REGIONAL MEDICAL RESEARCH CENTRE

(INDIAN COUNCIL OF MEDICAL RESEARCH)

BHUBANESWAR, ORISSA

ANNUAL REPORT

1992-93

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Preface

The foundation of Regional Medical Research Centre, Bhubaneswar was laid on March 29, 1981 by the then Prime Minister Smt. Indira Gandhi. By early 1985, the centre was able to have a core faculty of 10 scientists and about 50 supporting staff housed in a rented accommodation and was directed to initiate research in filariasis, malaria, hemo-globinopathies and other major health problems of this region. The Centre started functioning from its own building at Chandrasekharpur only around January,1991. From the beginning itself lymphatic filariasis has been the major thrust area of this centre and comprehensive studies have been undertaken. Some of the achievements are the determination of the microfilaricidal dose of the new anti-filarial drug Ivermectin; reduction in the size of lympho-edematous limbs by DEC therapy; vector control by *B. sphericus*; production of antibodies to DEC and its cross-reaction with microfilarial sheath; demonstration of anti-malarial and anti-(adult) filarial activity in plant *Streblus asper*; immunodiagnostic utility of IgE and IgG4 levels; development of new technologies for vector control and successful isolation of natural juvenile hormones from Aphids.

A new animal model for filariasis in Mastomys natalensis has been developed by implantation of adult parasites of *S. digitata* which may prove immensely useful for undertaking experimental immunological and chemotherapeutic studies. The observation of cytotoxicity mediated by human malarial sera to filarial parasite is a novel finding. The induction of enhanced specific antibody production in microfilaraemic individuals following DEC therapy to a level comparable to that of endemic normals would suggest the importance of a purified antigen in protective immune mechanism. Studies have been initiated to prevent the occurrence of further attacks of adenolymphangitis in filariasis patients with Ivermectin therapy. Histopathological changes in filarial patients are being monitored.

The Centre has been identified by WHO, CDRI (CSIR) and NMEP for testing of biocides and other compounds against mosquitoes and a TDR supported project on large scale evaluation of *B. sphericus* is being carried out. Pockets of *B.malayi* infection in human communities have been detected and distribution of *Mansonioides* in various regions of Orissa is being mapped.

The Centre is actively engaged in studies on diarrhoeal diseases, thermophilic bacteria from hot springs of Orissa and plant products as anti-microbials. The Centre is involved in sentinel and blood donor surveillance for HIV infection in the region. These studies need to be expanded to cover port towns of two or three states of this region. The Centre is also imparting training to Ph.D students as a part of local human resource development programme in medical research. For different reasons this regional centre has not been able to take up active research on health and nutritional problems of backward districts like Kalahandi, Koraput and Phulbani. Attempts will be made to achieve a balance between laboratory based studies and community based studies, encompassing the whole range of health problems.

The staff and Directors of this Centre express their gratitude to Dr. S.P. Tripathy, Director General, ICMR and Dr. D.S. Agarwal, ECD chief, ICMR for their valuable guidance and kind support.

K. Satyanarayana

Place: Bhubaneswar
Date: 26.05.93

(Dr. K. Satyanarayana)
Director

Executive Summary

The variations in antibody prevalence to infective larvae of *W.bancrofti* with the age of normal individuals living in a filariae-endemic region (Khurdha district) of Orissa were determined. The sero-conversion to IgM occurred around 7th year, but the same process to other isotypes (IgG, IgG2, IgG4 and IgE) occurred later in life, after teenage.

Antibody level (IgG) to a purified filarial antigen was found to be very low in asymptomatic microfilaraemic individuals (AS) in comparison to the amicrofilaraemic groups of chronic patients and endemic normals. Such AS individuals would appear to have an inability to produce antibodies to this antigen. However after treatment with DEC, the treated individuals exhibited 10 fold enhancement in the antibody level. It appears that the mechanism responsible for this phenomenon may be playing a protective role in mediating antimicrofilarial immunity.

Antibodies to DEC raised in experimental animals were found to react with a soluble antigen of *S. digitata*. This antigenic component also precipitated strongly with wheat germ agglutinin (WGA), a lectin that recognizes N-Acetyl D-glucosamine residues. The antigen has been affinity purified using WGA-Sepharose for further characterization.

A new animal model has been developed for induction of microfilaraemia. Adult stage parasites of *Setaria digitata* on intraperitoneal implantation in *Mastomys natalensis*, induced microfilaraemia by 7th day, which lasted for nearly 105 days. This has opened up avenues for undertaking immunological and chemotherapeutic studies in experimental filariasis. The existing laboratory animal models require an incubation period of at least 100-120 days before the appearance of the first microfilariae in circulation.

Tropical Pulmonary Eosinophilia (TPE) is considered to be a hypersensitive reaction to microfilarial antigen primarily affecting the respiratory system of humans living in filarial endemic areas. Longitudinal studies on TPE were continued at the outpatient department of Unit III Govt.

dispensary. The aim is to evaluate the clinical manifestations, pattern of recurrence, concomitant immunological alterations and response to therapy.

A double blind chemotherapy trial with Ivermectin or DEC versus placebo is being carried out in the field with the financial support from WHO/TDR. The aim is to prevent future occurrence of filarial adenolymphangitis in patients with lymphatic filarial infection. Trial drugs are given in single monthly doses to each case enroled. A total of 58 patients were registered for therapy.

Cryptosporidium was not found in 47 diarrhoeal and 106 non- diarrhoeal subjects attending paediatrics clinic catering to middle income group. In a hospital based study, eighteen blood samples from 6 patients with subacute endocarditis were examined. Bacteria could be grown from samples of five patients. Serological testing of samples obtained from a field study on tribal population showed 60.6% carrier status for *Salmonella typhi*. During the year a total of 3327 samples were screened for sero-positivity to HIV/AIDS. Six were positive by ELISA, but only one was positive by Western blotting. Plant products that impart flavour/odour (Essential oils) have been screened for antibacterial activity against 17 bacteria representing 8 genera. From the natural hot springs of Orissa bacteria were isolated which tolerated a temperature of 60^0 C for 3 hours.

DNA extraction and sequencing was done in G-6-PD deficient and normal subjects. A single base change was found on Codon 44 producing a substitute of alanine to glycine at this portion. However, some normal subjects had also shown this mutation. Attempts are under way to search for the causative mutation.

Development of a cat model for experimental filariasis studies was achieved. Two cats which had microfilaria at about 18 mf /20 cmm of blood are under observation. In a third cat which died before developing microfilaraemia in the blood, an adult worm was retrieved from the thoracic cavity on autopsy. In a hospital based study histopathological changes and antibody levels were examined in the synovial tissue, synovial fluid and serum from the patients suffering from filarial arthritis.

Limited studies on cross-sectional basis were undertaken on diarrhoeal disorders, prevalence of malaria and health perceptions in some areas.

Qualitative survey was attempted on the seasonal food supplies of Koraput tribal populations. Inadequate availability of food, seasonal epidemics of diseases, morbidity and mortality patterns of weaker sections of society are to be documented in the backward districts of this region.

The project on present status of *Mansonioides* and *B. malayi* was continued during the year. Mosquito fauna in five villages of Puri (endemic for malaria) and Balasore (endemic for filariasis) revealed eight species of Anophelines and nine species of Culicines. Blood meals of mosquitoes belonging to various species were analyzed by gel diffusion technique.

Large scale field evaluation of *B. sphaericus* (WHO/TDR) against *Cx. quinquefasciatus* was initiated by the Centre in Khurdha area (highly endemic for filariasis). Pipili area was taken as the comparison area. The values of LD_{50} and LD_{90} for the biocide supplied by WHO were determined along with its recycling capacity in the laboratory and field. It recycles in the laboratory for 6 months and under field conditions for more than 6 weeks at a lower dose.

The centre also carried out laboratory and small scale field studies on *B. sphaericus* produced by the CDRI Lucknow. Four strains have been screened and among them strain 'B' appears to be effective against mosquitoes. A small scale field trial of this strain indicated that the larval density decreases soon after the application of the biocide, but larvae reappeared after three to four days of application. In a seperate study on marine products supplied by the CDRI Lucknow tested, only two viz., Zoanthus and unidentified corals were found to be toxic to mosquito larvae. Studies on aphid extracts (natural juvenile hormone) and OMS compounds (synthetic juvenoids) have been continued.

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(upto 11th January 1993)

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(from 17th May 1993)

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B.Pradhan	Insect Collector
C.S.Tripathy	Insect Collector
S.S.Beuria	Insect Collector
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K.C.Dalai	Generator Operator
J.Behera	Pump House Operator
Md.Daulat Khan	Driver
Sibaram Patra	Driver
R.Pradhan	Driver
Anakar Nayak	Driver
A.R.Khan	Driver
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A.Senapati	Animal House Attnt.
S.C.Das	Animal House Attnt.
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R.C.Dash	Office Attendant
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Banamali Naik	Sweeper
K.G.Samal	Sweeper
Banamali Sahoo	Gardener
R.S.Rai	Watchman
Sankar Pr.Sharma	Watchman
Som Pr.Sharma	Watchman
M.B.Thappa	Watchman
T.Bahadur	Watchman
R.S.Bahadur	Watchman

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I. PARASITE IMMUNOLOGY

1.1 Immunological Studies of Filariasis:

Scientist	:	Dr. Manoj K. Das
Technical Staff	:	Dr. M.K. Beuria
		Lt. Col. Dr. A.K. Praharaj (Guest worker on study leave from Indian Army)
Starting date	:	July 1985

Objective

To study immunological responses to infective-larvae (L_3) of *W. bancrofti* in endemic population.

Progress

We have shown earlier that antibody responses to L_3 of *W. bancrofti* in endemic normals follow an age dependent pattern (RMRC Annual Report-1991). An attempt has been made recently to describe the distribution of anti L_3 isotypic (IgG, IgG_2,IgG_4, IgE and IgM) titer in endemic normals (104 numbers, Bajapur village, Khurda District) of different age classes. Such analyses will indicate the age at which seroconversion (L_3 seropositivity) occurs or the majority of people will become antibody positive. It is found that L_3 conversion for all antibody isotypes, except IgM which peaked at a much earlier age (about 7 yrs) is attained in early adult class of 16-21 years (18.6 ± 1.6). Children below 5 yrs are seronegative to all antibodies other than IgM. The maximum values of antibody prevalence are 100% for IgM, IgE and IgG_2 ; 50% for IgG and 28% for IgG_4 in endemic normals (Fig.1). These values do not appear to decline, rather persist in older age groups (30+ yrs). The increased antibody response to L_3 stage in normal adults, a target group potentially important for evaluating protective immunity, could be a contributory factor towards the resistance against filarial infection.

The centre has described earlier about the isolation of an allergen from *S. digitata* and its immune response in human filariasis. (vide publication : *J. Biosciences* (1991) 17: 453-463). The IgE response to this antigen in endemic regions closely parallels to that of much difficult to get L_3 antigens of *W.bancrofti*.

Fig. 1 : Age-dependency of prevalence levels for anti-infective larval antibodies in normal individuals of filarial region

These studies led us to look for other antigens which could be isolated by different biochemical procedures. Antibody response to a detergent-soluble antigen of *S. digitata* (DSSd) is found to be particularly suppressed in asymptomatic mf-positive individuals (n=23) compared to endemic normals and chronic filarial cases. On the other hand, after treatment of AS individuals with diethylcarbamazine a dramatic enhancement (about 10 fold) in antibody response was registered. The enhanced IgG level to DSSd antigen became comparable to that of endemic normals. These results indicate the importance of antibody response to DSSd antigen in mediating filarial immunity in humans. Immunochemical characterization of DSSd antigen will be attempted.

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

Scientist	:	Dr. Manoj K. Das
Technical Staff	:	Dr. Dasarathi Das
Starting Date	:	February, 1987

Objective

To elucidate the mechanism of antifilarial activity present in the plant *Streblus asper*. As there are few drugs available at present with activity against adult filarial parasite, the toxic effect of *Streblus asper* should warrant detailed investigations for macrofilaricidal property.

Progress

We have earlier demonstrated potent toxicity activity of *Streblus asper* bark aqueous extract against microfilariae of *Wuchereria bancrofti* and adult worms of *Setaria digitata*.

The activity is retained even after the dialysis of the extract (against phosphate buffer pH 7.2) indicating the active principle to be of high molecular wt. Gel filtration data suggest that the molecular weight would be in the range of approximately 1,00,000. The active component was purified. Whereas proteolytic enzymes did not affect the killing, periodate oxidation of the agent abolished microfilaricidal activity. It indicates the importance of carbohydrate moieties of *S. asper* in the killing activity. However simple sugars viz., D-Glucose, Galactose, Mannose etc. (at 0.1M) do not inhibit the killing. It was also found while studying kinetics that purified component killed fairly rapidly-showing 100% mortality by 2 hrs. The substance is active at very low

concentration ($2X10^{-11}$ M) against microfilariae. The active substance is devoid of RBC-lytic activity. The extract in a range of 0.5 to 1.5 mg (sufficient for an adulticidal effect) could not release haemoglobin from erythrocytes.

In order to elucidate the mechanism of filarial killing by *S. asper*, the release of lactate dehydrogenase (LDH; a cytosolic enzyme) from microfilariae was studied. Microfilarial killing by the extract did not release LDH activity indicating that the killing process does not involve "pore" formation in the parasitic membranes.

It is further demonstrated that filarial killing is temperature dependent. For example, efficient killing could be carried out readily at 37° C or at 25° C; but at a lower temperature of 4° C, killing does not occur even after 18 hrs of incubation.

1.3 Antigenicity of filarial enzymes in endemic population.

Scientist	:	Dr. Manoj K. Das
Technical Staff	:	Ms. Madhusmita Bal
Starting Date	:	October 1991

Objective

The theme of the project is to :

1. detect and isolate biologically important enzymes such as superoxide dismutase, glutathione-S-transferase and proteinases of filarial parasites; and
2. investigate their immunological roles in human filariasis.

Progress

Superoxide dismutase : This enzyme is found in almost comparable levels in B. malayi L3 and *S. digitata* adult worms, but in significantly reduced quantity in microfilarial stages of filarial parasite (*S.digitata* and *W. bancrofti*). The enzyme is also detected in excretory and secretory (E-S) products of worms. A partial purification of (5 fold) enzyme activity is achieved from *S. digitata* adult worms. The activity is heat labile; heating at 100° C for 3 min. completely abolished the enzyme activity. The purified SOD appears to be immunogenic in filarial patients.

Glutathion-S-transferase (GST):

This enzyme is detected in adult stage of *S. digitata* but found to be absent in microfilariae. It is also absent in ES products in contrary to superoxide dismutase which is secreted by filarial (adult) worms.

These enzymes can be classified broadly as antioxidant enzymes and are not studied well in filariae. GST has been known as a vaccine candidate immunogen in a related parasitic disease e.g., schistosomiasis and would therefore be ideally useful to study as a purified immunogen in human population in Orissa.

Lecture

Dr. M.K. Das delivered a lecture entitled " Dynamics of human immune response to filarial parasites" in the Dept. of Biochemistry and Biophysics, University of Delhi (South Campus) on 11th August 1992.

Other important events

Sri M.K. Beuria, M.Sc., Research Asst. received Ph.D. degree (in Zoology) from Utkal University in November 1992 under the supervision of Dr. M.K. Das. The title of his Ph.D. thesis was "Role of adjuvants in the immune response of parasitic antigens".

Dr. M.K. Das was a Co-director of a laboratory training programme sponsored by Department of Biotechnology, Govt. of India on "Immunological Concepts in Tropical Parasitic Diseases" conducted by the Immunology Division, RMRC, Bhubaneswar, 9th-27th, November '92.

Publications

1. M.K. Beuria and M.K.Das (1992) Immune response to an allergenic fraction of *Setaria digitata* in human filariasis. *Journal of Biosciences* **17** : 453-461.

2. M.K. Das, M.K. Beuria and A.P. Dash (1992) Immunoglobin E and G4 antibodies to infective larvae in *Wuchereia bancrofti* endemic populations. *International Archives of Allergy and Immunology*, **99** : 118-122.

II. APPLIED IMMUNOLOGY

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

Scientists	:	Dr. B. Ravindran
		Dr. A.K. Satapathy
Technical Staff	:	Mr. P.K. Sahoo
Starting Date	:	November, 1990

Antigenic mimicry between the anti-filarial drug Diethylcarbamazine (DEC) and microfilarial sheath of *W. bancrofti* were reported by us in earlier years. Antibodies reacting with DEC were raised using various haptens such as methyl piperazine carboxylic acid (MPCA), piperidine carboxylic acid (PCA) or piperidine propionic acid (PPA). The reactivity of anti-DEC antibodies to microfilarial sheath was demonstrated by immunofluorescence assay. The antibodies were also found to be reactive to microfilarial and adult stage parasites of *Brugia sp*. This observation is significant since the biological role of these antibodies *in vivo* can not be studied with *W. bancrofti* since animal models are not yet available for this parasite. In order to perform *in vivo* investigations with anti-DEC, *Mastomys natalensis* infected with *Brugia malayi* have been obtained and cyclical passage of the parasite through *Aedes aegypti* (Liverpool strain) has been performed into fresh animals. About 30 *Mastomys sp*. have been so far infected with infective larva of *B. malayi* and peripheral microfilaraemia has already been demonstrated in eight of these animals. The incubation period for appearance of microfilariae in circulation varied from 95 to 130 days. It is proposed to undertake experiments on passive transfer of rabbit anti-DEC antibodies into infected *Mastomys* in order to monitor the effect of such passive immunity on microfilaraemia in the near future.

Further attempts were made to identify the nature of parasite antigen(s) recognised by anti-DEC antibodies. Since anti-DEC antibodies were found to react with surface carbohydrates on microfilariae and the lectin, wheat germ agglutinin (WGA) also react with mf surface, a solubilized adult extract of *S. digitata* was tested by precipitation in agarose against WGA. One antigenic component was found to react strongly with WGA. The WGA binding antigenic component was then affinity purified by passing the crude antigenic extracts of

S. digitata through a column of WGA-Sepharose. The affinity purified antigen was found to react with Mastomys anti-DEC as revealed by ELISA.

Anti-DEC have also been successfully raised in *Mastomys natalensis* using MPCA as a hapten. They were found to cross react (unlike rabbit raised anti-DEC) with microfilarial and adult stage parasites of *Setaria digitata,* a bovine filarial parasite. This has been a significant finding since it is now possible to test the *in vivo* effects of anti-DEC in *Mastomys natalensis - Setaria digitata* model also. Results of our successful attempts on the induction of a long lasting microfilaraemia of *Setaria digitata* in *Mastomys natalensis* are presented under project no 2.2.

2.2 Development of a laboratory animal model for *Wuchereria bancrofti.*

Scientists	:	Dr. B. Ravindran Dr. A.P. Dash Dr. A.K. Satapathy
Technical Staff	:	Miss S. Mukhopadhyay
Starting date	:	November 1991

Wuchereria bancrofti the most widely prevalent human filarial parasite has not been adapted in any of the laboratory animals so far. The aims of this project are :

i) development of infective larvae (L_3) to 4th stage larvae (L4) of *W. bancrofti* in multimamate rats, *Mastomys natalensis,* and

ii) development of a microfilaraemic model by passive transfer of purified microfilariae in mice.

The strategy for the first objective was to implant intraperitoneally L_3 stages of the parasite (derived from mosquitoes) in plexiglass chambers sealed on open sides with semipermeable membranes- such a system is needed since L_3 of *W. bancrofti* do not survive for 10-15 days in *Mastomys natalensis* to develop into L4 stages.

For the second objective, the strategy was to use CBA/N strains of mice which are genetically deficient in immune response to T-independent antigens - such responses have been suspected to be responsible for immune elimination of transfused mf. of *W. bancrofti* in mice. Plexiglass chambers have been locally fabricated and attempts are underway to procure CBA/N strain of mice from U.S.A, as these are not available in any of the laboratory animal facilities in India.

In the meantime work was initiated during in the last 6 months of the year under report, for intraperitoneal implantation (in *Mastomys natalensis*) of adult stage parasites of *Setaria digitata*, a filarial parasite of cattle. This exercise was considered essential to standardise all the methodologies required for work on *W. bancrofti* - the scarce availability of L_3 stages and microfilariae purified from human subjects would not allow different combinations (doses, routes etc.) of injections and implantations. The following is the summary of the work performed so far :

a) When three female gravid adult stage parasites of *S.digitata* were implanted intraperitoneally 90% of *Mastomys sp.* (63 out of 70) were found to have microfilaraemia from 6-7th day till about 100-105 days. The mf density were variable from 5 to 80 per 20 µl of blood - the mean peak parasitaemia (about 25 mf/20 µl) was seen around the third week after implantation.

b) The implanted adult parasites were not found to be viable when tested 7-14 days after implantation. However, the percentage survival of parasites was significantly high in animals administered daily doses of cyclophosphamide for 15 days prior to implantation. Understanding of various components of the mechanisms of adult stage parasite survival in such immunosuppressed animals will be crucial for understanding the underlying phenomenon of parasite killing in this model.

c) High titres of antibodies were demonstrable (in the implanted animals) by ELISA to the somatic antigens of the adult stage parasites. Studies performed so far have failed to demonstrate the appearance of anti-microfilarial sheath antibodies in the implanted animals.

d) The microfilariae that appear in the peripheral blood of implanted animals were found to be infective to *Aedes aegypti* (Liverpool strain). The complete development from mf to L_3 stages was seen and attempts are underway to infect normal *Mastomys* with these L_3 stages of *Setaria digitata* for establishment of a new animal model for filariasis.

e) Attempts are underway to induce microfilaraemia for *S. digitata* by intraperitoneal injection of *in vitro* released microfilariae. These studies are being performed in normal as well as cyclophosphamide treated animals. The results of these investigations will be utilized for induction of microfilaraemia of *W. bancrofti*.

2.3 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, 1986

In studies performed earlier in our laboratory, antibodies to the microfilarial sheath were demonstrated to play a critical role in elimination of circulating microfilariae in human Bancroftian filariasis. There was a clear inverse correlation ($r = -0.78$, $p < 0.01$) between the presence of circulating microfilariae and the absence of anti-microfilarial sheath antibodies in human sera. Higher level of antimicrofilarial sheath antibodies were associated with absence of microfilaria in the peripheral blood. Although such a statistical correlation was a significant finding, there were always about 15-20% of cases where anti-sheath antibodies could be detected in microfilariae carriers. We have attributed these observations to

i) the absence of the adequate amounts of particular sub-class of IgG with anti-sheath activity and/or

ii) the possible existence of antigenic polymorphism of mf sheath components of different strains of *W. bancrofti*.

Investigations towards the second possibility were undertaken. Sera of microfilaraemic and amicrofilaraemic individuals from one geographical area (Jatni) were tested against microfilariae (by indirect immunofluorescence assay) collected from the same area i.e., Jatni and also against microfilariae (purified) from blood samples obtained from another geographical area namely, Gania. The inverse correlation between anti-sheath antibodies and amicrofilaraemia could be demonstrated only if the sera were tested against microfilariae collected from the same area. These investigations have clearly indicated the possible existence of antigenic polymorphism of mf sheath components. Studies are now underway to quantify anti-sheath antibodies in a number of mf carriers against autologous microfilariae, the results of which may even lead to sero-typing of the various strains of *W. bancrofti*.

2.4 Studies on serum cytotoxic factors in malaria

Scientists	:	Dr. B. Ravindran Dr. A.K. Satapathy
Technical Staff	:	Mr. P.K. Sahoo
Starting date	:	July, 1991

One of the outcome of immune response in human malaria has been the induction and release of a variety of soluble molecular mediators in circulation. They have been collectively designated as 'crisis form factors' since they induce both *'in vivo'* and *'in vitro'* 'crisis forms' of the erythrocytic stage parasites of *P. falciparum*. The 'crisis forms' have been demonstrated to be the degenerating stages of the parasites that have lost their infectivity both *'in vitro* and *'in vivo'*. The soluble serum factors have been demonstrated to act on the intra erythrocytic stages of parasites, unlike serum antibodies which act only on extracelluar stages of the parasites. The constituents of the 'crisis form factors' have been mostly speculative although Tumour necrosis factor, gamma interferon, lipid peroxidation products and even the role of endogenous phospholipase A_2 have been suggested.

Our interest in these factors in malarial sera were primarily due to accidental observations made in the laboratory during our studies on cerebral malaria (CM). Nearly 65 percent of sera collected from CM cases were found to be cytotoxic to microfilariae of both *W. bancrofti* and *Setaria digitata* as shown by an *'in vitro'* cytotoxicity assay (Fig.2). Similarly 67% of sera samples from children who had non-cerebral malaria (NCM) also showed cytotoxicity towards the microfilariae of that parasite. The cytotoxic factors were of large molecular weight (> 10 kD), precipitable by ammonium sulphate and moderately heat stable (56°C for 30 min). The presence of mf cytotoxic factors was independent of anti-mf sheath antibodies in sera. Sera collected from patients with acute or chronic filarial symptoms or asymptomatic mf carriers were not found to contain such mf cytotoxicity factors. Preliminary investigations have indicated that at least some of mf cytotoxicity (in cerebral malarial sera) is associated with low density lipoproteins indicating a possible role for lipid peroxidation products.

The above observations raised the possibility of studying the role of such cytotoxic factors produced during acute malarial infections in mediating the elimination of circulating microfilariae in the event of concurrent infections (of

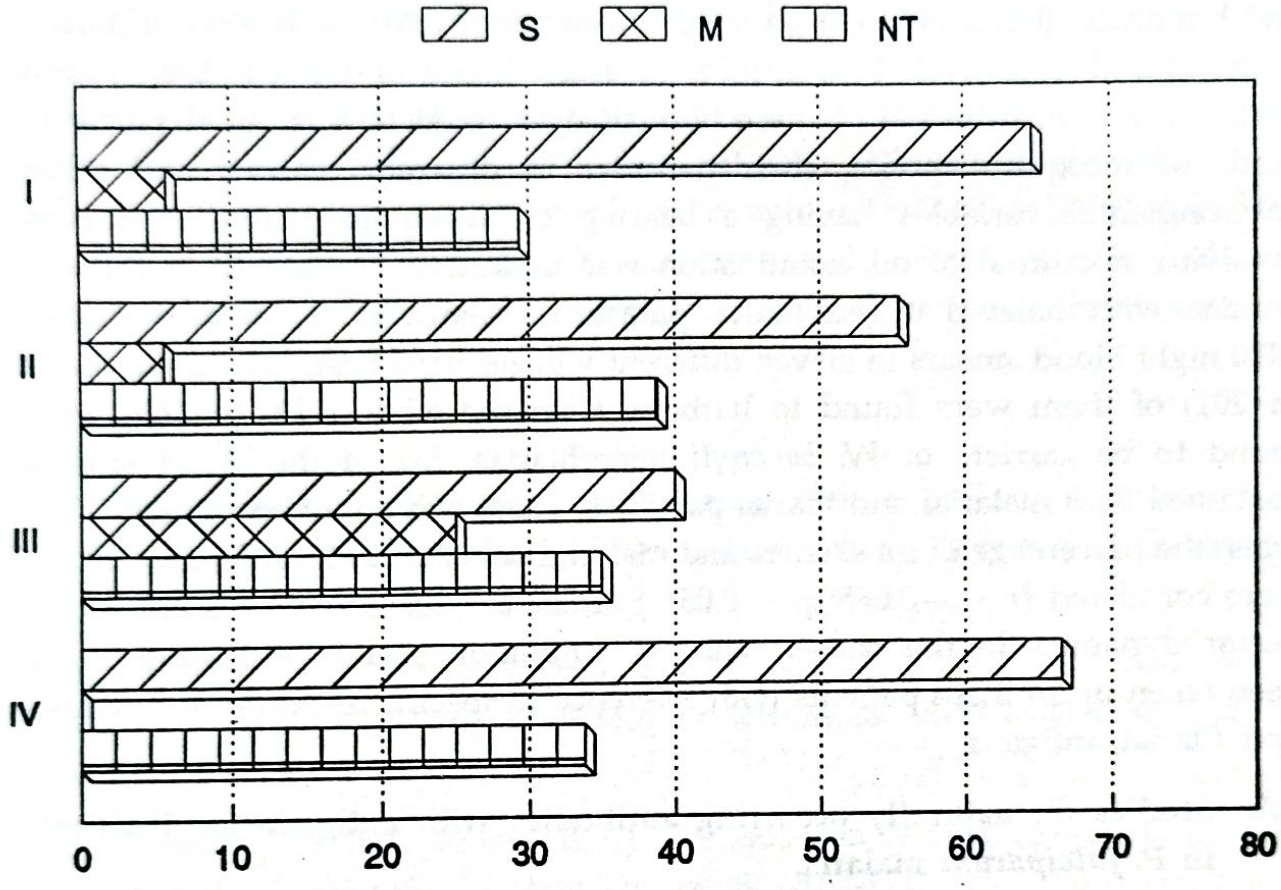

Fig. 2 : **Malarial serum induced *in vitro* cytotoxicity to microfilaria of *S. digitata***

CM : Cerebral malaria; Pf +ve, n = 17

PCM : Presumptive cases of CM; Pf —ve, n = 18

NCM (A) : Non Cerebral malaria (adults); Pf +ve n = 20

NCM (C) : Non Cerebral malaria (children); Pf +ve, n = 24

S : Severe cytotoxicity- > 80%

M : Moderate cytotoxicity- 50-80%

NT : No significant cytotoxicity- < 50%

malaria and filariasis) in human communities. Empirical epidemiological observations do indicate the near mutual exclusiveness of *P. falciparum* malaria and lymphatic filariasis in Orissa. High incidence of filariasis is seen primarily in the coastal areas while *P. falciparum* malaria is highly endemic in the western parts. Such a distribution of these two diseases could be attributed partly to kinds of mosquito species, their densities in different seasons and other environmental variables having a bearing on mosquito fauna. A survey involving nocturnal blood examination was undertaken to identify subjects/ patients with malarial and/or filarial parasites in Gania block. So far a total of 2400 night blood smears in eleven different villages have been examined - 8.3% (n=201) of them were found to harbour *Plasmodia* while 6.7% (n=163) were found to be carriers of *W. bancrofti* microfilariae. Six of the blood smears contained both malarial and filarial parasites. There was an inverse relationship when the percentage of mf carriers and malarial parasites in 11 different villages were correlated ($r = -0.685$; $p < 0.05$). Studies are underway to examine the vector dynamics in the chosen villages. Immunological investigations have been taken up in these patients with reference to specific responses to malarial and filarial antigens.

2.5 Studies on naturally occurring antibodies with anti-galactosyl activity in *P. falciparum* malaria.

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

Technical Staff : Mr. P.K. Sahoo

We had earlier demonstrated antimalarial antibodies in Cerebro-spinal fluid of cerebral malaria patients and had proposed a role for them in the pathogenesis of human cerebral malaria. Since no breach of blood CSF barrier is known to occur in cerebral malaria the antimalarial antibodies in CSF were attributed to intrathecal synthesis of immunoglobulins within the tissues of the CNS.

The possible synthesis of auto-antibodies in CNS of cerebral malaria patients was investigated with a view for (i) development of an immunodiagnostic test for cerebral malaria and (ii) for finding a possible cause-effect relationship between autoantibodies and cerebral malaria. Antigalactosyl antibodies in CSF were found to be significantly high (about 70%) in cases of cerebral malaria or presumptive cases of cerebral malaria in comparison to

normal controls. The antibodies were of mostly IgG isotype. The details of the findings have since been published in *Trans. Roy. Soc. Trop. Med. Hyg.* (please see under publications). The precise role played by anti gal in pathogenesis of cerebral malaria is still conjunctural. Antigal affinity purified through a column of Synsorb (that contains appropriate ligand of the trisaccharide residue) was found to react with soluble extracts of different parts of human brain tissue as revealed by ELISA. The reactivity of these antibodies could be absorbed significantly by preincubation with rabbit erythrocytes. Attempts are under way to cytochemically localize α-galactose determinants in neural tissue using fluorescenated lectin probes.

Publications

1. B. Ravindran and B.K. Das (1992) Human cerebral malaria-α-galactosyl antibodies in cerebrospinalfluid. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **86** : 132-133.

2. T. Hussain and B. Ravindran (1992) Antigenic mimicry between piperazine derivatives and *W. bancrofti* microfilariae. *Acta Tropica* **50** : 353-356.

3. B.Ravindran and E.Devaney (1992) The isolation of sheath/epicuticle of *Brugia pahangi* microfilaria. *Acta Tropica* **51** : 167-171.

Meetings

1. Dr. B. Ravindran was invited by the Department of Science and Technology, New Delhi to deliver a special lecture on "Immunological studies in human lymphatic Filariasis" during the project advisory committee meeting for 'Medical Biology and Immunology' held at the National Institute of Oceanography, Goa, Jan 22-23 1992.

Other activities

Dr. B. Ravindran was a Co-Director of a laboratory training programme sponsored by Department of Biotechnology, Govt. of India on "Immunological Concepts in Tropical Parasitic Diseases" conducted by the Immunology Division of RMRC, Bhubaneswar from 9th to 27th November, 1992.

Mr. Prakash K. Sahoo, Lab. Asst. was sent in Dec. '92 for a training programme on "Short Term Technician Training Course in Biotechnology" at Dept. of Biotechnology, Madurai Kamaraj University, Madurai.

Mr. A.K. Satapathy, Research Assistant, submitted his Ph.D. thesis entitled "Studies on determinants involved in host-parasite interactions in mammalian malaria" to the Utkal University, in the month of June, 1992

Ms. T. Hussain, Senior Research Fellow, submitted her Ph.D. thesis entitled "Immunological studies on antibodies to Diethylcarbamazine with reference to Filariasis" to the Utkal University in the month of August, 1992.

Membership

Dr. B. Ravindran was nominated and subsequently elected as a regular member of the Society of Experimental Biology and Medicine (New York) in June, 1992.

III. CLINICAL DIVISION

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

Scientist	:	Dr. S.K. Kar
Technical Staff	:	Dr. J. Mania Mr. T. Moharana Mr. K. Dhal Mr. R.N. Nayak
Starting Date	:	December, 1991

Objectives

A double blind placebo-controlled study 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 its future episodes.

Progress

135 dosages of drug were received from WHO/TDR for trial patients in August '92. Before the arrival of the drug, screening of study cases with history of ADL was continued in the field according to the criteria. The pre-drug assessment included analysis of blood, urine and stool for baseline parameters of health. Besides, clinical history, physical examination, parasite count (by Membrane Filtration Technique, mf/ml) & tonometry of the affected limb in study cases were also carried out. The subjects with prior history of at least two ADL attacks and currently manifesting ADL as per criteria was selected for the study. After pre-drug assessment, they were given cotrimaxazole (b.i.d) and paracetamol (t.i.d.) for initial five days prior to institution of trial drug which would be commencing from the 6th day. Subsequently each selected patient was to be administered with a single oral dose of the drug (Ivm., DEC or Placebo) every month, for 12 consecutive months. They were being treated with antibiotics and paracetamol for initial five days followed by single oral dose of one of the following in a double blind fashion, i.e. neither the patient nor the investigator is aware of the actual contents of the capsules.

i) Ivermectin (400 µg/kg wt.)

ii) DEC (10 mg/kg wt)

iii) Placebo

The trial drugs were prepared in different body weight ranges of adult subjects (30-39 kg, 40-49 kg, 50-60 kg & 61-65 kg). For operational convenience in the field, patients were taken in small batches after screening for drug administration. The post drug monitoring of vital signs, physical examination and blood analysis for safety are being carried out for each batch of patients as per the criteria laid down in the protocol.

Results

After pre-drug assessment the eligible cases in the villages Malipada & Patrapada with filaria related adenolymphangitis (ADL) were brought under chemotherapy in the trial villages which began in Sept.92. Till date 58 cases from different weight groups have received the drugs for periods ranging from one month to seven months. No serious untoward side reactions were encountered.

Reasons for continuation

i) 77 more cases will be included in the study for chemotherapy according to different weight groups as per protocol.

ii) The cases (n=58) who have already received the first dosages of drug are being followed up and subsequent monthly drug administrations are continued regularly.

iii) After the administration of 12 doses, all the cases will be followed up for a further period of 1 year, to record the occurrence of Adenolymphangitis attacks among the various groups of subjects belonging to all treatment categories. Besides, the clinical follow up examination for ADL, blood samples will be collected from each study case during pre-drug period and on day 14, 180 and 360 post-drug period. These will be analyzed for circulating filarial antigen and their filaria specific antibody status, besides repeating other tests, carried out during pre-drug period.

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

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

This ongoing clinical study is being carried out at Unit-III Government Dispensary, Out Patient Department, Bhubaneswar, twice a week. Suspected cases of TPE are referred to this OPD from other OPDs. During the year, 1064 cases were examined. Based on the presence of symptoms/signs, 308 cases were suspected as cases of TPE. Further haematological examination revealed eighteen confirmed cases of TPE (AEC > 3000 cells/cm). These were then assessed by clinical examination, Spirometry & Electrocardiogram. Sera were collected from them 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 level, lung function and immune responses and recurrence pattern of cases were recorded.

Out of the 18 confirmed cases of TPE recorded last year 83.3% were males and 16.7% were females. Their median age was 25.5. The pre-drug total leucocyte count was 7594.61 + 7829.6. The AEC count averaged to 4252.4 + 5006.23 cells per mm^3. The average eosinophil count as percentage of total leucocyte count was 49.9% + 16.7. Out of TPE cases 66.7% and 94.5% were positive for circulating filaria specific IgG and total IgE respectively. The mean circulating immune complex level was very high. Post drug assessment at 1st month revealed significant fall in their average eosinophil percentage ($p<0.01$) (Fig.3). The percentage of cases positive for filarial specific IgG, total IgE and CIC level decreased following therapy (Fig.4). Fifteen cases showed improvement in their lung functions following 1st month of DEC. Cases registered last year also attended the OPD for their regular periodic follow up examinations.

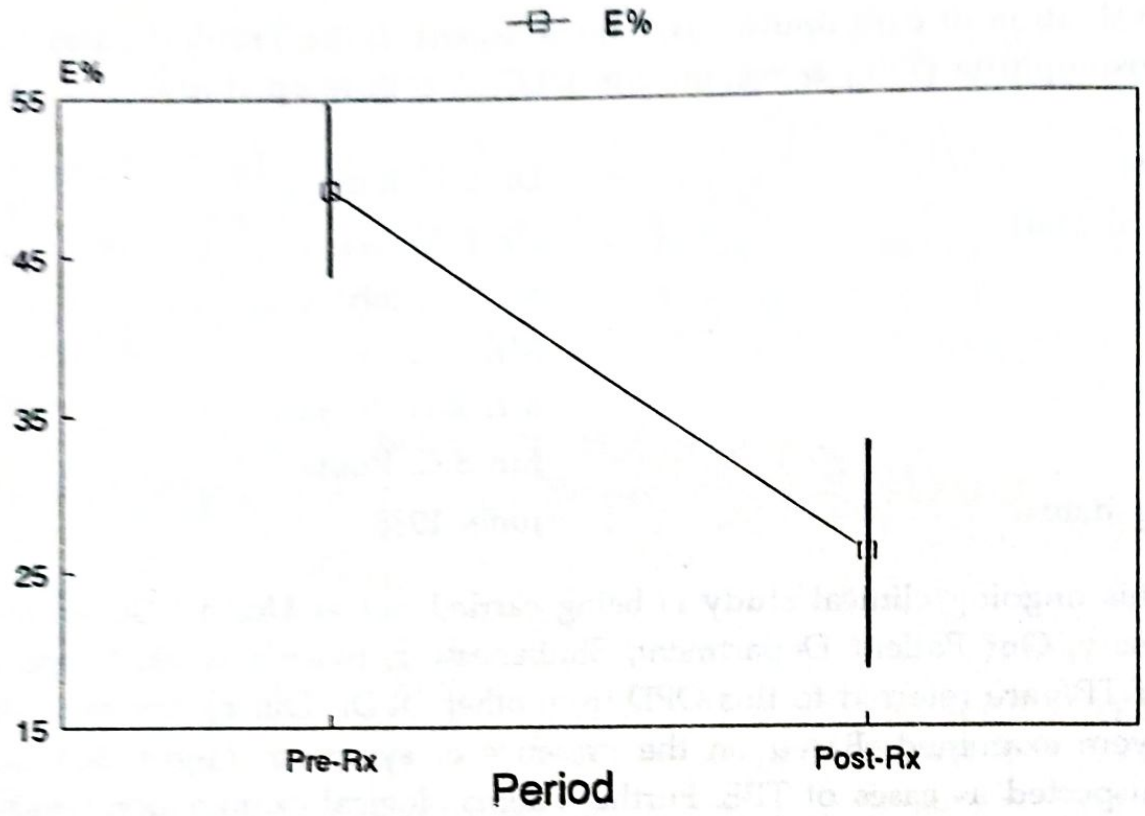

Fig. 3 : Eosinophil (E%) response of TPE Cases (n=18) before (pre-Rx) and after therapy (Post-Rx)

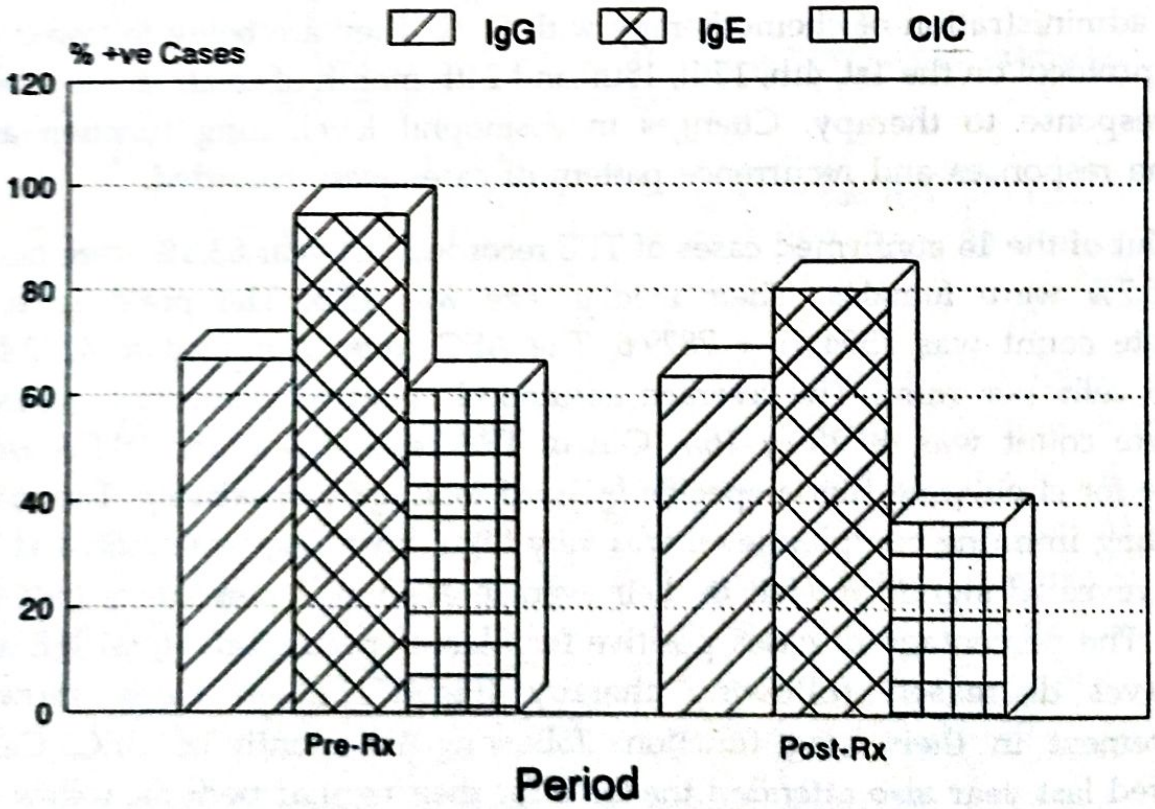

Fig. 4 : TPE cases with +ve immune response before and after DEC therapy (1 month)

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

Scientific Conference/Workshop/Seminar attended :

Name	Seminar attended Date and place	Papers presented
Dr.S.K.Kar	"Meeting on clinical investigation in Ivermectin trials against Lymphatic filariasis" held at Penang, Malayasia, from 27th to 30th January. 1992.	i. Epidemiological study of Filarial Adenolymphangitis. ii. Ivermectin in the treatment of bancroftian filarial infection in Orissa, India.
Dr.S.K.Kar	Joint ICMR/WHO/TDR workshop on "Social and Economic aspects of Lymphatic filariasis" at Bhubaneswar from 3rd Feb.92 to 7th Feb. '92.	i. Relationship between Social & Economic factors & levels of endemicity in lymphatic filariasis. ii. Basic information checklist on filaria endemic villages, Bhubaneswar, Orissa
Dr.S.K.Kar	Annual Conference of Research Society for study of Diabetes in India, held at Puri from 14th to 16th Nov. '92	Nil

2.C. Papers published/accepted :

1. Kar S.K., Kar, P.K., Mania J. (1992) Tissue Tonometry: A useful tool for assessing filarial lymphedema. *Lymphology* **25** : 55-61.

2. Kar S.K., Mania J., Baldwin CI., Denham D.A. The sheath of the microfiariae of *Wuchereria bancrofti* has albumin and immunoglobulin on its surface. *Parasite Immunology* (In Press).

3. Kar S.K., Mania J., Kar P.K. Humoral immune response during filarial fever in Bancroftian filariasis. *Transaction of the Royal Society of Tropical Medicine and Hygiene* (In Press).

4. Kar S.K., Pattnaik S., Mania J., Kumaraswami V. Ivermectin in the treatment of Bancroftian filarial infection in Orissa, India. *The South East Asian Journal of Tropical Medicine & Public Health* (In Press).

Any Other important event :

Dr.(Miss) J. Mania, R.A. was awarded a Doctorate degree in Science (Zoology) from Utkal University, Orissa in 1992. Her topic is entitled, "Studies on the Biochemical tissue composition of air breathing catfish Clarias batrachus (Linn.) in relation to artificial feed and its reflection on growth".

IV. MICROBIOLOGY

4.1 Bacteriology of subacute endocarditis

Scientists : Dr V.R.Subramanyam
Mr C.C. Rath

In collaboration with the Dept. of Cardiology, S.C.B. Medical College, Cuttack.

Starting date : May 1992

This project was undertaken at the initiation of the Prof. of Cardiology, SCB Medical College because no information exists on the range of bacteria associated with subacute endocarditis, in patients of this region.

Three samples of blood (5ml.) at one hour intervals are collected from each patient and inoculated into 50 ml of TSB. The blood cultures are incubated at 37° C and subcultured onto blood agar and MacConkey agar plates at the end of 1, 2, and 7 days of incubation. Any growth on the plate was examined and cultures identified by standard bacteriological methods. So far samples from six patients have been examined.

Patient No	Seq. sample No			Organism	Antibiogram*	
	1	2	3		Sensitive to	Resistant to
1	-	-	-	-		
2	+1d	-	+7d	Coagulase -ve *Staphylococci*	Em, Gm	Pn, Km,Tc
3	+1d	+7d	+7d	*Salmonella typhi*	Sm, Tc, Ap	Sz
4	-	+1d	+1d	Biochemically *Salmonella*	Na, Tc,Gm	Sm,Gm,AP
5	+1d	-	+1d	i. Coag. Neg. *Staphylococci*	Tc,Sm,Gm, Km, Em	Cl
				ii.Biochemically *Salmonella*	Tc, Gm, Sm Km	
6	+1d	+1d	+1d	Coag. Neg. *Staphylococci*	Tc, Em, Gm Km, Sm	Cl

* Pn : penicillin; Em: Erythromycin; Gm: Gentamycin;
Sm: Streptomycin; Tc: tetracycline; Ap: ampicillin;
Na: Nalidixic acid; Km: kanamycin; Cl: cloxacillin;
Sz : Sulfa diazene

4.2 Microbiology of local hotspring waters:

Scientists : Dr. V.R.Subramanyam
Mr. C.C.Rath
Ms. Vidhya Ramamurthy,
Research Student (honorary)

Starting date : September 1992

Hot springs are traditionally credited with medicinal properties. In addition the hot springs offer an unique opportunity to study thermophilic microbes. This project was initiated to study the prevalent microbes in the local hot springs.

We have been able to isolate three types of bacteria. They have been tentatively identified as Bacillus sp. Pseudomonas sp. and Gram positive cocci.

Detailed characterisation is in progress. An interesting finding is the fact that these organisms remain viable even after subjecting to a temperature of 60°C for 3 hours.

4.3 Establishment of a mycology facility

Scientists : Dr. V.R. Subramanyam
Mr. C.C.Rath

Starting date : September, 1992

There is no record of any systematic study on medically important mycoses in this area. One possible reason for this is the non-availability of a diagnostic mycology facility. The Division of Microbiology, has been striving to offer referral service in the field of diagnostic microbiology.

Recently we isolated a fungus from the chronic non healing ulcers on the lower limb of a 21 year old male. Based on growth characteristics and morphology, the fungus has been identified as Curvularia sp. Prior to our mycological diagnosis the patient had been treated variously with antibacterials (including anti mycobacterials) with disappointing results. After initiating antifungal therapy the patient has had remarkable recovery.

4.4 Microbiological studies of diarrhoeal diseases

Scientists : Dr. V.R. Subramanyam

Mr. C.C. Rath

Starting date : August, 1991

Diarrhoea is a problem of great concern and outbreaks of diarrhoeal diseases are not uncommon in this region due to various factors. Last year, it was proposed to take up an active project to study this problem in great detail. However, due to lack of funds and staff, it was not possible to contemplate a systematic study. But in the wake of news paper reports about the outbreak of diarrhoea /gastroenteritis in the nearby areas, a limited study was undertaken for the purpose of bacteriological documentation. The outbreak was reported from Khurda and Jatni areas, in Aug.-Sept. '92. Samples (rectal swabs/stools) were collected from 10 patients admitted in hospitals. In addition, the village Tapang was visited because the hospital sources indicated that most cases were from this village. Two water samples (one from the drinking water source- a

well, and the other from one affected house-hold) were collected from this village.

Bacteria suggestive of *Shigella* were isolated from 2/10 subjects' samples and from both the water samples. Out of the 5 representative isolates sent to the National Shigella Centre, Lucknow, only one (from a 65 year male patient) has been confirmed to be *Shigella dysenteriae* type 1. This is sensitive to kanamycin(Km), gentamicin(Gm), nalidixic acid(Na), ciprofluoxacin(Cp), and resistant to ampicillin(Ap), tetracycline(Tc) and streptomycin(Sm).

In addition to this, organisms biochemically resembling *Salmonella* and NAG Vibrios were isolated from 2 out of 10 human samples and from one of the 2 water samples. Representative isolates of putative *Salmonella* and NAG Vibrios were sent to CRI, Kasauli, and to NICED, Calcutta respectively, for confirmation. The report from Kasauli indicates that the isolates are not Salmonella. The report on the putative NAG Vibrios is awaited. A point of significance is that even these bacteria are found to be resistant to a variety of drugs such as Ap, Sm, (biochemically *Salmonella*) and to Na, chloramphenicol(Cm), Ap, Tc and Sm(? NAG Vibrios). This could be a potential source for acquisition of drug resistance by important enterobacteria.

4.5 Febrile agglutinins in sera from healthy tribals

Scientists	:	Dr. V.R. Subramanyam Keshar K. Mohanty (Feb.-May '92)
Starting date	:	Feb. '92
Completion	:	May '92

In the diagnosis of febrile ailments measurement of serum levels of agglutinating antibodies plays an important role. However for these agglutination test results to be relied upon, it is important to have information regarding the baseline prevalence of these agglutinins in the population. Additionally, the titre of antibodies to *Salmonella typhi* Vi antigen is indicative of the prevalence of *S. typhi* carrier state.

We screened 33 serum samples collected from healthy individuals from a tribal area for agglutinins against *S. typhi* Vi and against *Brucella melitensis*. Two fold dilutions were made in a tube dilution method. Results are given in the table.

Antibody titre (reciprocal)*		No positive
S. typhi Vi		
	≥ 40	3
	20	3
	10	14
	5	8
	< 5	5
Br. melitensis	0	

* Serum dilution tested were 1:5- 1:40 for *S. typhi* Vi and 1:80-1:1280 for *Br. melitensis.*

An antibody titre to *S. typhi* Vi antigen, of 1:10 or more is suggestive of carrier state for *S. typhi.* Our results imply that 20 out of 33 i.e. 60.6% of the tribal subjects investigated have possible carrier status for *S. typhi.*

4.6 A search for the presence of Cryptosporidium in paediatric practice.

Scientist : Dr. V.R. Subramanyam
Dr. B.B.Pal
In collaboration with Dr. G. Sarangi, Paediatrician, Cuttack.

Starting date : May '92

Completion : July '92

We had earlier reported on the prevalence of Cryptosporidiosis in children attending the local hospital (Annual Report 1986-87 p.39 and *Annals of Trop. Paediatrics* (1989) 9: 122-125). The present study was undertaken to see if our earlier observation would hold true even for a private clinic which usually caters to a clientele of different kind, i.e., generally of a better socio-economic status. Forty seven stool samples from diarrhoea cases and 106 stool samples from children having no diarrhoea were examined microscopically for the presence of Oocyst of Cryptosporidium by Safranin staining method. None of the samples was positive for cryptosporidium in this series, while our earlier study had shown a positivity rate of 12.9% in acute diarrhoeal cases.

4.7 Genetic location of HCH metabolising activity in *Pseudomonas* isolates

Scientist	:	Dr. V.R. Subramanyam In collaboration with Dr. N. Sethunathan (CRRI, Cuttack)
Starting date	:	August, 1991
Completion	:	1992

The Scientists at CRRI had isolated *Pseudomonas* sp. from sugarcane rhizosphere and from flooded rice soils treated with HCH, a commonly used pesticide. These isolates have the ability to metabolize HCH. In our laboratory, we had isolated and demonstrated the presence of a plasmid DNA band from these isolates, by agarosegel electrophoresis. In an attempt to link the HCH metabolizing activity with the presence of the plasmid, HCH nondegrading (HCH -ve) variants were derived from HCH +ve strains by treatment with acidine orange (work at CRRI). Subsequently the HCH +ve and HCH -ve strains were examined in parallel for the presence of plasmid DNA (work at RMRC). Plasmid band was seen in both the HCH + ve and HCH -ve strains, indicating that the HCH degrading activity is not coded for by this plasmid.

4.8 Antimicrobial efficacy of essential oils

Scientists	:	Dr. V.R. Subramanyam Ms. Smaranika Pattnaik In collaboration with Dr. C.R. Kole, OUAT
Starting date	:	Sept '91

Plant products that impart characteristic odour and flavour are referred to as essential oils (derived from the word 'essence' meaning a flavouring agent). Essential oils are important constituents of plants and are gaining importance as biologically active substances. We are interested in the antimicrobial activity of essential oils. In an initial screening we tested 10 essential oils (hydrosteam distillates) and 2 water - extracts (Champa and Kevda) against 17 bacteria representing 8 genera. The results indicate that the water extracts of Champa and Kevda have no antibacterial activity. All the 10 hydro steam distillates tested showed antibacterial activity, though the range of bacteria against which they were effective varied. Three of the essential oils (eucalyptus, aegale and

orange) were effective against all the 17 strains of bacteria; three essential oils (palma rosa, peppermint and lemon grass) were active against 16 out of 17 test bacteria; oils of patchauli, ageratum, citronella and geranium ranked next, being effective against 15, 13, 10 and 8 bacteria respectively. Results are tabulated.

Bacteria	Strain No.	Response to Essential Oils											
		1	2	3	4	5	6	7	8	9	10	11	12
Ps.aeruginosa	VR-6	S	R	S	R	S	R	R	R	R	S	S	S
Esch. coli	VR-12	S	S	S	S	S	R	R	R	S	S	R	S
Klebsiella sp.	VR-13	S	S	S	S	S	R	R	R	R	S	R	S
Citrobacter sp.	VR-14	S	S	S	S	S	R	R	R	R	S	R	S
Shigella boydii	VR-15	S	S	S	S	S	R	R	R	R	S	S	S
Shig. flex.	VR-16	S	S	S	S	R	R	R	R	R	S	R	S
ETEC	VR-17	S	S	S	S	S	R	R	R	S	S	S	S
EPEC	VR-18	S	S	S	S	R	R	R	R	R	S	S	S
S.typhimurium	VR-19	S	S	S	S	S	R	R	R	R	S	S	S
Sal. typhi.	VR-20	S	S	S	S	S	R	R	S	S	S	S	S
V. cholerae	VR-65	S	S	S	S	S	R	R	S	S	S	S	S
V.cholerae	VR-75	S	S	S	S	S	R	R	S	S	S	S	S
V. cholerae	VR-78	S	S	S	S	S	R	R	S	S	S	S	S
V.cholerae	VR-80	S	S	S	S	S	R	R	S	S	S	S	S
Ps.aeruginosa	SP-10	S	S	S	S	S	R	R	S	S	S	S	S
Staph aureus	SP-1	S	S	S	S	S	R	R	S	S	S	S	S
Esch coli	SP-11	R	S	S	S	S	R	R	S	S	S	S	S

Essential Oils: 1.Palma-rosa, 2.Lemon grass 3.Eucalyptus 4.Peppermint 5.Patchauli 6.Champa 7.Kevda 8.Geranium 9.Citronella 10.Aegale 11.Ageratum 12.Orange

S: Sensitive R: Resistant.

4.9 An investigation into the reported occurrence of anthrax in Koraput District.

Scientists	:	Dr. K.A.V.R. Krishnamachari Dr. V.R. Subramanyam Mr. C.C.Rath
Starting date	:	July, 1992
Completion	:	December '92

There were reports in local press about occurrence of human anthrax in Muniguda PHC area. A visit to the area followed by enquiries revealed the following.

Eleven cattle had died unexpectedly, the first death being on 11.06.92 and the last on 6.7.92. Coincidentally there have been 6 human deaths in the same area, at about the same period. Two of these were perhaps due to old age but the other four could not be explained satisfactorily. Vaccination of cattle, sheep and goats has been undertaken by the veterinary surgeon, against anthrax, the disease having been suspected on circumstantial evidence.

We saw 6 persons with lesions on limbs at various stages of necrosis/ healing/exudation. The lesions were about 10-15 days old (elicited history).

We collected samples from the lesions of two affected persons, in addition to their blood for culture. Blood culture yielded no growth.

Microscopic examination revealed an occasional Gram positive rod in only one of the two samples. A heat treated sample (washings of the lesion) from the same person also yielded a single colony of non motile, spore-forming Gram positive rods. The culture was sent to CADRAD, IVRI, Izatnagar for identification. It has been reported to be not *B. anthracis*.

4.10 HIV Sero surveillance work:

Scientist	:	Dr. V.R. Subramanyam Dr. B.B.Pal
Starting date	:	January, 1987

Though the sero surveillance was initiated at this centre in 1987, the number of samples screened in the early years has been very small. In the

previous years only a passive surveillance was done as and when samples were sent to RMRC for testing. Sustained efforts are being made by this Centre to expand the surveillance activities. In Dec. 1991 as per the suggestion of the Director General, ICMR, this Centre started the sentinel surveillance activity in addition to screening of donated blood samples. This Centre is also supported by the National AIDS Control Organization by being recognised as a Surveillance Centre.

Orissa state has a long coastal belt with a commercial port, has a major pilgrim centre, a lot of migrant labour population and is a favourite visiting place for foreign tourists. All these factors make Orissa a potential target for HIV infection. Keeping this in mind, we have been actively involved in IEC programs organised by the local Government and NGOs. We have delivered non technical talks at such gatherings as the seminar on 'Drug abuse prevention' organised by the CYSD (an NGO) and at the seminar organised by the Collectorate of Central Excise and Customs, on International Day against drug abuse and illicit drug trafficking. We had also organised an exhibition of posters (made at this Centre) followed by a popular lecture program and a question-answer session with the audience, on World AIDS Day. Contacts have been established with industries that employ a large number of frequently transferred workers such as security personnel.

A 'module' for HIV testing activities in this state, detailing the required infrastructure, materials, man power as well as training components has been prepared and submitted to the State Govt.

There has been a good improvement in the number of samples screened this year. A total of 3327 samples were tested this year. Out of these, six were positive in ELISA test on one or more occasions but only a single sample was confirmed to be positive for HIV antibodies by Western blotting (performed at NICED, Calcutta). This positive sample was from a resident of Andhra Pradesh who seems to have acquired the infection during his stay at Bombay. The reasons for higher rate of false positive ELISA testing are not clear.

The stage is now set for this Centre to undertake more active surveillance programs with the help of Governmental and non governmental organisations.

Summary of HIV tests done

		Blood donors	Foreigner Survei-llance	Tribals	Sentinel	Others	Totals
1987	T	0	14	0	0	344	358
	E +		0			0	0
	W +		0			0	0
1988	T	0	10	0	0	365	375
	E +		0			0	0
	W +		0			0	0
1989	T	7	0	0	0	0	7
	E +	0					0
	W +	0					0
1990	T	0	0	0	0	0	0
	E +						
	W +						
1991	T	989	0	340	30	13	1372
	E +	1		0	0	0	1
	W +	0		0	0	0	0
1992	T	1517	4	834	971	1	3327
	E +	2	0	1	2	1	6
	W +	0	0	0	0	1	1

1 T:number tested; E+.numbers ELISA +VE; W+: numbers Western Blot +ve

Other activities:

1. Dr. V.R. Subramanyam attended the Annual Conference of Association of Microbiologists of India at Madurai(10-12 Jan 1992)

2. Dr. V.R. Subramanyam participated and gave a talk on "HLA and human disease" at the JBS Haldane birth centenary celebrations held during the "International Symposium and 17th Annual Conference of Indian Society of Human genetics, Utkal University (22-25 Jan '92)

3. Dr. V.R. Subramanyam attended the meeting of workers on HIV at ICMR Hq (21 May '92).
4. Dr. V.R. Subramanyam gave an invited lecture on immunology of tuberculosis in the tuberculosis symposium organised by the Association of Physicians of India, Orissa State Branch at Talcher (31st May 1992).
5. Dr. V.R. Subramanyam gave two lectures on concepts of gene regulation and mechanism of gene action and genetic code to M.Sc students of Puri college (8th and 9th June '92)
6. Dr. V.R. Subramanyam was an invited speaker at the seminar on Drug abuse prevention Organised by the CYSD, Bhubaneswar at Soochna Bhawan on World Drugs Day (26th June '92). Topic: Drugs and AIDS.
7. Dr. V.R. Subramanyam was a guest speaker at the seminar organised at the Collectorate of Central Excise & Customs, Bhubaneswar on International Day against drug abuse & illicit drug trafficking (26 th June '92).
8. Dr. V.R. Subramanyam gave two lectures (DNA fingerprinting; HIV/ AIDS) to the participants of NCERT'S Teachers Reorientation Course organised by Regional College of Education,Bhubaneswar. (23rd Sept., '92).
9. Dr. V.R. Subramanyam participated as a resource person in the ICMR-WHO workshop on Surveillance methodology on HIV" organised by the ICMR unit for Research on AIDS in North Eastern States of India, Calcutta. (19-26 Oct.,1992)
10. Dr.V.R. Subramanyam was a faculty and gave two lectures (Nucleic Acid Methodology in Parasitology) for the participants of RMRC-DBT Course on Immunological Concepts in Tropical Parasitic Diseases (17-18 Nov '92). Division of Microbiology organised a popular lecture programme and an Exhibition at Soochna Bhawan as a part of World Aids Day Observance, in collaboration with the Indian Red Cross Society, Orissa State Branch and the Rotary Club, Bhubaneswar North (1st Dec. '92).
12. Dr. V.R. Subramanyam participated in the workshop on AIDS awareness in Orissa organised by the Dept of Health & FW, Govt of Orissa and British Council, Bhubaneswar (2nd-4th December, 1992). Gave a talk on "HIV surveillance work at RMRC, a 5 year experience."
13. Ms. Keshar K. Mohanty was awarded the Ph.D. degree of Utkal University for her thesis on "Characterization of filarial antigen in bancroftian filariasis" under the guidance of Dr. V.R. Subramanyam.

V. PATHOLOGY

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

Investigator	:	Dr. G.P. Chhotray
Co-investigator	:	Prof. Luccio Luzzatto
Starting date	:	April 1986

Having established the high prevalence of G-6-PD deficiency in a malaria endemic tribal area of Orissa (IJMR [B] 92 Dec 1990 pp 443-446), 10-20 ml of venous blood was collected from 27 persons having severe G6PD deficiency and 8 non deficient persons of the same area (to serve as control).

The G-6-PD enzyme activity, Km for G-6-PD was estimated and electrophoretic characterization was also done. From these data it was apparent that the majority of deficient samples have similar properties with G6PD activity of about 10-20% of normal, and "B" like electrophoretic mobility.

DNA extraction was carried out in the deficient samples and was sent to Prof. Luccio Luzzato's lab at Hammersmith hospital, Haematology Dept. for further analysis.

DNA analysis was carried out on 20 samples. From 3 of the G6PD deficient samples the entire coding was amplified in segments and each one was analysed for single strand confirmation polymorphism. In all the three cases the DNA segment containing exons 3 and 4 showed an abnormality and was therefore sequenced. A single base change was found in codon 44 (GCC to GGC) which would predict a substitution of alanine to glycine in that position. Having found this mutation it was observed that this would cause a destruction of an Hae III restriction enzyme site. Accordingly the same fragment from all other samples were analysed with a Hae III and the results are shown in the table I. It was observed that all the G6PD deficient samples had the mutation but interestingly three samples (1,16 & 17) which had normal G-6-PD activity also had this mutation. These observations indicate that codon 44 replacement is not responsible for G-6-PD deficiency and this must be explained by a second mutation which has so far eluded us.

Justification for continuation of the project:

Since it is a new trend setting finding that codon 44 is not responsible for G6PD deficiency, a search for second mutation will be carried out.

A search for the second mutation will eventually reward with establishing a new finding that the codon 44 would then be a heitherto totally unknown polymorphism of normal G6PD. (Just like a variant in Africa) and in this respect would be a new mile stone in the history of haematology.

Table-1

Sample No	G-6-PD IU/gHb	Hae III digest
1	8.84	M
X1	7.40	N
2	9.4	N
2A	9.69	-
X2	1.4	M
3	2.01	M
X3	1.4	M
4	2.18	M
5	1.33	M
6	1.98	M
7	1.56	M
8	2.34	-
11	3.4	M
12	3.6	M
13	2.6	M
14	2.8	M
15	2.4	M
16	9.6	M
17	7.3	M
18	2.2	M
19	2.0	M
20	1.9	M

- : Failed to amplify
M : Mutant (codon 44 Ala-Gly GCC- > GGC)
N : Normal

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

Scientist	:	Dr. G.P. Chhotray
Technical Staff	:	Dr. M.R. Ranjit Dr. M. Mohapatra Mr. B.N. Sethi Mr. H.K. Khuntia
Starting date	:	November, 1990

In order to establish the natural infection of *B. malayi* amongst the cat population in the existing isolated pockets of *B. malayi* of Orissa, 259 domestic cats (Felis catus; 44 male and 215 female) were screened, but none of them showed the microfilariae.

Simultaenously 1838 individuals (1116 males and 722 females) of various age groups ranging from 1-75 yrs form 16 villages of the same area i.e. Satyabadi Block of Puri district were screened for microfilariae in their night blood smear. The mf positivity rate amongst the population studied so far was found to be 13.93% of which 10% were *W. bancrofti*, 3% were *B. malayi* and the rest were having mixed infection.

In the absence of occurrence of the natural infection amongst the cats, in our study areas 4 kitten were inoculated with infective larvae (L_3) dissected from *Aedes aegypti* Liverpool strain mosquitoes fed with infected *Mastomys* for experimental filariasis study. The cats were monitored at regular intervals for the appearance of microfilariae in their peripheral blood. Simultaenously the total and differential white blood cell count (W.B.C.) was also undertaken.

Of the four cats that were infected experimentally with microfilariae, one died after 76 days of 1st inoculation without showing microfilariae in the peripheral blood. Autopsy was performed which did not reveal any gross pathological findings. Histopathological study of various organs (liver, spleen, lymph-nodes etc.) did not reveal any significant pathological findings except chronic inflammatory cell infiltration.

The second cat died after 87 days of infection. On autopsy it was observed that the superficial lymphatic channels were tortuous, dilated, engorged and cord like which was distinct to the naked eye. There were fine grannulomatous areas beneath the skin and subcutaenous tissue. The superficial regional

lymphnodes were enlarged. On gross examination of various visceras, there were areas of grannulomatous changes over the surface of heart and liver. A portion of the lungs showed atelactesis and another part showed emphysematous changes. The peritoneal and thoracic cavity was filled with amber coloured fluid. An adult worm could be retrieved from the thoracic cavity. Testes and epididymis was enlarged and the spermatic cord was thickened. Paraffin sections were made and stained with H and E stain for histopathological study. Histopathologically noncaseating grannulomatous reaction was observed. There were dilated spaces lined by single layer of endothelial cells. There were varying degrees of chronic inflammatory cell infiltration like lymphocytes and plasma cells. The other two cats developed microfilariaemia on 80-90 days of first inoculation and are still maintaining their microfilarial status. They are being monitored regularly.

Justification for continuation :

Having established cat as a successful animal model for *B. malayi* infection, it will now be possible to study the early and late pathological changes occurring in cats due to *B. malayi* infection which is more akin to human *B. malayi* infection. Efforts will be made to transmit cat to cat infection with this particular strain, which is adopting itself to cats of Orissa for the first time as per our observation.

5.3 Study of Urogenital and Renal involvement in a filarial endemic area of Orissa

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

The project was initiated with the following aims and objectives :

1. To determine the frequency of urogenital and renal abnormality occurring in the patients suffering from filariasis and to determine antigen in the biopsy specimens of selected cases.

2. To assess the potential of filarial infection to account for a portion of aetiologically undetermined urogenital and renal involvement seen in endemic areas.

3. To evaluate the functional and structural status of kidney by light microscopy and immunopathological techniques in patients showing urinary abnormalities.

1315 persons (M = 706, F =609) with the ages ranging from 10 to 65 years from 8 villages of the study area, i.e. Sakhigopal area of Puri district were screened for mf positivity. Out of 1315 persons (135) persons were found to be mf positive. A detailed clinical examination was performed on 142 persons consisting of asymptomatic and symptomatic carrier stage, acute and chronic cases. 90 of them were mf positive (M = 50 and F = 40). Morning midstream urine of these patients were collected and examined at the RMRC laboratory.

Out of the 90 urine samples examined 21 samples revealed asymptomatic microsocpic haematuria of Grade I degree i.e. 0-8 RBC/HPF. One case showed haematuriae Gr. II and mild proteinurea, two cases showed only proteinurea. None of the cases had chyluria or any other urinary abnormality.

Justification for continuation:

Although renal involvement is not well appreciated as a concommitant event of bancroftian filariasis yet there have been several reports describing the occurrence of glomerularnephritis, proteinurea and haematuria with or without chyluria. In patients having one or the other form of filarial infection. Moreover studies in experimental animals have also documented glomerular involvement. Hence a detailed study on this aspect will add to the knowledge of clinical spectrum of the disease process.

5.4 A histopathological correlative study of arthritis in filariasis patients.

Scientists	:	Dr. G.P. Chhotray Dr. S.S. Mohanty (S.C.B. Medical College, Cuttack)
Technical Staff	:	Mr. B.N. Sethi Mr. H.K. Khuntia
Starting date	:	December, 1992

The study has been initiated in collaboration with Orthopaedic department of S.C.B. Medical College, Cuttack having an aim to establish the correlation between histopathological and clinical presentation of filaria specific arthritis and to distinguish these from other forms of arthritis and to follow up of these cases before and after treatment.

So far 6 cases have been registered from amongst the patients presenting with arthrirtis of various forms to the out Patient Deartment of S.C.B. Medical College. A detailed clinical history was taken and they were admitted for routine pathologcal, haematological, biochemical and radiological examinations including mf in night blood. Special investigation like R. factor, CRP, VDRL ASO titre etc. were performrd to exclude non filarial aetiology.

Synoveal tissue and Synovial fluid of these patients were collected by closed needle biopsy and were preserved for histopathological and imunological examinations.

Justification for continuation:

To establish the role of filariasis in the aetiology of arthritis, estimation of filaria specific antibody titre in synoveal fluid by ELISA using mf excretory secretory antigen will be undertaken. Immunoperoxidase and immunofluorescent staining will be performed on histopathological sections of synoveal tissues. This will contribute significantly to the understanding of clinicopathological profile of the disease in an endemic area.

5.5 Immunological status of patients with sickle cell anaemia.

Scientist	:	Dr. G.P. Chhotray
Technical Staff	:	Dr. M.R. Ranjit Mr. B.N. Sethi Mr. H.K. Khuntia
Starting date	:	April, 1990
Completion	:	February, 1992

Serum immunoglobulins (IgG, IgA, IgM) and complement (C_3 and C_4) levels were estimated by radial immuno diffusion method in 14 cases of sickle cell anaemia (HbSS), 15 cases of sickle cell trait (HbAS) and was compared with that of 14 age and sex matched control cases (HBAA). It was observed that the IgA and IgG values were increased which was statistically significant ($P<0.01$) in sickle cell anaemia when compared with the control cases. The C_3 level of HbSS and HbAS cases was significantly lower ($P<0.01$) whereas IgM and C_4 level did not reveal any significant change. The present study gives some indication about the pattern of changes in serum immunoglobulin and complement levels in sickle cell disease patients from the Western part of Orissa.

Papers submitted for publications:

1. Serum immunoglobulins and complement level in sickle cell disease in western Orissa. Chhotray, G.P. and Ranjit, M.R. - *IJMR.*
2. Asymptomatic microscopic haematuria in a filarial endemic area of Orissa. Chhotray,G.P. and Ranjit, M.R. - *IJMR.*
3. Periodicity of microfilaria in Orissa. Chhotray, G.P. - *The J. of Communicable Diseases.*

Scientific Conference/Workshop/Seminars attended:

1. Dr. G.P. Chhotray attended the XX Annual all Orissa chapter meeting IAPM Nov 1992. SCB Medical College Cuttack - presented a paper entitled Experimental filariasis in cat model.

VI. ENTOMOLOGY

6.1 Present status of Mansonioides and *B. malayi* in Orissa.

Scientist : Dr. A.P. Dash

Technical Staff : Mr. R.K. Hazra

Starting date : September, '89

6.1.1 Distribution of B. malayi and Mansonioides

Blood samples were collected from four districts of Orissa viz., Puri, Balasore, Mayurbhanj and Bolangir. Sample size from two districts was less than 40. The results of the survey are presented below.

District	Total No of blood smear collected	Total smears +ve for microfilaria							
		B.malayi		*W.bancrofti*		W.b+ B.m		Total +ve smears	
		No	%	No	%	No	%	No	%
Puri	2766	135	4.8	154	5.5	6	0.2	295	10.6
Balasore	344	-	-	23	6.6	1	0.3	24	6.9
Bolangir	37	2	5.4	-	-	-	-	2	5.4
Mayurbhanj	29	-	-	2	8.9	-	-	2	8.9

The Mansonioides mosquitoes were also collected from the abovedistricts. Four species of Mansonioides viz., *Ma. annulifera, Ma. uniformis, Ma. longipalpis* and *Ma. indiana* were encountered in the survey villages along with two species of Coquillettidia. All species were found in human dwellings and cattlesheds.

Ma. annulifera and *Ma. indiana* were found to be endophilic and endophagus in nature while *Ma. uniformis* and *Ma. longipalpis* were mainly exophilic and exophagus.

6.1.2 Seasonal prevalence and vectorial capacity of Mansonioides

Out of four species of Mansonioides mentioned above *Ma. annulifera* and *Ma. uniformis* are dominant species, both being found throughout the year with a peak in September/ October. The man hour density (MHD) of *Ma. annulifera* varies from 3.9 to 18.7 reaching its peak in October and lowest in July. The man hour density of *Ma. uniformis* varied from 0.61 to 21.5 reaching its peak in September and lowest in June.

Vectorial Capacity:

All the three developmental stages of *B. malayi* were encountered in *Ma. annulifera*. The infection rate was highest in October (2.8%) and lowest in February (0.4%). Rest of the months showed varying rates between of 1.14% to 1.4%. The annual infection rate was found to be 0.76%. Filarial infective mosquitoes were found only in four months of the year viz. February, September, October and November. The infectivity rate varied from 0.4% to 0.96%. Average number of L3 per infective mosquito was 1.1.

6.2. Development of filarial parasites in Aedes aegypti Liverpool strain

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

6.2.1 *Mastomys natalensis* infected with *Brugia malayi* were fed to black eyed *Aedes aegypti* (Liverpool strain) in successive batches throughout the year. Total 3199 of mosquitoes (27 batches) were fed and these mosquitoes were kept separately under optimal conditions for 1 to 16 days for the development of infective larvae (L3). The mosquitoes were dissected and 2237 number of L3 were obtained, which were further used for reinfection (mechanical, intra peritoneal routes etc.) of fresh Mastomys and cat, without involving the vectors for transmission.

6.2.2 Aedes aegypti liverpool strain were also fed to M.natalensis infected with Setaria digitata from September 1992. Total ten batches comprising of 1609 number of mosquitoes were fed and 477 number of L3 were procured. Development of Parasite from microfilarial stage to infective stage took about 14 to 15 days.

Results of the development of both the parasites are tabulated below.

Filarial parasite	Mf density /20 µl (range)	Total No of mosquitoes fed	Total No of mosquitoes dissected	Total No of mosquitoes +ve for L_3	Total No of L_3
B. malayi	25-130	3199	1219	554	2237
S. digitata	7-124	1609	862	248	477

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

Following mosquitoes were collected from different villages of Puri and Balasore districts (Endemic for filariasis & malaria). The species composition is tabulated with realtive density.

Species	A R E A			
	Khurda (PMHD)	Gania (PMHD)	Balasore (PMHD)	Pipili (PMHD)
An. annularis	3.3	18.3	3.2	3.8
An. 'hyrcanus' group	7.9	5.0	—	8.0
An. subpictus	6.2	9.6	3.4	8.1
An. vagus	5.3	8.3	4.2	5.2
An. pulcherimus	-	-	-	0.1
An. culicifacies	1.1	15.2	-	1.1
An. varuna	1.2	-	-	2.0
An. aconitus	2.0	-	-	3.5
Ae. aegypti	1.1	2.1	1.2	1.9
Ae. vittatus	0.1	-	-	0.2
Ae. lineatopenis	-	-	-	0.1
Cx. quinquefasciatus	52.2	24.2	6.7	48.5
Cx. tritaeniorhynchus	16.7	10.1	2.1	14.8
Cx. vishnui	8.8	2.1	1.2	6.5
Cx. gelidus	3.1	-	-	2.1
Ma. annulifera	1.9	-	26.5	19.7
Ma. uniformis	1.2	-	17.2	25.4

PMHD : Per Man Hour Density: Relative density of mosquitoes: collected by one man in one hour.

Blood meals of 1716 mosquitoes belonging to eight species were analyzed by gel diffusion technique. The anthropophilic indices were found to be 7.1%, 7.6%, 5.0%, 93.1%, 11.5%, 3.15%, 65.5% and 56.6% respectively for An.hyrcanus

group, *An.vagus, Cx.gelidus, Cx.quinquefasciatus, Cx.tritaeniorchynchus, Cx.vishnui, Ma.annulifera* and *Ma.uniformis. Cx. quinquefasciatus* had the highest anthropophilic index (93.1) and Cx. vishnui had the least index value (3.15%)

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

The project work started on March 1992. Two areas viz., Khurda and Pipili area were taken as experimental and comparison area respectively. Preliminary data viz., demography, Mf rate and mosquitogenic conditions of both the areas were recorded. All the houses of both the areas were numbered and a detailed map was drawn showing all the details of the breeding spots.

Fortnightly larval collections were done. The vector breeding spots encountered are cess pits, cess pools, drains, temporary latrines, faulty septic latrines, unused wells and ponds. Baseline data of experimental and control area were comparable.

Laboratory Studies: Bioassay tests of B.sphaericus were done in the laboratory by standard WHO method. LD50 and LD90 dosage were determined to be 0.0039 PPM and 0.014 PPM respectively.

Recycling and persistence study under laboratory condition revealed that the biocide recycles in dead cadavers upto five months and can cause 100% mortality to fresh larvae.

Field Study:

In the field it has been observed that the biocide persists till sixth week in Cess pits, Cess pools and unused well. The study is under progress.

6.5 Small scale field trial of *B. sphaericus* (CDRI) Lucknow Strain

Scientists	:	Dr. A.P. Dash Dr. N. Mohapatra
Technical Staff	:	Mr. R.K. Hazra Mr. H. K. Tripathy Ms. Saswati Rup Ms. Rajashree Mohapatra
Starting date	:	July 1991
Completion	:	October 1992.

Laboratory Study:

Four different samples of *B. sphaericus* (A,B,C & D) were supplied by CDRI Lucknow for laboratory evaluation. Bioassay test of these four samples against three species of mosquitoes viz., *Aedes aegypti, Culex quinquefasciatus* and *Anopheles stephensi* have been carried out.

LD_{50} and LD_{90} values of these four samples are given below:

Mosquito species	Sample-A		Sample-B		Sample-C		Sample-D	
	LD_{50} in PPM	LD_{90} in PPM	LD_{50} in PPM	LD_{90} in PPM	LD_{50} in PPM	LD_{90} in PPM	LD_{50} in PPM	LD_{90} in PPM
Cx. quin.	8.269	14.469	5.185	10.541	9.939	18.460	8.358	20.162
An. steph.	5.753	11.510	7.422	11.305	6.425	19.410	7.00	18.92
Ae. aeg.	2.205	8.166	2.698	10.832	3.601	7.385	1.557	3.872

Field Study:

The preliminery results from field experiment showed that the biocide reduced the larval density in the nature significantly, immediately after application. But after three to four days, reappearance of larvae occured and further mortality did not occur. This strain seems to have no recycling effect.

The detailed observations are given in the following table. B.sphaericus (CDRI strain) field trial in Bajapur.

Sl No	Types of breeding spot and area	Dose	Averege larval and pupal density per dip															
			Before treatment Number of			After treatment												
						24 hour Number of			48 hour Number of			72 hour Number of			96 hour Number of		
			E	L	P	E	L	P	E	L	P	E	L	P	E	L	P
1.	Pit attached to drain	2g/lit	-	10	-	-	-	-	7	-	-	2	23	-	1	45	-
2.	Pit with highly polluted water	0.5 g /500ml	-	24	-	-	3	1	3	1	-	-	18	-	2	29	5
3.	Pit with polluted water	0.5g/500ml	-	13	-	-	-	-	2	-	-	8	-	-	10	-	
4.	Pit attached to drain	0.5g/500ml	10	300	-	-	5	-	-	5	-	1	3	2	1	15	-
5.	Pit	0.5g/500ml0	20	169	-	-	9	-	-	2	-	-	5	1	-	23	3
6.	Pit	2 g/ lt	30	321	70	-	19	38	-	-	-	7	125	13	2	230	4

E: egg; L: Larvae; P: Pupa

6.6 Mosquitocidals from Plants : Their Bioactivity and Bioefficacy

Scientist : Dr. A.P. Dash

Technical Staff : Ms. U.L. Mohanty

Starting date : February 1992

6.6.1 Indigenous plants:

About thirty two indigenous plant species of different families were collected from different locations of Orissa. Their bioactivity against three laboratory reared vector mosquitoes viz., *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus* were tested using their different crude extracts. The solvents are used were Acetone, Hexane, Diethylether, Methanol and Chloroform etc. After screening, different parts of the selected plants were used for solvent extraction by Soxhlet method.

Among the three plants tested, crude ether and Hexane extract of cotyledon of *Pongamia glabra* (L) of family Papilionaceae was found to be toxic to the above three mosquito species tested. All types of solvent (S) extracts of *Anacardium occidentale* L of family *Anacardi aceae* were also highly toxic to these

mosquito species both at the larval and pupal stages. Also it has been found that plants like Azadirachta indica *A. Juss.* (Meliaceae), *Andrographi paniculata Ness* (Acanthaceae). *Clerodendrum inerme* (L) Gaertn. (Verbenaeceae), *Thuza occidentalis* L. (Pinaceae), *Momordica charantia* L. (Cucurbitaceae) and *Vitex negundo* L. (Verbenaceae) were showing JH mimics causing larval-pupal intermediates or causing death with abnormalities in pupal stage. This work is in progress.

6.6.2 Mosquitocidal effects of marine products

The following marine products viz., *Bullia vittata, Modiolus striatulus, Rhizotoma* species, *Zoanthus* sp. *Onchidium verruculatum, Sigmadocia pumila, Callyspongia spinosissima, Gracilaria crassa* and unidentified corals were supplied by CDRI Lucknow for laboratory evaluation. The methanolic and chloroform-methanolic extracts of these Zoanthus and unidentified corals were found to be toxic to larvae of *Cx. quinquefasciatus, Ae.aegypti* and *An. stephensi.* The chloroform-methanolic extracts of unidentified coral were highly toxic to *An. stephensi* (LD_{50}/LD_{90} is 20.29 + 0.187 ppm/68.71 + 0.071 ppm).

Some other marine products supplied by CDRI Lucknow are under study.

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 92

Bioassay of the juvenoid OMS 3031 was conducted under laboratory conditions at 26-28°C and 86% RH, against early fourth instar larvae of *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus.*

The EC_{50} and EC_{90} concentrations were calculated by Logit analysis.

Table: EC_{50}/EC_{90} values for OMS 3031 against different mosquito species.

Mosquitoes species	Six hour exposure		Continuous exposure	
	EC_{50}	EC_{90}	EC_{50}	EC_{90}
Ae. aegypti	$3.88X10^{-27}$	$4.58X10^{-7}$	$7.8X10^{-5}$	$8.11X10^{-3}$
An. stephensi	$4.5X10^{-6}$	$4.41X10^{-3}$	$1.07X10^{-4}$	$2.8X10^{-2}$
Cx.quinquefasciatus	$8.327X10^{-18}$	$1.63X10^{-5}$	$3.85X10^{-27}$	$4.58X10^{-7}$

During the bioassay tests several morphological anomalies in mosquito larvae like curved abdomen, reduction in body length, melanization of tail portion were observed.

6.8 Laboratory Biology and Colonization of Mosquitoes OF MOSQUITOES (Cyclic Process)

Cyclic colonies of the following species are maintained in the laboratory.

i) *Aedes aegypti*

ii) *Aedes aegypti* (Liverpool strain) (black eyed)

iii) *Aedes vittatus*

iv) *Anopheles stephensi*

v) *Culex quinquefasciatus*

vi) *Culex vishnui*

vii) *Ma.annulifera*

viii) *Ma.uniformis*

Laboratory bred mosquitoes and larvae are supplied to different research workers, including Utkal and Calcutta Universities. Mosquitoes are also used for developing filaria larvae through membrane feeding.

Seminar/Symposia Attended

Name	Seminar attended	Paper presented
Dr. A. P. Dash	Rice Field Ecosystem'Cuttack, May, 1992	Rice Field Ecosystem and Disease transmission
	Third National Symposium on Vectors and Vector Borne Diseases, Bombay, Sept. 1992	Guest lecture delivered on "Insect hormones and their role in vector management
	Second Convention on role of voluntary agencies in national disaster reduction, Konark, Sept. 1992	Participated in discussions
	Health System Research, New Delhi, Dec. 1992	Participated in discussion
Dr. N. Mahapatra	Third National Symposium on Vectors and Vector Borne Diseases, Bombay, Sept. 1992	Feeding patterns of eight mosquito species in Orissa
Mr. R. K. Hazra	Third National Symposium on Vectors and Vector Borne Diseases, Bombay, Sept. 1992	Seasonal prevalence and vectorial capacity of *Mansonioides* in Orissa
	WHO/NICD workshop on "Alternative methods of Vector Control of Malaria and other mosquito borne diseases", Jagdalpur, Jan. 1992	Participated in discussion

Full Length Papers Published :

Dash, A.P. and Ranjit, M.R. (1992)- Comparative efficacy of aphid extracts and some juvenoides against the development of mosquitoes. *J. Am. Mosq. Cont. Assoc.* **8** : (3) : 247-251.

Mohanty, U.L., Dash, A.P. and Ranjit, M.R. (1992) - Mosquitocidal activity of *Pongamia pinata* (Linn.) extracts. *Asian J. Zool. Science,* **1** : 15-18.

Other Activities :

1. Dr. A.P. Dash has been awarded the Dr. T.R. Rao award for 1991.
2. Dr. A.P.Dash graced the seminar on "Rice field ecosystem" as the Guest of Honour, organised by the Zoological Research Association in May 1992 and addressed the participants on "Rice field ecosystem and disease transmission".
3. Dr. A.P. Dash was a Ph.D. examiner of University of Bombay and Utkal University during 1992.
4. Dr. A.P. Dash was invited as a faculty by the UNICEF/NMEP-Project at Keonjhar in July 92.
5. Dr. A.P. Dash acted as the chairman of the scientific sessions VI (Vector ecology & Control) of the 3rd National Symposium on Vectors and Vector borne diseases, held at the Haffkine Institute, Bombay during September,'93.
6. Dr. A.P. Dash acted as a faculty member of DBT sponsored course held at RMRC, Bhubaneswar during Nov., 1992 and delivered a lecture on Insect Immunity in relation to parasitic diseases.

VII. FIELD STUDIES AND BIOSTATISTICS

7.1 A qualitative study on the dietary habits of tribals of Koraput

Scientist : Mr. Amarendra Mohapatra

Starting date : July,1992

Tribal population of Koraput district are presumed to be under the stress of inadequate food supplies during lean seasons. Repeated enquiries have been made regarding alleged mortality due to shortage of adequate amount of food supplying good nourishment. We have made a preliminary assessment of dietary habits of tribal groups during our visit to Koraput district. A total of 12 PHCS were covered during our visit in two seasons. These PHCs were Rarnaguda, Boipariguda, Khairput, Gunupur subdivision hospital, PadmapurGudari, Ramnaguda, Kolnara, Laxmipur, Boriguma, Papdahandi and Bonda hills. Discussions were held with the local medical officers, district medical officers and the district magistrates to elicit their viewpoint on tribal health, nutrition and food habits. Koraput district has 51 tribal groups. Mostly they do shifting cultivation (Podu chaso) and live on hunting and food gathering type of economy. The staple food is rice and they are non-vegetarian by dietary habits. Besides rice they do cultivate pulses also. They collect many fruits and tubers from the forest to live upon. Since they are non-vegetarian in their dietary habits they eat meat, beef, chicken, fish and many more animals of forest.

Drinking water:

The tribals have a habit of drinking surface water in preference to under ground water. Many tube wells were rejected or used for other purposes and surface water which is muddy during the rainy seasons is preferred for drinking purposes because of taste. This practice is not safe from health point of view.

Cereals and pulses:

Rice, Ragi and Maize were the main cereals cultivated in thearea. During post-harvest seasons they subsist mainly on cereal diet of maize, ragi or rice which ever is available.

Green gram (Mung) and Bengal gram (Arhar) are the two main pulses grown by them. Surprisingly, it is reported in some areas and at times they can survive on Bengal gram diets during harvest season when cereals are out of stock/less in amount.

Fruits:

During seasons of fruits they depend on fruits heavily and they do preserve the fruits to some extent. Sun drying method is used to preserve the fruits. The mango seed-cotyledons are also consumed in season after processing.

Animal proteins:

The tribals are non-vegetarian in their dietary habit. They eat meat from goats, sheep, cows, buffalos, chicken. Their food habit include consumption of fish, egg and many more animals.

During or before harvest they observe Puja in which buffalo or animal sacrifice is a ritual compulsion accompanied with feasting and drinks. During this period if there is surplus meat, they do preserve the meat, by sun drying and later on with fumigation. The method used for the latter process is crude, i.e near the hearth of the house.

Houses normally have a single room with a small 3-4 feet partition for the kitchen. The sun-dried meat is pierced into a string (like a meat garland) and it is hung over the hearth. It is observed to emit slightly offensive smell during drying. Before actual consumption is done they wash the dried meat thoroughly and soak it in water and then cook the reconstituted meat.

It is suspected widely by the medical staff that the meat is one of the main cause of different infections. The meat is not properly dried because usually the surplus meat and festivities happen during rainy season. Hence half dried and fumed meat when consumed with local drinks may lead to infections. However there are no empirical studies on this aspect.

The rice liquor and Mohua liquor which they prepare in each village may be another source of infection in rainy seasons. However, detailed studies have to be carried out along with diet survey to measure food and nutrient intakes in various seasons to assess the magnitude of undernutrition in lean seasons. Physical anthropometric indices will help in quantifying the nutritional status

of tribal population in this crucial district which is reportedly ravaged by frequent large scale occurrence of gross under nutrition.

7.2 Investigation in to Cuttack Liquor tragedy.

Scientists : Dr. K.A.V.R. Krishnamachari
Dr. S. Sriramachari
Dr. G.P. Chhotray
Dr. S.K. Kar.

As directed by ICMR this centre had initiated scientific investigation into the unfortunate spurious liquor tragedy at Cuttack. Associated Prof. K.K. Mishra and his colleague from Forensic Medicine & toxicology department had performed the medicolegal autopsies on the victims. A small portion of various viscera from liver, spleen, kidney, lungs, brain, stomach, and a loop of the intestine were collected from the 6 autopsy cases who died during 9th & 10 May 1992. The gross and histopathological finding reports were shown and discussed with Dr. S. Sriramachari who was present during the investigations. 45 urine samples from these patients were examined for routine & microscopic examination, specific tests for detecting aldehyde by Schiff's reagent and specific quantitative test to detect formaldehyde by Chromotropic acid was also performed. The inference of the test results are as follows : Formaldehyde was not detected in any of the urine samples. Presence of aldehydes (Formaldehyde + acetaldehyde) was detected in 9 samples and continued to persist on repeated examinations. All these investigations along with the clinical profile leads to the conclusion that the tragedy was due to the adulteration of methyl alcohol.

7.3 A study on outbreak of epidemic of gastroenteritis in Koraput district.

Scientists : Dr. G.P. Chhotray
Dr. V.R. Subramanyam
Dr. S.S.S. Mohapatra

A team from this centre had conducted a study in Koraput district to find out the epidemiological situation and possible aetiology of the outbreak of the present epidemic of diarrhoeal diseases sweeping the district.

The study revealed that there was an outbreak of epidemic of diarrhoeal diseases affecting 33 Primary Health Centres of Koraput district since 28th June,1991. The affected persons constituted about 5% of the population of

affected Primary Health Centres. It appears that the epidemic is caused by mixed infection of Vibrio cholerae and other enteropathogens pending bacteriological confirmation.

The study of the epidemiological data of gastroenteritis for the past 3 years (1988-1990) indicates that the district is endemic for the disease having peak incidence during the monsoon (July- August). A detailed report is enclosed in a seperate sub section based on secondary data collected from PHCs.

Considering the scarcity of the safe drinking water sources in the villages and the food habits of the inhabitants it appears that the disease has multiple sources of infection which requires an in depth study and constant monitoring to assess the correct picture of the disease in this region.

7.4 Situational analysis of occurrence of diarrhoeal diseases in Koraput district

Scientists	:	Mr. Anil Kumar Dr. S.S.S. Mohapatra Mr. A. Mohapatra
Starting date	:	July 1992.
Completion	:	September 1992.

Reports of outbreaks of diarrhoeal disorders during July- September of each year are a common phenomenon for the Koraput District of Orissa.Our team had undertaken preliminary assessment of the situation in July.1991 as indicated in the above sub-section. A detailed investigation of the epidemic was also carried out by NICED,Calcutta (Annual Report,1991-92). Subsequently an RMRC, Bhubaneswar team visited some of the affected PHCs in July-September, '92 and obtained secondary data for the year 1991-92 from medical officers and others personnel. The team covered 11 PHCs of Koraput district and collected information on villages affected, no of cases (attacks) and deaths attributable to gastro- enteritis and other diarrhoeal disorders. The secondary data indicated that the Boriguma PHC area registered limited number of cases during January 1991. Kosagumuda PHC area was affected in June '91. Sixty one villages of this PHC had reported 222 cases and 33 deaths. In all 11 PHCs have reported 3142 cases of diarrhoeal diseases in 1991 with 382 deaths. The case fatality rate (CFR) was 121.6 per 1000. The graph (Fig.5) shows the sudden

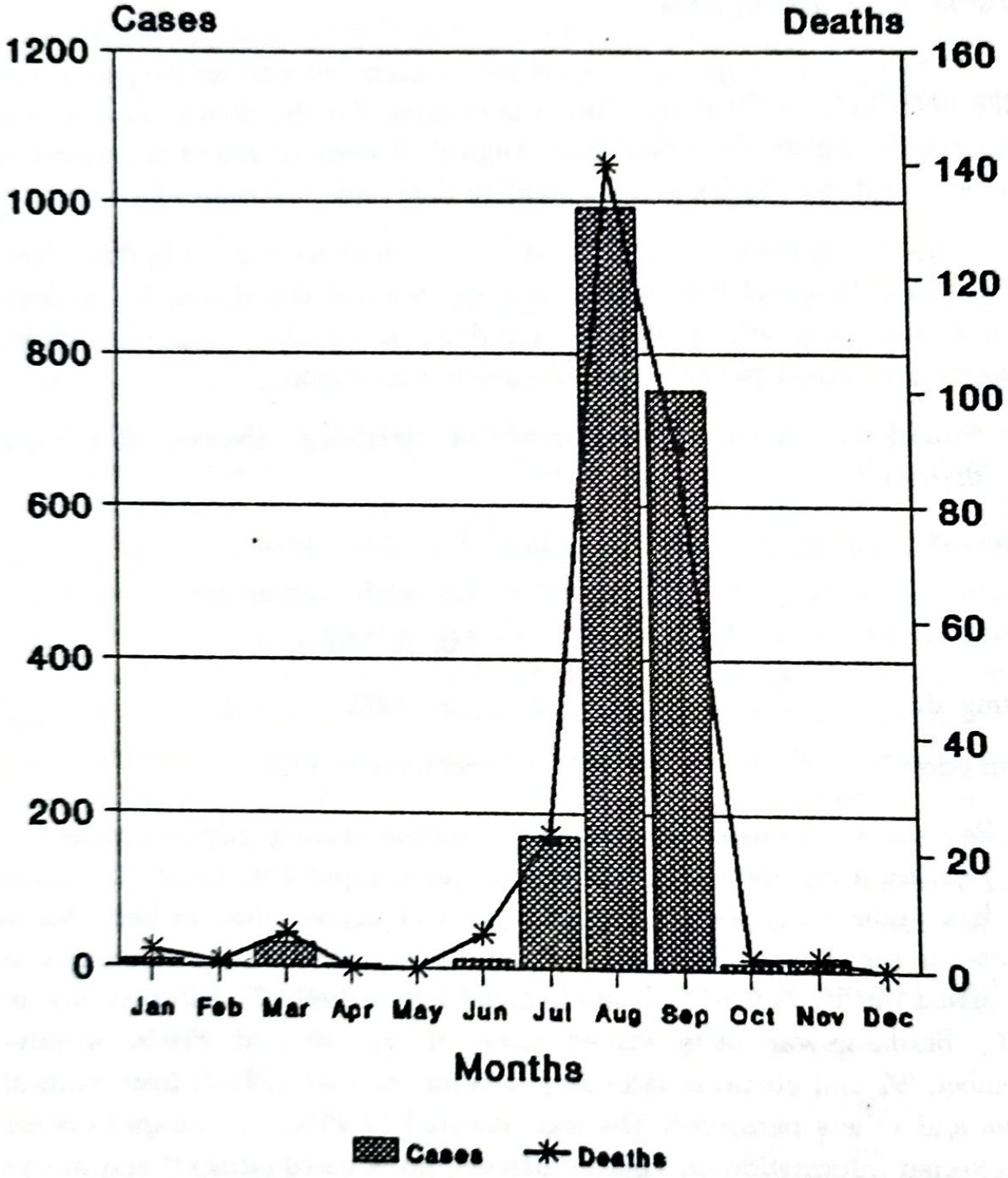

Fig. 5 : Monthly reported cases and deaths of diarrhoeal diseases in Koraput district, Orissa (1991)

outbreak of diarrhoeal diseases in July, reaching a peak during August, showing recession by September and reaching a very low level by Oct. '91.

7.5 Epidemiological study of malarial infection along with vital health parameters of Gania block, Puri district.

Scientist	:	Mr. Anil Kumar
Starting date	:	March 1992.
Completion date	:	December 1992

A demographic household survey was undertaken in 9 tribal villages of Gania block, Puri district with the help of Gania Unnayan Committee (GUC) - an NGO. However blood smear survey could be carried out in 6 villages only. The aim was to assess demographic indicators of mortality and health perceptions, particularly regarding malaria. The survey was carried on a random sample of 25 households in the nine villages selected. Blood smears were obtained from all cooperative volunteers and also those who had fever and were examined (by Immunology Division) for the presence of malarial parasite. The preliminary data indicate the following.

The socio-economic levels are low. Household economy is based on tendu leaf collection for about half a year. Health facilities are not adequate in the surveyed villages. The communication links are cut off during monsoon. Vital statistics were computed from the data available on about 1300 individuals drawn from about 200 household of 9 villages. The sample size is not fully adequate for such computations. However, the following vital rates have been given to show the direction of results from this limited study. The IMR was found to be 81/1000 live births, crude death rate was found to be 10/1000 and birth rate was found to be 29 per 1000 population It is, however, noted that child,particularly infant deaths are often not recounted by the families and this could be one of the reasons of lower infant mortality noticed. The indirect estimates of mortality, based on proportion of children ever born to those now surviving indicated very high mortality. The expectation of life at birth was computed to be very low from the collected data. About 85% of head of the households perceived malaria as a major disease in the area. Of the 17% who had reported to be currently ill, 12 percent reported to be suffering from malarial fever.

Out of the 235 persons whose blood smears were examined for malarial parasite, 14.9 percent were found positive. The main parasite was P.falciparum (86%). None of the household has reported use of mosquito nets or repellents, with a few exceptions of using smoke, as personal protection measures against mosquitos. About 2/5th felt mosquitos to be the causative agents of malarial spread. Almost everybody expressed willingness to use mosquito net, if provided free of cost. It was also found that illiterate persons had almost twice the risk of getting malaria than the literate persons. As was the case in other areas almost half of those diagnosed positive for malaria parasite did not present themselves with any symptoms of malaria fever (asymptomatic parasitaemia). This may indicate the nature of endemicity of malaria in such areas. In Assam,30% of the school children had asymptomatic parasitaemia in the Boku PHC study (Annual Report. RMRC, Dibrugarh, 1991)

Training Programme Offered :

I. Mr. Anil Kumar conducted a two weeks extensive computer training programme for a batch of Junior/Senior Research Fellows recruited in various divisions at RMRC.

II. Mr. Anil Kumar taught a basic course of Biostatistics to all research students at RMRC.

Abstract:

An abstract "HIV spread: Role of behavioral factors" appeared in Abstract book of International Congress on Aids in Asia and the Pacific, held at Delhi.

Publications :

1. A. Mohapatra (1992) A community based study of the Sociological Aspects Night Blindness in the Desert Region of Rajasthan. *The Indian Journal of Social Work.* Vol. XL 111 No.1.

2. A. Mohapatra (1992) Calorie and protein consumption pattern among Desert people of Rajasthan during drought. *Annals of Arid Zone* 31 (1) 25-32.

3. A. Mohapatra (1992) "An analysis of knowledge attitude and practice regarding some common disease in rural part of Jodhpur district". *The Indian Journal of Preventive & Social Medicine,* Vol. 23, No.2.

4. A. Mohapatra (1992) Socio-demographic profile of tribal population in three Rajasthan Districts. *The Eastern Anthropologist,* Vol. 45 : 3.

5. A. Mohapatra. Profile of Opium Addiction in Western Rajasthan". *Indian Journal of Human Ecology* (In press).

CONFERENCES ATTENDED:

Mr. A. Mohapatra attended the international symposium on science and society & XVIIIth Annual Conference of Indian Society of Human Genetics. Jan.22-25, 1992, Utkal University, Bhubaneswar. and presented a paper "Status of women and state of child health". Mr. A. Mohapatra attended the ICMR/ WHO/TDR Workshop on Social and Economic Aspects of Lymphatic Filariasis, 3-8, Feb.92, RMRC, Bhubaneswar and presented a paper "Socio-economic profile of a village in Orissa endemic for lymphatic filariasis".

VIII. ACTIVITIES OF THE FIELD STATION, LOCATED AT V.S.S. MEDICAL COLLEGE HOSPITAL, BURLA

Staff :

i. Dr.R.S. Balgir, Assistant Director & Team leader.

ii. Mr B.P.Dash, Research Assistant

iii. Mr R.K.Das, Research Assistant

iv. Mr.P.C.Nayak, Senior Stenographer

v. Mr Chakradhar Naik, Sweeper-cum-Attendant

Research & Collaborative Activities :

The collaborative activities were continued during the period under report as per the previous practice. A total of 1885 cases were referred to Dr. B.C. Kar for expert opinion, of which 1508 were new cases and 377 were the old followed up cases.

i. Sickle Cell Clinic: The haematological investigations were done for screening 1508 cases. In addition to above, 377 old cases were followed up and investigations were repeated as and when required by Dr. B.C. Kar.

ii. Natural History of Sickle Cell Disease: For the study of Natural History of Sickle Cell disease 377 old cases were followed up and laboratory investigations were done as following: 327 haemoglobin estimations, 41 fetal haemoglobin estimations, 169 reticulocyte counts, 36 packed volumes, 36 mean cell haemoglobin concentrations and 92 serum bilirubin estimations by both direct and indirect methods. With the addition of 296 new sickle cell diseases/sickle cell -β- thalassaemia cases, haemoglobin estimation, fetal haemoglobin, PVC, MCHC, retics and serum bilirubin estimation were done for 169, 108, 73, 163 and 108 cases respectively.

iii. Diagnostic Facilities: For welfare and proper treatment of the patients, diagnostic facilities were provided by doing the electrophoresis both in acid and alkaline medium for screening the patients. During the screening, 296 sickle cell disease/sickle cell-β-thalassaemia, 900 sickle cell trait, 301 normal cases were detected. There were three suspected cases of AD, 2 SD, 1 HB. E-thalassaemia, 3 cases of beta-thalassaemia major and 2 cases of beta thalassaemia trait were provisionally diagnosed. Family studies

were completed on 45 cases of sickle cell disease/sickle cell-β-thalassaemia. However screening tests for other family members were partial. For all the cases, sickling tests were performed to detect the Sickle Cell hemoglobinopathy.

Other Research Activities :

A study of 190 mothers in relation to sickle cell hemoglobinopathy was carried out to know the differential reproductive outcome. The reproductive wastage (abortions and still-births) has been found to be higher in mothers afflicted with sickle cell anaemia than the sickle cell heterozygote and control mothers.

An attempt was also made to find out the relationship between sickle cell hemoglobinopathy and age at menarche in Orissa. The study shows that menarche is delayed by one year in subjects afflicted with sickle cell disease (14.9 years) as compared to controls (13.6 years). In case of sickle cell trait, the mean age at menarche has been found to be 14.0 years.

Training Given :

i. Dr. (Mrs) T. Bhuyan, Medical Superintendent (Pathology), Regional Hospital, Talcher, SECL had undergone training on "Diagnosis of Sickle Cell Anaemia by Electrophoresis Techniques" at Field Station, R.M.R.C., Bhubaneswar, V.S.S. Medical college Hospital, Burla with effect from 4.5.1992 to 8.5.1992.

ii. Dr. D.B. Sarkar, Assistant Research Officer (H), Clinical Research Unit (T) for Homeopathy, Ministry of Health, Sambalpur has taken exposure at this Field Station, Burla to "advanced techniques such as electrophoresis, pathological investigations like reticulocytes counts, differential counts, peripheral smear. etc employed in sickle cell anaemia" with effect from 21.10.1992 to 24.10.1992.

Publications :

Balgir, R.S. (1992) Distribution of Serogenic markers in the tea garden tribal population of Dibrugarh District, Assam. Bull Dept. Anthropol. Dib. Univ. **19** : 29-38.

Balgir, R.S.(1992) Clinical profile and fertility of Hemoglobin E afflicted mothers in North Eastern India. *Med. Surg.* **31** : 16-18.

Balgir, R.S. (1992) Serogenetic variations in a Mechkachari Tribal Population. *Man In India* **72** : 293-301.

Balgir, R.S. (1992) Reproductive profiles of mothers in relation to haemoglobin E genotypes. *Indian J. Pediatr.* **59** : 449-454.

Balgir, R.S. (1992) Current status of hemoglobinopathy and G6PD deficiency in Orissa: Some further considerations. *Man in Society* : 76-78.

Dash, B.P., Mitra A. and Kar, B.C. (1992) G6PD deficiency and sickle cell disease in India. *Indian J. Hemat. Blood Transfus.* **10** : 85-88.

Papers Presented in Conferences :

i. Dr. R.S. Balgir, presented a paper entitled "Genetic markers in the Tribal Health, Development and Welfare of North-Eastern India" in the 79th Indian Science Congress held during 3-8 January, 1992 at Baroda.

ii. Dr. R.S. Balgir, Asst. Director, submitted a paper entitled " Genetic Variation in a Mechkachari Tribal population of Dibrugarh district in Assam" in Professor JBS Haldane Birth Centenary International Symposium on Science and Society held during 22-25 January 1992, Bhubaneswar.

Awards Received :

Dr. R.S. Balgir, Assistant Director & Team Leader was awarded the prestigious ICMR Prize for Biomedical Research conducted in underdeveloped areas of India on the Subject " Human Genetics in the Tribal Health of North-Eastern India" for the year 1991.

IX. MEMBERS OF THE SCIENTIFIC ADVISORY COMMITTEE

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

2. Dr D.S.Agarwal
   ECD Chief
   Indian Council of Medical Research
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3. The Addl. Secretary
   Health & Family Welfare
   Govt. of Orissa
   Bhubaneswar

4. The Director
   Health Services Heads of dept. Building
   Bhubaneswar

5. The Director
   Medical Education and Training
   Head of Dept. Building
   Bhubaneswar

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

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

8. The Principal
V.S.S.Medical College
Burla, Dist. Sambalpur

9. Dr S.K.Sood
Prof. & Head
Department of Pathology
University College of Medical Sciences
Shahdara
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10. Dr V.P.Sharma
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Malaria Research Centre
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New Delhi 110 054

12. Dr I.C.Tiwari
Advisor (Health)
Planning Commission
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13. Dr Kalyan Banerjee
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15. Dr V.K.Vinayak
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16. Dr B.B.Tripathy
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17. Dr U.C.Chaturvedi
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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. The Director
Regional Medical Research Centre for Tribals
Medical College
Jabalpur 482 003

22. The Director
Regional Medical Research centre
Chandrasekharpur
Bhubaneswar 751 016 (Member-Secretary)

X. DISTINGUISHED VISITORS

Drs S.P. Tripathy, DG, ICMR; S. Pattanayak, Consultant W.H.O., Delhi; Olavi Elo, W.H.O. Representative Delhi; C.P. Ramachandran W.H.O., Geneva; M. Tanner, Swiss Tropical Institute; B. Greene, Alabama; M. Weiss, Boston; E.A. Ottesen, N.I.H.; D. Denham, London School of Tropical Medicine & Hygiene; S. Sriramachari, Ex-ADG ICMR; S.C. Pal, Ex-Director NICED, Calcutta; R.Gisler, Basel Institute of Immunology; J.P. Gupta, Director, NIHFW Delhi; Harcharan Singh, Former Advisor, (Health) Planning Commission; S.Basu, Director, National Institute of Immunology, Delhi; Sushil Kumar, Director, HRD Group, CSIR Delhi; Amit Ghosh, Dy.Director IMTEC (CSIR) Chandigarh; V.Dhanda, Director, VCRC; Kodakovich, WHO; Nirbhaya Kumar, Johns Hopkins Univ.; MVVL Narasimham, Director, NMEP, Delhi; Pali Sen, University of Florida; S.K. Mahajan, Molecular Biology Division BARC Bombay; R.Prabhakar, Director, TRC; K.K. Mohanty, Director, Tribal and Harijan Research and Training Centre, Bhubaneswar; Mr. N.K. Das, Director, ORSAC; Mr. K.L. Pujari, Soil Conservation Officer, Bhubaneswar; Mr. A. Yuster, UNICEF; Mr. Ian Patt, British Council and Mr. S.K. Mishra, Dy. General Manager, NALCO.

XI. DEVELOPMENTAL ACTIVITIES

In addition to reinforcing the existing roads on the campus, new roads have been laid to enable better access to the garages and residential blocks. All the buildings have been given a face-lift by way of colour washing of exteriors. Inauguration of the RMRC office building will be taken up soon.

A new block of 9 'D' type quarters has been added and is almost ready for occupation, thus increasing the number of residential quarters on the campus to 52, offering accomodation to almost 70% of the permanent staff. Sheds for cycles (24) and scooters (6) have been constructed for the use of campus residents. Five garages for residents' vehicles and five for office vehicles are under construction.

To make the campus more secure, a proposal has been sent for the Council's approval for raising height of the boundary wall and by providing street lights along the boundary wall.

The Annual Day function was celebrated by organizing various cultural programmes and competitions in which both the staff and their families participated.

XII. OTHER ACTIVITIES

1) An International Workshop on "Socio-economic aspects of ymphatic filariasis", sponsored by ICMR/WHO/TDR was held in RMRC, Bhubaneswar, 3rd to 7th February 1992. The meeting was attended by about 25 scientists from abroad (USA), UK, Switzerland, Phillipines, Brazil, Kenya, Tanzania, Ghana) and 15 scientists from the Indian institutions and was inaugurated by Dr S.P. Tripathy, DG,ICMR.

2) A Department of Biotechnology (Govt of India) sponsored course entitled "Immunological concepts in Parasitic Diseases" was conducted at the Immunology Divisions of the centre from 9th to 27th November, 1992. Twelve young scientists from various institutions of the country (NIN, Hyderabad, VCRC, Pondicherry, NII, INMAS, and AIIMS, Delhi, IVRI, Izat Nagar, University of Indore, Sanjay Gandhi Institute of Medical Science, Lucknow, Utkal University, OUAT & CIFA, Bhubaneswar) attended the course.

MEETING/SEMINARS OF THE DIRECTOR

Dr. K.A.V.R. Krishnamachari participated in the state level review meeting on leprosy, chaired by the Secretary, Health & Family Welfare, Govt. of Orissa on 17th April 1992.

- attended a meeting convened by Secretary, Rural Development and UNICEF jointly on 11-12 April at Berhampur to discuss the strategies for CDD and WATSAN for Ganjam district, Orissa during the coming years.
- delivered a lecture on "Crystal Structure of bone and its relevance to Human health" in the Physics Department, Utkal University on 18th April 1992.
- gave a key note address at NALCO., Angul in the symposium on "Health of industrial workers" with particular reference to "Fluoride industry" on 11th May, 1992.
- delivered a lecture at the 13th Disaster Management Course at the Asian Institute of Technology, Bangkok, Thailand 10-12 June.

-he was invited the Chief Guest at the Regional Research Laboratory, (C.S.I.R), Bhubaneswar on 26.09. 1992 and delivered C.S.I.R. Foundation Day lecture.

- received Dr. YELLA PRAGADA SUBBAROW Memorial Oration Award Gold Medal for 1992 from the Indian Medical Association on 18.10.1992.
- was invited to participate in the Research Advisory Group Meeting of Andhra Pradesh school Health Project at Hyderabad on 21st and 22nd October, 1992.
- was the Guest of Honour at the All India Convention of Society for Research in Diabetes in India held at Puri on 14.11.92.