REGIONAL MEDICAL RESEARCH CENTRE

BHUBANESWAR

ANNUAL REPORT

(1986 - 87)

INDIAN COUNCIL OF MEDICAL RESEARCH • NEW DELHI •

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NEWDELHI

REGIONAL MEDICAL RESEARCH CENTRE

(INDIAN COUNCIL OF MEDICAL RESEARCH)

NANDANKANAN ROAD
BHUBANESWAR - 751005

ANNUAL REPORT

(1. 4. 86 to 31. 3. 87)

AR-35

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RMRC, Bhubaneswar.

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Director

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PREFACE

The annual report (1986-87) covers the period from 1st April' 86 to 31st March 1987. The scientific activities at the Regional Medical Research Centre are presented under individual projects carried out during the above mentioned period. Some of the earlier projects were completed during the year under report and the results are indicated under "Projects Completed". The ongoing research programme is given under "Projects in hand" (as on 31st March,1987) and most of these activities continue during the current financial year. However, the results obtained during the year of report are given under each project. It is also proposed to take up some new areas of research during the current year and these are given separately as "New Projects Proposed to be Undertaken". The scientists working at this centre are collaborating in several projects. In some projects, they are the principal/ sole investigators. Other projects are collaborative in nature with multidisciplinary approach. It is the team work and collective effort to tackle a health problem.

The main areas of activity cover human lympahtic filariasis, leprosy and malaria; the three endemic diseases in this region of the country. For the clinical and epidemiological studies field trips are organised under leadership of the scientists and with the help of the supportive staff (technical and non-technical). Laboratory work is carried out by the scientists mostly using the materials collected during the field trips. Since the insect vectors play a very important role in the epidemiology of filariasis and malaria the main thrust is also to understand and tackle the entomological aspects of these diseases. For filariasis the entomological studies are confined to the villages around Bhubaneswar. For malaria the entire work is done at the field unit functioning at Jeypore (Koraput district) with active participation of the team from V.C.R.C., Pondicherry.

Besides the above mentioned diseases, helminthic and protozoal infections seen in young school going children have also been studied with the main aim of assessing the impact of these infections on the health (growth & development) of children. Study of the incidence of cryptosporidiosis in young children suffering from diarrhoea has also been carried out.

In Orissa cases of G-6-PD deficiency and sickle cell disease have reported from time to time. This centre has, therefore, organised a sub-centre for study of sickle cell disease/trait in the western districts of the state, at V.S.S. Medical College, Burla, with a separate grant from the Council and as a time bound programme. The technical staff were recruited and have joined the laboratory set up for this purpose. But no suitable candidates were found for the scientist's job, for clinical and laboratory work. The Government of Orissa has been requested to depute suitable candidates for this work

Studies on G-6-PD deficiency are being carried out in Keonjhar district endemic for malaria. This is an ongoing programme till a large sample is analysed to draw meaningful conclusions.

The study of Cardio-pulmonary involvement in Tropical Pulmonary Eosinophilia, organised at Chest diseases department of the S.C.B. Medical College, Cuttack, had to be abandoned in October 1986, as the Research Officer (Clinical) was relieved of his duties to join the state medical services. Due to the continuing ban on recruitment and appointment of scientific staff the project remained suspended.

The Scientific Advisory Committee of the centre met for the first time on the 24th January 1987. Its recommendations are being implemented for the ongoing projects. Lack of adequate laboratory space in the present set up (in the Drug testing laboratory) and adequate power supply for the equipments are hindrances against expanding the scientific activities. It is hoped that these problems will be solved when the laboratories start functioning in the Centre's own laboratory building which is under construction in the R.M.R.C. Campus.

The Library of the centre has been further enriched by receiving a donation of 45 volumes of scientific books from the British Council. Another lot of 60 Volumes is expected soon. Forty foreign journals are being subscribed by the centre, besides the W.H.O. publications and a few selected Indian journals of medical importance. Most of the earlier volumes of journals received so far are now available in bound form in the library for consultation.

(Prof. L. N. Mohapatra)
DIRECTOR

PROJECT IN HAND ON 31.3.1987

A. LEPROSY & MYCOBACTERIA

A. 1. Immunology of Mycobacterial Infection in Man and Animals.

Scientist	:	Dr. Santosh Kumar Kar Assistant Director
Technical staff	:	Mr. Amiya Ranjan Nayak Research Assistant
Starting date	:	April, 1985

Objectives :

a) To characterise the *Mycobacterium leprae* specific antigens circulating in the body fluids of leprosy patients and to explore the possibility of development of an early diagnosis for leprosy based on antigen detection.

b) To understand how mycobacterial antigens modulate the immune response of the infected host.

c) To understand how mycobacteria survive inside macrophages.

The Approach

This study involves isolation and biochemical characterisation of *Mycobacterium leprae* specific antigens from infected human tissue and body fluids and understanding of their immunological characteristics while in circulation. It also involves use of cultivable mycobacteria such as *Mycobacterium kansasii, Mycobacterium vaccae, Mycobacterium marinum, Mycobacterium smegmatis* and *Mycobacterium fortuitum* for the isolation of different antigens, analysis of their surface architecture and understanding the mechanism of their survival in the mouse and human macrophages.

During 1985-86 the work was mostly focussed on developing methods for isolation of *M. leprae* specific antigens from infected human tissue and serum. For this no standard method was available. Since treatment of patients alters the dynamics of the mycobacterial antigens circulating in the body fluids and deposited on the tissue for correct understanding of the process, one needs to study patients who have had no treatment for leprosy. Such patients are becoming increasingly difficult to find due to introduction of

mass therapy. Our preliminary study during 1985-86 with human tissue and serum derived phenolicglycolipid-I (PGL-I) antigen which is specific to *M. leprae*, had shown that the chemical nature of the PGL-I from human source could be different from armadillo tissue derived PGL-I. Interestingly further work during 1986-87 has shown that both the PGL-I have identical elution profile in HPLC and in their reactivities towards polyclonal anti PGL-I antibodies raised in rabbits or antibody obtained from human serum. To establish the chemical nature of the PGL-I molecule isolated from human sources a detailed chemical as well as spectroscopic analysis is necessary. For this atleast 10 mg. of pure PGL-I is needed. To obtain this much of PGL-I sufficient tissue from untreated leprosy patients is not readily available. However, our effort is continuing. During this study it was found that Dapsone, the drug used for treatment of leprosy patients, has strong affinity for mycobacterial lipids and is found to be associated with PGL-I isolated from treated leprosy patient. This property of dapsone is being studied in greater detail with a view to understand how Dapsone acts on *M. leprae*.

Serum and punch biopsy from untreated leprosy patients belonging to TT and LL category have been collected and the level of PGL-I in them has been measured by using HPLC and Enzymelinked immunosorbent assay. The level of PGL-I correlates very well with the bacteriological index for lepromatous leprosy patients. In the case of tuberculoid leprosy patients the level of PGL-I in the serum did not correlate with the bacteriological index. When the same patients were given multi drug therapy and their serum and biopsy were examined, the amount of PGL-I deposited in the tissue or circulating in the serum was found to be reduced drastically indicating that the treatment is eliminating *M. leprae* from their body. This method can now be employed for monitoring the effect of treatment and also for the identification of drug resistant cases of leprosy. Serum from twenty contacts of infectious leprosy patients, who are exposed to them every day and who have not yet developed any clinical symptoms of leprosy, has been collected. Four out of the twenty contacts have PGL-I circulating in their serum. Three out of four of these PGL-I positive cases are lepromin negative (3 weeks Mitsuda reaction). It is proposed to continue this study with a larger number of contacts and measure the PGL-I in their serum at different time points to find out about the applicability of this system for early diagnosis of leprosy.

Reactions in leprosy (type I and II) are believed to be 'Immunological complications' arising out of the presence of large amount of *M. leprae* antigens in the body fluid. Using an Enzyme linked immunosorbent test it has not been possible to detect the presence of any mycobacterial proteins in the serum of patients with reaction. Western blotting technique involving biotinylated antibody and enzyme conjugated avidin is being used to detect protein antigens. This work is in progress. There is evidence for the presence of mycobacterial lipids in the serum of patients having reaction. The role of such lipids in bringing about immunological complication is being studied.

Suppression of cell mediated immune response to *M. leprae* derived antigens is an unique feature of lepromatous leprosy. It is believed that mycobacterial lipids play a very important role in causing immunological suppression. The ability of the phenolicglycolipid-I antigen to cause immunological suppression in a mixed leucocyte reaction is being tested. There appears to be suppression in mixed leucocyte reaction in a dose dependent fashion. This work is continuing.

Lipids from cultivable mycobacteria like *M. kansasii, M. vaccae, M. marinum, M. smegmatis* and *M. fortuitum* has been isolated by extraction with chloroform methanol mixture (2:1) and purified by passing through silicic acid column. These lipids have been tested for their immunogenicity in mice which were sensitised with live mycobacteria. The lipids were able to elicit delayed type of hypersensitive reactions (DTH) in mouse foot pad. In a limited study involving human volunteers from a leprosy endemic area these lipids were found to be equally immunogenic when given intradermally as skin test antigen. We are now trying to fractionate these lipids to find out which component is highly immunogenic and whether they can be used in protection experiments in mice. Since the surface of mycobacteria plays very important role in the initial interaction of the organism with the immune system of the host, methods for the analysis of their surface architecture by using surface probes has been developed. Surface radioiodination of mycobacteria by Na I (Iodine Isotope 125)using iodogen method and analysis of the radiolabelled surface antigens either by sodium dedecylsulphate polycrylamide gel electrophoresis (SDS PAGE) or by thin layer chromatography (TLC) followed by autoradiography reveals that there are several lipid molecules present on the surface which could be labelled with radioactive iodine. In contrast to this, very few, if any, proteins could be labelled by surface iodination. This again establishes the fact that mycobacterial surface is very hydrophobic and is different from other bacteria. We are doing further study to identify the exact nature of the lipid molecules present on the mycobacterial surface and their role in enabling the mycobacteria to survive in the host.

Justification for the Continuation of the Project :

Development of an early diagnosis for leprosy based on detection of *M. leprae* specific antigens circulating in the serum of infected persons will contribute significantly towards control of leprosy. The same method also can be used for assessing effective chemotherapy of leprosy by monitoring elimination of *M. leprae* from the body. Therefore, the work on the detection of PGL-I in contacts of leprosy patients is of considerable importance and should be continued. Our understanding of the mechanism of survival of *M. leprae* and other mycobacteria in an infected host is meagre. It is very likely that the molecules present on the mycobacterial surface play a very important role in enabling the mycobacteria to survive in an immune competent host. Therefore, study on the mycobacterial surface antigens and their role in immuno modulation should be continued.

A-2. Mycobacterial Genetics

Scientist : Dr. V. R. Subramanyam

Technical staff : Ms. K. K. Mohanty (since Dec'86)
Mr. B. B. Pal

Starting date : In early 1985

Progress :

As a prerequisite for further genetic studies, standard strains of *Mycobacterium* were mutagenised using NTG to obtain auxotrophic markers. The general methodology has been outlined in the previous annual report. Updated results are given in Table-1.

Justification for continuation :

A large number of strains with different markers is the raw material of any genetic studies. Hence more mutants need be found. Simultaneously experiments are underway to effect gene transfer between marker strains of mycobacteria, by spheroplast fusion.

Table - 1

Micobacterial mutants

Strain	No. of colonies * screened	No. of mutants obtained	Mutant Designation	Markers **
M. fortuitum (NIHJ 1615)	178	2	SM2	Arg
			SM3	Pur
SM2	1914	1	SM29	Arg, Leu, His
SM3	4129	5	SM24	Pur, Met
			SM25	Pur, Met
			SM26	Pur, Met
			SM27	Pur, Met/Cys
			SM28	Pur, Cys/Met
M. smegmatis (NIHJ 1628)	903	6	SM10	Lys
			SM13	Leu
			SM19	Pur
			SM20	Lys
			SM21	Pur
			SM22	Arg
SM10	650	2	SM33	Leu, ?
			SM34	Leu, ?

* After treatment with NTG, bacteria were plated on a nutritionally rich medium. Discrete colonies from this medium were tested for auxotrophy on different media.

** Three letter codes indicate auxotrophy for the particular aminoacid or nucleotide base; ? indicates experiments in progress.

B. FILARIASIS

B-1. Long term follow-up of asymptomatic microfilaraemics in an endemic area with reference to their peripheral eosinophilia and antibody level.

Scientist : Dr. Shantanu Kumar Kar
Technical Staff : Miss. J. Mania
Mr. K. Dhal
Mr. R. N. Nayak
Mr. T. Moharana
Mr. S. Rout

Starting date : November 1985

Progress :

a) In order to identify the asymptomatic carriers in endemic population for study, population from two villages (Kumarabasta and Panichhatra) were screened by clinicoparasitological examination and 114 subjects were selected for follow-up,

b) Blood was collected (5 ml.) for detection of

i) mf (by nuclepore membrane filtration technique),

ii) Differential Leucocyte count, Absolute eosinophil count,

iii) Detection of filaria specific antibodies (IgG, IgE, IgA) and

iv) Immune complexes circulating in blood.

c) Blood was also collected from non-endemic population for comparison from Phulbani and Bolangir districts.

The one year follow-up examination was completed and results are summerised below. The second year of follow-up was initiated in November, 1986.

a) 34 sera were tested for detection of filaria specific antibodies (IgG) using *W. bancrofti* mf.

b) Immune complexes circulating in their blood was tested by PEG precipitation technique in 11 cases of which 2 were positive.

c) The age, sex distribution and eosinophil count of the carriers are summerised in table below. (Table-2)

Table-2

Age group	A.M.C. Studied		Total	Eosinophil count/ cub mm blood	
	M	F		3000	3000 or more
01—14	20	21	41	40	1
15—29	18	18	36	36	0
30—44	7	12	19	19	0
45 +	6	12	18	16	2
Total	51	63	114	111	3

Justification for continuation :

The above study will highlight the serial immunologic changes occuring in carrier stage during continuation or conversion of the carrier stage to amicrofilaremic or disease state. Their peripheral eosinophil responses related to filarial infection can be evaluated. It was presumed that lymphatic alteration occurs and progress during asymptomatic carrier stage. The tonometric assessment will reveal the above informations. Hence the study is important and should continue.

B-2. Study of arthritis with relation to filariasis in filaria endemic area.

Scientist : Dr. Shantanu Kumar Kar
Technical Staff : Miss. J. Mania
Mr. K. Dhal
Mr. R. N. Nayak
Mr. T. Moharana
Mr. S. Rout

Starting date : March 1986

Progress :

a) Screening of the endemic population of two villages by clinical and parasitological examination revealed that 61 out of 997 subjects had features of arthritis not pertaining to any specific etiology (Rhumatoid, tuberculosis, syphilis, osteo-arthritis, spesis)

b) Age, sex matched endemic normals (56) were taken from same endemic area as controls who do not show any evidence of arthritis. Approximately 25 individuals with arthritis (of specific cause) will be selected in patients attending orthopaedics OPD in Hospitals.

c) Parasitological examination for detection of mf (by membrane filtre technique), blood examination (DC, TLC, AEC) were carried out in above subjects.

d) Detection of filaria-specific circulating antibodies (IgG) was carried out using bancroftian microfilaria E.S. Ag by ELISA in 26 subjects of arthritis of which 22 became positive for presence of antibodies. 18 out of 28 sera from endemic normals taken as controls were positive for antibodies. Sera were also collected, stored in above study population and controls for detection of IgE, IgA antibodies and circulating immune complex.

e) Test for circulating immune complex deposits was carried out so far in sera of 5 cases (by PEG precipitation) which revealed one positive case. One out of 13 endemic normals sera had immune complex.

f) Joint fluid collected and tested in 3 subjects with effusion in knee joint revealed high antibody titre, (IgG) against filarial (ES) antigen.

g) Biopsy was taken from synovial membrane from above two cases so far.

Histopathology of synovial membrane, detection of immune complex deposits in membrane (by immunoperoxidase and Immunoflurescence method) and their filarial specificity will be carried out.

The preliminary results obtained so far is presented in the table below. (Table 3)

Table-3

Age group in Years-	No. of sub. with arthritis M	F	Total	No. of cases associated with filarial disease.	Nos. mf. + ve	Nos. with monoarth-ritis	Nos. with effusion in Joint
01—19	4	3	7	2	1	4	1
20—39	7	6	13	3	3	9	4
40 +	19	22	41	7	2	24	21
Total	30	31	61	12	6	37	26

Justification for continuation :

Few reports have shown the association of filariasis with arthritis in endemic area. Filarial etiology has not been conclusively established so far. Here an attempt has been taken to examine cases of non-specific arthritis prevalent in endemic areas and to study in depth its strengh of association with filariasis.

B-3. Clinico pathological study of Lymphatic nodules in human filariasis.

Scientists : Dr. Shantanu Kumar Kar.
Dr. G. P. Chhotray.

Technical Staff : Miss. J. Mania.
Mr. K. Dhal.
Mr. R. N. Nayak.
Mr. T. Moharana.
Mr. S. Rout.

Starting date : March 1986

Progress :

a) 68 subjects from filarial endemic area manifesting with lymphatic nodules and 48 age, sex matched endemic control s were selected for study.

b) Detailed clinical examination and parasitological examination (MF technique) were carried out in study subjects and controls.

c) Filaria—specific antibody (IgG) was observed in 14 out of 18 number of subjects tested in cases with nodules.

d) Lymphatic nodules from two subjects were excised and subjected to histopathological study. This showed evidence of filarial infection with adult filarial worm in one of the sections.

The age, sex distribution of the study subjects are summerised in table below. (Table 4)

Table-4

Age group in Years	No. of subjects with nodule M	F	Total	Nos. mf. +ve	Nos. associated with filarial disease.
01—19	21	8	29	3	16
20—39	14	0	14	5	11
40 +	16	9	25	2	20
Total :	51	17	68	10	47.

Justification for continuation :

Results of the previous epidemiological study carried out at this centre indicated that a group of endemic population manifest with lymphatic nodules in their

extremities which appeared to migrate. It is relevant to know whether this group is in any way different than endemic normals or the group showing obstructive pathology. Histopathological studies around the developing adult worm will show associated pathological changes which is important in understanding pathogenesis of the nodule formation.

B-4. Immunology of *Wuchereria bancrofti* infection in humans.

Scientists : Dr. Santosh K. Kar.
Miss. Beenu Joshi (SRF)

Starting date : June 1985

Objectives :

a) To characterise *Wuchereria bancrofti* antigens circulating in the serum of filaria patients and to develop a diagnostic method for microfilariae carriers on the basis of antigen detection.

b) To understand the effect of Diethyl carbamazine citrate (DEC) on *W. bancrofti* parasite (microfilariae and adult) and on the immune system of the infected humans.

c) To make DNA library of *W. bancrofti* microfilariae in a suitable host for isolation of parasite specific DNA fragments and for possible synthesis of parasite antigens.

The approach :

This study involves analysis of serum samples from a large number of persons living in a bancroftian filariasis endemic area with the following clinical status.

a) Endemic normals without any clinical symptoms of filariasis and without microfilariae in their night blood samples.

b) Endemic normals without any clinical symptoms of filariasis and with microfilariae in their blood.

c) Acute cases of filariasis.

d) Chronic cases of filariasis.

e) Non-endemic normals.

Treatment of a population living in a bancroftian filariasis endemic area with microfilariae in their blood using one regimen of Diethyl carbamazine citrate (DEC) and study of the effect of the treatment on parasite clearance and immune response of the infected host against parasite and other antigens are under study. This study also involves isolation of microfilariae from the night blood samples of microfilariae carriers and extraction of DNA from the parasite for making DNA library and synthesis of parasite antigens.

The serum and plasma samples that were collected during 1985-86 from persons living in several bancroftian filariasis endemic areas covering the entire clinical spectrum of filariasis were used to develop an enzyme linked immunosorbent assay (ELISA) system for the detection of filaria parasite antigen. The same plasma samples have now been affinity purified on concanavalin A columns and the eluted antigens have been used in a western blot to detect parasite specific antigens. Usiug biotinylated human immunoglobulin isolated from the serum of chronic filaria patients, anti *W. bancrofti* infective L3 antibodies and anti *Dipetalonema viteæ* antibodies and avidin-HRPO sytem, several antigens are being detected on the western blot in the plasma of microfilariæ carriers which are not present or present in reduced amount in the plasma of normal persons without microfilariæ. This system is now being tested with many serum and plasma samples to establish the authenticity of the antigens detected as parasite specific. A diagnostic system could be developed to test its applicability in the field condition for detection of microfilariæ carriers. The only problem encountered here is lack of *W. bancrofti* antigens to establish the identity of the antigen detected on the western blot.

One hundred ten persons from a filaria endemic village without any clinical symptoms of filariasis but having microfilariæ in their night blood (asymptomatic carriers) have been treated with 300 mg of DEC for 21 days. Eighty of them cleared microfilariæ after treatment. These persons are being followed up for their ability to remain free of microfilariæ while living in an endemic area. Serum from asyptomatic microfilariæ carriers before and after treatment with DEC (the above regimen) when tested for antibody against microfilariæ antigen did not show much difference in antibody titre. Using various skin test antigens like PPD, Lepromin, *D. viteæ* antigen etc., it was also observed that treatment with DEC did not alter very drastically the cellular response of asymptomatic carriers to filaria parasite and other antigens.

The isolation of DNA from *W. bancrofti* microfilariæ in sufficient quantity has not yet been possible due to the contamination of the parasite with host lymphocytes and non-availability of sufficient material.

Justification for continuation :

Development of a diagnostic system for the detection of asymptomatic microfilariæ carriers is a very important requirement for filaria control. Therefore work in that aspect should continue. The effect of DEC *in vivo* on the *W. bancrofti* microfilariæ and adult and on the immune system of the host is not well understood. This understanding will be crucial for the development of an effective regimen for chemotherapy of filariasis. Therefore work on the effect of DEC on the parasite as well as on the immune system of the host should continue. The DNA library of *W. bancrofti* microfilariae will be useful for typing different isolates of the parasite from different geographical locations and therefore effort in that direction should continue.

B.5. Culture of *W. bancrofti* microfilariae

Scientists	:	Dr. N. M. Pattnaik
Technical Staff	:	Mr. Dasarathi Das
Staritng date	:	September 1985

Progress :

An improved method for microfilariæ (mf) purification using polycarbonate membrane filter has been standardised and adopted for routine use. The recovery as well as purity of the mf obtained by this method are far superior to that from the sedimentation method used earlier.

When mf are incubated at 37° in DME/F12 medium without any serum they remain metabolically active and secrete substantial antigenic material into the medium. A culture of 10,000 mf/ml produces approximately 200 ug protein/ml over a 24 h. incubation period and with very little nucleic acid contamination. This has been used as "ES antigen" in other studies.

Justification for continuation :

1) This provides us with "ES antigen" material routinely for use with other projects. 2) With the availability of insect tissue culture media the emphasis is now to be shifted to the development process of mf to the infective larval stage.

B. 6. Biochemical anyalysis of filarial antigens.

Scientist	:	Dr. N. M. Pattnaik
Technical Staff	:	Mr. Dasarathi Das
Strting date	:	October 1986

Progress :

Methods have been standardised for the extraction of antigenic material from the circulating microfilariae using detergents and alkali either alone or in combination. Antibodies to these antigens have been raised in rabbits and purified from sera of patients. Using enzyme conjugates of these antibodies immunoassays have been developed to detect and quantitate the antigens in circulation as well as during the course of a purification procedure.

The antigen sources currently under investigation are 1) the blood sera of various categories of patients, 2) the mf culture media and 3) hydrocele fluid.

Justification for continuation :

Biochemical characterization of the molecules eliciting any host immune response will help in following the course of events in the disease process.

B. 7. Evaluation of alternate medicines for filariasis.

Scientists	:	Dr. N. M. Pattnaik. Dr. V. R. Subramanyam.
Technical Staff	:	Mr. Dasarathi Das.
Starting date	:	February 1986

Progress :

In collaboration with the clinical research unit (CRU) of the Central Council for Research in Homœopathy the vallage of Beldal, near puri town, was surveyed for filariasis. The detected cases are currently under treatment with Homœopathic drugs and are being followed up periodically. On the completion of one year of medication in April a detailed follow up through clinical as well as night blood smear examination is planned. *In vitro* studies on the microfilaricidal properties of some useful drugs are detailed in Dr. V. R. Subramanyam's report (see B-8).

Justification for continuation :

In view of the continuing prevalence of filariasis and acceptance problems of DEC therapy a search for effective alternatives must continue.

B. 8. Effect of Homoeopathic drugs on microfilariae *in vitro*.

Scientists	:	Dr. V. R. Subramanyam. Dr. N. M. Pattnaik.
Technical Staff	:	Mr. Dasarathi Das
Starting date	:	February 1987

Progress :

Based on the experience of the staff of Filariasis Clinical Research Unit (CRU), Central Council for Research in Homœopathy (CCRH), Puri, it was decided to see the action, if any, of certain drugs on microfilariae *in vitro*.

Rapid immobilization and presumably killing of *W. bancrofti* microfilariæ (mf) takes place when incubated at 37°C with mother tinctures of *Apis mel* and *Rhus tox*, at a concentration of 20 µl/ml (final alcohol content 2%). At this concentration mother tinctures af *Bryonia, Hydrocotyle, Natrum mur* and *Microfilariae* (nosode) as well as 30 and 200 centecimal potencies of all the tested drugs were not effective in killing mf.

In another experiment, to avoid alcohol in the system, aliquotes of the mother tinctures were allowed to dry in the wells of a tissue culture plate and then mf in 1 ml culture medium were introduced. Under these conditions, with 20 µl of the drug per

well, killing was observed in 10 min. with Hydrocotyle, in 1 hr. with Rhus tox, in 2.5 hr. with Apis and in 5 hr. with Bryonia. Even 2 µl of these drugs effects nearly total killing in 24 hrs. Interestingly, Bryonia appears to have a better killing effect at a lower concentration (0.2 to 0.5 µl/well) than at higher concentrations.

Justification for continuation :

Because of certain known short comings of DEC therapy for filariasis, alternative medicines need be explored.

B-9. Circulating Immunocomplexes (CIC) in filariasis.

Scientists	:	Dr. V. R. Subramanyam. Dr. M. K. Das. Dr. N. M. Pattnaik. Dr. B. Ravindran. Dr. S. N. Das.
Technical Staff	:	Mr. B. B. Pal.
Starting date	:	January 1986.

Progress :

In continuation of our earlier work on CIC, we studied CIC in cases of Tropical Pulmonary Eosinophilia (TPE). All of the 45 TPE sera showed considerably high levels of CIC by the PEG assay method, vis a vis 48 sera from endemic normals (Table-5). In the same samples, antibodies reacting with *W. bancrofti* antigens have been quantified (see projects completed item No. 5)

Table-5

		C. I. C.		
Category	No.	Range	Mean	S.D.
Endemic Normal	48	0.01—0.17	0.05	0.04
T.P.E.	45	0.11—0.72	0.46	0.14

Specificity of the antigen/antibody in CIC is being investigated by an ELISA system.

Justification for continuation :

CIC could be a relatively easy source of antigen/antibody if the specificity is ascertained.

B-10. Detection and characterisation of antisheath antibodies in Bancroftian Filariasis.

Scientists	:	Dr. B. Ravindran. Dr. N. M. Pattnaik. Dr. M. K. Das. Dr. V. R. Subramanyam.
Technical Staff	:	Mr. Ashok K. Satpathy.
Strting date	:	October 1986

Progress :

Antibodies to the microfilarial sheath have been incriminated in the immune elimination of microfilariae from circulation. Their role in Brugian filariasis has been established by earlier workers, but has not been explored in detail in Bancroftian filariasis. In the present investigation Indirect immunoperoxidase assay (IPA) and indirect fluorescent antibody assay (IFA) have been performed in 118 sera collected from subjects living in an endemic area near Bhubaneswar. The distribution of anti-sheath antibodies in different clinical spectra of Bancroftian filariasis cases was investigated. The following is the brief summary of the study.

a) Nearly 80% of chronic filariasis cases and 75% of endemic normal sera had anti-sheath antibodies. The above subjects were amicrofilaraemic.

b) Only 10% of microfilaræmic asymptomatic carriers had demonstrable antisheath antibodies.

c) Statistical evaluation revealed a very significant negative correlation between the presence of antisheath antibodies in serum and microfilaraemia.

d) There was 80% correlation between the two assays IPA & IFA.

e) The determinants on the sheath that reacted with antisheath antibodies were found to be carbohydrate in nature—they were heat stable (100 C for 30 mins), sensitive to Sodium periodate treatment and resistant to pronase treatment.

The project is in progress to investigate the role of IgE antibodies with reactivity to microfilarial sheath.**

** The findings on antisheath antibodies were presented at the annual meeting of Indian Immunology Society held at New Delhi in Dec. 1986. The preliminary findings are in press in the Journal of Tropical Medicine and Hygiene. The manuscript of characterization of antisheath antibodies in Bancroftian filariasis is in preparation.

B-11. Production and characterisation of rabbit antibodies with reactivity to *Diethylcarbamazine*.

Scientists	:	Dr. B. Ravindran. Dr. N. M. Pattnaik.
Technical Staff	:	Mr. Ashok K. Satapathy. Mr. Dasarathi Das.
Starting date	:	July 1985

Progress :

Antibodies with reactivity to DEC have been raised with the view to develop a sensitive immunoassay for quantitative measurement of DEC in body fluids and also to use it as a tool in investigations on the mode of action of DEC against filarial parasites. The following are the results of the study.

a) DEC free base was hydrolysed with 70% Sulphuric acid and the product 4-methyl piperazine-1-carboxylic acid (MPCA) was coupled with Bovine serum albumin (BSA) by carbodiimide reaction. The resultant conjugate MPCA-BSA was used for immunization of rabbits in conjunction with Freund's complete adjuvant. Antibodies to MPCA-BSA in sera collected at different intervals were monitored by ELISA *(Fig.1)*. The antibodies were found to be mostly of IgG in nature.

b) The specificity of the antibodies was assessed by a modified ELISA. Purified IgG (Protein-A Sepharose column) from immunized rabbit sera was coated on the ELISA plate, to which lat er a conjugate of MPCA-alkaline phosphatase (MPCA-AP) was added. This reaction between anti-MPCA and MPCA-AP was competitively inhibited by various concentrations of DEC, MPCA and piperazine citrate. The standard curve *(Fig.2)* also indicates that DEC at a concentration of 0.5 µg/ml could effectively inhibit the reaction—such inhibition ELISA could thus be effectively used for quantitation of DEC in unknown samples.*

c) Rabbit antibodies that react with DEC were also found to react with the microfilarial sheath of *W. bancrofti* as shown by indirect immunofluorescent assay (IFA) and indirect immunoperoxidase assay(IPA). The reaction could be very effectively inhibited by 1.25mM DEC and 5mM MPCA as shown by IFA or IPA using immune sera after preincubation with the respective haptens. The determinant on the microfilarial sheath that reacts with Anti- MPCA was found to be resistant to heat and Sodium periodate treatment. Further studies are in progress to investigate the immunological cross reactivity between microfilarial sheath and DEC.

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* The above results were presented at the annual conference of the Indian Immunology Society held at New Delhi in Dec. 1986. The paper on the production and characterization of antibodies with reactivity to DEC is to be published shortly in Med. Sci. Res. (In press)

B-12. Determination of immunological parameters of *Wuchereria bancrofti* infected sera.

Scientists	: Dr. M.K. Das. Dr. N.M. Pattnaik. Dr. V.R. Subramanyam. Dr. B. Ravindra.
Technical Staff	: Mr. M.K. Beuria.
Starting date	: July 1985.

Objective :

Filariasis caused by *W. bancrofti* presents a wide spectrum of clinical manifestations in man. It is necessary to determine quantitatively various parameters namely the level of antibodies to mf and L3, ES— antigens and the L3 antigen assay in order to correlate immunological data with the stages of infection. Such a composite study may prove useful in immunodiagnosis.

Progress :

This is an on-going project, some aspects that were completed are given under (''Project completed'', project No 4 & 5). At present antibody to L3 and ES— antigens is being performed by ELISA. An assay for the determination of L3 antigens is in the process of development.

B-13. Cellular aspects of immunomodulator action : application in malaria and filariasis.

Scientists	: Dr. M.K. Das. : Dr. B. Ravindran.
Technical Staff	: Mr. M.K. Beuria.
Strarting date	: June 1986.

Objective :

Role of different adjuvants that were non-toxic to man to elicit desired immune response is a major subject of investigation. An area of considerable uncertainity is cell-mediated immunity, especially in malaria and filarial infection. This project would try to evaluate liposomes and other adjuvants (saponin, alum etc.) for their effectiveness in disease-infected experimental animals.

Progress :

Antiserum against L3 of *W. bancrofti* was raised in rabbits by both liposomes and Freund's adjuvant. The relative increase in titre is being checked by ELISA. *Plasmodium berghei* antigens are isolated from the erythrocytes of infected Balb/C mice (% parasitaemia 50%). The antigen will be used for CMI studies.

B-14. Studies on mosquito antigens eliciting immune response in man.

Scientists	: Dr. M. K. Das.
	Dr. A. P. Dash.
Technical Staff	: Ms. Anindita Mishra (JRF)
Starting date	: April, 1986

Objective :

Culex quinquefasciatus is a vector of filarial parasite *W. bancrofti* which is endemic in Orissa. A local cutaneous reaction is common in man following mosquito bites. Although it is assumed to be immunologically mediated the underlying mechanism is not clear at present. The immune response in man specific to mosquito antigens needsto be studied. Such a study will help in understanding host-vector relationship and vector biology.

Progress :

Antibody to *Culex quinquefasciatus* has been demonstrated in man for the first time and is reported under "Project completed". Antiserum to culex has been raised in rabits and the cross-reactivity with other species of mosquitoes is being investigated. Salivary gland antigens from the mosquitoes are isolated.

C-ENTOMOLOGY

C-1 Present status of *Anopheles sundaicus* in Orissa.

Scientist	: Dr. A. P. Dash.
Technical Staff	: Mr. R. K. Hazra.
	Mr. G. D. Mansingh.
Starting date	: October 1986

Progress :

Extensive and intensive surveys are being carried out in the Chilika area, Paradeep and other coastal areas of Orissa to study the present status of *A. sundaicus* in the state. During the surveys in the Chilika lake area (including the islands in the lake) the following 21 species of mosquitoes were found including 13 anopheline species.

Anopheles aconitus, An. annularis, An. barbirostris, An. culicifacies, An. 'hyrcanus' group, *An. jeyporiensis, An. karwari, An. philippinensis, An. ramsayi, An. subpictus, An. tesselatus, An. vagus, An. varuna, Aedes aegypti, Armegeres theobaldi, Culex epidesmus, Cu. gelidus, Cu. quinquefasciatus, Cu. 'vishnui'* group, *Mansonia annulifera* and *Manosonia uniformis.*

Justification for continuation :

The search for *A. sundaicus* is on progress. Conclusions will be drawn after completing the survey covering all the seasons of an year.

C-2 Comparative vactor studies in Kumarbasta.

Scientist : Dr. A. P. Dash.

Technical Staff : Mr. R. K. Hazra.
Mr. G. D. Mansingh.

Starting date : May, 1986

Progress :

The RMRC, Bhubaneswar was carrying out clinical studies on filariasis in the village Kumarbasta, Puri District having a population of 2020. The tribal population (about 320) is isolated from the main village and was reported to have less filarial incidence. Weekly mosquito collections were made from these areas following statistical random sample. The perman hour densities were as follows, (Table-6)

Table-6

Months of the year	Non-tribal			Tribal		
	Total Mosq.	Anophelines	*Culex quinquefasciatus*	Total *Mosq.*	Anophelines	*Culex quinquefasciatus*
06/86	14.4	0.4	13.0	18.5	3.0	6.5
07/86	41.12	6.0	33.0	15.6	4.0	9.2
08/86	65.8	26.7	29.2	25.6	17.6	4.0
09/86	30.76	9.0	21.6	50.2	30.2	0.5
10/86	21.7	3.42	12.5	12.0	1.3	4.0
11/86	24.0	4.8	13.3	12.0	11.0	4.0
12/86	42.2	2.2	40.2	24.0	5.3	13.3
01/87	25.3	0	25.0	22.0	10.0	10.0
02/87	33.0	0.5	32.0	0.2	0	0

Justification for continuation :

The project is nearing completion and will be closed during the next month. The data will be analysed.

C.3-Effect of aphid extracts on the development of mosquitoes.

Scientists	:	Dr. A. P. Dash. Dr. G. P. Chhotray.
Technical Staff	:	Mr. M. R. Ranjit.
Starting date	:	January 1987

Progress :

The project has just started. Initial experiments showed that mosquitoes proved to be sensitive to the aphid extracts in the water environment. Different techniques are being standardised.

Justification for continuation of the project :

This project is initiated for the point of view of the juvenilizing effect and the protection of natural environment. The project will be undertaken at three stages :

a) Assessment of juvenile hormone in the extracts from different aphid species.

b) Action of aphid extracts and synthetic juvenile hormone on the development of mosquitoes.

c) Histopathological studies of the developmental stages of mosquitoes in relation to the application of aphid extracts.

The work is on progress. The project has been accepted for registration for the Ph.D. degree of the Utkal University.

C-4. Biology of *Culex* mosquitoes in relation to the development of *W. bancrofti* in Orissa.

Scientist	: Dr. A. P. Dash.
Technical staff	: Ms. Namita Tripathy.
Starting date :	October, 1986

Progress :

The project was recently started after the joining of a Senior Research Fellow. Three forms of *Culex quinquefasciatus* have been identified. A simple technique has been standardised for membrane feeding of *C. quinquefasciatus* in the laboratory using locally available artificial membrane. It will help to study the development of filaria parasites in mosquitos *in vivo*.

Justification for continuation of the project :

The project is at the initial stage. The work is on progress. Techniques are being standardised for the study. The project has been accepted and registered for the Ph. D. degree, under Utkal University.

D-PATHOLOGY

D-1. A Study on erythrocytic G-6-PD deficiency in a malarial endemic tribal Population of Orissa.

Scientist	: Dr. G.P. Chhotray.
Technical Staff	: Mr. M.R. Ranjit.
	: Mr. H.K. Khuntia.
Starting date	: April, 1986

Progress :

A study area, Banspal block of Keonjhar district was selected. Banspal block and PHC area is situated 28 KM from the Keonjhar district headquarter and is mostly inhabited by tribals having high incidence of malaria. Total population is 67,237, scheduled tribe 47,664, scheduled caste 2,698 and general 16,865.

a) Total number of cases studied so far from April 86 to March 87 is 166 out of which 90 cases are from scheduled tribe mainly Kolha and Bhuyan, 26 cases are from scheduled caste mainly Patra and Dasa and 50 cases are from General Caste.

b) Various haematological investigations were performed as per the table-7, showing rationale of investigation.

c) Out of 166 cases studied severe G-6-PD deficiency was detected in 16 cases (9.6%) and intermediate G-6-PD deficiency was detected in 41 cases (24.7%). Combining together 57 cases (34.34%) of G-6-PD deficiency was detected so far.

d) Out of 57 cases of G-6-PD deficiency 47 were male and 10 were female patients.

e) The incidence of G-6-PD deficiency is found to be significantly more 45.56% (33.33% intermediate +12.23% severe) in comparison with the general population i.e- 16% (4% severe +12% intermediate).

f) The incidence of G-6-PD deficiency in scheduled caste is also more 30.77% (severe 11.54% + 19.23% intermediate) in comparison to that of general population.

g) Out of 166 cases studied malaria parasite (all *P. falciparum*) was found in only 38 cases.

h) Amongst 57 G-6-PD deficiency cases studied malaria parasite was positive in 10 cases (17.54%) and negative in 47 cases (82.46%).

i) From 57 G-6-PD deficiency cases studied 23 cases (40.35%) were found to be anaemic i.e. haemoglobin level below 11 g./dl and 34 cases (59.65%) were above 11g./dl.

j) Not a single case of Haemoglobinopathy is detected so far by starch-Agarose gel electrophoresis.

k) Sickle cell anaemia was not detected in any of the cases studied.

l) Osmotic fragility was found within the normal range in almost all cases of G-6-PD cases studied.

m) Faetal Hb (Hb F) in all the cases of G-6-PD cases studied are within normal range i.e. below 1%.

Justification for continuation :

a) To establish the incidence of G-6-PD deficiency in the study area minimum 325 samples are required as calculated statistically.

b) Since the incidence so far detected is quite high 34.34% out of which intermediate positive being 24.7% the following further detailed studies are indicated such as :

i) Quantitative estimation of G-6-PD deficiency.

ii) Identification of G-6-PD variant.

D-2 Prevalence of the intestinal parasitism and nutritional status of school going children in a semi-urban population of Orissa.

Scientist	: Dr. G. P. Chhotray.
Technical Staff	: Mr. M. R. Ranjit. Mr. H. K. Khuntia.
Starting date	: August 1986.

A study area was selected about 22 K.M. from Bhubaneswar situated besides the NH No. 5. The village Gangapada falls under the semi-urban area which has three Schools (the U.P.;M.E.; High) located in one campus. The pupils are from 5 to 20 years of age group.

Progress :

Total number of cases studied so far till March 1987 are 337. The stool samples were collected in plastic cups provided earlier and were transported to the laboratory where it was examined by formol-ether concentration technique and by normal saline method. The individual students were examined and the various anthropometric data were taken at the field site. The pupils were also screened for other associated diseases and accordingly the treatment was given after getting the stool examination report.

a) Out of the 337 patients examined the overall parasitic prevalence (Helminthic, Protozoa[and Combined) in various age group ranging from 5 to20 is found to be 216 (64.09%)

b) Only Helminthic infection was detected in 15 cases (33.3%) in the age group of 15-20 years in comparison to 5-10 years, Where it is (9.4%) and (16.4%) in 10-15 years of age group.

c) Round worm (R.W.) infestation was found to be the commonest infestation among the Helminths present which constitutes 22 out of 216 cases (10.18%).

d) Hook worm (H.W.) infestation was found in 5 cases and both RW & HW in 16 cases.

e) Giardiasis is found to be the commonest infestation among the protozoan infection constituting 65 cases (29.16%). The next common infection was found to be the EH.

f) The various anthropometric measurement like Height and Weight on the 337 cases examined are noted and the median (50th percentile value) was obtained from the NCHS standard prescribed by WHO. Taking these values as the 100%, our results obtained from the studied population the following results were made.

i) Taking weight for height as the criteria to assess the nutritional status (it has been observed that only 7 cases (2.07%) out of 337 cases were found to be above normal in nutritional status and 178 cases (52.81%) were found to be malnutrited. 152 cases (45.10%) of the studied population are found to be within normal limit.

ii) Taking weight for age criteria (Gomez category) it has been observed that 214(63.49%) of the total cases studied falls under the malnutrited category.

iii) Taking Ht. for age as the criteria it has been observed that 263 cases out of the total cases studied (78.03%) are found to be malnutrited.

Justification for continuation :

i) At this stage of the study it is difficult to assess any correlation between the intestinal parasitism and the nutritional status of the same population studied.

ii) After a lapse of three months of giving first deworming treatment the second round of stool examination will be carried out and various anthropometric data will be taken simultaneously.

E. MALARIA

E-1. Studies on naturally occuring antibodies with Alpha-galactocyl activity in *P-falciparum* Malaria.

Scientist : Dr. B. Ravindran.
Technical Staff : Ashok K. Satapathy.
Starting date : March 1986

Progress :

The development of acquired immunity to malaria in experimental models has been demonstrated to result in the production of various autoantibodies, particularly those which are directed towards host erythrocytes. Anti erythrocytic autoantibodies in sera have also been demonstrated in human malaria and malaria endemic areas. The precise role of such autoantibodies in malarial immunology has not been understood, nor their molecular specificity elucidated. The present investigation was initiated by the reported role of antibodies with Alpha-galactocyl specificity in immune opsonization and elimination of senescent erythrocytes under physiological conditions. Such autoantibodies constitute nearly 1% of total IgG in normal human serum. *P. falciparum* infected erythrocytes are known to express on their surface galactose determinants. In this aspect they resemble senescent erythrocytes.

Brief results of the present study are given below :

i) Conventionally anti Alpha galactose antibodies in human sera are detected by a rosette assay involving rabbit erythrocytes and a human myeloid cell line K562. A modified ELISA has been developed at this centre for quantitation of anti-gal antibodies. In the test a monolayer of rabbit erythrocytes is fixed to polystyrine plates and the endogenous peroxidase activity is eliminated by treatment of the monolayer with 0.5% Hydrogen peroxide in methanol. The test sera are then titrated and the bound antibodies are detected by the use of antihuman IgG conjugated to peroxidase. The above reaction is inhibited when the test sera are preincubated with melibiose.

ii) The levels of anti-gal autoantibodies were quantitated in 85 sera collected from a) *P. falciparum* infected patients b) Normal subjects living in *P. falciparum* endemic areas c) Normal subjects living in non-endemic areas. The anti-gal levels were found to be four fold more during the acute phase of infection as compared to non-endemic normals.

iii) The anti-gal IgG was affinity purified using rabbit erythrocytes and is being tested for its binding property to *P. falciparum* infected erythrocytes. Further investigations with the purified anti-gal antibody are in progress to study its *in vivo* role in *P. falciparum* malaria.

E-2. Production and characterization of rabbit antibodies to antimalarial drugs chloroquine and primaquine.

Scientists : Drs. B. Ravindran, M.K. Das & N.M. Pattnaik.
Technical staff : A.K. Satapathy.
Starting date : July 1985

Progress :

The necessity of developing sensitive immunoassays for quantitation of chloroquine (CQ) and primaquine (PQ) has been stressed far too often, although no attempt has been reported in literature regarding production of antibodies against these drugs. Successful production of specific antibodies to durgs are a prerequisite for development of immunoassays. Detailed procedures for the production of antibodies against CQ and PQ were given earlier (vide RMRC annual report, 1986). Monospecific antibodies (purified IgG) to primaquine were used in an inhibition ELISA for quantitation of PQ in unknown sample. The methodology, using a PQ alkaline phosphatase conjugate, was essentially the same as described earlier for DEC. The assay revealed that PQ in unknown samples could be detected in nanogram quantities. Further work is in progress to translate the findings for the detection of PQ in urine and serum.

E-3. Role of carbohydrate determinants in the interaction between *P. berghei* infected erythrocytes and macrophages from spleen, bone-marrow and peritoneum of BALB/C mice.

Scientists : Dr. B. Ravindran, Dr. M.K. Das.
Technical staff : Ashok K. Satapathy.
Starting date : March 1986.

Progress :

Carbohydrate determinants on the surface of various bacteria have been extensively studied for their role in interaction with surfaces of macrophages. They have been incriminated as 'recognition molecules' for effective phagocytosis. Endogenous lectins and specific antibodies have been shown to play an additional role in these interactions. Malarial parasites, by virtue of their intracellular localization in erythrocytes, interact with macrophages in many organs. Parasitized erythrocytes have been shown to express a number of carbohydrate determinants on their surface-determinants which are not expressed on normal non-parasitized erythrocytes. The present study is aimed at analysing the factors and carbohydrate determinants involved in the interaction

between *P. berghei* infected mouse erythrocytes and macrophages from spleen, bone-marrow and peritoneum of BALB/C mice. An *in vitro* phogocytic assay has been standardized to study such interaction. A preliminary *in vivo* experiment was performed to study the role of various carbohydrates (after oral administration) on the course of *P. berghei* infection in mice. The results indicated that galactose and N-Acetyl galacto samine would increase the degree of parasitaemia and result in early mortality.

E-4. The chloroquine sensitivity studies in *P. falciparum* is continuing in other PHC areas specially the area contiguous to the already discovered resistant pockets with the idea of maping out such foci of resistance. Presently the study is being taken up in Mathili PHC which is adjacent to Malkangiri PHC area.

Scientist	:	Dr. S. S. S. Mohapatra.
Technical Staff	:	Mr. H. K. Tripathy.
		Mr. Prakash K. Sahoo.
		Mr. Harisankar Nayak.
Starting date	:	January 1986

E-5. Study on the Incidence of malaria.

The incidence of malaria is being studied in the limited 15000 population of Borigumma PHC by fortnightly surveillance- The positive cases are given prompt radical treatment and contact cases are also screened by blood smear examination.

Scientist	:	Dr. S. S. S. Mohapatra.
Technical Staff	:	Mr. H. K. Tripathy.
		Mr. Prakash K. Sahoo.
		Mr. Harisankar Nayak.
Starting date	:	November 1986

Justification for continuation :

Koraput is one of the highly malarious areas of the State having *P. falciparum* as the predominant species (more than 90%) and reporting many deaths due to malaria. The emergence of chloroquine resistant strains of *P. falciparum* is one of the major causes of resurgenc of malaria as far as the parasite vector is concerned. Therefore the monitoring of drug resistance is *P. falciparum* can not be over emphasised.

Table-7

RATIONALE OF INVESTIGATION *(Item-D-1)*

Sample	Anticoagulant used for Preservation	Tests Performed	Method	Reference
Blood Collected by Venepuncture in Disposible Syringe	EDTA (1.5 mg/1 ml)	(a) Hb Estimation	Cynomethaemoglobin Method	Wintrobe-1981
		(b) TLC	Visual Method	Dacie-1980
		(c) PCV	Wintrobe's Method	Dacie-1980
		(d) Osmotic Fragility	Saline Concentration Method	Whitby & Brilton-1969
		(e) TRBC	Visual Method	Dacie-1980
	ACD (1 ml/5 ml)	(a) Haemolysate Preparation	—	Wintrobe-1981
		(b) Starch-Agarose Gel Electrophoresis		Dash & Dash *etal* 1978
		(c) MR Test for G-6PD Deficiency		Brewer *etal* 1962
		(d) HbF Estimation	Alkali Denaturation Method	Singer, K. *etal* 1951
	DIRECT	(a) Sickling Test	Sodium Metabisulphite Method	Dacie-1980
		(b) Differential Count		Wintrobe-1981
		(c) Malaria Parasite	From this Smear By Wright's Stain 3% Giemsa Stain	

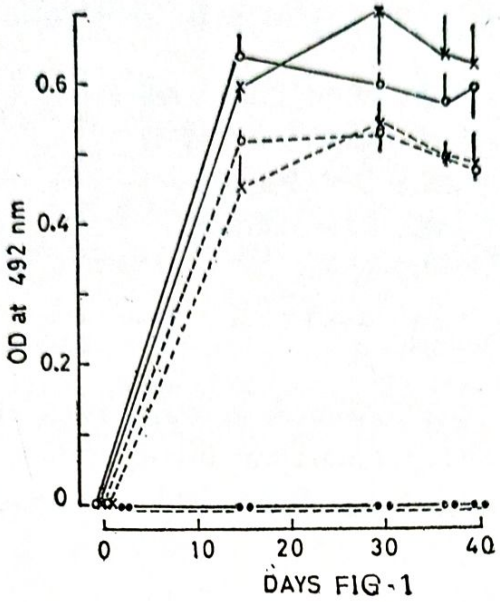

Fig 1 : Demonstration of antibody activity to MPCA-BSA in immunized rabbit sera by ELISA I : R-901 untreated serum o——o; R-901 serum treated with 2-ME o.....o; R-305 untreated serum x——x; R-305 serum treated with 2-ME x.....x; R-901 ●——● and R-305 ●.....● tested against BSA coated plates. Mean ± SD of quadruplicate values. Curve connected by eye.

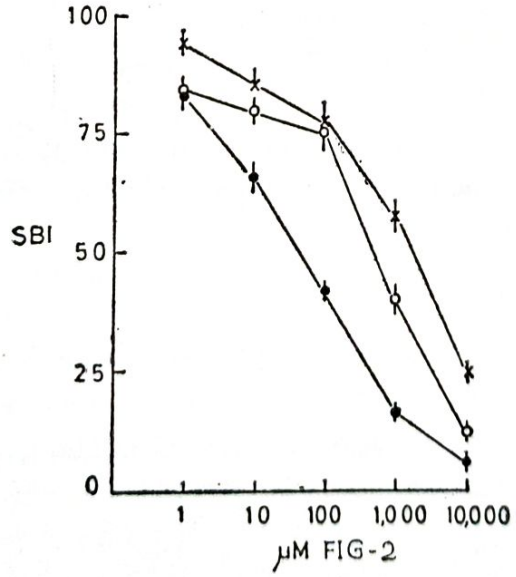

Fig 2 : Competitive inhibition of AP-MPCA reactivity with antibodies in ELISA II by DEC ●——●, MPCA o——o or piperazine x——x, SBI - specific binding index; Mean ± SEM of three independent experiments, each performed in quadruplicate wells (i.e.n = 12). Inhibition curve connected by eye.

PROJECTS COMPLETED

1. Asymptomatic microfilaria carriers— A 3 Years follow-up report.

Scientist : Dr. Shantanu K. Kar.
Technical Staff : Mr. K. Dhal.
Mr. R.N. Nayak.
Mr. T. Moharana.
Mr. S. Rout.
Period of study : July 1983 to July 1986

The above study was initiated in July 1983 at Patrapada village of Puri district and the previous follow-up report was incorporated in 1985-86 Annual Report. This follow-up report is after 3 years and detailed results of the completed study are given below.

Objectives :

The exact course of disease development in lymphatic filariasis is not fully understood. It is not known whether clinical manifestation of filarial disease are always preceded by an asymptomatic microfilarial carrier stage. Asymptomatic microfilarial carriers (A.M.C.) form an important group for study of the course of the disease. Besides, the duration of microfilaraemia in a carrier reflects the duration of parturating ability of female adults. Duration of microfilaraemia need to be understood to determine the transmission potential in an area. Previous studies have shown that the state of microfilaraemia due to *W. bancrofti* could last 5 years, 25 years or as long as 4o years. The above studies are not based on regular follow-up but an observation made in two points of time. Therefore a prospective study in a rural community was undertaken on about 163 A.M.C. subjects in Patrapada and Bhagabanpur villages of Puri district of Orissa endemic for filariasis.

Results :

Out of de-jure population of 2102 in both villages 1773 (84.3%) were covered by both clinical and parasitological examinations. This resulted in detection of 163 (9.1%) asymptomatic microfilaria carriers (A.M.C.). The quarterly clinical and parasitological examinations were conducted in 143 (87.7%) carriers for 3 year (July' 83 to July 86).

The distribution of carrier state was more prevalent in younger age group (46.8%) and did not show any sex predilection (P is less than 0.5). Table-8

During the course of follow-up it was observed that 23 (16.1%) subjects had discontinuous carrier stage with or without experiencing any disease, which was more

pronounced in older age group. Only 14 (9.8%) subjects became spontaneously amicrofilaraemic without experiencing any disease in three years follow-up period.

During follow-up, 34 (23.8%) subjects developed clinical signs and had acute attack of clinical filarial disease, of which only 9 (26.5%) became amicrofilaræmic in subsequent follow-up examinations. Disease conversion was more pronounced in younger age group ($P < .02$) and was progressively lower in higher age groups. The incidence of disease in males was more than females ($P < .05$) during follow up period.

There was a progressive drop in the number of mf carriers during successive 12 re-examinations (Table-9). The discontinuous mf carrier status was observed to be 7%, 12.6% and 16.1% at the end of 1st, 2nd and 3rd year respectively.

The cumulative conversion to disease was observed in 18.2%, 23.1% and 23.8% in three successive years.

Filarial fever was consistently observed in all 34 individuals converted to clinical filarial disease. Adenolymphangitic attack pertaining to limbs alone was seen in 12 and in rest, the genitals were affected in form of orchitis, funiculitis and / or epidedymitis. Filarial abscess was observed in 4 subjects. Fifteen out of 34 subjects showing clinical manifestations, had multiple attacks of lymphangitis during the follow-up period.

Lymphatic nodules were observed in 19 subjects in their extremities during follow-up. Arthralgia pertaining to limbs and malaise were the commonest symptom observed amongst the carriers particularly with subjects who subsequently manifested with clinical disease.

A considerable proportion of asymptomatic microfilaria carriers (83.9%) continued their microfilaræmic status for 3 years under observation. The microfilaria rate increased with age and then levelled off. There was no sex difference of A.M.C. stage. The discontinuous carrier stage was more pronounced in older age group and higher percentage of young population amongst A.M.C. showed disease manifestation. The discontinuation of microfilaræmic status without experiencing any clinical manifestation was probably due to natural host defence mechanism developed to clear the system of microfilaræmia. The study of immunological changes occuring during this conversion stage is essential to understand natural host defence mechanisms. This study reflects the minimum patency period of the adult parasite. It has been assumed in this study that the microfilaræmia continued to exist in carriers during 3 months interval of two consecutive observations. The possibility that a small proportion of A.M.C. might have dropped the infection and acquired a fresh infection remains though very remote. Yet another possibility may be that carriers have more than one adult worm contributing to microfilaræmia.

2. Prevalance of Tropical Pulmonary Eosinophilia (TPE) in a filaria endemic community.

Scientist	:	Dr. Shantanu K. Kar.
Technical Staff	:	Mr. K. Dhal.
		Mr. R. N. Nayak.
		Mr. T. Moharana.
		Mr. S. Rout.
Period of Study	:	1985-86

Objectives :

This pilot study was carried out in Patrapada village in 1985-86 and after analysis, detailed results are communicated below.

To determine the prevalence of Tropical Pulmonary Eosinophilia in the above study population, the following methods were followed.

Results :

1926 subjects were screened by detailed clinical examination for presence of symptoms or signs of TPE.

i) H/O cough and/or noctural wheeze for two months or more.
Suspected TPE subjects thus screened, were subjected to

a) Blood examination (DC, TLC, AEC)

b) Stool examination for presence of helminths (by floatation technique) like Ascariasis, Ankylostoma and Strongyloid.

Those subjects showing helminth infestation as above were given de-warming agents and repeat stool examination conducted after 3 weeks and absolute Eosinophil count was reassessed.

Subjects whose absolute Eosinophil count showed 3000 or more Eosinophils/Cmm of blood were considered as cases of TPE and were subjected to large plate x-ray chest examination.

The above study revealed that only 18 persons (0.93%) had manifestations of Tropical Pulmonary Eosinophilia in the endemic area.

Prominent broncho vascular marking was demonstrated in 12 subjects and milliary mottling was seen in only 5 cases (27.7%) of TPE.

Further study is needed to determine their hyper responsiveness to microfilarial antigen like high IgE, IgG titre in order to get a true prevalence.

AR-35

3. Lipid and Lipoprotein levels in *W. bancrofti* filariasis :

Scientist : Dr. N. M. Pattnaik
Technical Staff : Mr. Dasarathi Das
Period of study : September 85 to March 87

Results :

The individual blood lipids were analysed after extraction and separation using colorimetric methods. High density lipoproteins (HDL) were separated from the low (and very low) density lipoproteins (LDL) by dextran sulfate precipitation. Samples were analysed in terms of their total cholesterol content. Distribution of lipoprotein classes were monitored qualitatively by0.5% agarose gel electrophoresis and Fat Red 7B staining. The results obtained are summarised below (Table-10).

Table-10

Filarial Status	Chol/pl	CE/UC	HDL/LDL
Endemic normal (n = 10)	1.26 ± 0.14	2.04 ± 0.90	0.49 ± 0.14
Microfilariæ carriers (n = 7)	1.34 ± 0.11	2.03 ± 0.21	0.28 ± 0.05
Chronic Patients (n = 13)	1.08 ± 0.25	2.16 ± 0.57	0.39 ± 0.16

Notes : Chol = Cholesterol total, Pl = Phospholipids total
CE = Cholesterylesters, UC = Unesterified cholesterol

Summary :

The cholesterol content of per unit phospholipids and the ratio of esterified to unesterified cholesterol were similar in the various sera. The HDL/LDL ratios, however, appeared to be lower in the microfilaremic subjects compared to that in normal individuals or chronic patients.

4. Immunoassays for the detection L3 antibodies in filarial sera.

Scientists : Dr. M. K. Das.
Dr. A. P. Dash.
Technical Staff : Mr. M. K. Beuria.
Period of Study : Nov. 85 to Dec. '86

Results :

A comparative study has been made using L3 and mf antigens of *W. bancrofti* to determine the respective antibody level by IHA in filarial sera (Table-11).

Table-11

Results of IHA in the detection of L3 and mf antibody in Filarial sera.

Sera	No.	Positivity * (%)		Antibody titre (G. M)	
		L3 antibody	mf antibody	L3	mf
Asymptomatic carrier	28	64	81	24.93	38.85
Chronic	33	50	88	18.53	66.72
Endemic Normal	48	34	58	16.22	32.50
Non-endemic Normal	7	0	0	2	2

* Sera having titre $_2{}^4$ are considered as positive.

It is seen that the presence of L3 and mf antibody in endemic normals are 34% and 58% respectively. The corresponding values in asymptomatic carriers are 64% and 81%. Non-endemic normals (from U. K.) are antibody negetive to both the stages of *W. bancrofti*.

5. Quantification of mf antibody in TPE patients.

Scientists : Dr. M. K. Das.
Dr. S. N. Das.
: Dr. V. R. Subramanyam.
: Dr. N. M. Pattnaik.
Duration : March—Sept. '86

Results :

Sera from TPE individuals having high circulating eosinophil counts ($>$ 2,000 /mm^3, eosinophil ranges from 25 to 75%) were checked for antibody to *W. bancrofti* mf by IHA. The results are compared with that of normal endemic population (Table-12)

Table-12

Determination of mf antibody and immune complex (IC) level in TPE sera.

Sera	No.	mf antibody titre (G. M.)	IC level*
TPE	45	75.8	$0.46 \pm .14$ (0.11—0.72)
Endemic normal	48	32.5	0.05 ± 0.04 (0.01—0.17)

* Values in parenthesis represent the range.

The mean titre of mf antibody in TPE cases was 75.8, whereas in endemic normal the titre was 32.5. The immune complex (IC) level as performed by PEG assay was also determined. Four out of 20 TPE sera were found to be positive for RF (Rheumatoid factor). No correlation seems to be present between eosinophil content and RF positivity, although RF positive sera have very high eosinophil and IC level.

6. Demonstration of antibodies to *Culex quinqefasciatus* in man.

Scientists : Dr. M. K. Das.
Dr. A. P. Dash.
Period of Study : Aug. '86—Oct. '86

Results :

Culex antigen was prepared from the whole body extract of laboratory-bred female *Culex quinquefasciatus*. This was used in indirect haemagglutination (IHA) to determine the level of antibody in normal and filarial infected people (Table-13).

Table-13

Titres of Human Antibody against *Culex* antigens

Sera	n	Reciprocal antibody titre 2	4	8	16	32	64	128	256	512	G.M. titre
Normal	45			2	1	16	13	7	5	1	60.17
Filariae infected	53		2		2	12	13	18	4	2	71.06
Children (British)	7	7									4.00

The difference between the two groups is not significant. *Culex* antigens were treated with various agents like protease, heat and periodate. The effect of such modification on the antigenicity was investigated (Table-14).

Table-14

Effect of various modifications on the antigenicity of *Culex* homogenates

	Reciprocal antibody titre								G.M. titre
Antigen	4	8	16	32	64	128	256	512	
Untreated	—	--	--	--	2	8	7	3	187.40
Protease	3	6	10	1	-	-	-	-	10.93
Heat	-	-	2	7	5	5	1	-	55.71
Periodate	-	-	1	9	6	3	1	-	41.98

A marked reduction in antigenicity was noticed in all these cases ($P < 0.01$, student's 't' test). It suggests that antigenic determinants are composed of peptides and carbohydrates.

7. Drug Sensitivity Studies :

Scientist : Dr. S. S. S. Mohapatra.
Technical Staff : Mr. H. K. Tripathy.
Mr. Prakash K. Sahoo.
Mr. Harisankar Nayak.
Period of Study : January '86—Feb. '87

Remarks :

i) Chloroquine sensitivity (28 days) tests in *Plasmodium falciparum* by *in vivo* method (WHO standard Field Test 1973) were carried out in three primary health centre areas of Koraput district namely Borigumma, Lamtaput and Malkangiri.

1500 mg of chloroquine base (per adult) was used in selected population.

In Borigumma PHC the Parasite *P. falciparum* is found to be sensitive to chloroquine.

In Lamtaput PHC parasite resistance at R1 level delayed type was found in 17. 1 % of cases.

In Malkangiri PHC parasite resistance at R1 (delayed) and R11 level was found in 11.8% and 8.8% of cases. However in all these studies, considering the field situation the possibility of reinfection in these cases showing recrudescence, can not be ruled out.

ii) In Boipariguda PHC a 7 days *in vivo* test of 1500 mg chloroquine base was carried out during the early part of this year the result of which was sensitive/R1. This test was mainly done as a part of orientation training to the new recruits in the line of the study. The sample size was small due to non availability of adequate no. of participants in the test.

iii) In Kundra PHC 600 mg of chloroquine base (per adult) was used as presumptive dose to fever cases. The cases found positive among them were followed up on 7th day by a blood smear examination to see the presence of asexual parasites. The test revealed 100% clearance of asexual parasitaemia on 7th day with the 600 mg presumptive dose. (Table-15)

8. Study on prevelence of malaria in a limited area of Borigumma PHC involving 15000 population.

Scientist	:	Dr. S.S.S. Mohapatra.
Technical Staff	:	Mr. H. K. Tripathy.
		Mr. Prakash K. Sahoo.
		Mr. Harisankar Nayak.
Period of study	:	Nov. '86 to march '87.

Results :

A mass blood survey operation in this PHC area was started from 15th Nov '86 and 6493 blood smears collected so far revealed 263 positives, of which 243 are *P. falciparum*, 19 are *P. vivax* and 1 was *P. malariae*. This shows the SPR value to be 4.05, SFR 3.74 and species infection rate of *P. falciparum* is 92.39%. Thus *P. falciparum* is found to be the predominant species. The parasite incidence per thousand population is 31.7.

8. A comparative field experiment on the efficacy of different larvicides to control *Culex quinquefasciatus*.

Scientist	:	Dr. A. P. Dash.
Technical Staff	:	Mr. R. K. Hazra.
		Mr. G. D. Mansingh.
Period of study	:	September '85 to Aug. '86

Results :

The two villages i.e. Bhagabanpur and Patrapada of Puri district, which were taken under a pilot study on filariasis by this Centre, were taken for larvicide application

and an adjacent village, Sijua was taken as control. To start with all the breeding places were surveyed and mapped. The base line data on both larval and adult mosquito densities were collected before the larvicide application. The following larvicides were applied : Pyrethrum, a synthetic pyrethroid was applied in half of patrapada; Baytex, a chemical (O—P compound) was applied in the rest of Patrapada, *B. sphaericus* (powder form), a biocide was applied in half of Bhagabanpur and *B. sphaericus* (liquid form) was applied in rest of Bhagabanpur. Another village 'Sijua' was taken as control. The spray operation was continued till April/May, 1986. The results obtained in a nut-shell is as follows. (Table-16)

Table-16

Larvicide	Per man hour densities			
	Pre Spray		Post Spray	
	Total Mosquito	*C. quinque-fasciatus*	Total Mosquito	*C. quinquefa-sciatus*
Nil (control)	34	29.2	28.0	23.0
Pyrethrum	33.8	32.2	9.1	8.2
Baytex	31.1	29.9	7.3	7.0
B. sphaericus (liquid)	47.0	46.0	27.6	27.3
B. sphaericus (powder)	46.5	46.5	26.8	26.3

It is obvious that *B.sphaericus* definitely helps in checking mosquito populations. But what intrigues is that it does not compare well with the other agents applied and baytex was found to be most effective against *C. quinquefasciatus*.

Both the formulations of *B. sphaericus* (liquid and powder) were obtained from V.C.R.C., Pondicherry. Weekly expenditure on pyrethrum and baytex was Rs. 36/- and Rs. 2.60 respectively.

The larvicides were applied in all the breeding places except the wells. The temporary indoor breeding places (earthen pots etc.) were taken care by the villagers, school students themselves through health education and peoples participation.

Statistical analysis :

By applying analysis of variance (two way classification) and applying critical difference it is found that :

i) Baytex and Pyrethrum are significantly different from others. *B. sphaericus* powder and liquid formulations are not significantly different from control.

ii) Baytex is prefered to any other spray and pyrethrum can be substituted for baytex.

Summary and conclusions :

A comparative field experiment was carried out in a small area on the efficacy of pyrethrum, baytex and *Bacillus sphaericus* (both powder and liquid form) to control *C. quinquefasciatus*. The post-spray density was compared with the pre-spray density and Baytex was found to be most effective.

9. Bionomics and vectorial capacity of the mosquitoes in Puri district, Orissa with reference to filariasis.

Scientist : Dr. A. P. Dash.
Technical Staff : Mr. R. K. Hazra.
Mr. G. D. Mansingh.
period of Study : Jan, '85 to Dec. '86.

Results :

C. quinquefasciatus, the known filaria vector was the dominant species in all the places. It was found throughout the year with a peak in January. Three morphological variants of the species have been detected. *C. vishnui* group also occurs in good numbers next to *C. quinquefasciatus*. The monthwise densities of these species are shown in table-17. Calculating the correlation coefficients and then applying the students 't' test, it is fonnd that there is significant relationship between *C. vishnui* and relative humidity (RH) (at $P > 0.05$) i.e., when R.H. increases, *C. vishuni* density increases and vice versa.

Vectorial capacity :

Mosquitoes collected from the endemic areas were dissected for detection of firfst stage (L1), second stage (L2) and third stage (L3) infective larvae. The L3 of filaria parasite have been detected in *C. vishnui* group in addition to *C. quinquefasciatus*. The details of the results are tabulated in Tables-18 and 19.

Susceptibility Status :

Susceptibility test of adults and larvae of *Culex quinquefasciatus* to discriminative doses of various insecticides revealed that the adults were highly susceptible to Malathion and K-othrine and resistant to DDT, while the larvae were susceptible to malathion, fenitrothion (baytex), fenthion and temephos (abate). The mean mortality in different insecticides was as follows : Table- 20

Table-20

Adult/larvae	Insectivide	Concn	Mortality
Adult	D.D.T.	4%	12.5%
—do—	Malathion	5%	100%*
—do—	K-othrine	0.025%	100%
Larvae	Malathion	1%	100%
—do—	Fenitrothion	0.125%	97.5%
—do—	Fenthion	0.05%	97.5%
—do—	Temephos	0.02%	85%

* The mean mortality of Puri town populations of adult *C. quinquefasciatus* in malathion 5%, was however 25%.

Precipitin test :

336 blood meals of *C.quinquefasciatus* were tested out of which 322 (98%) showed positive reactions to human blood. The species showed a high preference for human blood, the anthropophilic index being 98% (table 21). Necessary facilities are now available for precipitin test of mosquito blood meals by gel diffusion technique.

Table-21

Species	Number tested	Positive for human	Positive for cow	Anthropophilic index
C. quinquefasciatus	336	322	14	98 %
A. 'hyrcanus' group	8	0	8	8%

Gonotrophic cycle :

The gonotrophic cycle in different populations of *C. quinqefasciatus* have been studied under laboratory conditions at 25.5. C. The results were as follows :

Bhubaneswar populations	: 72 hours
Patrapada populations	: 96 hours
Puri populations	: 120 hours

Summary & Conclusions :

The susceptibility status, vectorial capacity, anthropophilic index and gonotrophic cycles of *C. quinquefasciatus* have been studied. In Puri district, the mosquito species is found throughout the year with a peak in January. The infection and infectivity rates of *C. quinquefasciatus* varies from 2.59 to 25 and 1.29 to 12.5 respectively. The infectivity rate is the highest in January (12.5%). Infective larvae of filaria parasite has also been detected in *Culex "vishnui"* group. *C. vishnui* group were also found in considerably good numbers in some areas in Puri district. There is a significant relationship between *C. vishnui* and relative humidity. The number of L3 in individual *C. quinquefasciatus* varies from 1 to 18 with a mean of 4.4. The anthropophilic index of *C. quinquefasciatus* was 98%. The species is highly resistant to D.D.T. The gonotrophic cycle varies in different populations.

10. Incidence of Cryptosporidium in children with diarrhoea.

Scientist	:	Dr. V. R. Subramanyam.
Technical Staff	:	Mr. B. B. Pal.
Period of Study	:	Nov. '85 to March '87.

Results :

Results are summerised in table below. (Table-22)

Table-22

Incidence of Cryptosporidium in children with diarrhoea.

Age	Male		Female	
	No.	Positive	No.	Positive
6 months	6	3	6	2
6—12 months	30	2	3	0
13—18 ,,	7	0	3	1
2 Years	7	0	2	1
3—8 ,,	6	1	6	0
	56	6	20	4

Summary & Conclusions :

Of 76 diarrhoeal samples tested, 10 were positive for cryptosporidium Oocysts. Safranin staining method of Baxby *et al* (1984, J. Hyg., Camb; 92 : 317) was used for detecting Oocysts in the faeces.

Table - 8

Status of Asymptomatic microfilaria carriers with relation to age and sex during 3 years follow-up.

Age group (in years)	Initial A. M. C. cases followed-up			AMC discontinued carriers stage (3years)			AMC converted to Disease in 3 years			No. of cases discontinued mf with disease		
	M	F	Total	M	F	Total	M	F	Total	M	F	Total
0-19	28	39	67	6	4	10 (14.9)	13	9	22 (32.8)	5	1	6
20-39	16	27	43	1	4	5 (11.6)	3	5	8 (18.6)	0	1	1
40 +	7	26	33	4	4	8	1	3	4 (12.1)	1	1	2
Total	51	92	143	11 (21.5)	12 (13.00)		17 (33.3)	17 (18.5)	34 (23.8)	6	3	9 (26.5)

M- Male F- Female
(Figures in parenthesis indicate percentages.)

Table-9

Course of Asymptomatic microfilaria carriers during 3 years follow-up.

No. of Exam.	July' 83 (1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	July' 86 (12)	(13)
INITIAL MF positive	143	143	141	138	133	133	131	127	125	122	120	120	120
% of Initial mf + ves	100	100	98.6	96.5	93.0 7%	93.0	91.6	88.8	87.4 12.6%	85.3	83.9	83.9	83.9 16.1%
Disease conversion (%)	6 (0)	19 (13.3)	2 (1.4)	3 (2.2)	2 (1.5)	1 (0.8)	3 (2.3)	3 (2.4)	— (0)	— (0)	1 (0.8)	— (0)	— (0)
Cumulative conversion to disease %	0 (0)	19 (13.3)	21 (14.7)	24 (16.8)	26 (18.2)	27 (18.9)	30 (21)	33 (23.1)	33 (23.1)	33 (23.1)	34 (23.8)	34 (23.8)	34 (23.8)

Table–15

Results of *Invivo* (28 days) Chloroquine sensitivity Test in *P. Falciparum* in Koraput District

Sl. No.	P.H.C Area	Period of test	Type of test	D0	D1	D2	D3	D4	D5	D6	D7	D14	D21	D28	REMARK
1.	Baipariguda (With 1500mg base of Chloroquine)	May' 86	7 days	3/3	3/3	0/3	0/3	0/3	0/3	0/3	0/3				'S' or 'RI'
2.	Boriguma (With 1500mg base of Chloroquine)	July'86	28 days	33/33	25/33	9/33	0/33	0/33	0/33	0/33	0/33	0/33	0/33	0/33	All 'S'
3.	Lamtaput (With 1500mg base of Chloroquine)	Sept. & Oct-86	28 days	38/38	21/38	7/37	3/36	3/35	1/35	0/35	0/35	5/35	4/35	4/35	S = 29 (82.9%) R1 (delayed type) Or Reinfection = 6 (7.1%) Discontinued = 3
4.	Malkangiri (With 1500mg base of Chloroquine)	Oct & Nov-86	28 days	38/38	30/38	20/38	12/38	8/38	6/38	3/37	3/36	4/36	4/34	5/34	S = 27(79.4%) R1 (delayed type) = 4(11.8%) Or reinfection. R1 = 3 (8.8%) or reinfection. Discontinued = 4
5.	Kundura (With 600mg base of Chloroquine)	Dec '86	7 days	17/17							0/17				Complete clearance (100%) asexual stages on 7th day.

Numerator = Positive cases, Denominator = Cases Examined.

Table - 17

Seasonal Densities of mosquitoes in Patrapada and Bhagabanpur of Puri District

Months	Density of mosquitoes (Per Man Hour)			Temp.	R. H.
	C. quinque fasciatus	*C. vishnui* group	Other speeies	oC	%
January	61.3	--	--	26.9	72.0
February	23.4	0.33	6.0	29.3	65.0
March	37.2		1.44	33.8	61.0
April	33.3	0.99	1.33	39.1	60.7
May	26.63	1.33	0.99	37.4	68.0
June	21.2	0.99	0.99	34.8	65.0
July	31.5	3.0	3.5	31.5	85.5
August	31.8	--	11.5	32.5	86.0
September	30.5	3.2	0.34	31.6	88.6
October	24.29	3.38	2.72	30.6	80.0
November	32.6	1.6	3.96	29.1	73.3
December	37.15	0.31	1.05	26.2	70.0

Table - 18

Infection and infectivity rates of mosquitoes (total)

Species	Total No. dissected	Positive for L-1 & L-3	Infection rate	Positive for L-3	Total No. L-3	Infectivity rate
C. quinquefasciatus	1463	108	7.38	79	290	5.4
C. 'vishnui' group	31	4	12.9	3	9	9.6
C. elegans	5	-	-	-	-	-
C. gelidus	5	-	-	-	-	-
An. 'hyrcanus' group	10	-	-	-	-	-

Filariasis is one of the major public health problems in India. As early as 600 B.C. Susruta, a physician mentioned about the disease while Madhavakara, a pathologist described the signs and symptoms of filariasis later in 700 A.D.

Orissa is an endemic home for falariasis. Although it can not be said as to when and how the disease came into existance in the state, the inscription with genital swelling was found in the stone carvings of the fomous Sun Temple of Konark built in 13th century A.D. This gives an idea that filariasis was prevalent in the state from very olden times and people were aware of its clinical manifestation.

Table-19

Monthwise infection and infectivity rates of wild caught *C. quinquefasciatus* in Puri district, during 1986.

Months of 1986	Total Mosq. dissected	Positive for L1 & L2	Positive for L3	Total No. of L3	Infection rate	Infect-tivity rate	Average No. of L3 per pos.
January	120	12	15	54	10.0	12.5	3.6
February	46	4	2	3	8.6	4.34	4.0
March	40	10	1	2	25.0	2.5	2.0
April	55	3	1	2	5.4	1.8	2.0
May	102	1	0	0	0.9	0	0
June	77	2	1	1	2.59	1.29	1.0
July	80	3	4	6	3.75	5.0	1.5
August	165	9	5	7	5.45	3.0	1.4
September	20	2	1	3	10.0	5.0	3.0
October	45	9	1	3	20.0	2.2	3.0
November	120	10	4	6	8.3	3.2	1.5
December	363	34	33	202	9.0	9.0	6.12
TOTAL	1233	96	68	294	7.8	5.5	4.32

NEW PROJECTS PROPOSED TO BE UNDERTAKEN IN 1987-88

O-1. Sciatica like syndrome in Filariasis.

Scientist : Dr. Shantanu K. Kar.

Scientific-background and justification :

A variety of syndromes like tenosynovitis, lateral nerve palsy, dermatosis have been suggested as being manifestations of filariasis. It has been observed during our survey work, that a segment of endemic population complain of neuralgic pain originating at a focus approximately one and half to two inches from midline of L3-L4 vertebra level radiating downwards along sciatic nerve distribution. These symptoms being waxing and waning type persist for 2-3 years and incapacitate the individuals from routine work for few days during acute epidodes. Nodules are often observed at the "focus" which is tender and painful. Few patients who received DEC treatment were relieved of pain and their nodules regressed in size. Routine B complex therapy did not show any significant clinical response. Hence to evaluate the observational data, it is essential to know whether nerve is involved in inflammatory process or by mechanical pressure by nodule and the association of these symptoms with clinical filarial disease.

This study will envisage evaluation of neurological deficit (EMG, Nerve Conduction, Nerve biopsy), clinico parasitological examination, detection of filaria specific antibodies, detection of filaria specific Ag in nerve tissue in above cases in a carefully designed study.

This work will be carried out with collaberation of Prof. R. N. Sahu, professor of Neurology, S. C. B. Medical College, Cuttack.

O-2. Chemotherapy study on Filariasis : Comparative study on efficacy of DEC in different dosage schedule.

Scientist : Dr. Shantanu K. Kar.

The presently applied DEC dosage shedule (6mg/KG/body wt. for 12 successive days) in cases of chronic filariasis does not show promising results. Studies carried out in Indonesia (*B. timori* filariasis) and China have suggested better therapeutic response of elephantiasis cases with high dose DEC therapy. It is also experienced during our survey work that long term treatment of DEC with bandaging of limb and regular active physical exercies show beneficial result in treating chronic cases of filariasis. Hence, an attempt will be taken to evaluate clinical, parasitological, immunological and other responses in cases of filariasis treated with various dosage shedule of DEC.

O-3. Immunologic status of patients with sickle cell anaemia.

Scientist : Dr. G. P. Chhotray.

Scientific back ground and justification :

Despite extensive research on many aspects of patients with sickle cell anaemia including clinical studies and evaluation of haemoglobin and membrane of the RBC, relatively little is known about the immunologic status of these patients. Studies on T. Lymphocytes have been limited and have shown variable results. Similarly no information is available regarding the antibody producing capacity of B. Lymphocytes in sickle cell anaemia cases.

Orissa especially the western parts have a wide belt of sickle cell disease beginning from Dhenkanal District covering Sambalpur, Sundargarh, Kalahandi, Phulbani and Balangir.

A new sickle cell unit has been set up by ICMR funding at V.S.S. Medical College, Burla. The above project is proposed to be undertaken through the active co-operation of the sickle cell unit.

O-4. Effects of acetone extracts from aphids on the development of mosquitoes.

Scientists : Dr. A. P. Dash.
Dr. G. P. Chhotray.
Mr. M. R. Ranjit.

Scientific background and justification :

The effect of acetone extracts from aphids on the development of mosquitoes will be studied for the juvenalizing action of the aphid extracts. Besides other studies the histo-pathological studies of the development at various stages will be elaborately studied. This project is taken up for a student for the Ph. D. degree of the Utkal University.

O-5. Studies on *Culex 'vishnui'* group in relation to the development of filaria parasites, in Orissa.

Scientist : Dr. A. P. Dash

Scientific background and justification :

While carrying out studies on the development of filarial parasites in *Culex* mosquitoes in nature, development of the parasite was detected in *C 'vishnui'* group of

mosquitoes. It is felt necessary to continue the work on *C. vishnui* in different endemic areas of Orissa to find out its possible role as a secondary vector of filariasis.

These aspects will be carried out in the field as well as in the Laboratory. The wild caught *C. vishuni* will be dissected for detection of different larval stages of the filarial parasites; their contact with man will be assessed by precipiti n tests and their age will also be determined. These species will be reared in the laboratory, the gonotophic cycles will be recorded. The development of microfilaria will be studied in the laboratory through membrame feeding technique which will help in the correct identification of parasite species.

O. 6. Feasibility of filariasis control by integrated methods in some endemic villages of Orissa.

Scientiest : Dr. A. P. Dash.

Scientific background and justification :

Control of vectors is an important factor for the control of filariasis transmission. Though the main vector *C. quinquefasciatus* has very high reproductive potential and is a ubiquitous breeder, it was shown that a patent case of filariasis is the result of very large numbers of repeated infective bites. Hence, it was not necessary to eradicate the vector species but would only be necessary to reduce its density to a considerably low level i.e. less than 3.4 perman hour density.

Therefore, an attempt will be made to study the feasibility of controlling the vector species by integrated control methods, in the rural endemic areas of Orissa, after mapping the breeding places carefully. Baytex R 1000 will be used as the chemical insecticide alongwith environmental management and source reduction. (The detailed protocol has already been submitted to the I.C.M.R. head quarters for approval and funding, since quite a good number of personnel are required for such type of project.)

O. 7. Studies on mosquitoes of Orissa with special reference to filariasis and malaria.

Scientist : : Dr. A. P. Dash.

Scientific background and justification :

Our present knowledge on the taxonomic aspects of Indian mosquitoes is more than 50 years old. Orissa is highly endemic for both the mosquito borne diseases, filariasis

and malaria. There has not been any systematic study on mosquito fauna of Orissa and consequently our knowledge on mosquito fauna of the state is poor. In view of the importance of the two mosquito borne diseases there is an urgent need to study the mosquito fauna, their habits, behaviourial status and vectorial capacity in the state. The study would provide informations on i) changes that have taken place in the mosquito fauna over the years ii) ecological succession of mosquito species, iii) Basic knowledge on morphometry iv) reveal naturally occuring variation within each taxon and v) information on age composition, susceptibility status etc. The study would also help in the control / containment of filariasis and malaria in the following manner :

a) Stratification of region as per the relative abundance would help in aligning control measures so that efforts directed on the target species and not uniformly.

b) The study would be of great help in evolving alternate methods of vector control, in particular environmental control.

c) It would provide information on the role of secondary vectors in epidemiology of filariasis and malaria.

d) The study would reveal information on the distribution of masquitoes in the state and their susceptibility status which will help in adopting control measures.

(The proposal has been submitted to the I.C.M.R. head qurarters for approval and funding.)

O. 8. Feasibility of control of *Culex quinquefasciatus* using indegenous agents.

Scientists : : Dr. A. P. Dash.
Dr. V. R. Subramanyam.

Vector resistance to the safer and cheaper insecticides, increased cost of insecticides and oppositions to the use of chemicals by the environmentalists are some of the major problems contributing to the break down in vector control. These factors have promoted advances in vector control using biological agents like fish, fungi and bacterial agents etc. Orissa is rich in fish, fungi and bacteria fauna. The study is proposed to be undertaken to find out the mosquito control potential of some species of indegenous biological agents.

(The project has been submitted to the I.C.M.R. head quarters. for approval and funding)

O-9. Development of anti-idiotypic antibodies for *P. berghei* and *P. falciparum*.

Scientists : Dr. B. Ravindran.
Dr. N. M. Pattnaik.
Dr. Manoj K. Das.

Idiotype network have been shown to regulate the immune response to a variety of antigens. Anti-idiotypic antibodies have thus opened the way for experimental and therapeutic manipulations of the immune system. Anti-idiotypes mimic the original antigen and induce an immune response very similar to the antibody response to the antigen. Experimental idiotype vaccines have been developed during the last few years for a variety of infectious agents such as hepatitis B virus, rabies virus, poliovirus, pneumcoccus, l isteria, trypanosomes etc. There has been no attempt so far on the production of idiotype vaccines for plasmodia. The present investigation proposes to :

a) Raise protective antibodies to *P.berghei* in rabbits by following established procedures.

b) Affinity purify the anti parasite antibodies and raise anti-idiotypic antibodies in rabbits. The immune response will be the monitored by ELISA.

c) Immunize mice with anti-idiotypic antibodies and to monitor the production of anti-malarial antibodies and to challenge with live parasites to look for protective immunity.

d) Immunize rabbits with a synthetic peptide (representing the RESA antigen of *P. falciparum*) conjugated to a carrier molecule and raise anti-idiotypic antibodies and to use the anti-idiotypes to raise anti-RESA antibodies in rabbits. The synthetic peptide, an octapeptide will be synthesized and supplied by Dr. R. Nagaraj of Centre for Cellular and Molecular Biology, Hyderabad.

O-10. Development of an anti-mosquito vaccine for blocking transmission of malarial and falarial parasites from vertebrate hosts.

Scientists : Dr. B. Ravindran.
Dr. A. P. Dash.

Since Plasmodia are obligate parasites and do not have any intermediate animal host, human beings (those who are harboring gametocytes or microfilariae in circulation) are the only reservoir of these parasites in human communities. Thus blocking the transmission of parasites is considered more important than chemotherapy for infected individuals or the use of vaccines which promise to offer clinical immunity at the individual level. Although gamete vaccines aim at achieving the objective, the limitations need to be compromised. The possibility of developing antivector vaccine although not new has been paid very little attention so far. The present investigation proposes, to study the development of sporogonic cycle in mosquito fed on experimental animals im munized with gut tissue and infected with *P. berghei*

PUBLICATIONS

A. Papers :

1. Detection of antibodies to *Culex quinquefasciatus* in man.
   M. K. Das and A. P. Dash.
   IRCS Med. Sci. **14**, 1190-1192 (1986)

2. Tunicamycin—resistant mutants of *Bacillus amyloliquefaciens* are deficient in amylase, protease and penicillinase synthesis and have altered sensitivity to antibiotics and autolysis.
   V. R. Subramanyam.
   Jour. Appl. Bacteriol. **60** : 271-275 (1986)

3. Host selection pattern of five mosquito species of Orissa.
   A. P. Dash,
   J. Zool. Soc. India. **37** : 111—116 (1986)

4. A note on the use of cross-linked starch in microbiology with special reference to detecting amylase production.
   D. C. Modi, Y. B. Bhatt, M. K. G. Shikh, V. R. Subramanyam and F. F. Dias.
   Jour. Appl. Bacteriol. **61** : 315—318 (1986)

5. Atypical features in Lymphatic filariasis.
   Shantanu K. Kar.
   Ind. J. Med. Res. **84** : 270 (1986)

6. Health for all by the year 2000.
   L. N. Mohapatra.
   Souvenir-38th Orissa Annual State Conference of Indian Medical Association—December 1986

B. Letters :

1. Response to lepromin unaltered by application of zinc to the skin in lepromatous leprosy patients.
   V. R. Subramanyam.
   Leprosy Rev. **57** : 73 (1986)

2. Role for carbohydrate moieties in immune response to malaria.
   B. Ravindran and M.K. Das.
   J. Immunology. **137** : 1091 (1986)

C. Abstracts :

1. Modulation of Immune response by *Mycobactorium kansaii* lipids : Studies using an *in-vitro* antigen presenting system.

   Amiya Ranjan Nayak, Kodukula Krishna and Santosh Kar.
   Abstract No. 5:17:17 at the 6th International Congress of Immunology, Toronto, Canada, July 6-11, (1986)

2. Asymptomatic microfilaria carrier - A 3 years follow-up study.
   Shantanu K. Kar.

   Abstract No. **134** Souvenir, XLII joint Annual Conference of the Association of Physicians of India, Madurai, Jan. 87, P. 68 (I987)

D. Papers accepted for publication (in Press) :

1. A study of the antigens, antibody and immune complex levels in *Wuchereria bancrofti* filariasis with reference to clinical status.

   M. K. Das, V. R. Subramanyam, B. Ravindran and N. M. Pattnaik.
   J. Tropical Med. Hyg.

2. Production and characterisation of rabbit antibodies with reactivity to diethylcarbamazine.
   B. Ravindran, D. Das and M. N. Pattnaik.
   Med. Sci. Research (Aprii, 1987)

3. The use of starch and skim milk in the regeneration of *B. amyloliquefaciens* and *B. subtilis* protoplasts.

   Y. B. Bhatt, V. R. Subramanyam and F. F. Dias.
   Lett. Appl. Microbiol.

4. Evaluation of aspiration cytology in the diagnosis of metastatic lymphadenopathy.
   G. P. Chhotray.
   Ind. J. Med. Res. (June, 1987)

5. Trichomonas Vaginitis : Evaluation of Serological tests and identification of Immune reactive surface peptides.

   G. Satapathy, S.K. Kar, J.C. Samantray and S.K. Panda.
   British Journal of Dermatology.

SCIENTIFIC CONFERENCES/WORKSHOPS/SEMINARS ATTENDED

Name of the Scientist	Scientific conferences/workshops/seminars attended with date	Papers presented, if any.
Dr. Santosh K. Kar	Indo - US Symposium on Immunology and Molecular Biology of Leprosy, New Delhi, January 27-30, 1986.	Interaction of human Immune System with *Mycobacterium leprae* antigens Activation of the antibody producing cells.
	Indo UK Symposium on Leprosy Research, 7-12th April, 1986, JALMA, Agra.	The structure of Mycobacterial cell wall using glycosylation inhibitors.
Dr. Shantanu K. Kar	International symposium on Filariasis at CIBA-Geigy Research Centre, Bombay 12th May, 1986.	Epidemiology and clinical aspect of Filariasis.
	42nd conference of Association of Physicians of India at Madurai, 23rd to 27th Jan. 87.	Asymptomatic microfilaria carriers A 3 yr. follow up study.
	Workshop on communication on Bio-medicine held at Patna, 10-12th Feb., 1987.	—
Dr. Aditya P. Dash	Workshop on communication on Bio-medicine held at Patna, 10-12th Feb., 1987.	—
Dr. V. R. Subramanyam	Eastern Regional Workshop on AIDS at NICED, Calcutta 14-15 Nov '86.	—
Dr. G. P. Chhotray	XXXV Annual conference Indian Association of Pathologist & Microbiologist, 28-30 December, 1986.	Evaluation of aspiration cytology in the diagnosis of metastatic lymphadenopathy (Poster presentation).
	Workshop on communication on Bio-Medicine held at Patna, 10-12th Feb., 1987.	—

Name of the Scientist	Scientific conferences/workshops/seminars attended with date.	Papers presented, if any.
Dr. Manoj K. Das.	"Biochemical Education" workshop of SBC, Trivandrum, Dec. 13-17, 1986.	Participated as a teacher on immunochemistry.
Dr. Nikhil Mohan Pattnaik.	SBCI, 55th annual convention, Trivandrum Dec. 15-17th, 1986.	—
	"BEST Programme" - invited instructor Trivandrum, Dec. 13th & 14th, 1986.	—
Dr. B. Ravindran.	Indian Immunology Society 13th Annual meeting held at National Institute of Immunology, New Delhi 6-8th Dec., 1986.	1. Production of rabbit antibodies with reactivity of Diethyl carbamazine.
		2. Detection and characterisation of anti-sheath antibodies in Bancroftian Filariasis.

Name of the Scientist	Conferences/seminars/workshops attended with date	Papers presented, if any.
Dr. L. N. Mohapatra	V Conference of Association of Physicians of India, Orissa State Branch at MKCG Medical College, Berhampur, 12th April 1986.	Guest Speaker "Opportunistic infections in humans."
	X Annual Conference of Indian Association of Preventive & Soc. Medicine, S.C.B. Medical College, Cuttack, 8th Nov. 1986.	Chief Speaker: "Health for all by the year 2000."
	Association of Obstetricians & Gynæcologists of Orissa, Annual Conference, M.K.C.G. Medical College, Berhampur, 30th Nov. '86.	Guest Lecture: "Viral aetiology of uterine cervical cancer".
	Association of Orthopaedicians, Orissa State Branch, at Annual meeting Dhenkanal, 15th Feb. 1987	Guest Lecture on "Mycetoma."
	Doctors India International 2nd Annual Convention, Cuttack, 7th March 1987.	Guest Speaker: "Achievement of Health for all by 2000 AD through primary health cancer."
	National Workshop Deptt. of Preventive & Social Medicine, S.C.B. Medical College, Cuttack on 10th March 1987.	Guest Speaker: "Training of Medical students."
	Annual Day, Academic Society, S.C.B. Medical College, Cuttack on 11th March 1987	Chief Guest : "Scientific basis of modern Medicine".
	Annual Commemoration Day, V.S.S. Medical College, Burla on 14th March 1987.	Chief Speaker "Undergraduate Medical Education in India and 21st Century."

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    St. Louis Mo. 9.3.87.

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