an important source of infection.

Earlier reports exist documenting the survival of vibrios in a variety of food stuffs such as rice, barley, wheat, potato, shell fish etc. However, there is no published report regarding the survival of vibrios or other medically important bacteria in "ragi" gruel, a staple food of the people belonging to lower socio-economic status in this tribal dominated region.

It is usually believed that vibrios survive for prolonged periods in presence of a high salt concentration or at alkaline P^H values (around 8). Our finding is of interest because the medium in our experiments had a mere 0.5% of NaCl and the initial pH was 6.5 ± 0.1. Even under these conditions, the medium could support the growth of a variety of bacteria including *V. cholerae*.

4.4. Development of an ELISA for subcutaneous *Curvularia* infection.

Scientists:	Dr V.R.Subramanyam
Tech. Staff:	Chandi Charan Rath
Started :	March'93
Completed:	May'93

We had isolated a *Curvularia* sp. from a subcutaneous lesion (RMRC, Bhubaneswar Annual Report 1992-93 p.23). From this isolate a crude soluble antigen was prepared by sonication and clarification. This antigen did not show any precipitin line in agarose gel diffusion test against the patient's (homologous) serum. We then standardized an enzyme immuno assay, by coating this antigen on to the wells of a microtiter plate. Antibodies in patient's serum were detected using anti-human IgG coupled to peroxidase. When tested at a dilution of 1:50, the patient's serum gave a value of A492 = 0.642, as compared to the mean A492 value of 0.411 ± 0.075 of 10 normal human sera. This is possibly the first report of ELISA being used to confirm any subcutaneous mycosis (vide Mycoses Vol.36, in press).

4.5. Applied Microbiology

In addition to engaging in diagnostic microbiological investigations of health problems in this region, this Centre is actively pursuing research topics of applied interest, as detailed below.

4.5.1. Microbiology of local hot springs.

Scientists	:	Dr V.R.Subramanyam
Tech staff	:	C.C.Rath
Started	:	Sept. 1992
Completed	:	Aug 1993

Hot springs offer a unique opportunity to study thermophilic microbes. The objective of this project was to study the microbes in the local hot springs. The temperature of the hot springs ranged from 45-60° C and the pH at ambient temperature was 6.7-6.8.

We have isolated 35 strains of bacteria and 26 strains of fungi, from 3 hot springs.

4.5.1.1. Characterisation of the isolates:

Gram staining, motility, pigmentation, catalase, oxidase, nitrate reduction, lysine decarboxylation, gelatin liquefaction and acid production from glucose, lactose and mannitol were tested for all the bacteria. Slide cultures were done for examining the microscopic morphology of fungal isolates.

4.5.1.2. Thermotolerance of the bacteria:

All the bacteria could tolerate a temperature of 60°C for 3 hours. In addition, the Gram positive cocci and Gram positive rods could tolerate 70° C for 10 minutes and 100°C for $\leq$ 10 minutes respectively.

The Gram positive cocci could grow at a temperature of 60° C and the Gram positive rods at 75°C (maximum tested). But the Gram negative rods did not grow beyond 45°C.

4.5.1.3. Growth under various conditions:

The bacteria and fungi have been tested for growth on a variety of media ranging from simple minimal media to routine laboratory media. The bacteria have also been tested for their ability to grow at pH values from 4-11. In addition, the bacteria have been tested for growth in presence of varying concentrations of metal ions such as cadmium, chromium, cobalt, copper, lead, manganese, mercury, nickel, silver and zinc.

4.5.1.4. Kinetics of growth:

Representative bacterial strains have been tested for their growth rate constants and the generation times have been calculated at different temperatures.

4.5.2. Metabolic propensity of microbes from hot springs:

Scientists: Dr V.R.Subramanyam
Tech. Staff: C.C.Rath
Started : Jan'93
Ongoing.

Microbes from hot springs offer potential sources for industrially important bioactive substances such as enzymes.

4.5.2.1. Enzyme activities of hot spring bacteria:

The bacteria have been screened for various enzymes such as amylase, protease, lipase, glutaminase, cellulase, ß-lactamase and DNase at different temperatures (37,40,45,50 and 60° C). Some of the enzyme activities have been tested by altering the temperatures of growth and the assays.Some of the bacteria have been shown to elaborate thermostable enzymes.

4.5.2. Enzyme activities of hot spring fungi.

The 26 fungal isolates have been screened for amylase, protease, lipase, glutaminase and cellulase activities. All the isolates were negative for protease, lipase and glutaminase activities under the test conditions. At room temperature, Amylase and cellulase activity was found in 15 and 8 strains respectively. Three strains showed the activity even at a temperature of 80°C at a pH of 11.

4.5.3. Demonstration of plasmid DNA in hot spring bacteria.

Scientists : Dr V.R.Subramanyam
Tech. Staff : C.C.Rath
Started : March'93
Completed : October'93

Plasmids are basic tools of molecular biology. For applying recombinant genetic techniques to manipulate any bacteria, plasmids are indispensable. The rich potential that thermophilic bacteria offer in terms of industrially important bioactive substances, prompted us to examine these isolates for any resident plasmids. Plasmids, if found, would be very useful as vectors in genetic manipulations of these bacteria.

All the isolates were screened for plasmid DNA by a miniprep method followed by agarose gel electrophoresis. Plasmid DNA was demonstrable in 18 bacteria.

4.5.4. Antimicrobial efficacy of essential oils.

Scientists	:	Dr V.R.Subramanyam Ms. S. Pattnaik, Research Scholar (honourary) in collaboration with Dr C.R.Kole OUAT.
Started	:	Sept'91
Completed	:	Dec'93

We had earlier documented the antibacterial activity of several essential oils (Annual Report RMRC,Bhubaneswar 1992-93; p 26-27). The study has been extended to cover antifungal activity. Components of essential oils have also been tested for antibacterial and antifungal activity. The results demonstrated the utility of essential oils and their components as antimicrobial agents. Their activity spectrum includes human and plant pathogens.

4.5.4.1. Influence of genetic differences in the genus *Cymbopogon* on the antimicrobial activity of its essential oils.

Scientists	:	Dr V.R.Subramanyam Ms Smaranika Pattnaik,Res.Scholar In collaboration with Dr C.R.Kole,OUAT and Dr S.Sahoo,RRL, Bhubaneswar
Started	:	Jan;93
Completed	:	Dec'93

Essential oils are secondary metabolites of aromatic plants. It is generally known that the genetic make up of a plant would influence most of its attributes. We have examined essential oil extracted from 6 genetic varieties of the plant genus *Cymbopogon*, for antimicrobial activity. Eighteen bacteria and 12 fungi were tested against each of the 6 essential oils.

Results of antibacterial activity are given in Table 1 and those of antifungal activity are given in Table 2. Two varieties differed from other varieties and 6-8 bacteria were found to be resistant for them (Table 1).

Table 1: Comparison of antibacterial activity of essential oil from six varieties of *Cymbopogon*.

Variety*		No. of bacteria sensitive	No. of bacteria resistant
1.	CKP - 25	18	0
2.	RRL - B16	17	1
3.	RRL - B18	10	8
4.	RRL - B15	12	6
5.	RRL - B77	18	0
6.	RRL - B69E	17	1

* 1 = *Cymbopogon pendulus;* 2 = *C.flexuosus;* 3&4 = *C. winterianus;* 5 & 6 = *C. martinii.*

In tests for antifungal activity, all fungi were sensitive to undiluted oil. Hence dilutions were tested to discern quantitative differences in the antifungal activity of the essential oils (Table 2). Two of the fungi tested had shown lower sensitivity for most of the varieties tested. Genetic varieties have shown varying levels of inhibition zones at equivalent dilutions.

4.5.4.2. Essential oil - bacterium interactions.

Scientists	:	Dr V.R.Subramanyam Ms Smaranika Pattnaik, Hon.Res.Scholar
Tech. Staff	:	C.C.Rath
Started	:	Jan'93
Completed	:	Dec'93

The antibacterial activity of essential oils is fairly well-known. However, the interaction between essential oils and bacteria, that ultimately leads to the antibacterial activity has not been well understood.

We examined the possible mode of action of essential oils (Lemon grass, *Palma rosa,* Peppermint and Eucalyptus) against the bacterium *E. coli.* The oils were bactericidal at a

Table 2: Influence of genetic background of essential oils of genus *Cymbopogon* on antifungal activity.

Sl. No.	Fungus	Strain	Oils from *Cymbopogon* varieties					
			CKP-25	RRLB-16	RRLB-18	RRLB-15	RRLB-9E	RRLB 77E
1.	Ca.alb	SP-14	34	54	27.5	20	14	12
2.	Cr. neo	SP-16	24	42	18	12	10	38
3.	Sp. sch	SP-15	8+	9+	11+	S/R+	S+	S+
4.	Al.cit.	SP-6	50+	42+	40+	41+	43+	40+
5.	As.fum	SP-13	30*	S/R*	30	34	36	S/R*
6.	As. ory	SP-2	28	20+	20+	32+	14+	30+
7.	Fu. oxy	SP-4	42+	29+	20+	34+	10+	22+
8.	Fu. sol	SP-8	30+	16+	21+	34+	9+	20+
9.	He. com	SP-9	8+	28+	14+	12+	18+	25+
10.	Ma.pha	SP-3	20	30	8	26	R	18
11.	Sc.rol	SP-5	50*	40*	42*	50*	46*	44*
12.	Tr. men	SP-12	35*	39*	17*	8*	8.5*	7*

Table shows the inhibition zones (diameter in mm); The essential oils were tested undiluted, or at a dilution of 1:5* or 1:10+; S, Zone sizes not measurable; R, Resistant (no zone).

concentration of 1.66 ul/ml *(Palma rosa,* Lemongrass and Eucalyptus) or 2.5 ul/ml (Peppermint). This bactericidal effect was observed both at 4°C and at 37°C, i ndicating that the killing effect is not energy - dependent. The cidal effect was seen without any lag period. The cidal effect was not due to osmotic shock because the presence of 0.5 M sucrose (an osmoprotectant) did not prevent the death of *E. coli.*

Palma rosa and Peppermint (but not Lemongrass or Eucalyptus) oils induced the formation of elongated filamentous forms of *E. coli* cells that reached a length of 60-70 um (normal *E. coli* cells measure about 3-5 um in length).

4.5.4.3. Genetic studies on resistance to essential oils in *Pseudomonas aeruginosa*

Scientists	:	Dr V.R.Subramanyam Ms Smaranika Pattnaik,Hon.Res.Scholar
Tech. Staff	:	C.C.Rath
Started	:	March 93
Completed	:	Dec'93

Pseudomonas aeruginosa is an important bacterium, notorious for being resistant to several antimicrobial agents. In our studies on antimicrobial efficacy of essential oils, it was observed that *Ps. aeruginosa* (strain VR-6) was not inhibited even by 20 ul/ml of four essential oils *(Eucalyptus, Palma rosa,* Lemongrass and Peppermint). The fact that resistance to many antimicrobial agents is encoded by plasmid genes prompted us to explore the possibility in this context. We could demonstrate the presence of plasmid DNA in the bacterium VR-6. Next, we subjected the bacterium to acridine orange treatment (known to eliminate plasmids from bacteria). On screening 1220 clones, a single clone (VR6-AO-1) was obtained that was susceptible to just 16.6 ul/ml of *Palma rosa* oil indicating an increased susceptibility (or decreased resistance) to *Palma rosa* oil as compared to the parental strain VR-6. However, when this clone (VR6-AO-1) was examined further, it still harboured plasmid DNA. The results indicate that resistance to *Palma rosa* oil is not plasmid - mediated in *Ps. aeruginosa* (VR-6).

Publications:

1. V.R.Subramanyam, K. Ramaswamy and A.P.Dash Characterization of *Bacillus* sp. isolated from mosquito larvae in a filariasis endemic area. Jour Comm Dis (1992) 24:60-61.

2. C. Kole, S.Pattnaik, V.R. Subramanyam. A Narain . Antifungal efficacy of oil and its genetic variability in citronella. Crop Res (1993) 6:504-512

3. V.R. Subramanyam, C.C. Rath, M.Mishra and G.P.Chhotray Subcutaneous infection due to curvularia sp. case report. Mycoses (in press).

Conferences etc. attended:

V.R.Subramanyam

1. "HIV/AIDS: an International Social Disaster". Invited speaker, at the meeting of Rotary Club, Bhubaneswar North (15 Jan 1993).

2. "HIV/AIDS: the importance of HIV antibody testing". Invited lecture at the Conference of Blood Bank Officers, Indian Red Cross Society, Orissa State Branch, Bhubaneswar (27 Feb 1993).

3. Resource Person; WHO-ICMR Workshop on Biomedical Communication,Bhubaneswar (Sept 1993).

4 Resource Person; NACO-State AIDS Cell sponsored workshop on Safe Blood Banking: HIV/AIDS (14 Oct 1993).

5. Presented a paper at the 2nd Indian Conference on Hospital Infection; Bangalore (Nov 1993).

6. Technical Advisory Committee to Govt. of Orissa, on HIV/AIDS. Participated in meeting (Aug 1993).

B. B. Pal

1. National Environmental Science Academy; 8th Conference; Bhubaneswar (Sep 1993).

2. Participated in the WHO-ICMR Workshop on Biomedical Communication,Bhubaneswar (Sept 1993).

C.C.Rath

1. Participated in the workshop on molecular biology at Institute of Life Sciences, Bhubaneswar (22-29 Nov 1993).

2. National Environmental Science Academy; 8th Conference, Bhubaneswar. Presented a paper and won Junior Best Paper II Prize (Feb 1993).

3. Associations of Microbiologists of India. 34th Annual Conference, Ludhiana. Presented a paper (February 9-11, 1994).

Other activities

V.R.Subramanyam

1. Visited NACO, New Delhi for discussions and IEC material (May 1993).

2. Organised the First Training Programme on 'HIV testing in the context of blood transfusion safety', for NALCO & HAL hospital personnel (5-8 Jan 1993).

3. Organized the Second Training Programme on 'HIV testing in the context of blood transfusion safety', for NALCO & HAL hospital personnel (11-12 March 1993).

4. Organized an IEC Program on HIV/AIDS at 120 Inf. Bn.; Bhubaneswar (June 1993) (K.Satyanarayana, B.B.Pal, C.C.Rath).

5. Organized an IEC program on HIV/AIDS for the benefit of prison inmates, Choudwar (Nov 1993; K.Satyanarayana, B.B.Pal, C.C.Rath).

6. Prepared an audio cassette in Oriya language, on HIV/AIDS (C.C.Rath).

7. Prepared informative posters on HIV/AIDS in Oriya language (C.C.Rath).

8. Organized an exhibition on HIV/AIDS at the Rally organized by Territorial Army (Oct 1993; B.B.Pal, C.C.Rath).

9. Prepared "Guidelines for HIV testing", for the State AIDS Control Organization.

10. Delivered 2 lectures on basic microbiology at the P. G. Dept. of Zoology, Utkal University, Bhubaneswar in connection with the refresher course organized by the Academic Staff College of the Univ. A practical demonstration was also organized on "Microbes and their activities" (Nov 1993).

11. Delivered a Guest lecture "Genetics of bacterial drug resistance" at the BMS College for Women, Bangalore (Dec 1993).

12. Organized a demonstration of equipment and kits for HIV testing; Blood bank officers' conference (NACO-State AIDS Cell sponsored) 14 Oct 1993; (B.B.Pal).

Smaranika Pattnaik:

Submitted Ph.D thesis "Antimicrobial efficacy of some essential oils", to Utkal University (Co-guide: V.R. Subramanyam).

V. CLINICAL PATHOLOGY DIVISION

5.1 Study of erythrocytic G-6-PD deficiency and its variants at molecular level in a malaria endemic tribal population of Orissa.

Scientists	:	Dr. G. P. Chhotray
		Dr. M. R. Ranjit
Co-Investigator	:	Prof. Luccio Luzzatto
Technical Staff	:	Mr. B.N. Sethi
		Mr. H. K. Khuntia
Starting date	:	April 1986

In addition to 20 DNA samples analysed earlier, 7 more new DNA samples from G-6-PD deficient tribals and 2 repeat G-6-PD normal samples were further carefully analysed in search of a second mutation.

The results of 20 DNA samples analysed earlier proved to be most interesting. The sequence analysis of 3 deficient samples revealed previously un-described base change in codon 44 (GCC to GGC) predicting a substitution of amino acid, alanine to glycine in that position. The remainder of the samples including the normals were tested for this base change with the enzyme Hae III. All samples apart from 2 samples (which failed to amplify) revealed fragments consistent with the base change. Surprisingly out of 6 DNA samples having normal G-6-PD activity, 3 revealed data consistent with the above base change at codon 44.

Out of these 3 cases with normal G-6-PD activity, 2 could be traced and a repeat analysis was carried out along with 7 new samples (Table 1). The result can be summarized as follows:

i The two persons who had normal G-6-PD activity and who exhibited second mutation at codon 44 on repeat analysis failed to show the second mutation.

ii. Out of the seven new G-6-PD deficient samples, 4 have shown evidence of the Orissa mutation (GCC to GGC) at codon 44 and three didn't show this change.

iii. The characterisation of the 3 samples that did not show the Orissa mutation is in progress.

Justification for continuation of the project:

Having proved the previously un-described base change in codon 44 (Orissa mutation) in the DNA analysis of G-6-PD deficient tribals of Keonjhar district of Orissa, it is proposed that similar surveys to be undertaken in most of the tribal inhabited districts of Orissa so as to have a clear and detailed mapping of the situation.

TABLE - 1

Sl No.	Sample No.	Name	Ethnic group	Langu-age	G-6-PD activity (IU / gHb) Normal 7-11	Km for 6-6-PD (fm) Normal 55-61	Source (blood donor/ patient etc.)	Clinical Manife-station	Hb g/dl of whole blood	Hb % of Haemo lysate
1	21	A.M.	S. T.	Tribal	1.7	38	N.H.P.	Nil	10.4	0.14
2.	22	N.N.	S.T.	Tribal	2.8	38	N.H.P.	Nil	11.4	0.18
3	23	N.P.	S.T.	Tribal	2.1.	42	N.H.P.	Nil	12.2	0.19
4	24	D.N.	S.T.	Tribal	1.9	45	N.H.P.	Nil	10.8	0.14
5	25	G.N.	S.T.	Tribal	1.3	47	N.H.P.	Nil	10.6	0.14
6	26	G.P.N.	S.T.	Tribal	1.7	43	N.H.P.	Nil	10.4	0.13
7	27	P.K.K.	S.T.	Tribal	2.4	47	N.H.P.	Nil	12.6	0.18
					Samples collected for repeat analysis					
8	16	G.C.N.	S.T.	Tribal	10.1	54	N.H.P.	Nil	14.2	0.26
9	17	S.C.	S.T.	Tribal	10.0	56	N.H.P.	Nil	14.0	0.26

S.T.: Scheduled Tribe or *Adivasi*

N.H.P.: Normal Healthy Person

Sample no 16 and 17 are the repetition samples of DNA bearing same sample no 16 and 17 sent earlier.

5.2 A chronological study of the histopathology of malayan filariasis in a cat model.

Scientists:	Dr. G.P.Chhotray
	Dr. M.R. Ranjit
Technical Staff:	Dr. M. Mohapatra
	Mr. B.N. Sethi
	Mr. H.K. Khuntia
Starting date:	November 1990
Completion date:	January 1994

Felis catus (cat) has been shown to be a good experimental animal for *B.malayi* infection and the manifestations are more akin to human *B.malayi* infection. We have reported successful establishment of this model at our laboratory last year. Four animals could be infected and two animals died last year. The other two animals expired this year. The following observations were made during the course of infection when the animals were alive and after autopsy.

1. Microfilarial status and course of mf infection in the experimental animals inoculated with infective larvae.

2. Periodicity of microfilariae in the experimental model.

3. Gross and microscopic pathological changes.

Microfilarial status:

Microfilariae appeared in the peripheral blood of the cats after 77 to 90 days of inoculation of infective larvae (L_3) subcutaneously. There was a slow progression of mf count after they appeared in the peripheral blood during initial 3 months. There was a rapid progression of mf count up to 6 months, after which it remained almost static till the cats died after 10 months to 1 year of appearance of mf in peripheral blood.

Periodicity of mf:

It was observed that the infected strain was nocturnally periodic and the peak count was observed at 23 hrs.

Autopsy findings:

Autopsy revealed dilated superficial lymphatic channels of abdominal and thoracic region and enlargement of regional lymphnodes. The peritoneal and thoracic cavities were filled with amber coloured fluid and an adult worm could be retrieved from thoracic cavity of one cat (Annual Report 1992- 93). In the male cats both testes and epididymis were enlarged and the spermatic cord was thickened.

Paraffin sections were made from enlarged lymphnodes and stained with H&E stain for histopathological study. Microsections revealed reactive lymphnode with chronic inflammatory cell infiltration of varying degree, dilated lymphatic spaces, histiocytes and macrophages. It was further observed that although the target organs were lymphatics, lymphnodes, testes & epididymis, yet there were no signs of lymphoedema or obstruction, by the time the animals expired.

Present status:

In spite of our best efforts it was not possible to maintain the infected cats beyond one year and the animals died due to various reasons. Many of the sequential immunological and pathological studies that were planned could not be completed.

5.3 A clinico - pathological correlative study on arthritis with special reference to filariasis.

Scientists:	Dr. G.P.Chhotray
	Dr. S.S. Mohanty (SCB Med. Coll. Cuttack)
	Dr. M.R. Ranjit
Technical Staff:	Dr. M. Mohapatra
	Mr. B.N. Sethi
	Mr. H.K. Khuntia
Starting date:	December 1992
Completion date:	March 1994

A total of 194 cases presenting with arthritis of various forms were selected from the orthopaedic and medical out patients department of SCB Medical College, Cuttack. Out of these, 80 cases having monoarticular involvement were taken up for study. A detailed clinical

history and clinical examination were performed. When required the patients were admitted to indoors. Routine haematological, pathological, biochemical and radiological investigations were carried out. Special investigations like R.factor, VDRL, ASO titre, CRP and Mantoux test were performed to exclude other aetiological diagnoses. In all the 52 cases who had effusion, synoveal fluid was aspirated and was subjected to routine, microscopic biochemical and immunological investigations. Synoveal tissue was obtained in all cases by closed needle biopsy/open biopsy/excisional biopsy techniques whichever was found suitable and was subjected to histopathological examination.

To establish the correlation of filarial arthritis with other forms of arthritis the following investigations were carried out. :

- Screening for mf by night blood smear.
- Estimation of anti-filarial antibody titre in blood and synoveal fluid by ELISA using soluble mf antigen.
- Assay of circulating immune complex (CIC) in serum and synoveal fluid by spectrophotometer.
- Immuno peroxidase staining of synoveal tissue sections for histopathological examination.

The distribution of various monoarthritis cases were as follows:

1.	Septic arthritis	8	5. Metabolic	22
2.	Tubercular	12	6. Nonspecific arthritis	15
3.	Rheumatoid	13	6.1 Filarial associated	11
4.	Degenerative	20	6.2 Filarial not associated	4

Attention was focussed on 15 cases where no definite aetiological diagnosis could be established. A careful history taking regarding filarial attacks and the presence of filarial disease was sought for. Eleven cases out of 15 cases of nonspecific arthritis could be classified in this group (labelled as filarial associated) on the basis of history and clinical examination.

Results:

Out of 194 cases studied, 41.2% had monoarticular involvement. Majority of them (62.5) belonged to 21-40 years of age with a male preponderance (58.8%). Knee joint was mostly

affected (91.3%) with pain (87.5%), swelling (81.3%) and tenderness (75.0%) being the chief symptoms. Routine laboratory parameters were found to be normal except leucocytosis in septic, rheumatoid and metabolic arthritis cases and a high ESR in septic and tubercular arthritis. Radiological findings varied in different types of arthritis.

About 19% of monoarticular arthritis cases were classified as nonspecific arthritis, as no aetiological diagnosis could be established. Out of these 15 cases, 11 had history of association with filarial disease (73.3%) in this endemic area. Two cases had microfilaria in their peripheral blood. Maximum number of patients were in 21-40 years of age group (53.3%) with a male preponderance (80.0%) and presented with pain, tenderness, swelling and effusion. Routine laboratory parameters were normal except mild rise in ESR. Radiology was normal. Synoveal fluid analysis and biopsy revealed nonspecific findings. Immunological parameters revealed increased level of antifilarial antibody titre and circulating immune complex levels both in serum and synoveal fluid when compared to normal values.

All the filarial associated arthritis cases have clinically responded well to recommended doses of antifilarial treatment. In the absence of any specific filarial diagnostic test, the alleviation of the clinical symptoms may be considered as a positive correlation.

5.4 Study of urogenital and renal involvement in a filarial endemic area of Orissa.

Scientists	:	Dr. G.P.Chhotray
		Dr. M.R. Ranjit
Technical Staff	:	Dr. M. Mohapatra
		Mr. B.N. Sethi
		Mr. H.K. Khuntia
Starting date	:	September 1992

A total of 1378 individuals (748 males and 630 females) between the ages ranging from 7-70 years from 8 villages of Sakhigopal and Pipili area in Puri District were examined to determine the frequency of undetermined urogenital and renal involvement associated with filariasis (Table 1).

Out of these 1378 individuals screened, 135 individuals (9.8%) were found to be microfilaraemic. A detailed clinical examination was performed and urine samples were collected from 228 cases, comprising of endemic normals, asymptomatic mf carriers, acute and chronic symptomatic cases. Ninety (39.5%) cases out of the 228 cases examined had microfilariae in the peripheral night blood smear and rest were amicrofilaraemic.

Table 1

Particulars of the study sample

Study area	8 villages namely Ahmadpur, Panchukera, Badahat, Birabalbhadrapur, Malatipatpur, Bhatpur, Basukera and Birapratappur of Puri, Sakhigopal area and patients attending Pipili hospital.
. Total population	6,100
. Persons screened	1378, Males: 748, Females : 630
. mf positive cases	135, Males: 67, Females : 68
. Urine samples collected	228

The morning midstream urine samples were collected from these patients and the samples were transported to RMRC laboratory for detailed analysis. Simultaneously blood samples were collected for various immunological and pathological examinations.

Table 2

Percentage with haematuria in different categories of individuals

Individuals	Number tested	Haematuria Grade			
		Gr. I	Gr. IV	Total all grades	% of total
Endemic normals	43	--	--	--	--
Asymptomatic mf carriers	50	8	--	8	16.0
Past hisotry of filariasis	46	1	--	1	2.2
Acute cases	70	7	-	7	10.0
Chronic cases	19	5	3	8	42.1
Total	**228**	**21**	**3**	**24**	**10.5**

Of the 228 urine samples examined, 24(10.5%) samples revealed asymptomatic microscopic haematuria of various degrees (Table-2). None of the cases had chyluria or any other abnormalities. Grade-I haematuria (1-8 RBC/HPF) predominated and 21 out of 24 belonged to this category. There were no cases with Grade-II or Grade-III haematuria. Only

three persons had Grade-IV haematuria. All the three cases with grade-IV haematuria (field-full of RBC/HPF) had chronic filarial manifestations. Also 42.1% of chronic cases group suffered from microscopic haematuria of various degrees, which was 3 to 4 fold higher than other categories such as acute disease (10.0%) or mf carriers without any signs and symptoms (16.0 %). All the persons in the asymptomatic mf carrier group and 40% of persons with acute disease had shown mf in circulation, while none of those with chronic disease had shown mf in circulation. Haematuria was more common in the chronic patients group and they did not currently harbour mf in the circulation. Thus haematuria is not positively associated with the presence of mf in circulation and probably has its aetiology in the later stages of disease process in the natural history of filariasis. Therefore the prevalence of haematuria was observed to be higher as the disease progressed to a chronic stage.

Justification for continuation:

Various biochemical examinations of blood like blood urea, creatinine, blood sugar etc., immunological tests like CIC and antifilarial antibody level in the serum and urine are in progress to establish a possible correlation of various parameters with asymptomatic microscopic haematuria associated with filarial disease in this endemic area.

5.5. The 4th National Network Workshop on Haemoglobinopathies and allied disorders.

In connection with the 4th "Meeting of National Network on Haemoglobinopathy", a field based workshop was organised by RMRC(ICMR), Bhubaneswar during 4-6th March 1994 at village Kania in Angul District of Orissa. This was co-sponsored by the Orissa Volunteers Health Association(OVHA) and Centre for Action on Rural Development (CARD). This area falls near industrial township of Talcher and National Thermal Power Centre. Participants were as follows:

1. Dr.D.Mohanty, Director, Institute of Immunohaematology(ICMR),Bombay (Coordinator)
2. Dr.R.Colah, A.D., I.I.H. (ICMR), Bombay
3. Dr.R.Z.Patel, Associate Professor, Dept. of Pathology, Bardoli, Gujrat
4. Dr.Jyotishi Patel, Paediatrician, Medical College, Baroda
5. Dr. Y.Z.Italia, Valsad, Gujrat
6. Dr. S. L. Kate, Ex-Professor, Biochemistry, Dhule Med. College, Emeritus medical scientist
7. Dr.K.Ramasamy, Ooty, Tamilnadu

8. Dr.P.K.Das, Reader, Dept. of Anthropology, Utkal University, Bhubaneswar
9. Dr.G.P.Chhotray, A.D., R.M.R.C.(ICMR), Bhubaneswar (Secretary)
10. Dr.R.S.Balgir, A.D., R.M.R.C.(ICMR), Burla

Workshop participants 5 and 7 were from NGOs.

A camp was organised on 5th March 1994 in the Kania Block, where detailed clinical examination was performed on 101 persons of either sex from the surrounding villages who attended the camp having complaints of anaemia (pallor), joint pains, body ache and weakness. Some of them were also referred for expert opinion and advice-after being suspected as cases of sickle cell disease, haemoglobinopathies and allied haematological disorders.

Various laboratory investigations such as haemoglobin estimation, haematological indices, solubility test for sickle cell disease, DCIP test for G-6-PD deficiency, Hb electrophoresis by cellogel strips for detecting haemoglobinopathies were performed on the spot in 100 blood samples collected.

Table 1: Percentage with haemoglobinopathies in the Kania Survey

Caste	Total screened (M/F)	Haemoglobin type by electrophoresis AA No. (%)	AS No. (%)	SS No. (%)	G6PD deficiency No.(%)	Hb (g/dl) Mean ± S.D.
General	73 (51/22)	39 (53.4)	25 (34.3)	09 (12.3)	3 (4.1)	10.0 ± 3.3
SC *	24 (16/8)	17 (70.8)	06 (25.0)	01 (4.2)	02 (8.3)	10.2 ± 2.6
ST * *	3 (3/0)	02	01	--	02	10.8 ± 3.4
Total	100 (70/30)	58 (58.0)	32 (32.0)	10 (10.0)	07 (7.0)	10.6 ± 2.9

* Sc = Scheduled caste

* * ST = Scheduled tribe

The results of the analysis have been depicted in Table 1. Of the 100 cases, 42%, were detected of having sickle cell gene by solubility test which was confirmed by Hb electrophoresis. Among the 42 with sickle cell gene, a sizable number (32 out of 42; 76.2%) were found to be heterozygous (AS) and 10 (23.8%) were homozygous (SS) for sickle cell gene. Majority of the subjects as well as cases detected belonged to the general castes as compared to Scheduled Caste. However, the number of Scheduled Tribe patients being 3 only it was difficult to arrive at a conclusion regarding the caste wise prevalence of the disease.

The mean Hb% was found to be 10.0 g/dl amongst the general caste population as compared to 10.2 g/dl in SC category. G-6-PD deficiency was observed in 7(7.0%) cases studied.

The patients diagnosed of having haemoglobinopathies were given medical advice, social and genetic counselling to improve upon their health awareness. Such type of camps arranged in future will help in alleviating the problem.

5.6 Investigations on reported endemic fluorosis in an isolated pocket involving two adjacent villages namely Balsing and Singhpur of Bolagarh block of Khurda district:

Scientists	:	Dr.Anasuya Das, DD, NIN, Hyderabad (Co-investigator)
		Dr.G.P.Chhotray
		Dr.S.S.S.Mohapatra
		Mr.Anil Kumar
		Mr.A.Mohapatra
		Dr. M.R. Ranjit
Technical Staff	:	Dr. M. Mohapatra
		Mr. B. N. Sethi
		Mr. H.K. Khuntia
Starting date	:	June 1993

The 2 villages Balsing and Singhpur, which were affected with endemic fluorosis are twin villages separated by a street. They are situated in the Bankoi PHC area of Khurda District about 60 km from Bhubaneswar. There are 93 houses in the 2 villages and total population is 453. These villages are basically agriculture dependent, having no industries or river nearby. The socio-economic status of villages is generally similar to those residing in rural India.

These are isolated villages from other villages and are situated in a valley surrounded by hills on three sides. Although the villages are existing from 5-6 generations, the villagers have noticed, occurrence of lesions characterised by swelling of joints, bending of backbone, staining of teeth and deformity in the skeletal system since last 10 years.

A clinical survey was conducted in these 2 villages and 322 persons were examined. Among those examined 155 persons (48.1%) exhibited clinical signs and symptoms of fluorosis, as described below.

(a) Mottling of teeth was observed to be 35.8% and 48.5% in males and 28.2% and 28.8% in females in the two villages respectively. It appeared at about 5th year of age (6.0%) and reached the peak in 11-20 years of age (about 70%) and then gradually declined by 40 years of age (<10%). There was a slight rise in the prevalence of dental mottling among 41-60 years age group.

(b) Skeletal deformities; It appeared from 6-10 years of age (5%) and reached a peak prevalence (35%) in 51-60 years of age group. The manifestations were in the form of genu-valgum, Kyphosis, exostosis and arthropathy. Interestingly the prevalence of skeletal deformities declined in the 21-30 years age group. There appears to be a sex linked predisposition towards male sex and both the skeletal and dental abnormalities were found more frequently in both boys and men. Genu-valgum was more frequently seen in adolescent boys and attracted most attention and caused social problems.

(c) General examination revealed clinical evidence of anaemia in 11.8% of females and 7% of males besides other signs like xerosis, Bitot's spots and protein energy malnutrition with lower prevalence. This picture is similar to rural India.

The villages had common sources of drinking water, namely ponds and wells which were used for the past 5-6 generations, but only from the past one decade they have been using tube well water, dug by Govt. agencies for drinking and cooking purposes. The villagers claim that the manifestations of fluorosis are more pronounced ever since they have started using the tube well water.

Water samples were collected from all sources and were being analysed for their fluoride content by NIN, Hyderabad. Preliminary results indicated that most of the bore wells had safe levels of fluoride while conventional wells had higher levels of fluoride (4-6 ppm). In view of the lower prevalence of dental and skeletal abnormalities in men compared to adolescent boys, the changes in the trace element content of drinking water supply could be

of recent origin (10-20 years of duration). However water samples have been collected again with careful protocol for reanalysis. It is proposed to carry out health education campaign on discontinuation of sources with high fluoride levels even for parboiling of rice. This study is in progress in collaboration with NIN, Hyderabad. Attempts are also being made for surgical correction of the cases having bony deformity in collaboration with S.C.B. Medical College, Cuttack.

5.7 Investigations of an outbreak of encephalitis epidemic in Rourkela Steel City.

Scientists	:	Dr.G.P.Chhotray
		Dr.A.P.Dash
Technical Staff	:	Mr. R.K. Hazra
		Mr. B.N. Sethi
Period	:	July-August 1993

The findings of the investigative study conducted on a suspected viral encephalitis in the steel city of Rourkela is summarised as follows.

Present Outbreak:

Although there has been sporadic occurrence of encephalitis cases which are being admitted and treated in IGH Hospital from Jan.1992-June 1993, there was a sudden rise in the number of cases admitted during 1.7.93 to 25.8.93. During this period a total number of 42 patients were admitted out of which 9 died in the hospital with a high case fatality rate of 21.4%.

A detailed history and clinical examination revealed insidious onset of fever with rigor and chill associated with head ache and vomiting. Within 2-3 days, the patient became drowsy and rapidly progressed to coma. There was no evidence of neck rigidity or Kernig's sign. It was observed that young adults from both sexes were mostly affected. Various laboratory and radiological investigations did not reveal any significant findings to clinch the diagnosis. The clinical cases were exclusively found from the township and its outskirts. Twenty two serum samples collected from household contacts of index cases and two samples from patients were sent to NIV, Pune for sero-diagnosis. Results on sera samples from two convalescent patients proved inconclusive. The results from household contacts revealed that 36% had past flavi-virus infection and another 20% of them showed recent evidence of dengue infection.

Serum samples of patients sent for sero-diagnosis to NIV, Pune by the IGH, Rourkela

during the previous year (92-93) revealed that 22% had evidence of past flavi-virus infection and 2% had dengue infection. The prevalence of antibodies to dengue infection in household contacts is a new finding. However, patients in earlier years and household contacts at the end of current epidemic had evidence of past flavi-virus infection in one - fith and one-third of them respectively.

Although entomological data revealed the presence of 15 species of mosquitoes including Cx.*tritaeniorhynchus* and Cx.*vishnui*, yet *Ae.aegypti*, the known vector for dengue virus could not be encountered during this cross-sectional survey. Japanese encephalitis (JE) cases have been suspected earlier from this area. Vectors of JE virus (*vishnui* subgroup) were encountered in moderate numbers during the current survey (Section 6.3). Flavi-virus infections (JE, Dengue, West Nile) are difficult to separate, unless regular and serial investigations are carried out. About one-fifth of patients from earlier studies and one-third of contacts from this study had evidence of past flavi-virus infections (IgG). The current series showed evidence of recent dengue infection in one-fifth of close contacts, but we could not demonstrate the vector on the scene of subsiding epidemic. Clinical picture does not exclusively favour dengue infection, though household contacts had shown serological evidence. The picture gets more complicated because of frequent mobility of people from West Bengal districts where JE has been recorded. It was not possible to exclude JE epidemic as one- third of household contacts of patients had evidence of flavi-virus infections which includes JE also. Clinical picture also was not in favour of excluding JE epidemic.

5.8. **Diagnostic Facilities:** The clinical pathology division of RMRC, Bhubaneswar extended it's diagnositic services to the patients referred from local Capital Hospital and it's peripheral health centres, S.C.B. Medical College; NALCO hospital, Anugul and other far off places on histopathology and haematology, where these facilities are not available.

1. Histopathological examinations	120 tissues
2. Full haematological investigations	270 cases
3. Biochemical estimations	100 cases

A. Papers accepted for publication (in press)

1. Subcutaneous infection due to Curvularia species. V.R.Subramanyam, C. C. Rath, M.Mishra and G.P.Chhotray Mycoses; 36 (1994) .

2. Effects of Aphid extracts and juvenoids on the morphology and histopathology of developing mosquitoes. Ranjit, M.R., Dash, A.P., Chhotray, G.P. Asian J of Zoological Science 1994.

B. List of Scientific Conference/Workshop attended and papers presented

1. Attended the 3rd National Network group meeting and workshop at Bamni, Bardoli, Gujrat during September 11-12, 1993. Scientific paper presented : Clinical aspects of sickle cell disease in Orissa.
2. Attended the XXI Annual All Orissa Chapter meet of FAPM at Cuttack 23rd November, 1993
3. Attended the 42nd Annual All India Conference of FAPM held at SCB Medical College, Cuttack during 26-28th November 1993. Scientific paper presented : Adaptability of *B.malayi* filarial linfection in the cats (Felis Catus) an experimental study
4. Participated in ODA Workshop on "Health care for tribal communities in Orissa" 20th December 1993.

C. Other activities

1. Acted as a co-guide for a M.S.(Orthopaedic) Student of S.C.B. Medical College,Cuttack, for his thesis to Utkal University.
2. Recognised as a Guide, Utkal University for a Ph.D. student in medicine. The topic is "A comprehensive study on lymphatic filariasis-A clinico pathological perspective".
3. Dr. G. P. Chhotray was invited to deliver two lectures at Utkal University, Dept. of Anthropology in the Refresher Course for College teachers during March 1994.

VI MEDICAL ENTOMOLOGY

6.1 PRESENT STATUS OF *BRUGIA MALAYI* AND *MANSONIOIDES* MOSQUITOES IN ORISSA.

Scientist	:	Dr.A.P.Dash
Technical Officer	:	Mr.R.K.Hazra
Starting Date	:	September '89.

6.1.1 Distribution of *Mansonioides* and *B.malayi* :

B.malayi is essentially a rural infection with a tendency for localised distribution. Earlier workers reported that *B.malayi* infection had a localised distribution in Orissa, mainly in Balasore, Bolangir and Puri districts. Since there has not been any systematic study on the entomological aspects of filariasis in different parts of the state and consequently the knowledge on *Mansonioides* fauna of the state was poor. The present work was undertaken to report the distribution of *B.malayi* and *Mansonioides* in the state.

6.1.2 Prevalence of *B.malayi* :

A total of 3176 blood smears were collected from 4 districts. Out of these, 2766 and 344 blood smears were collected from Puri and Balasore districts, respectively.

In Puri district, out of 2766 blood slides collected 295(10.6%) were positive for mf out of which 154 were positive for *B.malayi* infection. *W.bancrofti* infection was detected in 135 blood smears and 6 slides were positive for both *B.malayi* and *W.bancrofti* infection. The mf rate of *B.malayi* and *W.bancrofti* were found to be 5.6 and 4.8%, respectively. In Balasore district out of 344 blood smears collected 24 slides were detected positive for mf out of which all but one belonged to *B.malayi* and the remaining one person showed infection for both *B.malayi* and *W.bancrofti*. The mf rate of *B.malayi* was found to be 6.7%.

Limited survey (less than 50 persons) was carried out in Mayurbhanj and Bolangir districts and mf positivity rate was noted at a moderate level of 5 to 8%.

6.1.3 Vector distribution:

Four species of *Mansonioides* have been identified in the present survey along with two species of *Coquillettidia*. *Ma.annulifera* and *Ma.indiana* were found to be endophilic and endophagous in nature while *Ma.uniformis* and *Ma.longipalpis* were mainly exophilic and exophagous.

District-wise mosquito collection data revealed the following patterns. In Puri district a total of 5862 *Mansonioides* were collected, out of which 3918 (66.8%), 1679 (28.6%), 147(2.5%), 92(1.6%) and 26(0.5%) were *Ma.annulifera*, *Ma.uniformis*, *Ma.indiana*, *Coquillettidia crassipes*, *Ma.longipalpis* , respectively. In Balasore district out of 1287 *Mansonioides* collected 774 (60.1%), 461 (35.8%), 17(1.3%), 6(0.5%), 29(2.3%) were *Ma.annulifera*, *Ma.uniformis*, *Ma.longipalpis*, *Ma.indiana* and *Coquillettidia crassipes* respectively. In Cuttack district, 175 *Mansonioides* were collected among which 108 (61.7%), 65 (37.1%) and 2 (1.2%) were *Ma.uniformis*, *Ma.annulifera* and *Ma.longipalpis* respectively. In Dhenkanal district, 86 *Mansonioides* were collected, out of which 52 (60.4%), 33 (38.4%), 1 (1.2%) were *Ma.uniformis*, *Ma.annulifera* and *Ma.longipalpis*, respectively. In Mayurbhanj district 211 *Mansonioides* were collected among which 129(61.1%), 82(38.9%) were *Ma.uniformis* and *Ma.annulifera* respectively. In Bolangir district 41 *Mansonioides* collected in which 22(53.6%), 17(41.5%) and 2(4.9%) were *Ma.uniformis*, *Ma.annulifera* and *Ma.longipalpis*, respectively. In Ganjam district out of 357 *Mansonioides* collected *Ma.annulifera*, *Ma.uniformis*, *Ma.longipalpis* and *Ma.indiana* were 135 (37.8%), 212(59.4%), 8(2.2%) and 2 (0.6%), respectively.

The study of the distribution pattern of four species of *Mansonioides* from Orissa state reveal the existence of two groups of species with similar distribution pattern depending on their adaptation to climatic condition and physiographic division. The present survey revealed that *Ma.annulifera* and *Ma.uniformis* were seen in all the four physiographical regions of Orissa except three districts i.e. Kalahandi, Phulbani and Keonjhar. These two species constituted about 90-95% of all *Mansonioides* collected. *Ma.indiana* and *Ma. longipalpis* were restricted to two coastal districts viz., Puri and Balasore.

6.1.4 Seasonality and vectorial capacity of *Mansonioides* :

Ludlow (1913) was the first to describe the role of *Mansonioides* in the transmission of filariasis. Though in Orissa *B.malayi* infection was first reported by Korke in 1928, no systematic study on seasonality and host efficiency of *Mansonioides* was undertaken. Mosquitoes were collected from January to December and monthly per man hour density were recorded. Mosquitoes were dissected in Haye's saline and examined under stereoscopic microscope for different larval stages of the filarial parasite.

6.1.5 Seasonality:

Four species of *Mansonioides* have been encountered during the survey, out of which *Ma.annulifera* and *Ma.uniformis* were found throughout the year with a peak in October and September, respectively. The man hour density of *Ma.annulifera* varied from 3.9 to 18.6 reaching its peak in October and lowest density was observed in June. The man hour density of *Ma.uniformis* varied from 0.6 to 21.5 reaching its peak in September, the lowest being in June. *Ma.indiana* and *Ma.longipalpis* were less in number and the latter was found only in April.

6.1.6 Ecology and Bionomics of *Mansonioides* Mosquito :

Mansonioides breed in hydrophyte infested water bodies which are predominantly found in rural areas. Ecological factors play an important role in the transmission of vector borne diseases. Information on vector bionomics is essential for a better understanding of the epidemiology of vector borne diseases. In order to plan for the control of this disease it is extremely important to know the bionomics and habitat of the *Mansonioides*. Ecology of *Mansonioides* has been studied using the following parameters:

1. Host feeding behaviour
2. Swarming, mating, feeding and oviposition
3. Biting activity

Host preference:

A total of 3242 blood meals collected (1540 *Ma.annulifera*, 1670 *Ma.uniformis*, 25 *Ma.indiana*, and 7 *Ma.longipalpis)* were subjected to gel diffusion technique to identify the nature of blood meal using known sera. Human blood meal was detected in majority of mosquitoes giving high anthropophilic index values for three species. The anthropophilic index for *Ma.annulifera, Ma.uniformis, Ma.indiana* and *Ma.longipalpis* were 65.5%, 56.9%, 48% and 28.5%, respectively.

Swarming, mating, feeding and ovipositing:

Male *Ma.uniformis* was found in the cow shed in routine collections. Higher incidence of male occurred in between 7.05 PM to 7.15 PM for both the species i.e. *Ma.annulifera* and *Ma.uniformis*. Male *Mansonioides* were also observed during the bait collection. Mating was observed near the bait.

Egg laying behaviour:

Maximum number of eggs were laid in the evening i.e., from 18.00 hrs to 20:00 hrs. and in the early hours i.e., between 3 AM to 6 AM.

6.1.7 Physico-chemical characteristic of breeding habitat:

From water analysis it was observed that when pH is > 7 (alkaline) the larval density is more and when pH is < 7 (acidic) larvae or egg rafts were not found. Similarly, larvae were not found in water having higher dissolved oxygen (7.7 to 8.9 mg/l) where as they breed in water with lower dissolved oxygen (0.95 to 3.17 mg/l); nitrate nitrogen content also indicate a positive correlation between nitrogen content and larval density. Larvae were not found in water with high chlorine content ranging from 15.6 to 21.7 mg/l, where as they were found in water with lower chlorine content i.e.,0.05 to 0.4 mg/l.

6.1.8 Seasonal biting frequency of Mansonioides:

During whole night collection a total of 4506 *Mansonioides* were caught. Out of these 1550 were from the outdoor, 1450 from indoors and 1506 from cattle baits. Maximum number of *Ma.annulifera* were collected between 2 AM to 4 AM and *Ma.uniformis* were collected between 6 to 8 PM. *Ma.uniformis* was found more in outdoor than indoor.

The distribution of *Mansonioides* between indoor and outdoor human bait and the cattle bait had shown seasonal differences. About 46% of *Ma.annulifera* were observed to land on the indoor human bait during the winter. During the summer about 33% of *Ma.annulifera* preferred indoor human bait. The percentages for the indoor human bait, outdoor human bait and cattle bait were 46.1%, 35.9% and 18.0%, respectively in the winter. The total number of *Ma.annulifera* caught in the winter were 410 and in the summer the number came down to 258 per night from the 3 baits. The percentages for the indoor human bait, outdoor human bait and the cattle bait were 32.6%, 38.8% and 28.6%, respectively in the summer season. Differences were also observed for the peak biting time in the different seasons. For *Ma.annulifera*, the peak was between 9 PM to 11 PM and 1 AM to 3 AM in the indoor and outdoor collections respectively in winter. In summer season the peak was found to be between 1 AM and 4 AM, while in rainy season the biting pattern distribution was uniform throughout the night. In *Ma.uniformis* the peak biting time was between 7 PM and 9 PM in winter; between 12 midnight and 2 AM in summer and the biting pattern was throughout the night in rainy season.

6.2. Development of filarial parasites in *Aedes aegypti* Liver-pool strain:

Scientists	:	Dr.A.P.Dash
		Dr.N.Mohapatra
Technical Staff	:	Mr.S.K.Parida
Starting date	:	October 1991.

6.2.1 *Mastomys* sp. infected with *Brugia malayi* were fed to black eyed *Aedes aegypti* (Liverpool strain) in successive batches throughout the year. Total 3922 mosquitoes (18 batches) were fed and kept separately under optimal conditions for 16 days for the development of infective larvae (L_3). The mosquitoes were dissected and 4383 number of L_3 were obtained, which were further used for reinfection (subcutaneous, intra peritoneal routes etc.) to fresh *Mastomys* and cat. *Aedes aegypti* liverpool strain were also fed on *Mastomys* infected with *Setaria digitata*. Twenty two batches comprising of 4507 number of mosquitoes were fed and 645 number of L_3 were procured (Table 1). Development of parasite from microfilarial stage to infective stage took about 14 to 15 days. Development of *S.digitata* in *Aedes aegypti* was found to be low as compared to *B.malayi*. It would appear that adaptation of *S.digitata* is not complete in this animal-vector model.

Table 1. Number of mosquitoes dissected and larvae obtained

Filarial parasite	Mf density/ 20 ul (range)	Total no of mosquitoes fed	Total number of mosquitoes dissected	Total no of L_3	L_3 load/mosq.
Brugia malayi	15-86	3922	2094	4383	2.09
Setaria digitata	12-48	4507	2171	645	0.30

6.3 Studies on mosquitoes of Orissa in relation to filariasis and Malaria/Mosquito Registry:

Scientists : Dr.A.P.Dash
Dr.N.Mohapatra

Technical Staff : Mr.R.K.Hazra
Mr.S.K.Parida
Mr.H.K.Tripathy

Starting date : June 1992.

Mosquitoes were collected from different areas of Puri District (endemic for filariasis) and Sundergarh district (Rourkela). The species composition reveals 21 species belonging to 6 genera (Table 2).

Table-2. Mosquito species collected from different areas

Species	AREAS			
	Pipili (PMHD)	Khurda (PMHD)	Puri Dist. (PMHD)	Rourkela (PMHD)
Ae. aegypti	1.1	1.2	–	–
Ae. albopictus	0.1	0.3	–	–
Ae. edwardis	–	–	–	0.12
Ae. vittatus	–	0.1	–	0.12
An. aconitus	0.2	1.3	1.0	–
An. annularis	2.1	1.9	2.5	0.5
An. culicifacies	0.3	0.1	0.2	0.3
An. hyrcanus	7.3	6.2	5.4	0.19
Ar. theobaldi	0.8	0.3	1.0	–
Ma longipalpis	–	0.1	–	–
Coq crassipes	0.3	–	–	–
Coq. novochraces	–	–	–	0.08
Cx. bitaeniorhynchus	–	–	–	0.19
Cx. epidesmus	–	–	–	0.11
Cx. (lutzia) fuscanus	0.7	1.8	–	0.19
Cx. quinquefasciatus	46.6	18.5	34.8	7.9
Cx. tritaeniorhynchus	0.7	11.2	–	3.08
Cx. vishnui	2.3	2.4	5.2	1.05
Cx whitemorei	0.3	0.7	–	–
Ma. annulifera	7.8	0.7	1.5	–
Ma. uniformis	7.3	0.1	3.8	–

The density of *Cx.quinquefasciatus* was the highest in Pipili and least in Rourkela collections. Rourkela study is for two days only, while studies in Khurda and Pipili are for the whole year. Khurda town had shown low level because of biocide applied there (Please see Section-6.4.). *Mansodnioides* were also found to be more in Pipili area. *Ae.aegy pti* were not seen in the Rourkela survey, where an epidemic outbreak of encephalitis was investigated (Section 5.7). Known vectors of J.E. were recorded in all the four places of survey. The density of these J.E. vectors was found to be higher in Khurda, A town where *Cx.quinquefasciatus* was controlled with biocide spraying. Known vectors of J.E. were recorded and known vector of dengue virus was not seen during the two day survey in Rourkela town.

Thus during the survey in Rourkela town, not a single larva or adult of Aedes aegypti was found by the team which surveyed the area while the epidemic of encephalitis was nearly subsiding. There were 9 deaths and 42 cases due to encephalitis epidemic in that town, just before the team visited that place (Section 5.7). One possibility is that the cross sectional (one point of time) surveys might miss the vectors due to seasonal changes and fresh rains. The other possibility is that local transmission of dengue virus may not be occurring in this town. Mobility of Rourkela population to neighbouring West Bengal districts is considerable. Sera from close contacts of patients were analysed by NIV, Pune. It was observed that one fifth of contacts had antibodies, indicating recent dengue infection and one-third had evidence of past flavi-viral infections (JE, Dengue and West Nile). The isolation of dengue virus antibodies in one-fifth of close contacts of patients could also be due to frequent movement of people to West Bengal, where they might have received the infection. From the clinical picture, J.E. epidemic could not be ruled out and one-third of household contacts had evidence of flavi-viral infection.

Feeding habits:

Blood meals of 1895 mosquitoes belonging to nine species were analysed by gel diffusion technique. The anthropophilic indices were found to be 5.2% for *An.hyrcanus*, 6.8% for *An.vagus*, 93.1% for *Cx.quinquefasciatus*, 9.7% for *Cx.tritaeniorhynchus*, 3.5% for *Cx.vishnui*, 2.8% for *Cx.pseudovishnui*, 30.2% for *Cx.epidesmus*, 63.2% for *Ma.annulifera* and 58.1% for *Ma.uniformis*.

The high anthropophilism of *Cx.quinquefasciatus* (93.1%) and *Mansonioides* (58.1% and 63.2%) shows a bias towards human blood, thereby acting as important vectors of both the filarial infections. *Cx.'vishnui'* group of mosquitoes were found to be highly zoophilic and their anthropophilic indices were less than 10%.

6.4 Field evaluation of *B.sphaericus* against *Culex quinquefasciatus* in Orissa:

Scientists	:	Dr.A.P.Dash
		Dr.N.Mohapatra
Technical Staff	:	Mr.R.K.Hazra
		Mr.H.K.Tripathy
		Ms.Saswati Rup
Starting date	:	March 1992

6.4.1. Field studies:

A large scale field study involving two towns, Khurda and Pipili, with about 75,000 population was taken up to evaluate the efficacy of *B.sphaericus* against *Cx.quinque*fasciatus and its ability to reduce filariasis transmission parameters. Khurda town with 45,000 population was selected as the experimental area and Pipili town served as the comparison area. This project was supported by WHO/TDR and the duration is 36 months from March 92. This study is part of a major effort by WHO/TDR with five Centres at global level. Baseline entomological and parasitological data were collected for 12 months up to March 93 and the areas were found to be comparable. About 9000 night blood smears were examined and the areas were found to be comparable for mf prevalence.

The intervention phase of the project was initiated in early April '93. All the breeding sites of the experimental area (Khurda) were treated with biocide *(B.sphaericus)* and routine monitoring of the larval density was carried out. Indoor resting collection, whole night man biting collection were carried out and transmission parameters were calculated for experimental as well as comparison areas. The spraying of breeding sites with the biocide was repeated at quarterly intervals, in the experimental areas. Results are given in Table 3 and salient findings are as follows:

Larval densities per dip were comparable for the two areas up to March '93. After application of the biocide the larval densities per dip have come down to single digit in April and May, while the control area, Pipili maintained more than 30 larvae per dip. Adult densities of *Cx.quinquefasciatus* mosquitoes have also followed the same pattern and in the experimental area the densities came down from about 40 per man hour to single digit figures. Similar trends were seen for number of mosquitoes landing per man per night. The original landing figures of about 250 per night had come down by 10 fold and reached a figure of 21 in May 93 in Khurda. On the other hand, the comparison area Pipili did not show such a dramatic decrease. Infection rate had also come down in Khurda by 10 fold and infectivity rate became nil, as there were no L_3 infective forms in wild caught mosquitoes dissected.

Table-3 : Larval and Adult Densities of *Culex quinquifasciatus* in Khurdha and Pipili Complex during 1993

Month	KHURDHA					PIPILI				
	Larval Density Per DIP	PMHD of resting adults	Man landing adults	Infection Rate	Infectivity Rate	Larval Density Per DIP	PMHD of resting adults	Man landing adults	Infection Rate	Infectivity Rate
January	41.5	48.3	278	9.95	5.4	40.1	48.7	243	8.1	3.4
February	39.5	41.2	263	9.62	5.3	28.4	40.9	229	7.2	3.1
March	31.6	37.6	247	4.4	2.7	27.2	33.4	217	5.6	2.2
April	2.6	8.2	58	3.1	1.6	18.6	30.1	175	5.3	2.4
May	1.7	5.3	21	1.1	-	34.8	29.8	153	3.8	0.5
June	12.3	18.2	60	0.6	-	36.2	52.3	196	1.0	0.5
July	15.5	18.8	63	1.1	-	39.2	60.4	318	10.6	2.4
August	4.6	11.6	60	2.2	-	34.8	53.5	360	13.2	3.2
September	4.2	8.2	38	2.4	-	43.6	53.8	443	9.3	3.4
October	1.8	4.9	17	-	-	49.8	55.8	341	12.3	5.4
November	1.6	4.5	18	-	-	45.2	53.0	270	8.4	4.8
December	5.7	12.3	32	-	-	41.8	47.5	248	5.2	2.3

Rainy season (June-August) had affected the results of biocide effectivity to a certain extent and larvae and adults of vector mosquitoes showed signs of recovery and reached 1/3 or 1/4 the levels of baseline values in Khurda, despite repeated focal spraying. However the comparison area had not shown much decline, rather adult densities increased over basal values. From October 93 onwards the results were more uniform, the larval and adult densities remained around single digit values and no infective larvae could be seen in mosquito dissections from experimental Khurda area. Number of mosquitoes visiting per man per night also stayed around 1/8th or 1/9th of baseline value. On the other hand in the comparison Pipili area all parameters studied during late 93 were similar to or more than baseline values seen in early 93. In the month of October '93, five mosquitoes out of 100 mosquitoes dissected had late infective (L_3) stages and 341 mosquitoes were landing on one man per night. Transmission potential is at its highest in the comparison area, whereas the transmission potential became zero in the experimental area.

Thus spraying of *B.sphaericus* at quarterly intervals and focal spray of the same in newer cess pools and drains, brought down the density and the transmission parameters of vectors to 1/8th or 1/9th of baseline values. Even in rainy season the reduction was about 60-70% of baseline values. Infective larvae of *W.bancrofti* in vector mosquitoes came down very much and mature L_3 larvae were practically absent, bringing down transmission potential to nearly zero or zero level in the protected town. The comparison area had 250-440 mosquito bites per man per night and about 5% of them carried L_3 larvae of *W.bancrofti*, with very high transmission potential of filariasis. Simple but regular spraying of *B.sphaericus* could protect the entire population of a town, though old cases of filariasis are not treated by special therapy. The study is in progress.

6.4.2. Persistence studies:

In the field, it was observed that the biocide persists till sixth week in cess pits, cess pools and unused wells, when applied at a dose of 2 ml/Sq. mtr. Higher dose has increased persistence of the biocide. The study is in progress.

Bioassay tests of 2nd batch of *B.sphaericus* were done in the laboratory by standard WHO method. LD_{50} and LD_{90} dosage were determined to be 0.037 ppm and 0.490 ppm, respectively.

Persistence studies under laboratory conditions have revealed that the biocide persisted in dead cadavers of larvae up to five months and caused 100% mortality to fresh larvae. Larval mortality was found to be more by applying larval cadavers of 48 hours than cadavers of 24 hours and '0' hour.

6.4.3 Effect of sublethal dose (LD_{25}) of *Bacillus sphaericus* on *Culex quinquefasciatus*:

Experiments were conducted by taking successive four batches of larvae. Around 200 larvae were taken in each batch. Significant differences were seen in the fecundity ($P < 0.001$) and gonotrophic cycle ($p < 0.05$) in the treated group. The sex ratio was found to be 0.82 in treated group, while it was 1.8 in control group. While about 70% of mosquitoes took the blood meal in both the groups, only 20% laid eggs in the treated group, while 73% (of those which had taken blood meal) laid eggs in the control group. It took 4 days duration for egg laying after blood meal in the control group. On the other hand it took four fold time (17 days) for egg laying in the treated group. Thus the whole reproductive cycle was altered in mosquitoes which had been exposed to sub-lethal doses of *B.sphaericus.*

6.4.4. Water analysis of breeding site :

Water samples were collected from three major breeding sites of *Culex quinquefasciatus* viz., cess pool, cess pit and drain. Biological oxygen demand (BOD), chemical oxygen demand (COD), P^H, total dissolved solids, suspended solids, alkalinity, Ca hardness and Mg hardness of the samples were determined.

BOD and COD levels were least in cess pool (11.0 mg/l and 12.8 mg/l), while COD levels were 320 mg/l in both cess pit and drain water. BOD levels were 200 mg/l and 160 mg/l in cess pit and drain water. Total dissolved solid content was very high (550 mg/l) in drain water while it was 370 mg/l in cess pit and 90 mg/l in cess pool water. Other parameters did not show much difference. The larval productions were found to be 11.2%, 26% and 57.1% in cess pool, cess pit and drain, respectively which showed positive correlation with total dissolved solid and BOD of respective breeding spots. Majority of the larvae (57.1%) that were collected from breeding sites came from highly polluted drains, with higher quantity of dissolved solids and greater demand for BOD and COD.

6.5 Testing of different strains of *B.sphaericus* :

Scientists	:	Dr.A.P.Dash
		Dr.N.Mohapatra
Technical Staff	:	Mr.R.K.Hazra
		Mr.H.K.Tripathy
		Ms.Saswati Rup
Starting Date	:	January 1992.

6.5.1. Laboratory Study :

Three different strains of *B.sphaericus* viz., Sphaerimos, Spherix and CDRI were tested against fourth instar larvae of *Cx.quinquefasciatus, An.stephensi* and *Ae.aegypti.*

Sphaerimos had lower LD_{50} and LD_{90} values (0.007 and 0.01 ppm) for *Cx.quinquefasciatus* than Spherix formulation (0.016 and 0.174 ppm) and CDRI formulation (3.9 and 11.0 ppm). For *An.stephensi* Sphaerimos (0.118 and 0.334 ppm) did not differ from Spherix (0.082 and 0.236 ppm), but CDRI strain required much larger quantities (18.6 and 62.8 ppm) for LD_{50} and LD_{90} action. Only for *Ae. aegypti* CDRI strain had some upper hand with LD_{50} and LD_{90} values as 4.0 and 8.2 ppm.

6.6. Mosquitocidals from Plants : their bioactivity and bioefficacy

Scientist	:	Dr.A.P.Dash
Technical Staff	:	Ms.U.L.Mohanty
Starting Date	:	January 1992.

6.6.1. Indigenous Plants:

Larvicidal activity of different solvent extracts of *Anacardium occidentale* (Cashew nut) and *Pongamia pinnata* (Karanj) have been tested following WHO standard bioassay method against *Cx.quinquefasciatus, An.stephensi* and *Ae.aegypti.*

Larvicidal activity of different solvent extracts of *Anacardium occidentale* L. *(Anacardiacea)* (Nut-shell) have been tested following W.H.O. (1973) standard bioassay method. It was found that *Anopheles stephensi* Liston and *Culex quinquefasciatus* say are most susceptible to *Anacardium* methanol extract (AME), whereas *Anacardium* ether extract (AEE) is most effective against *Aedes aegypti* (Linn). In order to find out the most useful fraction of the extract, the effective extracts were fractionated by column chromatography packed with silica gel and using ethyl acetate and hexane solvent system in different proportions. The bioactivity of these nine fractions have been tested against three mosquito larvae. It was observed that 20% EA/Hexane fraction and 5% EA/Hexane fraction of AME are active against *An.stephensi* and *Cx.quinquefasciatus*, respectively with potency 2.13 $\pm$ 0.12/8.2 $\pm$ 0.11 and 0.75 $\pm$ 0.48 / 5.0 $\pm$ 0.11. In case of Ae.aegypti, 100% Hexane fraction of AEE is highly active with potency (LD_{50} / LD_{90}) of 0.17 $\pm$ 0.04/0.32 $\pm$ 0.13. The bioactivity of LD_{50} doses of the effective extracts against the corresponding mosquito larvae is under study.

Larvicidal efficacy of *Pongamia pinnata L.* (Fabaceae) (Cotyledon) extracts have been tested and it was found that ether extract is least effective to *Anopheles stephensi* and not at all effective against *Culex quinquefasciatus* within the test concentration range (10 ppm. to 100 ppm). Amongst all the extracts, *Pongamia* methanol extract (PME) is active against the three mosquito species. So the extract was fractionated through column chromatography using silica gel and ethyl acetate-hexane solvent system in different proportions. The activity of these fractions is yet to be studied. The work is under progress.

6.6.2 Bioactivity of essential oils :

The bioactivity of two hydro-distillated essential oils Citronella and Pogostemon from *Cymbopogon* winterianus (Fam:Poaceae) and *Pogostemon Patchhouli* (Fam: Labiatae) respectively, has been tested against the mosquito vectors. It was observed that Citronella is highly active against *Culex quinquefasciatus* in comparison to *Anopheles stephensi* and *Aedes aegypti*, where as Pogostemon is highly active against *Aedes aegypti* and *Anopheles stephensi*.

6.6.3. Mosquitocidal effects of Marine Products :

About 15 marine samples, supplied by CDRI, Lucknow were tested for their bioactivity against the mosquito vectors. Most of them were not effective in the test concentration range (10 ppm to 100 ppm), but some were moderately effective. Five samples had LD_{50} value in the range of 40-50 ppm and LD_{90} value in the range of 80-200 ppm, against *Cx.quinquefasciatus*. Only two compounds had shown reasonable activity on *An.stephensi* and had values in the range of 80-125 ppm for LD_{50}. None of them were effective against *Ae.aegypti*.

6.7 Comparative efficacy of aphid extracts and juvenoid OMS 3031 against the development of mosquitoes.

Scientist	:	Dr.A.P.Dash
Technical Staff	:	Ms.Rajashree Mohapatra
Starting Date	:	October 1992.

6.7.1. Determination of EI_{50} and EI_{90} doses of different Juvenoids :

Bioassay of four Juvenoids viz., OMS 3031, OMS 2013, OMS 2012 and OMS 3040 were carried out in the laboratory at 26-28° C and RH 86%, against three species of mosquitoes. *An. stephensi*, *Ae.aegypti* and, *Cx.quinquefasciatus* following the procedure of WHO (1981).

The emergence inhibition (EI_{50} and EI_{90}) values of the four Juvenoids against the three species of mosquito vectors have been studied. At EI_{50} level OMS 3031 5% EC had an edge over the other three compounds for *An.stephensi*, (10^{-7} X 72 ppm for OMS 3031 versus 10^{-4} X 14 or 10^{-5} X 93 as EI_{50} ppm values for others). Similar picture is seen for EI_{50} of *Cx.quinquefasciatus* also (10^{-7} X 38 ppm for OMS 3031 versus 10^{-4} X 1 or 10^{-6} X 57 ppm for others).

For *Ae.aegypti* at EI_{50} level OMS 3031 5% EC (10^{-6} X 13 ppm) had an edge over only OMS 3040 (10^{-5} X 32 ppm). Among the two different products of 3031, 10% SC 3031 had better action on *Ae.aegypti* than the 5% EC 3031. This was not true for the other two mosquito species.

6.7.2. Preparation of Aphid Extracts :

The aphids, *Aphis neri* and *Aphis craccivora* were sampled from the infested *Calotropis procera* and *Lablab purpurens*, respectively and subjected to solvent extraction for the isolation of JHs following the methods of Bergot *et al.* (1981) with slight modification.

6.7.3. Toxicity effect of Juvenoid OMS 3031 on non-target species:

Different concentrations of the Juvenoid OMS 3031 were injected intramuscularly to a lower mammal (swiss mice) and the sublethal dose was calculated by probit regression equation.

LD_{50} = 40.0 mg/gm body wt.

LD_{90} = 181.0 mg/gm of body wt.

At higher dose (above LD_{50}) it shows paralytic effects on swiss mice.

6.7.4. Lipid estimation in *Aedes aegypti:*

The standard curve was obtained by taking double refined ground nut oil. Then 44 mg of mosquitoes were taken and the total lipid content was estimated by using the method of Van Handle (1985). The total lipid content of female *Ae.aegypti* was found to be 200 mg/gm of wet tissue.

6.8.Laboratory Biology and Colonization of Mosquitoes.(Cyclic Process)

Scientists	:	Dr.A.P.Dash
		Dr.N.Mohapatra
Technical Staff	:	S.K.Parida

6.8.1. Cyclic colonies of the following species of mosquitoes are being maintained in our insectary.

They are as follows.

1. *Aedes aegypti* (black eyed) liverpool strain
2. *Culex quinquefasciatus*
3. *Anopheles stephensi*

The eggs and larvae of above species were used for laboratory evaluation of different insect growth regulators (I G R), plant extracts etc. Laboratory bred mosquitoes and larvae were supplied to different research workers, including Utkal and Calcutta Universities, Mosquitoes were also used for developing filarial larvae through membrane feeding.

Seminar/Symposia attended.

Seminar attended	Paper presented
1. Dr.A.P.Dash	
WHO/TDR Workshop on use of "*B.sphaericus against Culex quinquefasciatus* in urban environment" in Cameroun April,1993	Field evaluation of *Bacillus sphaericus* against *Culex quinquefasciatus*
Vth National Symposium on advances in biological control of insects pests Muzaffar Nagar U.P. Oct. 1993.	Bio-control of insect vector with reference to use of *B.sphaericus* against *Cx.quinquefasciatus* in India.
2. Dr.N.Mohapatra	
"3rd ICMR/WHO Workshop on Biomedical communication" RMRC, Bhubaneswar, Sept.1993.	Participated in Discussion
IX Annual Conference of NESA,Hyderabad, December, 1993.	Effect of sublethal dose of *B.sphaericus* on *Culex quinquefasciatus.*
3. R.K.Hazra	
"3rd ICMR/WHO Workshop on Biomedical communication" RMRC, Bhubaneswar, Sept.1993.	Participated in Discussion

IX Annual Conference of NESA", Hyderabad, December, 1993.	Water analysis of *Culex quinquefasciatus* breeding sites.
4. S.K.Parida "3rd ICMR/WHO Workshop on Biomedical Communication" RMRC, Bhubaneswar, September, 1993.	Participated in Discussion
5. U.L.Mohanty "UIII Annual Conference of NESA", Bhubaneswar, February 1993. "IX Annual Conference of NESA", Hyderabad, December 1993	Bioefficacy of some marine products against *An.stephensi* Mosquitolarvicidal activity of two essential oils.
6. Rajashree Mohapatra "VIII Annual Conference of NESA", Bhubaneswar, February 1993. "IX Annual Conference of NESA", Hyderabad, December 1993.	Bioefficacy of three OMS compounds against three species of mosquitoes. Mosquito reproduction and Juvenile hormone.

Full length Papers:

Ranjit, M. R., Dash, A.P. and Chhotray,G. P. (1993). Effect of aphid extracts and juvenoids on morphology and histopathology of developing mosquitoes. Asian. J. Zool.Sci.2: (In press).

Ranjit,M.R. and Dash,A.P. (1993). Aphid extracts and juvenoids influence glycogen metabo lism Trop. Biomedicine: 11 (In press).

Mohapatra, N., Dash,A. P. and Hazra,R.K.(1993). Feeding patterns of 8 mosquito species in Orissa. Asian.J.Zool.Sci.:2 (In press).

OTHER ACTIVITIES:

Dr. A. P. Dash

- Graced the Annual function of Netaji Yubak Sangha and ecological awareness programme in Dhenkanal on 23.1.1993 as Guest of Honour.
- He was the Organising Secretary of VIIIth Annual Conference of NESA and Symposium

on "Environmental Management of preventable human problems" held on 16th to 19th Feb.1993., at Regional Medical Research Centre, Bhubaneswar.

- He was elected as a Fellow of National Environmental Science Academy.
- He was appointed as the examiner for doctoral scholars of Andhra University, Visakhapat nam, Madras University, Kerala University and Pondichery University.
- He was invited and delivered the Inaugural Lecture on 2.6.1993 at Tirupathi (Veterinary Science College, Parasitology Department) University of Tirupathi on the occasion of the inauguration of the ICAR sponsored Summer Institute on "Animal Parasitology".
- He received the Dr.T.R.Rao award from the Honourable Union Minister for Health and F.W. on July, 1993.
- He was invited to act as a resource person for preparation of an action plan for districts by the Regional Director, HFW Government of India on July, 1993. He also delivered Lecture on "Vector biology and control with special reference to Bio-environmental control of Malaria".
- He was invited and acted as the Chairman of Scientific session on Biotechnological consideration of insect pest control at the Vth National Symposium on "Advances in Biological Control of Insect Pests" held at Muzaffarnagar U.P. during October, 1993.
- He was invited by the Department of Zoology, Utkal University, Bhubaneswar to deliver lectures in the refresher course for senior college teachers during December, 1993.
- Dr.Dash was invited and acted as a Chairman of Scientific sessions of "Entomology and Vector Control" and acted as a Judge for Award session of IX Annual Conference of NESA in the National Conference of Environmental preservation role for every one.

Dr. N. Mohapatra

- She acted as treasurer of VIII th Annual Conference of NESA held at Regional Medical Research Centre, Bhubaneswar in the month of the February, '93.
- She was elected as the Fellow of National Environmental Science Academy.

Rajashree Mohapatra

- She received the Best Young Scientist Award in VIII th Annual Conference of NESA.
- She received the Best Young Scientist Award for Poster presentation in IXth Annual Con ference of NESA.

VII. COMMUNITY HEALTH AND NUTRITION

7.1. RMRC FIELD STATION, JEYPORE

Among the several communicable diseases prevalent in Orissa, malaria, diarrhoeal disorders and filariasis have been on the forefront as major health problems. The state of Orissa bears disproportionately higher disease burden of India (14-15% of total) as against the lower representation of population (less than 4% of India's Population). While the Eastern Ghat regions of the state having predominant tribal population have been the endemic home of malaria and diarrhoeal disorders, the coastal plains have been so for filariasis. Problems like fluorosis are limited to certain geographical areas.

During the year 1993-94 investigations were carried out on the above health problems of the state, as a prelude to detailed scientific studies.
They include the following.

Regarding the investigation of reported endemic fluorosis in an isolated pocket involving two adjacent villages, namely Balasing district and Singpur of Bolagad block of Khurda district. detailed presentation was given in Section-5.

Investigations of Diarrhoeal disorders in Koraput area.

The newly formed Nowrangpur district of the erstwhile Koraput district was most affected with the outbreak of diarrhoeal disorders. The onset of the outbreak was in April 93 affecting 3 blocks namely Jharigam, Papadahandi and Pujariguda and by August 8 blocks were found to be affected out of the total 10 blocks. There were 1512 attacks with 148 deaths in 128 villages by August 93. These findings were further supplemented with detailed investigations by a joint team of RMRC, Bhubaneswar and NICED, Calcutta which incriminated *Vibrio cholerae* 01 Inaba in rectal swabs and water samples. Details are presented under Section-4.

7.1.1.Sample survey of village Gaderkhai close to NALCO

Scientists : Dr.Saiyed, DD, RCOH, Calcutta (Co - investigator)
Dr.S.S.S.Mohapatra
Period : September.1993

The village is within 500 meter perimeter of the smelter plant of NALCO. It has 60 houses with 350 population. On the spot health check-up was carried out on 80 persons including 65 children. The following clinical picture emerged (Number of affected persons was given in parenthesis) : Mottling of teeth (4), Angular stomatitis (9), Hypopigmented skin patches all over body (14), Arthralgia (2), Clinical pallor (3). The most conspicuous complaint by the people was the patchy discolouration of the skin and clinical examination revealed its prevalence as 17%. The patches were hypopigmented, unevenly distributed throughout the body, mainly on face, back, abdomen and extremities. They are non-anaesthetic and non-itching in character varying in size from 1 cm to 10 cm in diameter. It may be noted that there are reports of similar skin affliction due to fluoride emission in gaseous form into the environment elsewhere in other parts of the world and that this type of dermatological disorders are not encountered with endemic fluorosis. The RCOH, Calcutta has taken up a detailed study of this problem in collaboration with NALCO, Anugul.

7.1.2. Investigation on the reported problem of tuberculosis in Syamsunderpur village.

Scientists : Dr.S.S.S. Mohapatra
Dr.V.R. Subramanyam

Period : September 1993

The village is located only a kilometre away from the state main highway, 3 km and 15 km away from Bolagad hospital and Bankoi PHC, respectively and connected with a good all weather road. The population comprises of settlers from Ranapur, Begunia and other areas of Puri district, migrated about 4-5 decades ago. Information gathered from the villagers points out that the illness was introduced into the village by a visitor who was suffering from chronic fever, cough and haemoptysis. The visitor stayed for few months and his host started having similar illness a few months later. The disease gradually affected some more persons of the village. The people in general in this village were found to be poor, illiterate and belong to backward community. They did not seek medical help which was available in a nearby peripheral health centre. Only a few among the affected have taken treatment at a TB hospital when the illness was beyond their endurance.

On the spot clinical examination of 41 persons available (out of total 333 population) showed that about one third of them have signs of respiratory diseases. Eleven persons had basal crepitations in lungs and five had clinical signs of cavity. Two children had cervical adenitis. Only 20 persons had evidence of BCG vaccination. Majority belonged to Scheduled caste who have poor knowledge of personal hygiene and health. They have an indifferent attitude towards utilizing the health care facilities available in their vicinity.

The clinical examination data showing 16 out of 333 population having pulmonary problems (if some are proved to be bacillary cases) may not be much different from the rural picture generally seen. However, detailed bacteriological examination is being taken up by the district tuberculosis officer, Puri. But it is imperative to study the reasons leading to changes in the perceived prevalence of pulmonary illness in an isolated pocket of this population.

7.2 FIELD STATION, RMRC(ICMR),V.S.S. MEDICAL COLLEGE, BURLA

7.2.1 Diagnostic facilities :

For the welfare and proper treatment of the patients who attended the Sickle Cell Clinic run by Prof. B.C.Kar (our collaborator), diagnostic facilities were provided to 361 patients of whom 58 were old followed up cases of sickle cell disease/sickle cell-beta-thalassaemia during the first quarter of the year under report. Screening of 303 new cases referred from various OPDs and wards of the Hospital was done for the detection of haemoglobin variants, employing the sickling test and haemoglobin electrophoresis in both acidic and alkaline media. Of these, 59 cases were detected to be normal (HbAA), 170 had sickle cell trait, and 74 were observed to be suffering from sickle cell disease/sickle cell-beta-thalassaemia.

7.2.2. A study of body mass index in sickle cell haemoglobinopathy

Scientists	:	Dr. R.S.Balgir
		Mr. B.P.Dash
		Mr. R.K.Das
Period	:	October '93 to March '94

The growth and development of persons with sickle cell anaemia is usually retarded, whereas in other conditions it may be similar to general pattern of the region. The retardation of growth in sickle cell anaemia cases, in addition to genetic constitution, seems to be influenced by the environmental factors. Studies in African Black populations have shown that the height and weight are reduced in sickle cell disease cases.

The body mass index (BMI), which is a measure of adiposity and body mass per unit area of an individual was investigated in the sickle cell affected cases to find any deviation in the bulk of tissues for unit height, compared with normal age-matched counterparts.

For this study, a total of 834 cases who attended the Sickle Cell Clinic and referred from various OPDs and wards of this hospital were included. Information available on each person

regarding age, sex, height, weight was used for this purpose. Analysis of the data was restricted to adults aged twenty years and above. Validity of BMI has been established to asess nutritional status of adults. Depending upon the BMI values, the subjects were classified into four categories namely, normal (18.5+), mildly undernourished (17.0-18.4), moderately undernourished (16.0-16.9) and severely undernourished (<16.0).

The mean values of BMI for men and women are given in Table-I. Sickle cell trait (HbAS) cases did not show any difference from the normal (HbAA) category in the mean body mass index in both sexes. However, the cases of sickle cell disease/sickle cell-beta-thalassaemia had considerably lower BMI values as compared to the normal and sickle cell trait cases, and the difference is statistically significant ($P<0.05$) except in females.

There were no significant differences between sexes for the mean value of BMI in normal, sickle cell trait and sickle cell disease/sickle cell- beta-thalassaemia (Table-I).

Table I : Mean body mass index of control and sickle cell cases.

Groups	Males		Females	
	Mean	S.D.	Mean	S.D.
Control (HbAA)	19.7 (N=38)	2.8	18.8 (N=31)	2.9
Sickle Cell Trait (HbAS)	19.4 (N=118)	2.8	19.0 (N=89)	3.1
Sickle Cell Disease (HbSS)	16.9 (N=20)	2.9	17.6 (N=10)	1.8

Males : HbAA Vs HbSS, $p<0.05$
HbAS Vs HbSS, $p<0.05$

Table-II gives the sex-wise distribution of 306 subjects into four BMI classes. Among various groups of men, only a small proportion (one-sixth) of patients with sickle cell disease had normal BMI values, whereas about 60% of control subjects and individuals with sickle cell trait had normal BMI values. Similar trend was observed in women, and only 30% of sickle cell

Table II : Distribution of Body Mass Index in Control and Sickle Cell Cases.

Groups & Body Mass Index	Male		Female	
	No.	%	No.	%
Controls (AA) :				
≥18.5	23	60.5	15	48.3
17-18.4	10	26.3	6	19.4
16-16.9	5	13.2	6	19.4
<16	0	0.0	4	12.9
Total	38	100.0	31	100.0
Sickle Cell Traits (AS) :				
≥18.5	71	60.2	49	55.1
17-18.4	22	18.6	13	14.6
16-16.9	15	12.7	15	16.8
<16	10	8.5	12	13.5
Total	118	100.0	89	100.0
Sickle Cell Disease/ Sickle Cell-Beta-Thalassaemia (SS) :				
≥18.5	3	15.0	3	30.0
17-18.4	7	35.0	4	40.0
16-16.9	5	25.0	2	20.0
<16	5	25.0	1	10.0
Total	20	100.0	10	100.00

Male : AA Vs SS, $p<0.01$; AS Vs SS, $p<0.01$.
≥18.5 = Normal, 17-18.4 = Mildly Undernourished
16-16.9 = Moderately Undernourished
<16 = Severely undernourished.

disease patients had normal BMI values. About one quarter of men with sickle cell disease had shown evidence of severe undernutrition (< 16.0, BMI), whereas none of the control group had such a low level of BMI. In case of women there were no differences between these groups for severe undernutrition.

The data indicate that severe undernutrition (BMI, < 16.0 in adults is nearly equivalent to emaciation and marasmus in young children) is frequent in men with sickle cell disease. Only less than one-third of men and women with sickle cell disease had normal BMI values, against half or more than half population with normal BMI in other categories. The significance of low BMI, if any, on the prognosis of haemoglobinopathies remains to be investigated.

Necessary health education and nutrition education and other positive health aspects need to be imparted to the public in general and to the sickle cell disease patients in particular to correct under-nutrition in this particular segment.

7.2.3 A study of menarche in sickle cell haemoglobinopathy.

Scientists : Dr. R. S. Balgir
Mr. R.K.Das
Mr. B. P. Dash

The age at the onset of menarche is dependent upon genetic as well as nongenetic factors including nutritional status. Among genetic factors, sickle cell haemoglobinopathies are known to influence the growth and development.

A study has been carried out in order to find out the association between sickle cell abnormality and the age of the onset of menarche.

The age of onset of menarche of 261 subjects attending the Sickle Cell Clinic at Burla was recorded from history. Screening of these cases for haemoglobinopathy was done following the standard procedure for haematological investigations. These cases were grouped into normal control (HbAA), sickle cell trait (HbAS) and sickle cell disease (HbSS) and the cases with other diagnoses were excluded from the study.

The mean menarcheal age of homozygous sickle cell disease patients (14.93 ± 1.56 years) was found to be higher as compared to sickle cell heterozygotes (14.09 ± 1.37 years) and normal controls (13.65 ± 1.37 years). The delay was found to be a year or more in the homozygous patients.

These findings are consistent with those reported from America and Jamaica.

7.2.4 A study of reproductive outcome of sickle cell afflicted mothers in western Orissa.

Scientist : Dr. R.S.Balgir
Mr. B.P.Dash
Mr. R.K.Das

The sickle cell haemoglobinopathy is a major cause of high morbidity and mortality in the affected families. No information is available from this part of India pertaining to the reproductive outcome of the sickle cell afflicted mothers.

An attempt was made to find out the differences, if any, in the reproductive performance of the mothers suffering from different sickle cell genotypes compared to their normal counterparts.

Haematological investigations were carried out on 190 mothers who attended the Field station laboratory at Burla. The information was recorded regarding the total number of conceptions, abortions, miscarriages, still- births and post-natal deaths, including number and ages of surviving children and the age at death by the retrospective method. The mothers were grouped according to the haemoglobin genotype, i.e. control (HbAA), sickle cell trait (HbAS) and sickle cell disease (HbSS) and the mothers with other haemoglobin genotypes were excluded from the study.

Statistically significant increase of spontaneous abortions/miscarriages, still-births and post-natal deaths and decrease of average number of live births and surviving children among the mothers afflicted with homozygous sickle cell genotype as compared to the heterozygote and control mothers was observed (Ind J. Paediat., in Press). These findings are in agreement with those reported from America and Jamaica.

Training of staff member

Mr B.P.Dash, Research Assistant was sponsored by RMRC, Bhubaneswar to participate in the workshop entitled "India-Japan International Workshop on DNA Diagnostics" held during 15-22 January 1994 at Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow.

Awards/Honours/Other recognitions :

i. Dr R.S.Balgir, Assistant Director, received "Award for Excellence in Medical Profession 1994" in recognition and appreciation of contribution rendered towards medical profession, awarded by the All India Medicos Society, Hoshiarpur at the VII National Medicos Congress held on 20th January 1994 at New Delhi.

ii. Dr R.S.Balgir, Assistant Director was conferred the Honourary Fellowship (Sr.No.131/94) of All India Medicos Society, Hoshiarpur at the VII National Medicos Congress held on 20th January 1994 at Taj Palace Intercontinental, New Delhi.

iii. Dr R.S.Balgir, Assistant Director has been made Life Member of the All India Medicos Society and a member of Scientific Committee, VII th National Medicos Congress held on 20th January 1994, New Delhi.

Conferences/Workshops etc. Attended and paper presented:

i. Dr R.S.Balgir, Assistant Director was invited to present a paper entitled "The Body Mass Index in Sickle Cell Hemoglobinopathy" in the Lecture Series on Frontiers of Anthropology held during 8-11 December 1993, Indian Statistical Institute, Calcutta.

ii Dr R.S.Balgir, Assistant Director presented a paper entitled "Genetic Epidemiology of Sickle Cell Anaemia in India" at the VII th National Medicos Congress held on 20th January 1994, New Delhi.

iii. Dr R.S.Balgir, Assistant Director participated as one of the organisers (Co-ordinator) in the IV National Network Group Meeting on Haemoglobinopathies and Allied Disorders held during 4-6 March 1994 at RMRC, Bhubaneswar.

Publications:

i. Balgir RS, Das RK and Dash BP (1993) Age of Menarche in Sickle Cell Hemoglobinopathy. Indian Practitioner 46:319-322.

ii. Balgir RS (1993) Sickle Cell Disease and Community Health Issues in Orissa. Indian Practitioner 46:427-431.

iii. Balgir RS (1994) The Prevalence of Sickle Cell Hemoglobinopathy in India. In : Encyclopedia of Dravidian Tribes. T.Madhava Menon (ed.) International School of Dravidian Linguistics, Trivandrum.

iv. Balgir RS (1994) Biomedical Anthropological Approaches in North - Eastern India: A synthesis of the Tea Garden Population of Assam. In : Studies in Anthropology of North Eastern India. Sarthak Sengupta (ed.).

v. Balgir RS, Dash BP and Das RK (1994) Reproductive outcome of Sickle Cell Afflicted Mothers in Western Orissa, India. Indian Journal of Paediatrics (in Press).

7.3 Bio-Staticstics Section

7.3.1 Prevalence of Filariasis: a survey.

Investigators	:	Mr. Anil Kumar
		Dr. A. P. Dash
		Mr. P. K. Jangid (upto Sept.'93)
Technical staff	:	Mr. G. D. Mansingh
		Mr. S. S. Beuria
Starting date	:	November 1992, ongoing

This field based epidemiological study was initiated with the following aims:

1. To estimate the filarial infection rate by age and sex at Thana/Tehsil/District level.
2. To study the effect of socio-economic variables and behavioural factors on the prevalence of disease parameters.
3. To study the man hour density of possible vector mosquitoes and transmission pattern.
4. Finally, to undertake statistical modelling to explore linkages between various disease parameters, social variables, behavioral patterns and entomological variables.

Villages and then households were selected using Proportionality Probable Sampling and systematic sampling (Ann. Rep 1992). The erstwhile Puri district has been recently (June 1993) reorganised into 3 new districts namely, Puri, Khurda and Nayagarh. Till January 1994, survey work could be completed in 46 villages (Table.1). According to SAC recommendation the study will now be limited to the coverage of 40 villages of restructured Khurda district. A total 26 villages were covered in this target district. This study will be concluded after covering remaining 14 villages of Khurda district. Before reorganization of the old Puri district, some villages were covered- 12 of them coming under Nayagarh district and 8 coming under re-organised and smaller Puri district.

So far 5118 persons have been examined for filarial parasite. Of the blood slides examined to date, average mf rates were found to be 7.8% in Puri, 9.4% in Khurda and 12.4% in Nayagarh. The overall mf rate was found to be 9.9 percent. These results suggest substantial

Table 1 : Number of villages covered, slides collected and mf Prevalence

	AREA			
	Khurda	Puri	Nayagarh	Total
Villages surveyed	26	8	12	46
BSC	2957	786	1375	5118
BSE	1996	676	955	3627
Mf rate (%)	9.4	7.8	12.4	9.9
BSC/Village	114	98	115	111

BSC : Blood Slides Collected
BSE : Blood Slides Examined

shifts of mf prevalence in the different geographical regions. Table.2 presents preliminary data by tehsils. The highest mf rate has been observed in Ranpur tehsil of Nayagarh district (16.8%) followed by Khurda (12.8%) and Pipili tehsils of Khurda district (10.1%). The main filarial parasite found was *Wuchereria bancrofti* (98.6%) and *Brugia malayi* is detected only in 1 village of Nimapara tehsil.

Table 2 : Mf prevalence by Tehsil 1993

Area	Vill	BSE	Mfrate	Parasite
A1 : Khurda Tehsil	7	828	12.8	Wb
A2 : Bhub. Tehsil	6	777	5.7	Wb
A3 : Tangi Tehsil	3	391	9.2	Wb
A4 : Pipili Tehsil	2	159	10.1	Wb
A5 : Nimapara Tehsil	3	321	4.4	Wb+Bm
A6 : Puri Tehsil	3	306	8.8	Wb
A7 : Nayagarh Tehsil	4	445	7.4	Wb

Further analysis of data on 934 persons from about 200 families drawn from 8 villages suggested that 15.6% of males and 15.7% of females were microfilaraemic with mean density of 6.4 and 5.7 per person, respectively. However more males (25.3%) than females (13.2%) had symptoms of acute and chronic filariasis. Among males, 8.4% each had symptoms of Lymphadenitis (local names: Balusahi, Bagi, Pichuli) and involvement of scrotum. Among 57 females with the disease, 40 (72%) had swelling of lower limbs (feet or legs).

Among males who used mosquito nets for personal protection, 15.1% were found harbouring microfilaremia, against 23.9% of those who never used mosquito net. Similarly 14.8% females using mosquito nets were observed to be microfilaraemic against 17.2% of those never used mosquito nets. This study is in progress to cover remaining 14 villages of Khurda district.

7.3.2 Simulation studies on estimating potential risk of HIV transmission in a cohort of normal adult population with majority practising heterosexuality.

The sero-positive individuals with HIV infection as well as full blown AIDS in India are showing rapid increase with time. It has been estimated that the numbers of HIV sero-positive cases will get doubled every 300 days if this trend continues. However, this is less likely. Present emphasis on screening of high risk groups and IEC programmes are probably responsible for changing the course of the epidemic. Since information on variables affecting HIV transmission is not available, an attempt is made to study the transmission dynamics of HIV in a cohort of normal adult population with majority practising heterosexuality and a minority practising homosexuality, by using a mathematical model.

If a cohort of adults is consisting of susceptibles (X), sero-positive infected persons (Y) and full blown cases of AIDS (Z) then $X + Y + Z = N$

If it is assumed that the full blown AIDS cases do not have any sexual activity and X and Y are homogeneously mixed since Y being asymptomatic carriers, do not get isolated. If infection in (X) spreads at the rate of 'a/year' and HIV carriers develops AIDS at the rate of 'b/year', then

$$\Delta x = - axy \ \Delta t \qquad (1)$$

and

$$\Delta y = (axy - by) \ \Delta t \qquad (2)$$

If no. of susceptibles, carriers are xo and yo (>0) at beginning of the cohort then it can be shown as

$$y = yo \exp (- bt)... \qquad (3)$$

and

$$x = xo (ayo / b. \exp (- bt -1) - \qquad (4)$$

The parameters a and b are crucial and need estimation. The parameter 'a'can be estimated as follows :

$$a = [1 - (p\,(1 - r)^{NU}\,(1 - 1 - e)\,r)^{U} + (1 - p)]^{m} \quad -(5)$$

a = Annual probability of transmitting HIV as a result of N sexual exposures.

where p = prevalence of HIV carriers at the beginning of cohort.

r = risk of transmission/sexual exposure

U = annual protected sexual exposures/partner

NU = Annual unprotected sexual exposures/partner.

m = acquired new partners/year

f = extent of protection used

e = effectiveness of protection used.

The maximum days/year when coitus can take place are estimated as 300 taking into account of 5 days of menstrual cycle i.e., 25 (30-5) per month. From various studies it is found that once infected, AIDS symptoms develop after an average period of 10 years. So the value of b is taken as 0.10/year. The results based on simulation are presented in Table 1.

A transmission rate of one new HIV infection per 1000 sexual intercourses (risk of 1 in 1000 if r = 0.001) was the basic assumption. Group-I persons were assumed to acquire two new partners per year and to practise sex on 300 days in a year. In such a population with the assumed prevalence of HIV sero-positives, the chances to acquire the disease without any protective measures are 1.4/1000exposures in Group I.

According to the model the risk of acquiring infection reduces by 79% if safer sex practices (condom) are used always and the risk rate then would be only 0.3 per 1000 exposures in Group I. Groups III, IV and V are constructed to simulate commercial sex workers. On the extreme end (Group V), a commercial sex worker may acquire 50 new partners per year and may take 6 partners per working day (300 days X 6 exposures per day and with 50 new partners per year). In such a group the odds go up very steeply and the risk is 8.8 chances of acquiring infection per 1000 exposures. The chances in this group may go up by nearly 10 fold (73.1 out of 1000 exposures) if the original risk of infection is 10 per 1000, instead of 1 per 1000. Figures based on this assumption were shown in the lower half of the Table 1.

This model predicts a reduction of risk by 80%, if condoms are used regularly (safer sex) and the risk in such a protected commercial sex worker could be 1.8 per 1000 exposures,

Table 1 : Annual Probability of transmitting HIV - Infection in five hypothetical groups of normal adult population

Prop. time protection used (f)	Gr.I	Gr.II	Gr.III	Gr.IV	Gr.V
		r = 0.001			
0	.0014	.0041	.0082	.0086	.0088
0.25	.0011	.0033	.0067	.0069	.0071
0.50	.0009	.0026	.0051	.0052	.0053
0.75	.0006	.0017	.0035	.0035	.0036
1.0	.0003	.0009	.0018	.0018	.0018
% Reduction	.7900	.7800	.7800	.7900	.8000
		r = 0.01			
0	.0078	.0207	.0410	.0579	.0731
0.25	.0070	.0190	.0376	.0503	.0609
0.50	.0059	.0164	.0326	.0410	.0476
0.75	.0045	.0128	.0254	.0299	.0331
1.00	.0026	.0075	.0150	.0164	.0172
% Reduction	.6700	.6400	.6300	.7200	.7600

Gr.I : Acquired new partners/year (m) = 2,
No. of sexual exposures = 1/day
Gr.II : m = 5, c = 3/d
Gr.III : m = 10, c = 6/d
Gr.IV : m = 20, c = 6/d
Gr.V : m = 50, c = 6/d
r = Efficacy of transmission/exposure; r = 0.001 = one new infection/1000 exposures

instead of 8.8/1000/year without the usage of condoms. Complete protection from the contact of HIV viruses particles to mucosal surfaces (by removing the possibility of contact of vaginal mucosa with probably infected semen was thus predicted to give enormous benefits by reducing risk of transmission and the risk is now not much different from unprotected group with just two partners per year and one exposure per day (1.4/1000 exposures).

This model predicts a 4-5 fold reduction in the transmission of HIV infection within any given group if they observe safer sex practices and also predicts a 6 fold higher risk for the

acquisition for HIV infection by any extreme group, Group V with multiple partners compared to Group I within a given category of safe sex practices (100% condom use or never used category). Further the benefits due to less number of sex partners and safer sex practices were assumed to have complimentary effect. This exercise relies on many assumptions. Some of the values have no Indian database to support. However within limitations mentioned the model quantifies the role of multiple sex partners (Group I to V) and the role of safer sex practices independently and jointly on HIV transmission patterns.

Paper Publication :

1. Anil Kumar and N. Mishra, The effects of homoeopathic treatment on filariasis : a single blind 69 month follow up study in an endemic village of Orissa. British Homeopathy Journal (in press)
2. Anil Kumar and A. P. Dash, The prevalence of filariasis in Ranpur Tehsil. J. Com. Dis., 25(4) 1993.

Conference attended :

- Mr Anil Kumar attended XVI IASP conference at Hotel Kalinga Ashoka, Bhubaneswar 18-19 Feb 93 and presented a paper "A Demographic study in an isolated rural population".
- Mr Anil Kumar attended 46th Annual conference of Indian Society of Agricultural Statistics, Feb 20-22, 93 at OUAT Bhubaneswar.
- Mr Anil Kumar attended XI Annual Conference of Indian Society for Medical Statistics, Oct 18-20,1993 at Dept. of Statistics, Vizag and presented a paper on Epidemiological model of HIV/AIDS : Issues of data needs, availability and projection.
- Mr Anil Kumar attended ICMR/WHO Workshop on Biomedical Communication. Sept.28-Oct.1,1993 held at RMRC, Bhubaneswar.

7.4.Anthropology and Nutrition.

7.4.1. An Anthropological Perspective of Filariasis.

Scientist	:	Mr.A.Mohapatra
Starting date	:	July 1992
Duration	:	2 years

The state of Orissa is known to be endemic for lymphatic filariasis and the costal districts namely, Cuttack, Puri, Balasore and Ganjam (undivided) are more affected. According to

Health Information of India - 1991, there were an estimated 1.40 million people affected by this disease and the estimated number of microfilaria carriers was about 2.23 million. The population at risk was estimated to be around 24.61 million, out of the total population of 31 millions.

The present study was initiated during July 1992 at Choudwar in Cuttack district and Patrapada village of Khurda district. Subsequently as per the recommendations of the SAC the study was extended to the villages and towns of Cuttack and Puri district. A total of 318 patients with elephantiasis have been covered for this social anthropological survey.

The study aims to document the peoples' perception and experience about filariasis within a given social system and cultural pattern in the different socio-economic strata of the society. The study will quantify the "Social stigma" attached to this disfiguring and disabling disease. The society, at large, attaches more stigma to the disease and at times the patients are housed separately. Social prestige of the individual affected by the disease comes down profoundly and the alliance pattern in the social hierarchy is also shattered. It is very difficult on the part of girl's parents to find a match, if a girl has visible disease.

The ethno-medical practices of filarial patients were investigated at length. It is observed that the people of the area do use many local medicinal substances, leaves and roots of different herbs and shrubs for local relief. In some cases, it is reported to have periodic symptomatic relief, but the usefulness of these ethno-medical practices has not been ascertained in this study.

It was observed that this disease has definite relation with the economic standard of the people. It affects mainly the economically poorer segments. However, in this series we have come across 22.3% of the affected patients from higher economic status. In depth studies revealed that these families too originated from economically backward segments and moved up in the economic level in the recent past.

Elephantiasis does not kill a man, but it cripples the family as a whole. The socio-economic structure of the family is affected by this disease. The patients were observed to spend about Rs.67/- to 80/- per each attack of exacerbation of the disease. These attacks are periodic and are irregular in nature. On the other hand, the "indirect cost" due to the disease involves loss of several weeks of productivity in the beginning and culminates in to the stage of complete loss of productivity.

7.4.2 Tribal food habits and its relation to health and disease.

Scientist	:	Mr.A.Mohapatra
Starting date	:	July 1992
Duration	:	3 years.

The study was initiated with the aim of finding out the crucial relation of the tribal nutrition pattern with that of the changing trends in food supplies and other socio-economic events. For this purpose the Koraput district was selected and the initial study was launched with the following aims-

(a) To find out the tribal food habits and dietary patterns.

(b) To ascertain the nutritional status of the tribal pre-school children.

(c) Food preservation and beverage consumption pattern, etc.

(d) Health and disease profile.

Out of 62 tribes in Orissa, Koraput district (undivided) has 51 tribal groups. Three to four predominant tribal groups will be selected for our surveys. Hence the profile of this study would be very useful to understand the tribal culture, dietary pattern and other socio-cultural milieus. During 1992-1993 a qualitative analysis of the dietary habits has been carried out and appeared in the annual report of that year. In that study it was observed that the water drinking habits (from unprotected wells and streams) were not safe especially during the rainy season. The staple cereals were rice, ragi and maize which were seasonal, in production and consumption, pulses used were green gram, bengal gram, etc as grown by them at household level. The seasonal fruit consumption was very high and they preserved fruit by Sun drying method.

Consumption of animal protein was mostly related to feasts and ceremonies. They eat meat of goat, sheep, cow, buffalo, chicken, fish, and many more animals and birds. The beverage consumption was high in males than females. The basic liquor they used were mohua and handia. Besides these some tribes eg. Bonda's prepare liquor from jackfruits, mango and banana ect.

The 8th SAC has recommended studies on the seasonal nature of diarrhoeal disorders and nutritional problems in the tribal districts. Such studies will provide authentic quantitative data on the nutritional profile of children and mothers of these deprived segments. The results from such surveys can be used for better planning and targeting of programmes for the improvement of tribal health and nutrition profile in Orissa.

7.4.3. Health and nutritional profile of school children.

Scientists	:	Mr.A.Mohapatra
	:	Mr.N.Marai
		Mr.J.Murmu
		Ms. G.Mallick
Starting date	:	March 1994

Nutritional status of school children is a convenient parameter to assess the nutritional status of a given area. Apart from vulnerable groups like pre-school children and pregnant and lactating mothers, school children constitute a major segment whose health and nutritional status will indicate the trends of changing profiles in the different regions. Six schools were covered this year as a beginning to document the nutritional status of school children by this Centre. Standard equipments were procured for the measurement of height, weight, mid-arm circumference and skin fold at triceps. Methodologies for these measurements were standardized. Clinical nutritional status parameters were standardized as per the protocol used for NNMB by NIN, Hyderabad. Haemoglobin (Hb) estimation was carried out by cyanmethaemoglobin method, using filter paper to transport 20 ul of finger prick blood samples as per NIN, Hyderabad field based method.

A total of 1143 school children aged between 5-14 years were examined for clinical nutritional status and nutritional anthropometry. Girls constituted 48% of the total. Bitots'spots were seen in 70 children, giving a prevalence of 6% for Vitamin A deficiency. Vitamin A deficiency prevalence was higher in the semi-urban Nandan Kanan High School. Angular stomatitis was seen in 80 children, giving a prevalence of 7% for B-complex deficiency. Prevalence of Vitamin A deficiency was found to be more than the general pattern seen in such surveys in other parts of the country.

A total of 1018 children were covered for Hb estimation. Only about 5% (4.7%) of these children had 12 gm Hb/dl of blood. About one-third (32.2%) had Hb values between 10-11.9 gm Hb/dl, indicating mild anaemia. About 40% (39.7%) had Hb values between 8-9.9 gm Hb/dl, indicating moderate anaemia. Nearly one-fourth (23.4%) had Hb values less than 8.0 gm Hb/dl, indicating severe degree of anaemia. Prevalence of anaemia and the magnitude of severity appears to be higher in these schools. Girls who constituted 46% of the sample covered for Hb collection, also showed similar profile for anaemia and 25.3% had severe degree of anaemia. Growth and development studies are in progress. These limited studies on school children had revealed that the nutritional plane in these children is lower than the general pattern seen elsewhere in the country.

List of Scientific Conferences Attended.

Mr.A.Mohapatra

-participated in the National Seminar on "Family Welfare, Population Education and Development" 11th July 1993.

- participated in the "VIII National Environmental Science Academy." February 1993, and presented a paper on "Physiological function in a hot desert environment and its relation to sex differences in physical fitness".
- participated in the ODA Workshop on "Health care for tribal communities in Orissa." 20th December 1993, at Hotel Swosti, Bhubaneswar.
- attended IX National Environmental Science Academy. 20th December 1993,Hydera bad
- participated in the ICMR/WHO workshop on "Biomedical Communication." 18th Sep tember to 1st October 1993, R M R C, Bhubaneswar.

List of publications during the year.

A.Mohapatra et al. "A retrospective study of opium addicts in Deaddiction Camps and rural community in Western Rajasthan." J Hum Ecology. Vol. 4(4): p.271-276, 1993.

A.Mohapatra et al. "Dietary intakes, knowledge of people about the disease and nutritional disorders in two desert districts of Rajasthan." Annals of Arid Zone, 32(1) 59-62, 1993.

VIII GENERAL SECTION

8.1 MEMBERS OF THE SCIENTIFIC ADVISORY COMMITTEE

(For the 8th SAC held on 13th Aug.1993)

1. Dr.S.P.Tripathy — (Chairman)
   Director General
   Indian Council of Medical Research
   Ansari Nagar, New Delhi 110 029

2. Dr.D.S.Agarwal
   Ex-ECD Chief
   Indian Council of Medical Research
   New Delhi 110 029

3. The Addl. Secretary — (Ex-Officio member)
   Health & Family Welfare
   Govt. of Orissa, Bhubaneswar

4. The Director — -do-
   Health Services, Heads of Dept. Building
   Govt. of Orissa, Bhubaneswar

5. The Director — -do-
   Medical Education and Training
   Head of Dept. Building
   Govt. of Orissa, Bhubaneswar

6. The Principal — -do-
   M.K.C.G. Medical College
   Berhampur, Dist.Ganjam

7. The Principal — -do-
   S.C.B. Medical College
   Cuttack, Orissa

8. The Principal (Ex-Officio member)
V.S.S. Medical College
Burla, Dist.Sambalpur

9. Dr.S.K.Sood
Prof. & Head
Department of Pathology
University College of Medical Sciences
Shahdara,Delhi 110 095

10. Dr.V.P.Sharma
Director
Malaria Research Centre
22, Shamnath Marg
New Delhi 110 054

11. Dr.M.V.V.L.Narasimham
Director
N.M.E.P., New Delhi 110 054

12. Dr.I.C.Tiwari
Advisor (Health)
Planning Commission
Yojana Bhawan, Sansad Marg
New Delhi 110 001

13. Dr.Kalyan Banerjee
Director
National Institute of Virology
20-A, Dr.Ambedkar Road
Post Box No.II
Pune 411 001

14. Dr.S.Pattnayak
W.H.O. (SEARO)
Indraprastha Estate
Mahatma Gandhi Marg
New Delhi 110 002

15. Dr.V.K.Vinayak
Addl. Professor
Dept. of Experimental Medicine
P.G.I.,Chandigarh 160 012

16. Dr.B.B.Tripathy
Retd. Prof. of Medicine
Saradiya Mission Road
Cuttack 753 001

17. Dr.U.C.Chaturvedi
Dept. of Microbiology
K.G.Medical College
Lucknow 226 003

18. Dr.M.K.K.Pillai
Dept. of Zoology
Delhi University, Delhi 110 006

19. Dr.V.Sitaramam
Dept. of Zoology
University of Poona, Pune 411 004

20. Dr.V.Dhanda
Director
Vector Control Research Centre
Medical Complex, Indira Nagar
Pondicherry 605 006

21. Dr.R.S.Tiwariy
Director
Regional Medical Research Centre
R.M.R.C. Complex, Nagpur Road
Jabalpur 482 003

22. Dr.K.Satyanarayana
Director
Regional Medical Research Centre
Chandrasekharpur
Bhubaneswar 751 016

(Member-Secretary)

8.2 Distinguished visitors

Dr.S.P. Tripathy, Ex-D.G., ICMR; Dr.G.V. Satyavati, Ex-Chief of P&I and D.G., ICMR; Dr.D.S. Agarwal Ex-ECD Chief, ICMR; Dr.S.K. Sood Univ. College of Medical Sciences, Delhi; Dr.M.V.V.L. Narasimham, Director, NMEP, New Delhi; Dr.S. Pattnayak WHO, SEARO, New Delhi; Dr.B.B. Tripathy Ex-Prof. of Medicine, Cuttack; Dr.M.K.K. Pillai, Delhi University, Delhi; Dr.V. Sitaramam, Univ. of Poona, Pune; Dr.B.K. Nanda, DMET, Govt. of Orissa, Bhubaneswar; Dr.K.K. Mishra, Principal, VSS MC, Burla; Dr.V. Dhanda, Director, VCRC, Pondicherry; Dr.Dipika Mohanty, Director, IIH, Bombay; Dr.Banu Iyengar, Director, IOP, Delhi, Justice R.N. Mishra, former Chief Justice of India; Sri.Prafulla Samal, Hon'ble Minister for Labour and Employment, Orissa; Prof.V.S. Ramamurthy, Director, Institute of Physics, Bhubaneswar; Dr.Parthasarathy, Secretary, Atomic Energy Regulatory Board, Bombay; Prof.P.S. Iyer, Head of Radiation Physics, BARC, Bombay; Dr.D. Kanungo, Central Insecticide Board, Delhi; Dr.S. Swarup, IVRI, Izzatnagar; Prof.B. Ananthraj, Madras; Dr.Rolla Rao, Hyderabad; Prof.Md. Hafeez, Tirupathi; Prof.K.C. Bose, Ranchi; Prof.T.R.C. Sinha, BETIAH; Prof.S.A. Salgare, Bombay & Prof.M.C. Dash. Dr.S.K. Satapathy, Regional Director, RDH&FW, Bhubaneswar; Dr.Randhir, Director, CIFA, Bhubaneswar; Mr.M. Lakshminarayana, Sr.DDG, ICMR; Dr.H.N. Saiyed, DD, RCOH, Calcutta; Dr.Anasuya Das, DD, NIN, Hyderabad; Dr.N. Medeppa, DDG (Sr.Gr.), ICMR; Dr.K. Satyanarayana, DDG, ICMR.

Other activities :

1. A four day ICMR/WHO Workshop on Biomedical Communication was organized by the P&I Division of ICMR at RMRC, Bhubaneswar between 28th Sept. to 1st October 93. Faculty included Dr.G.V. Satyavati, Sr.DDG (P&I); Dr.L.N. Mohapatra, Ex-Director, RMRC; Dr.N. Medappa, DDG (Sr.Gr.) (P&I); Dr.M.K. Das, DD, RMRC; Mr. N.G.K. Nair, DD, IRMS, Madras; Mr. R.F. Vallishayee, DD, JIL, F.U., Avadi; Dr.K. Satyanarayana, DDG, P&I, ICMR; Dr. B. Ravindran, AD, RMRC. A total of 25 participants from various states including 10 scientists from RMRC have participated in this workshop. Dr.Harcharan Singh, Ex-Advisor (Health), Planning Commission, inaugurated the workshop. Dr.L.N. Mohapatra, Ex-Director, RMRC, Bhubaneswar gave away the certificates.

2. The 4th National Network Meeting cum Workshop on "Haemoglobinopathies and Allied Disorders" was undertaken at this Centre. This was organized by Dr.Dipika Mohanty, Director, Institute of Immuno-Haematology, Bombay with the help of Dr.G.P. Chhotray, AD and Dr. Balgir, AD from this Centre. About 10 participants attended the meeting from different institutions. Dr.Dipika Mohanty had outlined various aspects of haemoglobinopathies and this was followed by discussions.

3. The 8th Annual Conference of the National Environmental Science Academy was held at RMRC, Bhubaneswar from 16th to 19th February 93. On this occasion seven symposia were organised under the main theme "Environmental Management of Preventable Human Problems". The annual conference and the symposia were inaugurated by Dr.S.P. Tripathy, DG, ICMR on 17.2.93. The valedictory function was held on 19. 2. 93 with Justice R.N. Mishra, former Chief Justice of India as the Chief Guest & Sri Prafulla Samal, Hon'ble Minister for Labour & Employment as the Guest of Honour. About 350 scientists from all over India and abroad participated in the above conference and as many as 176 research papers were discussed.

8.3 Meetings and seminars of Dr.K.Satyanarayana, Director

1. Organised 3rd National Network meeting on Haemoglobinopathies and Allied disor ders. About 10 workshop participants under took survey at Angul and a lecture series was arranged.

2. Organised ICMR/WHO Workshop on Bio-medical Communication for the Eastern Region on behalf of the P & I Division of ICMR. About 20 participants and 10 faculty members participated in this workshop.

3. Attended the 81st Indian Science Congress held at Jaipur, 2-6 January 1994 and gave a lecture on Nutritional requirements of sports persons in the Symposium **"Up-date on Nutritional Requirements"** organised by Dr. Rajammal P. Devdas.

4. Delivered a special Platinum Jubilee lecture on **"Nutritional profile in India"** on behalf of the committee on Home Science at the 81st Indian Science Congress, Jaipur 1994

5. An abstract was submitted to the International seminar on DHF/DSS held at NIV, Pune - 7-8th February 1994 - **"Flavi Virus Infections in some parts of North-East and Eastern India"** by Dr K.Satyanarayana, Dr H.C.Baruah, Dr A.P.Dash and Dr G.P.Chhotray.

6. Delivered a lecture on the Nutritional status of North-Eastern region at the local chapter meeting of NSI, held at Home Science College, Jorhat, Assam.

7. Attended the opening ceremony of Platinum Jubilee Celebrations of the National Institute of Nutrition, Hyderabad. Attended Platinum Jubilee Celebrations held during November 1993 and rapported one of the key sessions on Women's Health and Nutrition at the celebrations.

8. Participated in the IEC programme on HIV/AIDS carried out for the inmates of circular jail of Choudwar - November 1993. Also participated in the IEC programme on the prevention of HIV/AIDS for the staff of Infantry Battalion.

9. Participated and directed the organization of extension activities in connection with the National Science Day Celebrations during February 1994. Students from Sainik School were taken around the laboratories and highlighted the preventive health care aspects and disease causing agents to the school children. Dr M.K.Das,DD, Dr A.P Dash,AD and other colleagues had carried out programmes in Baripada and Bodasahi.

10. Delivered a lecture on the "Nutritional Profile of India with particular reference to Orissa" to the summer school students of Anthropology department of Utkal University.

8.4 Developmental activities :

Arrangements have been made to de-weed the campus by hiring labourers from registered contractors. Fresh estimates have been sent for increasing the height of the boundary wall, for providing street lights on the Western side of the campus and for the construction of scooter/cycle sheds near the main building and sanction of about Rs.6 lakhs was received from the Council for these works. Estimates for the construction of over head tank and digging of two bore wells was obtained from the CPWD authorities and forwarded to the Council. Executive Engineer of ICMR had suggested scaling down of the over head tank size and the CPWD authorities were approached for this purpose. Provisional estimate of Rs.5 lakhs was provided by the Council in the new budget for the year 94-95 for this purpose. Instrumentation Division of National Institute of Nutrition, Hyderabad has extended valuable help in the repair and maintenance of equipments at this Centre.

IX. EXECUTIVE SUMMARY

9.1 STUDIES ON IMMUNOLOGY:

A total of 18 asymptomatic mf carriers (AS) were treated with diethylcarbamazine (DEC). IgE antibodies to Fraction III (Fr.III) of bovine parasite *(Setaria digitata)* and *W.bancrofti* (most predominant human parasite) L_3 antigen were studied. The IgE antibodies increased transiently, followed by a sharp decline, more so for allergen Fr.III. In a field study, 103 individuals were tested for immediate type hypersensitivity (ITH) by using skin test reactivity to Fr.III - an allergenic fraction of bovine parasite. More than half of AS individuals showed positive ITH and in such positive persons IgE levels were higher and IgG4 levels were lower. However, this inverse nature of IgE and IgG4 levels in ITH positive persons were not seen in other filarial groups namely, chronic filariasis patients or endemic normals. Studies on filaricidal activity of purified active component of plant *Streblus asper,* indicated that 70 ug of purified agent could kill an adult *Setaria* parasite *in vitro.*

Separation and purification of enzymes superoxide dismutase (SOD) and proteases derived from *S.digitata* adult worm were investigated. SOD enzymic activity of both human and bovine parasites could be inhibited by incubation with IgG from chronic filarial patients. Higher sero-positivity to SOD was noticed in chronic filarial patients, while subjects from non-filarial region showed sero-negativity. Higher antibody levels to protease-I (SdP-I) were detected in most of filarial patients, while endemic normals and subjects from non-filarial regions were sero-negative. These results suggest a possibility for SdP-I (bovine parasite enzyme) for field use as a marker of filarial infections in human beings. Advanced studies on Fr.III indicated that this allergen is a metal dependent cysteine protease with a molecular weight of 30 KDa.

An antigen from somatic extracts of *S.digitata*, that could precipitate in agarose with wheat germ agglutinin (WGA), was purified and was found to react with anti-DEC antibodies. The WGA binding antigen was found to be heat stable, which had a large molecular weight and slow ß-electrophoretic mobility. Studies were carried out on mf clearance of *B.malayi* infected *Mastomys*, after anti-DEC antibodies were raised in them. Such animals did not show immediate mf clearance, but required only a small fraction of normal dose of DEC (1/20th) for subsequent mf clearance. Studies were carried out on the possibility of polymorphism for mf sheath antigens. Sera from seven amicrofilaraemic elephantiasis patients reacted uniformly

with all the five mf preparations. This was not the case in fifteen asymptomatic mf carriers who probably respond only to the variable antigen(s), while amicrofilaraemic chronic patients possess antibodies to the conserved antigens.

The cytotoxicity of malarial sera to filarial mf was examined further this year. This activity was reduced by the removal of low density lipoproteins and also by the addition of phospholipase A2(PLA_2) inhibitor. Both the mf and adult filarial bovine parasites were found to be susceptible to porcine pancreatic PLA_2. It is concluded that at least some of the mf cytotoxicity seen in malarial sera is mediated by PLA_2.

TNF - α has been shown to be responsible for many of the clinical manifestations seen in malaria. An exo-antigen containing phospholipid has been found to induce TNF - α in animal models. Anti-phospholipid (anti-PL) antibodies have been shown to offer protection (anti-toxic immunity) in murine malaria. These two parameters have been studied in twenty two human cerebral malaria patients. Anti-PL antibodies were significantly higher in patients with better prognosis. They were inversely related with TNF - α which is known to mediate harmful effects. Higher levels of anti - PL may be useful to predict better prognosis and survival in cerebral malaria patients.

Microfilarial model to maintain mf in circulation was standardized in *Mastomys* for *S.digitata,* by intraperitoneal implantation of gravid worms which can release mf. Such a procedure led to immunosuppression to tetanus toxoid in implanted animals. Antibody response to Fr.I and III of *S.digitata* was poor, when dead worms (cold stunned) were implanted. Earlier studies indicated that mf sheath antigens are carbohydrate in nature and may thus be T- independent antigens. In order to establish a model to sustain mf of *W.bancrofti* in circulation, genetically deficient (deficient for immune response to T-independent antigens) CBA/N strain mice were procured from USA, with the help of NII, New Delhi. This colony is flourishing (from 2 pairs) and it may be possible to maintain *W.bancrofti* mf for longer periods and to carry out *in-vivo* evaluation of anti-filarial drugs.

9.2 CLINICAL STUDIES :

A longitudinal study of 2 years duration on the efficacy of Ivermectin in the prevention of adenolymphangitis (ADL) attacks has been in progress at this Centre with WHO/TDR support. About 105 patients were registered for therapy with Ivermectin at a dose of 400 ug/kg on 12 occasions at monthly intervals. Majority (72.4%) were men and half of the men and women (48.6%) were aged below 30 years. A total of 54 subjects completed 12 months therapy course and entered the second year of the project for 12 months observation,

regarding ADL attacks. The second year of the study (94-95) will be crucial for this investigation. This being a double blind study the results will be available only at the end of the study i.e., by March 1995.

In a clinical survey of 4680 population, 1524 men were examined for various presentations of filariasis. A total of 439 (28.8%) had hydrocele. Apart from these, 6.8% of men had either lymphoedema of limbs alone or lymphoedema in combination with hydrocele. It was observed that half of the men above 30 years of age had hydrocele in this endemic area. About one- fifth of those who had shown hydrocele, did not give a history of filariasis and also did not show mf in night blood smears. This group was referred to as "idiopathic" group of non-filarial aetiology.

Using direct fluorescent antibody analysis, it was observed that the sheath of live mf of *W.bancrofti* as well as mf of *B.malayi* had human albumin. The mf of *W.bancrofti* had shown IgG1 (60%) and IgG4 (52%) on their surfaces. Similarly *B.malayi* mf were observed to have IgG (50%) on their sheath. These observations suggest the possibility that the mf may become coated with albumin soon after birth.

9.3 STUDIES ON MICROBIOLOGY :

This Centre had screened a total of 1624 blood samples from blood donors, TB patients and patients from OPDs of hospitals for seropositivity for HIV/AIDS during this year. There were 10 western Blot positive individuals, 6 from blood donors, 2 from TB patients and 2 from high risk groups, with a seropositivity rate of 6.16 per 1000 samples. The six years' cumulative figures upto March 1993 (1/5439) showed a low seropositivity rate of 0.18 per 1000 samples. The 7 years' cumulative figures including the current year (11/7063), registered a figure of 1.56 per 1000 samples screened. Majority of current year seropositive persons are Oriyas, who migrated to other cities for work and business. The Red Cross Blood Bank, Berhampur with 4 seropositive persons among its blood donors led this upsurge in infection of HIV/AIDS into this state. IEC programmes have been taken up for high risk groups at various locations.

Seasonal cycles of diarrhoeal disorders were common in the tribal regions of Orissa. This Centre had requested NICED, Calcutta for a joint survey of an epidemic involving 2636 persons with 285 deaths in the current year. About half of the diarrhoeal cases were registered in the month of August in which majority of deaths (60%) have occurred. *Vibrio cholerae* 01 Inaba were grown from 16 out of 34 rectal swabs and 3 out of 4 water samples collected - including one from a stream. The need for repeated chlorination of drinking water supplies, the need for basic sanitary arrangements and overwhelming need for health education for

improving personal hygiene were emphasized to the State Government authorities.

"Ragi" gruel supported the growth and survival of a variety of bacteria including *V.cholerae*, even at ambient temperature. "Ragi" gruel may thus have a potential role in transmitting infection between family members, as it is often made in bulk and consumed by all family members over a period of several hours.

Three strains of thermophilic fungi from hot springs showed enzyme activity (amylase) even at a temperature of 80° C or at a pH of 11. Eighteen bacteria and 12 fungi were tested against essential oils derived from six plants. The oils were bactericidal at a concentration of 1.66 ul / ml to 2.5 ul/ml. Studies carried out on possible mode of action revealed that *Palma rosa* and *Peppermint* induced formation of elongated filamentous forms of *E.coli* (by 10 fold).

9.4 STUDIES ON CLINICAL PATHOLOGY:

Cat as an experimental animal for *B.malayi* infection was studied. Microfilaria appeared after 2-3 months of subcutaneous injection of infective larvae. The mf counts reached peak levels by 6 months. The mf observed to have nocturnal periodicity with a peak at about midnight. Histopathological changes were recorded in the autopsies on the animals. In a hospital based study carried in SCB Medical College, Cuttack, 80 subjects with involvement of one joint (monoarticular arthritis) were investigated. After excluding specific diseases there were 15 cases with no specific cause and these were labelled as non specific arthritis. A careful history revealed that 11 of them had a history of filariasis and two of them had shown mf in peripheral blood. All these cases responded well to anti- filarial treatment. In a community based study a total of 1378 subjects were examined for signs and symptoms of filariasis with special emphasis on asymptomatic microscopic haematuria on a sub sample. A total of 24 persons (10.5%) out of 228 persons examined for microscopic haematuria had shown varying grades of haematuria. Excepting 3 individuals who had chronic filariasis and Grade IV haematuria (full of RBC/HPF), the rest of the 21 subjects had Grade-1 haematuria (1-8 RBC/ HPF). Haematuria is not positively associated with the presence of mf in circulation and it's prevalence is 3-4 fold higher in chronic patients (42.1%), who had no mf in circulation.

9.5 STUDIES ON MEDICAL ENTOMOLOGY :

A major 3 year study on the control of *Cx.quinquefasciatus*, by using the biocide *Bacillus sphaericus* is in its second year, with the support of WHO/TDR. Baseline data were collected in two towns with 75,000 population. About 9,000 blood smears were examined and bionomics of vectors and transmission parameters of filariasis were collected for 12 months and the two towns were found comparable. Intervention was carried out in one experimental

town Khurdha, from April 93, with quarterly spraying of the biocide and focal spraying of new cess pools and drains. Application of this biocide had reduced *Cx.quinquefasciatus* densities to 1/8th and 1/9th of baseline values. These gains of biocide were slightly altered in rainy season. However, the densities of larvae, adults, adult mosquitoes landing on man remained reduced to 60-70% of baseline values. After the monsoon the benefit was seen in full form and infective larvae (L_3) of *W.bancrofti* were practically absent in wild-caught mosquitoes of Khurdha town. This was true, though there were old filarial cases and no special therapy programme was offered to them. The control town Pipili still supported 20 fold level mosquitoes (at 200-440 mosquitoes/man/night) and about 5% of them had L_3 larvae of *W.bancrofti* awaiting transfer to human beings. Simple spray of breeding places with *B.sphaericus* could protect the entire population of one town from filarial transmission.

Laboratory studies on the effects of sub-lethal dose (LD_{25}) of *B.sphaericus* on *Cx quinquefasciatus* have revealed significant changes in the fecundity and gonotrophic cycle. Only 20% of treated mosquitoes laid eggs, while 73% of control group laid eggs. Reproductive performance and sex ratio was altered in the treated group and less number of male mosquitoes emerged. Analysis and experiments with water samples (from cess pools, cess pits and drains) were carried out. Out of the total larval collections, 57% were from drains and 26% were from cess pits and these two sources had very high level of pollution as indicated by total dissolved solids and biological oxygen demand (B.O.D). Cess pool water on the other hand had lower level of pollution and contributed only 11% of total larval collections.

Three different strains of *B.sphaericus* were assessed for their efficacy. CDRI formulation had very low efficacy and needed higher concentration (4 ppm) as LD_{50} value, against *Cx.quinquefasciatus*, as compared to 0.007 ppm of Sphaerimos and 0.016 ppm of Spherix. Similar differences were noted for *An.stephensi*. This Centre has also initiated investigations on innovative vector control agents, keeping environmental protection in mind. Studies on plant extracts revealed that *An.stephensi* and *Cx.quinquefasciatus* were found to be susceptible to methanol extract of *Anacardium*, whereas ether extract was effective on *Ae.aegypti*. *Pongamia* methanol extract was also found to be effective, but at a higher concentration. Bioactivity of hydro distillated essential oils, Citronella and Pogosteomon were tested and LD_{50} levels were found to be between 35 to 53 ppm for the three vectors listed above.

Several districts were covered in a comprehensive survey to understand the bionomics and feeding habits of *Mansonioides* mosquitoes, transmitting *B.malayi* filariasis. *Ma.annulifera* and *Ma.uniformis* predominated in all the collections, constituting about 90% of the total catch. *Mansonioides* reached their peak per man hour densities in the month of October and

the least densities were seen in June. This was due to growth of hydrophytes in water bodies and breeding of *Mansonioides* in such conditions at the end of monsoon. After the analysis of 1540 blood meals of *Ma.annulifera* it was observed that it preferred human blood on 57 occasions out of 100 and the rest 43 out of 100 blood meals were found to be from other species.*Ma. annulifera* visited man after midnight and changed its peak times and preferences according to the season.

9.6 STUDIES ON COMMUNITY HEALTH AND NUTRITION :

The 8th SAC of this Centre had given recommendations to carry out epidemiological studies on haemoglobinopathies and nutritional disorders in different areas of the region. About 70 persons belonging to general caste groups and 30 persons belonging to Scheduled Castes were screened for haemoglobinopathies in the village Kania, as part of a National Network meeting. More than one-third persons from general caste category had heterozygous sickle cell gene (AS) and another 10% had homozygous sickle cell gene (SS). In a hospital series 30 patients with sickle cell disease (SS), 207 patients with sickle cell trait (AS) and 69 control subjects were examined for body mass index (BMI). About a quarter of men with sickle cell disease had very low BMI values (<16.0), which indicated the presence of severe undernutrition in them.

Social, cultural and economic factors among 300 elephantiasis (filariasis) patients were investigated using an anthropological approach. Many of the patients have used variety of treatments, including herbal medicines. About one-fifth of patients belonged to higher economic status. An amount of Rs.70.00 was the direct cost to the patient for the treatment of each attack of acute exacerbation.

About 1160 school children were examined from upper primary and high schools in and around Bhubaneswar for their nutritional status. Vitamin-A deficiency as adjudged by Bitot's spots was prevalent in 6% of school children and was more frequent in semi-urban area. Growth and development studies are in progress. Attempts are underway to study the nutritional status of vulnerable groups from Gania Block of Nayagarh District and Nawarangpur area of erstwhile Koraput district.

Two villages namely Balasingh and Singhpur of Bolgarh block were affected with lesions similar to fluoride toxicity. Examination of 322 persons from these villages revealed clinical signs of dental and skeletal abnormalities attributable to fluorosis in 48.1% of the subjects. Dental mottling reached a peak at 11-20 years of age (72%). Skeletal abnormalities were more common in 51-60 years age group (35%). Analysis of drinking water supplies

revealed fluoride levels between 4-6 ppm in certain sources of drinking water. Detailed studies of water samples and food samples are in progress in collaboration with NIN, Hyderabad. Surgical correction of genu-valgum is being attempted in adolescent boys in collaboration with SCB Medical College, Cuttack.

A systematic study for the estimation of prevalence of filariasis in Khurda district revealed three fold variations in mf rates (5.7 to 16.8) in different Tehsils. Social, economic and personal protection habits are being studied simultaneously to develop a model.

DESIGNED & PRINTED BY CAPITAL BUSINESS SERVICE & CONSULTANCY, BHUBANESWAR