# REGIONAL MEDICAL RESEARCH CENTRE

## BHUBANESWAR

# ANNUAL REPORT

# 1988-89

# REGIONAL MEDICAL RESEARCH CENTRE

## ( INDIAN COUNCIL OF MEDICAL RESEARCH )

NANDANKANAN ROAD

BHUBANESWAR-751005

## ANNUAL REPORT

## ( 1.4.1988 to 31.3.1989 )







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## ACKNOWLEDGMENT

The Director expresses his gratitude to all scientists, technical and administrative staff of this centre for the work carried out during the year. Thanks are particularly due to Dr. M. K. Das, A.D., Dr. A. P. Dash, S.R.O. and Dr. G. P. Chhotray, S. R. O. for getting the annual report printed.



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# CONTENTS


- Preface 1
- Ongoing Projects
  1. Filariasis 3
  2. Malaria 16
  3. Clinical Pathology 19
  4. Entomology 21
  5. Mycobacterial Genetics 26
  6. Aids 29
  7. Sickle Cell Research Centre 31
- Projects Completed 32
- New Projects Proposed for 1989-90 46
- Papers Published 53
- Visitors 55
- Scientific Conferences/Workshops/Seminars Attended 56
- Ph. D. Programme under Utkal University 59
- Scientific Advisory Committee 61
- Ethical Committee 63
- Scientific Staff 64

# Preface

The Regional Medical Research Centre (RMRC) at Bhubaneswar was established by the Indian Council of Medical Research (ICMR) to generate regional capabilities for tackling local health problems. From its very inception (July 1982) the centre has taken up the study of human lymphatic filariasis covering its epidemiological, clinical, immunological, entomological and therapeutic aspects. Considerable amount of work has been done in these areas to gather the base line data in this endemic belt filariasis in the state. Several issues have emerged from these studies which need to be looked into indepth. Scientific acquisition of knowledge is a tedious process. Early diagnosis, the wide spectrum of host response, effective control measures and arresting the progress of the disease are some of the aspects on which emphasis is given over the past few years.

They know enough who know how to learn and research activity is the only road to knowledge. The centre had set up a field unit for study of Malaria in the tribal belt at Jeypore in Koraput district endemic for falciparum infection. After the preliminary study in collaboration with the Vector Control Research Centre, Pondicherry, the emphasis shifted to control of Malaria in the hilly terrain. Consequently this work was assigned to the VCRC which now is operating in that area with its field station at Jeypore. One Research Officer (Dr. S.S.S. Mahapatra) from this centre with considerable experience in the field of Malariology has been posted there to work with VCRC team.

The other areas of activity include study of G-6-PD deficiency in the Malaria endemic area at Keonjhar district which has a high tribal population. Having established the presence of this enzymatic deficiency the emphasis now is on study of the variants. The study of Sickle Cell Disease in the population in Western Orissa is continuing at the Sickle Cell Research Centre functioning at the V.S.S. Medical College, Burla, Sambalpur district.

A team from this centre (Dr. S.K. Kar, Asst. Director & Dr. G.P. Chhotray, S.R.O) visited several remote villages in Koraput district last year and submitted a report to the council on the epidemic of meningococcal meningitis there. It started in the winter of 1986 (February) and is still continuing in the three neighbouring districts of Koraput, Kalahandi & Phulbani. A copy of the report was submitted to the Director of Health Services, Orissa for his information and necessary remedial measures.

As has been the practice in the past the ongoing programme are given under different topics/disciplines. The projects which were completed during the year under

report are given serially in the next chapter. Several new projects which are proposed for the year 1989-90 are given separately with the objectives & brief description of the study planned.

The Bhubaneswar Development Authority has been entrusted to develop the entire 2 acre, RMRC campus, clear the shrubs & bushes, plant flowering trees and lay the lawns. The construction of laboratory building is nearing completion and is getting finishing touches. Dr. S. Jayachandran, Dy. Director who had joined this centre on transfer from V.C.R.C., Pondicherry in February 1988 has since been transferred to the ICMR headquarters (posted at the Anna University, Centre for Biotechnology, Madras) in December 1988. We welcome Dr. G.S. Bhattacharya, SRO (Clinical) and Mr. Anil Kumar, SRO (Statistics) who have recently joined this centre.

I would like to record my sincere thanks and appreciation of the co-operation I have received from the scientists, research staff, laboratory personnel and the staff in administrative wing to discharge my duties during the year. The spirit of team work, mutual co-operation among the scientific and research staff is our source of inspiration. The valuable advice received from the members of the Scientific Advisory Committee and support from the Ethical Committee are our guiding factors. The encouragement received from the Director General and Addl. Director General as the Chief of the ECD division are thankfully acknowledged. The support received from the headquarters office is very satisfying. Every year is a mile stone in the development and growth of this infant institution. Now that the teething troubles are nearly over we hope to discharge our duties and responsibilities to the best of our ability during the years to come.

**Prof. L. N. Mohapatra**
*Director*

# Ongoing Projects

## 1. FILARIASIS

### 1.1 Study of arthritis with relation to filariasis in filarial endemic area.

| | | |
|---|---|---|
| Scientist | : | Dr. S. K. Kar |
| Technical staff | : | Miss J. Mania |
| | | Mr. T. Moharana |
| | | Mr. S. C. Rout |
| | | Mr. K. Dhal |
| Starting date | : | March 1986 |

**Progress :**

Screening of the population in villages endemic for filariasis had earlier revealed 90 subjects with features of arthritis not pertaining to any specific aetiology (ruled out by clinical examination).

a) The mf status, filaria-specific antibody titre (using homologous mf Excretory-Secretory (ES) Ag by ELISA), mean circulating immune-complex and circulating filarial antigen (using Filaria Specific (FS) IgG by ELISA) were determined in 39 blood samples from study cases.

b) Microfilaraemia was detected in 16 (18%) of study cases. The mean eosinophil count percentage was 10.3 $\pm$ 5.57.

c) The circulating filarial antigen was detected in 26 (66.7%) cases. The mean circulating immune complex level of 0.108 $\pm$ 0.066 units was detected in 39 cases.

**Justification for continuation :**

It is proposed to carry out synovial membrane biopsy for study of filaria-specific immune complex deposits (by immunofluoresence and immunoperoxidase method) and histopathological changes. This in-depth study may reveal if there is any association with filarial aetiology.

## 1.2 Sciatica like syndrome in Filariasis

| | | |
|---|---|---|
| Scientist | : | Dr. S. K. Kar |
| Technical staff | : | Miss J. Mania<br>Mr. T. Moharana<br>Mr. K. Dhal<br>Mr. R. N. Nayak |
| Collaborator | : | Dr. R. N. Sahu<br>Prof. of Neurology<br>SCB Medical College<br>Cuttack<br><br>Dr. G. S. Acharya<br>Cancer Institute<br>Cuttack |
| Starting date | : | October 1987 |

**Progress :**

Seventy subjects presenting with neuralgic pain pertaining to lower/upper limb for six months or more were screened in a filarial endemic village. They were subjected to clinical, neurological, parasitological and immunological assessments.

a) Eight out of 70 cases were studied in detail for radiology of L3—L4 spine (A.P., lat view) to rule out any bony deformity, ultrasound scan of the nodules detected clinically (approx 2" lat to L3—L4 level), neurological manifestations and aspiration cytology of the nodules.

b) Well-defined nodules (1-5 cm—2.5 cm at L3—L4 level) were detected by scan in all the cases. Radiology of spine and neurological examination did not reveal any neurological deficit as to the cause of unilateral limb pain.

c) Aspiration cytology showed few lymphocytes and eosinophils not specifying any definite aetiology.

d) The response of these nodules to DEC therapy is being evaluated.

e) In study cases the mean Circulating Immune Complex (CIC) level was $0.092 \pm 0.061$ and 20 (80.0%) cases were positive for filaria specific antibody.

f) Biopsy of these nodules are being taken in a few cases for histopathological study. The study will be completed this year and detailed results will be analysed.

## 1.3 Chemotherapy of Filariasis : Comparative study on efficacy of DEC in different dosage schedule.

Scientist : Dr. S. K. Kar

Technical staff : Miss J. Mania
Mr. P. K. Kar
Mr. T. Moharana
Mr. K. Dhal
Mr. R. N. Nayak

Starting date : January 1988

**Progress :**

After screening the endemic population the subjects presenting with grade II and grade III lymphoedema and/or hydrocele were selected for the study (125 cases). Age, sex matched subjects with similar manifestations (32 cases) were taken as controls.

The study cases were re-grouped according to the regimen used (DEC and/or conservative physiotherapy). The regimens used were DEC 80 mg/kg wt/day for 3 weeks every month with or without conservative therapy. In controls, DEC was used 6 mg/kg/day dosage in similar fashion. Periodic clinical, parasitological, immunological (filaria specific Ag & Ab detection, immune complex assessment) and biochemical (SGOT, SGPT, Alk. phosphate, serum cholesterol) examinations are being carried out to ascertain any alteration during therapy. Limb circumference, tissue tonicity (using a tissue tonometer) at 3 fixed points were measured periodically to assess any alteration of lymphoedematous limb size. Analysis of the result will be carried out 1 year after completion of study.

**Justification for continuation :**

The periodic assessment and therapy need to continue for at least one year so as to get any effect on reduction of lymphoedematous limb size. Earlier pilot chemotherapy study reported from this centre (Annual Report 1987-88 p. 7) has shown beneficial results.

## 1.4 Evaluation of cardiopulmonary involvement in tropical pulmonary eosinophilia and response to DEC.

Scientist : Dr. S. K. Kar

Technical staff : Miss J. Mania
Mr. K. Dhal
Mr. R. N. Nayak

Starting date : June 1988

**Progress :**

Tropical pulmonary eosinophilia (TPE) is a hypersensitive reaction to microfilarial antigen. The prevalence of this syndrome was found to be 0.9% in a defined endemic population (RMRC annual report 1986-87 p-30). This study was initiated at Unit III dispensary, Bhubaneswar, where suspected cases of TPE are being referred to the clinic twice a week.

The initial screening process includes detailed clinical examination, blood (DC, TLC, AEC) and stool examination. The subjects with absolute eosinophil count of 3000 cells $mm^3$ or more with chest symptoms and/or signs are included in the study. The study cases are assessed by static and dynamic pulmonary function tests (FEV, FEV1%, FVC, TLC, PF, FEV/VC), and electro-cardiogram. Blood samples are taken for estimation of filaria-specific antibodies (IgG, IgM by ELISA), immune-complex and circulating filarial antigen. Radiological chest signs and heart size are also assessed (X-ray chest PA view) in study cases. The study subjects are treated with DEC and evaluated periodically for any alteration of clinical symptoms/signs, dosage requirement of DEC and for follow-up.

A total of 37 cases are included in the study so far with mean age 30.5 yrs $\pm$ 9.7 yrs, eosinophil percentage 40.34 $\pm$ 6.6% and absolute eosinophil count of 8181.1 $\pm$ 2113.3 cells per cub mm of blood at the initial period of study.

**Justification for continuation :**

The study will highlight the varied clinical presentation of TPE, alteration of lung functions and dosage requirement of DEC for reversibility of symptoms, eosinophil level, lung function and other immune responses.

## 1.5 Long term follow-up of asymptomatic microfilaraemics in an endemic area with reference to their peripheral eosinophil and antibody level.

| | | |
|---|---|---|
| Scientist | : | Dr. S. K. Kar |
| Technical staff | : | Miss J. Mania<br>Mr. P. K. Kar<br>Mr. R. N. Nayak<br>Mr. T. Moharana<br>Mr. K. Dhal<br>Mr. S. C. Rout |
| Starting date | : | November 1985 |

**Progress :**

Out of 87 subjects who co-operated for the 3rd year follow-up, 11 cases developed clinical manifestation of filarial disease. The majority 73 (83.9%) continued their microfilaraemic status. Circulating antigen (ELISA using filaria specific IgG) was detected in 45 (51.7%). Ten (11.5%) had positive filaria specific antibody (IgG).

**Justification for continuation :**

The above study will highlight the immunologic changes occuring in carrier state and conversion of carrier state to amicrofilaraemic or disease state. This will add to the knowledge of the course of carrier state and associated host response.

### 1.6 Immunological studies on filariasis

Scientist : Dr. M. K. Das

Technical staff : Mr. M. K. Beuria
Ms. A. Mishra

Starting date : July 1985

It is an attempt to study the immunological properties of filarial parasites, especially the infective larval ( $L_3$ ) stage, which has not been systematically investigated.

**Progress :**

In the initial phase of this study, (RMRC, 1987-88 annual report, p-10) various immunological parameters e.g. antibody, antigen and circulating immune complex levels of $L_3$ of *W. bancrofti* were measured in filarial sera. $L_3$ larvae were obtained by membrane feeding technique and from widely caught *Culex quinquefasciatus* in Puri district.

1. To develop an antigen-based immunoassay efforts are continuing to detect $L_3$ antigen in filariasis by ELISA for wider applicability. This was previously performed by counter immune electrophoresis using hyperimmune rabbit anti-$L_3$ serum. More of the antiserum is being produced. Availability of enzymes for conjugating to the sera is awaited.

2. IgE antibody is known to be of crucial importance in parasitic helminth infection where it plays protective and cytotoxic role. IgE levels to somatic antigens of $L_3$ were measured by ELISA in filarial infection. The highest IgE response is in patients of Tropical Pulmonary Eosinophilia (TPE) (n=22), the mean titer being 6 times higher than that of endemic normals. It is of interest to note that IgE titer in endemic normals (n=45) and chronic filariasis (n=40) are nearly equal whereas that of asymptomatic mf-carriers (n=25) is low. In non-endemic normals IgE response to $L_3$ is almost non-existent.

However amount of IgE-complexed Circulating Immune Complex (CIC) specific to $L_3$ in filarial sera was found to be rather low compared to IgG or IgM-CIC.

As a corollary to humoral IgE response, immediate type hypersensitivity is being studied with filarial antigens in experimental animals.

Work is in progress to fractionate and characterize defined antigen(s) of parasites for the induction of IgE. Cattle filarial worm *(Setaria)* which offers an abundant source of antigen is also being used for this purpose. Glyco-conjugate antigens will be analyzed to investigate the role carbohydrate residues play in filarial immunology.

3. IgG sub-class distribution of $L_3$ antibody response is being analyzed in details. IgG4 appears to predominate in asymptomatic carriers.

### 1.7 *in vitro* culture of microfilariae

| | | |
|---|---|---|
| Scientist | : | Dr. N.M. Pattnaik |
| Technical staff | : | Sri Dasarathi Das<br>Dr. Gouri Sankar Satpathy<br>Sri Hari Shankar Nayak |
| Starting date | : | September 1985 |

Conditions suitable for inducing development of the microfilariae in culture are not yet found. Following the lack of success with animal cell culture media and bacteriological media, alone or in combination, emphasis is being put on insect haemolymph as a media supplement. Use of whole haemolymph, however, proved toxic to the microfilariae (vide infra) irrespective of source (larvae or adult—mosquito, cocroach, tussar moth). The toxicity has now been found to be due to two components; (1) The high molecular weight polyphenol—oxidase (tyrosinase) and (2) a low molecular weight component. The latter, also lytic to red blood cells, is being studied in detail.

The residual haemolymph, freed of the above two is now being investigated for its growth promoting activity.

Maintenance of the purified microfilariae in serumfree medium ($L_{15}$ + Grace's) is being done as a matter of routine for the production of Excretory—Secretory (E-S) material. The microfilariae are also being used as a source for somatic antigen. Characterisation of secreted products is hampered because of dilution problem and is to be continued when lyophilisation facilities become available.

As an adjunct to the studies with *W. bancrofti*, microfilariae from *Setaria spp.* are now being used as a model for developmental and immunological studies. An obvious advantage with this model is that from the collected adult worms eggs as well as microfilariae are being produced in culture and thus are free from any serum component.

### 1.8 Characterisation of plant microfilaricidals.

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

Some plant extracts used in the Homeopathic and Ayurvedic systems were found to kill microfilariae in culture. Of these Shakhotak (sahada, *Streblus asper*) has long been in use as an antifilarial and hence was chosen for further study.

An aqueous extract of the bark or leaves was found to be microfilaricidal as well as lytic to red blood cells (rbc). The bark extract contained a higher concentration of these activities and is being used for purification of the active component.

Fractionation by gel permeation chromatography showed a small peak of high molecular weight and a much larger peak of lower molecular weight. The latter, containing mostly condensed tannin, was lytic to rbc but not microfilaricidal. The high molecular weight fraction was microfilaricidal but had no effect on rbc. This microfilaricidal fraction is now identified as a glycoprotein as judged by the presence of carbohydrates (phenol-sulfuric acid) and its degradation by trypsin.

Purification of the shakhotak microfilaricidal is now underway as a prerequisite to its characterization.

### 1.9 Role of Insect Haemolymph in microfilarial development in culture.

Scientist : Dr. N. M. Pattnaik
Technical staff : Sri Pramod Kumar Rana
Starting date : July 1987

In connection with the culture of microfilariae it was found that insect haemolymph from several sources are toxic to the microfilariae. Attempts to purify this component from the haemolymph of tussar moth, both larvae and adults, have pointed to two fractions

displaying this activity. The high mol. weight fraction contains polyphenol oxidase (tyrosinase) which is essential for insect defence mechanism through melanisation. This kills microfilariae in presence of tyrosine but is not lytic to red blood cells.

The second component is lytic to rbc and is routinely assayed this way. The rate of lysis is concentration dependent in a sigmoid fashion and displays a lag period before the onset of lysis. This is particularly pronounced at low concentrations of the toxin as well as at lower temperatures.

Another interesting facet of this toxin is that while it appears to have a very low molecular weight in the larvae it shows a much higher mol. weight in the adults. Whether it is due to a change in molecular forms or to its association with other macromolecules, particularly lipo-proteins, is under study.

This work will be helpful in understanding the developmental dynamics of the insect defence reactions as well as the role of haemolymph in growth of microfilariae.

## 1.10 Public Health perspective of filariasis and evaluation of alternate medicines.

| | | |
|---|---|---|
| Scientists | : | Dr. N. M. Pattnaik |
| | | Dr. V. R. Subramanyam |
| Technical staff | : | Dr. Gouri Shankar Satapathy |
| | | Sri Dasarathi Das |
| | | Sri Pramod Kumar Rana |
| | | Sri Hari Shankar Nayak |
| Starting date | : | February 1986 |

This project operates in three areas namely Jatni, Mendhasal and Puri. From our initial work at the Jatni PHC the problems of co-operation from the people in the night blood survey became apparent. This, combined with non-distribution of medicine in the village itself as well as the drug reactions, has made the PHC programme rather ineffective. Having made this observation we set out to find ways to overcome these difficulties as well as to look for control measures (parasitological) better accepted by the people. The latter includes shorter regimens of DEC and medicines already in local use.

At Mendhasal PHC the villages of Mendhasal, Jamujhari, Kantabada and Simulipatna have been or are being surveyed (Table 1) and medication started. Six-monthly and

annual parasitological/clinical follow ups are planned to monitor the efficacy of this intervention which appears promising in controlling microfilaria densities (Table 2).

Table 1

Filarial Profile around Mendhasal PHC

April-May 1988

| | Mendhasal | | | Jamujhari |
|---|---|---|---|---|
| | Muslim | Others | Total | Total |
| Study population (n) | 236 | 1296 | 1532 | 766 |
| Mf rate (%) | 17.4 | 10.7 | 11.8 | 16.5 |
| Disease rate (%) | 11.4 | 22.5 | 20.8 | 15.7 |
| Mean mf count | 22.0 | 14.6 | 16.5 | 23.3 |

n=number

Table 2

Effect of Treatment on Microfilaraemia

| Group | Treatment | Mean microfilaria counts (no. of carriers examined) | | |
|---|---|---|---|---|
| | (May-July '88) | April '88 | August '88 | March '89 |
| Mendhasal | | * | | |
| A | (Unani Med.) | 22.0 (41) | 27.3 (33) | 19.8 (20) |
| B | DEC, 1g (in 5 doses)** | 10.0 (70) | 2.8 (54) | 4.6 (47) |
| C | Placebo | 14.5 (37) | 18.5 (24) | 27.5 (16) |
| D | DEC, 1g (in 5 doses) | 24.8 (32) | 6.6 (23) | 6.5 (15) |
| A+B+C+D | — | 16.3 (180) | 12.3 (134) | 11.8 (98) |
| Jamujhari | DEC, 3.0g (in 15 doses) | 23.3 (127) | 5.7 (88) | 3.0 (78) |

* Pre-medication baseline data.

** All Dosage : 200 mg for adults (100 mg for children below 15) at bed time only.

A pilot sociological survey of our study population was undertaken in collaboration with the Xavier Institute of Management basing on which a larger study is planned.

At Mendhasal about 20% of the population belonged to the Muslim community which offered us two interesting sidelines : 1) to check up on the common (but firm) belief held in this area that Muslims as a class are less susceptible to filariasis than others— a view prevalent even among the local health workers and 2) to see the efficacy of Unani drugs commonly used by Muslims, against filariasis. As regards the first, we found no differences either parasitologically or clinically between the ethnic community and the rest of the village (Table 1). Regarding the second "Regional Research Institute of Unani Medicine", Bhadrak, of the Central Council for Research in Unani Medicine, has been treating this group with an herbal preparation "Daul Fil 8" and the progress will be known during the first annual follow-up this summer.

The Puri study started with the total population of two neighbouring villages and has been noteworthy for several reasons. The co-operation of the villagers has been unfailing in these three years and after two years of Homoeopathic treatment the patients feel "helped" although, statistically speaking, "clinical improvement" has been only marginally significant (paper communicated). It is important to understand such subjective

Table 3
Changes Microfilaria Densities at Beldal (Puri)
1986-88

| Group | Mean Microfilaria counts (No. of carriers examined) | | | |
|---|---|---|---|---|
| | Feb-March 1986 | March 1988 | August 1988 | November 1988 |
| Placebo | 35.5 (23) | 9.7 (17) | 9.6 (16) | 10.8 (17) |
| Medicine | 68 (33) | 26.8 (27) | 24.2 (24) | 34.0 (30) |
| Placebo + Medicine | 55.5 (56) | 20.2 (44) | 18.3 (40) | 25.6 (47) |
| Total village | 60.8 (59) | — | 15.3 (60) | — |
| Bairipur (A nearby village) | — | — | 35.6 (39) | — |

observations particularly for a chronic disease like filariasis whose course itself is ill-defined. Parasitologically the trend is similar to the last year's where the fall in mean microfilaria count is only marginally better in the medicine group compared to the placebo group. However, the observed downward trend in the mean microfilarial count of all groups appears to have stopped (Table-3).

At present this population is being kept under observation with periodic follow-ups and our direct involvement is minimal. It is proposed to continue our interaction with the CRU group as this has thrown up refreshingly new lines of thought.

### 1.11 Detection and characterization of anti-sheath antibodies in Bancroftian filariasis.

| | | |
|---|---|---|
| Scientist | : | Dr. B. Ravindran |
| Technical staff | : | Mr. Ashok K. Satapathy |
| | | Miss T. Hussain |
| | | Mr. Prakash K. Sahoo |
| Starting date | : | October 1986 |

Antibodies to the microfilarial sheath play a central role in elimination of circulating microfilariae. The kinetics of appearance and nature of anti-sheath antibodies in Bancroftian filariasis has not been studied so far. Characterization of the determinants on the sheath that are immunogenic is another aspect that needs to be understood. Studies conducted address these issues.

**Progress :**

a) Indirect immunofluorescent assay and immunoperoxidase assay were performed for quantifying total anti-sheath antibodies. An inverse relationship was demonstrated between microfilariaemia and presence of anti-sheath antibodies. A high percentage of endemic normals and chronic filariasis cases were demonstrated to have both IgG and IgM anti-sheath antibodies (vide RMRC annual report 1987-88 p. 18)

b) Acetone fixed sheathed microfilariae were treated separately with the following— trypsin, pronase, lipase, sodium periodate or heat (100°C for 30 min). The treated microfilariae were used as antigens to evaluate the anti-sheath antibody activity in a panel of filariasis sera. The studies indicated that anti-sheath antibodies react with mostly carbohydrate determinants on the surface of microfilarial sheath.

c) The carbohydrate nature of sheath antigens was further confirmed when subgroup typing was performed for IgG anti-sheath antibodies. The detailed results are shown in Table 4. IgG2 was the predominant antibody with anti-sheath activity.

d) About 30% of amicrofilaraemic individuals (chronic filariasis cases and endemic normals) had detectable IgE anti-sheath antibodies in comparison to about 8% seen in asymptomatic microfilariae carriers. IgE dependent platelet mediated cytotoxicity to microfilariae in Bancroftian filariasis is currently under investigation.

Table 4

IgG Subgroups with Anti-Sheath Antibody Activity +

| | Number (%) of samples | | | |
|---|---|---|---|---|
| | IgG1 | IgG2 | IgG3 | IgG4 |
| Chronic Filariasis n = 17 | 13 (76.4) | 16** (94.1) | 11 (64.7) | 9 (52.9) |
| Endemic normals n = 20 | 12 (60.0) | 17* (85.0) | 7 (35.0) | 5 (25.0) |

\+ Tested at a serum dilution of 1 : 5 by immunoperoxidase assay.
* Significantly more ($P < 0.05$) in comparison to activity in IgG1, IgG3 or IgG4.
** Significantly more ($P < 0.05$) in comparison to activity in IgG3 or IgG4.

### 1.12 Studies on antibodies with reactivity to Diethylcarbamazine.

Scientists : Dr. B. Ravindran
Dr. N. M. Pattnaik

Technical staff : Miss T. Hussain
Mr. Ashok K. Satapathy
Mr. Prakash K. Sahoo

Starting date : July 1985

Initially it was proposed to raise antibodies to DEC with a view to develop an immunoassay for measurement of the drug in body fluids. The anti-DEC antibodies raised were however found to crossreact with surface of microfilariae of *W. bancrofti*. This was a unique instance of antigenic mimicry between the anti-filarial drug and filarial parasite (details of the above findings were reported earlier and published in Medical Science

Research and in Immunology Letters—vide RMRC annual report 1987-88). Further studies were undertaken to characterize the reactivity of anti-DEC with other filarial parasites and to use it as a tool for developing an immunoprophylactic agent for blocking the transmission of microfilariae from vertebrate to mosquito hosts.

**Progress :**

a) Rabbit raised anti-DEC antibodies reacted with microfilarial surface of *W. bancrofti*, *Brugia malayi* (purified from patients or from experimentally infected animals) and *Setaria digitata*. Brief treatment of microfilariae with pronase increased their reactivity with anti-DEC. Microfilariae of *Dirofilaria immitis* and *Litomosoides carinii* did not react with anti-DEC.

b) Since antibodies to DEC raised in rabbits reacted with microfilariae, an attempt was made to detect and quantify anti-DEC antibodies in human filarial sera. The occurence of anti-DEC antibodies was more prominent in amicrofilaraemic individuals in comparison to asymptomatic microfilariae carriers in Bancroftian filariasis. A similar trend was seen in *B. malayi* infection also (sera from *B. malayi* infected patients were procured from WHO filariasis serum bank). Anti-DEC activity in the sera was mostly found in IgG2 subclass.

   Anti-DEC antibodies in pooled filarial sera were affinity purified by passing through a column of methylpiperazine carboxylic acid coupled to Sepharose 4B. The eluted fractions were found to react with DEC and more strongly with microfilariae of *W. bancrofti* indicating that the anti-DEC antibodies in human sera are primarily directed towards microfilariae.

c) Currently a mouse model has been developed for studying the microfilaraemia of *Setaria digitata* mf (a filarial parasite of cattle) in mice. Intra peritoneal injection of $6 \times 10^5$ *S. digitata* microfilariae (produced *in vitro* from gravid adult females) induced microfilaraemia in mice for 28-30 days. About 10-12 mf per 10 microlitre of blood could be demonstrated in mice during the microfilaraemic phase. Since anti-DEC cross reacts with *S. digitata* mf it is proposed to undertake active and passive immunization studies in mice to study the effect of anti-DEC on microfilaraemia in mice. Attempts are also underway to procure experimental animals *(Mastomys natalansis)* infected with *Brugia malayi* for undertaking *in vivo* studies on the crossreactivity of anti-DEC with microfilariae.

# 2. MALARIA

## 2.1 Induction of cell mediated immunity in Malaria by immunomodulators

Scientist : Dr. M. K. Das
Dr. B. Ravindran

Technical staff : Mr. M. K. Beuria
Mr. H. S. Naik

Starting date : June 1986

Cellular immune response (CMI), both antigen specific and non-specific are known to be depressed in malarial infection; possibly due to a defect in macrophage handling/ processing of antigen. The impairment of CMI is reflected most notably as the decreased DTH reactions to the antigen. The present project will utilise immunomodulators to check their efficacies in enhancing CMI in malaria.

**Progress :**

a) Immunological adjuvants that are relatively nontoxic like alum, liposomes and saponin were utilized. Soluble antigens of *P. berghei* were isolated from the erythrocytes of infected mice (NK 65 strain).

b) *P. berghei* antigen without any adjuvant did not induce DTH in infected mice. However measurable response could be induced when the antigen was mixed with adjuvant. MIF test, an *in vitro* counterpart of DTH, established similar result.

c) The increase in DTH reactivity began with the early rise in parasitaemia (15% parasitaemia, 8th day of infection) peaked around 10th day (35% parasitaemia) followed by a decrease with the subsequent rise in infection (14th day, 55% parasitaemia).

d) The enhancement in DTH response induced by adjuvants is specific as checked with BSA.

## 2.2 Studies on naturally occuring antibodies with α-galactosyl activity in *P. falciparum* malaria.

Scientists : Dr. B. Ravindran
Dr. M. K. Das

Technical staff : Mr. Ashok K. Satapathy
Mr. Ramakrishna Das
Mr. Prakash K. Sahoo

Starting date : March 1986

Autoantibodies with a distinct α-galactosyl specificity (anti-gal) in human sera have been implicated in elimination of senescent erythrocytes. Since terminal α-linked galactose determinants have been described in parasite specific glycoproteins the following studies were undertaken to investigate the relevance of anti-gal titres in malaria. We had developed a very sensitive cell-ELISA for monitoring anti-gal titres in human sera (vide RMRC Annual Report 1986-87 p. 23). Since monitoring anti-gal titres is becoming increasingly important for diagnosis or prognosis of many diseases such as leishmaniasis, rheumatoid arthritis, sickle cell anaemia, thyroid disorders and a host of other bacterial infections, an attempt has been made to evaluate cell-ELISA with immunoassays for anti-gal reported by other workers.

**Progress :**

a) Anti-gal titres were found to be elevated in a significant proportion of sera collected from *P. falciparum* infected (uncomplicated) patients or in subjects living in *P. falciparum* hyperendemic areas. However in infected patients with cerebral involvement anti-gal titres were comparable to that of non-endemic controls. Subclass typing revealed IgG2 to be the predominant antibody with anti-gal activity in non-endemic controls, while IgG3 anti-gal antibodies were increased many fold in *P. falciparum* infected patients' serum. The above details have been published in Immunology Letters (see under papers published).

b) To study the specificity of red cell associated IgG in *P. falciparum* malaria, the cell bound IgG was assayed by an anti-globulin ELISA and the levels compared with circulating anti-gal titres. There was no correlation between the two. Further, the anti-globulin ELISA could not be inhibited by preincubation of erythrocytes with melibiose. These studies indicate that erythrocyte associated IgG have specificity towards determinants other than galactose.

c) Since α-linked galactose determinants have been shown to be present in nervous tissues, studies were undertaken to detect and quantify intrathecal synthesis of anti-gal in cerebral malaria. Preliminary investigations indicate the presence of anti-gal in CSF collected from *P. falciparum* infected (with cerebral involvement) patients.

d) Cell-ELISA developed by us was evaluated for sensitivity, specificity and reproducibility with other assays namely enhanced agglutination test and lipid-ELISA. Cell-ELISA was found to be far more sensitive—interassay and intraassay co-efficient of variation revealed cell-ELISA and lipid-ELISA to be relatively more reproducible.

e) Anti-gal antibodies were affinity purified using a column of the adsorbent Gal $\alpha$ 1-3 Gal $\beta$ 1-4 Glu NAc—Synsorb. The eluate when tested by cell-ELISA revealed the presence of IgG, IgM and IgA antibodies with anti-gal activity. The antigenic determinant was sensitive to treatment with $\alpha$-galactosidase and not to $\beta$-galactosidase. The affinity purified anti-gal is now being used to study its reactivity to *P. falciparum* infected erythrocytes.

## 2.3 Role of carbohydrate determinants in the interaction between *P. berghei* infected erythrocytes and macrophages in Balb/c mice.

| | | |
|---|---|---|
| Scientists | : | Dr. B. Ravindran |
| | | Dr. M. K. Das |
| Technical staff | : | Mr. Ashok K. Satapathy |
| | | Miss T. Hussain |
| Starting date | : | March 1986 |

Intracellular plasmodia induce many changes on the surface of infected erythrocytes—these include expression of neo-antigens of the host. Studies on the interaction of these neo-antigens of infected erythrocytes with cells of the reticuloendothelial system are critical for understanding the development of acquired immune response in malaria.

**Progress :**

a) A cell-ELISA was developed to quantify antibodies to the surface of *P. berghei* infected erythrocytes. Most of the antibodies in infected sera reacting to parasitized erythrocytes were found to be auto-antibodies. They recognised sialic acid determinants present on parasitized or normal mouse erythrocytes. (vide RMRC Annual Report 1987-88 p. 22).

b) These auto-antibodies with specificity to sialic acid moieties also reacted with thymocytes but not with mature lymphocytes from spleen.

c) Antibodies to lipid antigens were also analysed (by ELISA) in *P. berghei* infected mice. Most of antilipid antibodies present in acute phase sera recognized normal mouse erythrocyte lipids. Fractionation and serological analysis of normal as well as parasitized erythrocyte lipids indicated the possible antigenicity of phospholipids of parasite origin. Further studies are in progress.

# 3. CLINICAL PATHOLOGY

## 3.1 Study of Erythrocytic Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency and its variants in a malaria endemic area with tribal population.

Scientist : Dr. G. P. Chhotray

Technical staff : Mr. M. R. Ranjit

Mr. H. K. Khuntia

Starting date : April, 1986

Based on the data collected last year (vide Annual Report 1987-88 p. 24) the study was further continued with a view to concentrate on the variants (as advised by Prof. Luzzatto). All the females and those males showing moderate G6PD enzyme deficiency were excluded from the present study. More male cases (50 tribals & 13 non tribals) were added to the lot. In the tribal group (207) severe G6PD enzyme deficiency was observed in 13.14% of cases as compared to 3.69% in non-tribals (108). No abnormal haemoglobin was detected in the study population. 15.87% of cases were positive for malaria parasite (*P. falciparum*).

10 ml of venous blood was collected from 6 persons having severe G6PD deficiency and was transported to RMRC laboratory in ACD anticoagnlant at 4°C within 36 hours of collection. The haemolysate was prepared. G6PD enzyme assay was carried out. The results were calculated by using the formula.

$$\text{Activity IU/g Hb} = \frac{(\text{OD/min}) 10^5}{6.22 \times \text{Hb}^* \times \text{enzyme volume}^{**}}$$

* Hb=g/100 ml.

** Enzyme vol. — $\mu$ of haemolysate/ml of reagent mixture.

Electrophoretic characterisation of G6PD enzyme was done by submerged Agarose gel electrophoresis using G6PD non deficient person's blood as control. In three of the deficient subjects no band was visible in electrophoresis on staining where as a faint band could be observed in three of the deficient cases. The mobility is less than in the control.

DNA extraction was carried out and the samples were stored at -70°C. G6PD variants will be analysed at the DNA level after being characterised biochemically and electrophoretically.

**Justification for continuation :**

The above research proposal was recommended by the Scientific Advisory Committee for a collaborative study with Prof. Lucio Luzzatto, Royal Postgraduate Medical School, Hammersmith Hospital, Haematology Division, Londown W OHS. It was also approved in the Health Ministry's screening committee meeting on 23.3.88 at the ICMR headquarters.

*On August 20, 1897 afternoon Ronald Ross wrote ''.....I must have examined the stomachs of a thousand mosquitoes by this time. But the Angel of Fate fortunately laid his hand on my head; and I had scarcely and almost perfectly circular outline before me of about 12 microns in diameter.....''*

*The next day he wrote to his wife, the following poem :*

*''This day relenting God*
*Hath placed within my hand*

*A wonderous thing; and God*
*Be praised. At His Command*

*Seeking His secret deads*
*With tears and toiling breath.*

*I find thy cunning seeds,*
*O Million-murdering Death.*

*I know this little thing*
*A myriad men will save.*

*O Death, where is thy sting ?*
*Thy Victory, O Grave ?*

*(EPIDEMICS AND OTHER TROPICAL DISEASES, O.P. JAGGI)*

# 4. ENTOMOLOGY

## 4.1 Present status of *Mansonoides* and *B. malayi* in Orissa.

Scientist : Dr. A. P. Dash
Technical staff : Sri R. K. Hazra
Sri H. K. Tripathy
Sri G. Mansingh
Sri M.N. Naik
Starting date : September, 1988

*Brugia malayi* infection was not known in India till Korke's report on its presence in Balasore district in 1929. It had a localised distribution in Balasore, Puri and Bolangir districts. A filaria survey carried out in 1979-80 revealed complete disappearance of *B. malayi* infection as well as of *Mansonoides* mosquitoes in certain areas of the State. Recently however this centre has detected *B. malayi* infection in Balasore district and some interior areas of Orissa and lot of *Mansonoides* have also been collected from coastal Orissa. Moreover, the seasonality of *B. malayi* vectors are changing over the years due to lot of ecological changes. Since systematic studies are lacking, there is an urgent need to study the present status of *Mansonoides* and *B. malayi* in the state.

The project was unanimously approved by the Scientific Advisory Committee in July 1988 and started from September, 1988.

**Progress :**

Entomological data have been collected from localities around Sakhigopal in Puri district and Soro in Balasore district. *Mansonoides* were the dominant mosquitoes which constituted 47.62 and 43.8% of the total collection in Puri and Balasore district respectively.

Per man house densities of *Mansonia annulifera* and *Ma. uniformis* in Puri district are tabulated below :

| Month and Year | Per Man House Density of | |
|---|---|---|
| | *Ma. annulifera* | *Ma. uniformis* |
| September 1988 | 12.3 | 80.0 |
| October 1988 | 2.0 | 42.4 |
| November 1988 | 6.0 | 26.0 |
| December 1988 | 5.3 | 12.0 |
| January 1989 | 10.5 | 9.1 |
| February 1989 | 18.0 | 5.0 |
| March 1989 | 6.5 | 24.5 |

The infection and infectivity rates of *Ma. annulifera* and *Ma. uniformis* studied upto March, 89 in Puri and Balasore district are as follows :

| Species | District | No. dissected | No. with $L_1$ & $L_2$ | Infection rate | No. with $L_3$ | Infectivity rate |
|---|---|---|---|---|---|---|
| *Ma. annulifera* | Balasore | 33 | 2 | 6.6% | 1 | 3.3% |
| *Ma. uniformis* | Balasore | 6 | 1 | 16.6% | 0 | 0 |
| *Ma. annulifera* | Puri | 10 | 0 | 0 | 0 | 0 |
| *Ma. uniformis* | Puri | 300 | 10 | 3.3% | 3 | 1% |

*B. malayi* is found in both the districts. The results of the blood slide examination in the said localities are presented below.

| Area | B.S. examined | Positive | *B. malayi* | *W. bancrofti* | Mixed |
|---|---|---|---|---|---|
| Sakhigopal | 293 | 45 | 11 | 32 | 2 |
| Soro | 91 | 7 | 7 | 0 | 0 |

Ectoparasites collected from *Ma. annulifera* and *Ma. uniformis* have been sent for identification.

**4.2 Effect of aphid extracts on the development of mosquitoes.**

Scientists : Dr. A. P. Dash
Dr. G. P. Chhotray

Technical staff : Sri M. R. Ranjit

Starting date : January, 1987

**Progress :**

**4.2.1. Laboratory evaluation of aphid extracts and some synthetic juvenoides against *C. quinquefasciatus* larvae.**

Laboratory evaluation of *Aphis gossypii* and *A. craccivora* extracts and some synthetic juvenoides like methoprene, OMS 3007 and Neporex was made against *Culex quinquefasciatus* by LOGIT ANALYSIS USING A FORTRAN PROGRAMME. The $LD_{50}$ and $LD_{90}$ values determined for each compound are as follows :

| Compound | $LD_{50}$ in ppm | $LD_{90}$ in ppm |
|---|---|---|
| *A. craccivora* | 30.84 ± 1.49 | 47.01 ± 4.01 |
| *A. gossypii* | 31.94 ± 2.43 | 49.81 ± 6.6 |
| Methoprene | 0.02029 ± 0.067 | 0.67107 ± 0.145 |
| OMS 3007 | 0.02063 ± 0.0043 | 0.08681 ± 0.0153 |
| Neporex | 0.018891 ± 0.053 | 0.94309 ± 0.173 |

(High lethal dose of aphid extracts are due to the use of whole body extracts without separation of juvenile hormones. Efforts are being made to separate the JH from these extracts for comparative study).

### 4.2.2. Effect of aphid extracts and some synthetic juvenoides on growth index and percentage of successful emergence inhibition of *C. quinquefasciatus.*

Effect of aphid extracts and synthetic juvenoides like methoprene, OMS 3007 and Neporex on the growth index and percentage of successful emergence of *C. quinquefasciatus* have been studied after treating eggs at different stages.

The following formula have been used.

$$\text{Growth index} : \frac{\text{Percentage of total adult emergence}}{\text{Average development period}}$$

$$\text{Successful emergence inhibition} : 100 - \frac{\text{No. of successful adults}}{\text{No. of pupae formed}} \times 100$$

The treatment remarkably decreased the growth index and increased the percentage of successful emergence inhibition in all the samples when compared with untreated and control ones. The extracts of both the aphids were almost equally effective as the synthetic juvenoid Neporex.

### 4.2.3. Effect of aphid extracts and some synthetic juvenoids on the Dyar's factor.

The effect of extracts of *A. gossypii* and *A. craccivora* and synthetic juvenoides like methoprene, OMS 3007 and neporex was evaluated on determining the Dyar's factor in *C. quinquefasciatus* larvae. All the compounds increased the Dyar's factor equally in case of thorax and length of abdomen.

### 4.2.4. Morphological abnormalities of *C. quinquefasciatus* pupae induced by the juvenoides and aphid extracts.

The above compounds are found to induce abnormalities in morphological characters in the pupae of *C. quinquefasciatus* like retention of larval hair in the pupal stage, the adults and pupae always remain with pupal and larval exuviae.

### 4.2.5. Effect of aphid extracts and juvenoides on the haemocyte count and glycogen content of *C. quinquefasciatus*.

The insect blood can be divided into two fractions, the fluid plasma and the cellular portion known as haemocytes. The haemocytes are known to play a vital role in the immune system of insects including mosquitoes. The study reveals that out of nine types of insect haemocytes, six are found in *C. quinquefasciatus* larvae viz., prohaemocytes, plasmatocytes, granulocytes, oenocytoids, spherulocytes and coagulocytes. All the compounds (aphid extracts and the synthetic juvenoides) significantly increased the plasmatocyte count and decreased the prohaemocyte and granulocyte counts.

The glycogen content of mosquito larvae decreased significantly in the treated samples in comparison to untreated and control samples.

### 4.3 Isolation of *Bacillus* species from mosquito larvae.

Scientist : Dr. A. P. Dash
Technical staff : Dr. K. Ramaswamy
Starting date : February, 1989

Vector resistance to the insecticides, increased cost of insecticides and oppositions to the use of chemicals by the environmentalists are some of the major factors which prompted research on biological control agents of mosquitoes. The sporeforming *Bacillus*, *B. sphaericus* and *B. thuringiensis*, are known to be effective against mosquitoes. Here an attempt is made to isolate the *Bacillus* species from the mosquito larvae collected from local habitats, after joining of a research assistant with microbiology background.

The growth of bacteria was studied in samples collected in and around Bhubaneswar. The colonies were irregular and opaque. Basic observations like motility, gram staining, spore staining etc. were made. Finally, on the basis of biochemical and physiological tests viz., catalase, growth in 7% NaCl, growth in 60°C, Voges Proskaur's reaction, growth anaerobic agar, acid and gas production, starch hydrolysis, casein

hydrolysis etc., the isolated bacteria are identified as *B. phaericus* (Courtesy : Dr. V. R. Subramanyam).

The study is in progress.

**4.4 Laboratory biology and colonization of mosquitoes.**

Cyclic colonies of the following species of mosquitoes have been established in the laboratory.

*Aedes aegypti* (obtained from VCRC, Pondicherry)

*Aedes aegypti* black headed Liverpool strain

*Culex quinquefasciatus*

*Culex tritaeniosrhynchus*

*Mansonia uniformis*

The laboratory colonized mosquitoes are used for various studies.

Of late in March 1988 efforts are being made to colonize *Aedes aegypti* black headed Liverpool strain for developmental studies of *B. malayi* (from *Mastomys*) and production of infective larvae.

**4.5 Characterization of immune response in man elicited by mosquitoes.**

| | |
|---|---|
| Scientists | : Dr. M. K. Das |
| | Dr. A. P. Dash |
| Technical staff | : Ms. A. Mishra |
| Starting date | : April, 1986 |

**Progress :**

Natural antibodies to *Culex quinquefasciatus* in normal human was determined by ELISA. Antigenic extract from salivary gland was found to be better regarding reproducibility compared to whole-body extract of the mosquito. Antibody titer to IgG and IgE isotypes is being determined.

# 5. MYCOBACTERIAL GENETICS

Scientist : Dr. V. R. Subramanyam
Technical staff : Mr. B. B. Pal
Ms. K. K. Mohanty
Starting date : January, 1985

**Progress**

## 5.1 Isolation of mycobacterial mutants

In addition to the several auxotrophic mutants of *M. smegmatis* and *M. fortuitum* reported earlier (Annual Report, 1987-88, p. 38), after screening 4444 colonies of NTG-treated *M. vaccae,* we obtained a single cytosine requiring auxotroph (SM69, *cyt-4*).

## 5.2 Viability and stability of the mutants

The mutants are stocked in glycerol-broth and stored at -80°C. Regular subcultures at 6 month-intervals are made to check for viability and stability of the mutants. All mutants are viable till date. However, a few of the mutants have lost their auxotrophic marker. The markers lost are *met-3*, *met-5*, *arg-2*, *pur-5* and *pur-6*, of SM26, SM28, SM22, SM40 and SM44 respectively.

## 5.3 Calculation of reverse mutation frequency

The conventional method for estimating the frequency of reverse mutation involves plating a large number of mutant bacteria on a medium selective for the revertant. A large number of plates would be required to avoid heavy inocula because the latter would interfere with results. We have explored the applicability of the multiple-tube most probable number (MT-MPN) method (which is a standard statistical method used in bacteriological quality control of drinking water) for estimating the reverse mutation frequency of mycobacterial auxotrophs.

One mutant each of *M. smegmatis* (SM19) and *M. fortuitum* (SM27) were tested by MT-MPN method and compared with the conventional plating method for reverse mutation frequency. Table 5 summarises the result obtained. The values of reversion frequencies by the two methods compare well. MT-MPN method offers certain advantages (over the conventional plating method) like : 1. simplicity and ease of performance, 2. larger inocula can be tested and 3. economises on the media and growth factors.

## 5.4 Resistogram of the mycobacteria and their mutants

Differential resistance to various agents can be exploited as a useful genetic marker. We screened three standard strains and all their auxotrophic mutants against various concentrations of $CuSO_4$, $ZnSO_4$, LiCl, and $NaN_3$. The minimum inhibitory concentrations (MICs) of these agents against the standard strains and such mutants that differed significantly from their parents are listed in Table 6.

Table 5

Reversion frequency of mutants by two methods

| Strain (marker) CFU/ml | SM19 (purine) $1.75 \times 10^9$ | SM27 (purine) $5.44 \times 10^{10}$ | SM27 (methionine) $5.44 \times 10^{10}$ |
|---|---|---|---|
| I. Plate method | | | |
| Inoculum | $4.36 \times 10^8$ | $1.11 \times 10^{10}$ | $1.11 \times 10^{10}$ |
| No. of plates | 17 | 17 | 17 |
| Revertants | 0 | 2 | 6 |
| Reversion frequency | $<2.3 \times 10^{-9}$ | $1.8 \times 10^{-10}$ | $5.4 \times 10^{-10}$ |
| II. MT-MPN method | | | |
| Inoculum (ml) | 1, 0.1, 0.01 | 1, 0.1, 0.01 | 1, 0.1, 0.01 |
| Replicates | 5, 5, 5 | 5, 5, 5 | 5, 5, 5 |
| Positive | 0, 1, 0 | 5, 3, 0 | 5, 3, 1 |
| MPN (Revertants per 100ml) | 20 | 790 | 1090 |
| Reversion frequency | $1.14 \times 10^{-10}$ | $1.45 \times 10^{-10}$ | $2 \times 10^{-10}$ |

Table 6

Resistogram of the Mycobacteria & Mutants

| Strain | MIC ($\mu$g/ml) of | | | |
|---|---|---|---|---|
| | $CuSO_4$ | $ZnSO_4$ | LiCl | $NaN_3$ |
| *M. smegmatis* | 128 | 4096 | 40,000 | 256 |
| SM12 | * | 512 | * | * |
| SM21 | * | * | * | 2048 |
| SM33 | * | 512 | * | * |
| SM34 | * | 512 | * | * |
| SM39 | * | * | 160,000 | * |
| SM40 | * | * | * | 1024 |
| *M. fortuitum* | 512 | 4096 | 40,000 | 512 |
| SM2 | 128 | * | * | * |
| SM3 | 128 | * | * | * |
| SM24 | 128 | * | * | * |
| *M. vaccae* | 128 | 512 | 40,000 | 64 |
| SM69 | * | 2048 | * | * |

* MIC values not significantly different from the parent strain.

**Justification for continuation :**

This is a long term project with the objective of isolating and characterising a number of mycobacterial mutants for use in gene transfer experiments.

## 6. AIDS

### Sero surveillance for Human Immunodeficiency Virus (HIV) infection.

Scientist : Dr. V. R. Subramanyam
Technical staff : Nil
Starting date : Early 1987

**Progress**

RMRC, Bhubaneswar is a part of the network established by ICMR for surveillance of HIV infection. So far, a total of 319 sera have been received and tested, All the sera were negative for anti-HIV antibodies by the Wellcozyme anti HTLV III ELISA kit. Details on the samples analysed are given in tables 7 and 8. Sixty six of the samples tested were from individuals at high risk.

Table 7

Source of the samples

| Year of collection | No. of Sera | Source |
|---|---|---|
| 1987 | 108 | SCB Medical College, Cuttack |
| | 13* | R. E. College, Rourkela |
| 1988 | 10* | —do— |
| | 180+ | SCB Medical College, Cuttack |
| | 1 | Capital Hospital, Bhubaneswar |
| 1989 | 7+ | MKCG Hospital, Berhampur |
| Total | 319 | |

* Foreigners, + details not furnished

In addition, another 200 sera (excluding the 237 reported in the previous year) collected from filariasis endemic areas were screened for anti HIV antibodies. All the sera were negative.

Table 8

Age/Sex distribution of sera screened

| Age group | Male | Female | Total |
|---|---|---|---|
| ⩽ 20 | 43 | 5 | 48 |
| 21-30 | 35 | 7 | 42 |
| 31-40 | 16 | 5 | 21 |
| ⩾ 41 | 16 | 3 | 19 |
| Not known | 2 | — | 189 |
| Total | 112 | 20 | 319 |

**Justification for continuation :**

Constant sero surveillance is essential for identifying virus-carriers and for monitoring the spread of the virus.

## WHAT IS AIDS ?

*AIDS stands for Acquired Immune Deficiency Syndrome. AIDS is caused by the Human Immunodeficiency Virus (HIV). The AIDS virus causes a break-down of the body's immune system, leaving it vulnerable to infections and cancers which normally do not affect healthy people. There is currently no cure for the immune deficiency itself, but there are limited treatments for the infections and cancers. The AIDS virus may also attack the nervous system and cause damage to the brain.*

## HOW IS AIDS TRANSMITTED ?

*Research has shown that AIDS is not contracted by being near, eating with, or touching a person with AIDS. AIDS is transmitted through semen, blood and vaginal fluids. Saliva and sweat have not shown to be means of transmission. The incubation period ranges from six months to more than five years. A person may spread the AIDS virus while showing no symptoms.*
*(San Francisco AIDS Foundation)*

AR-37



# 7. SICKLE CELL RESEARCH CENTRE, V.S.S. MEDICAL COLLEGE, BURLA.

## 7.1 Study of natural history of Sickle Cell Disease in Western Orissa.

By 31.3.88, 382 cases were recorded and are being followed up in this centre. 190 number of new cases have been added since April '88 till date thus bringing the total number of cases recorded to 572. These cases are attending the clinics at frequent intervals. On each visit their clinical assessment and necessary Haematological investigations are being done and recorded in addition to rendering necessary advice. Thus under this project 572 number of cases are being followed up and the total follow up period so far has been about 2-3 years in different cases.

Total attendance in the Sickle Cell Clinic since 1.4.88 up to date has been 2088 which includes number of new patients, their relations, suspected patients and old patients coming for follow up. These cases are referred from V.S.S. Medical College Hospital, from other Hospitals of the state and patients from Madhya Pradesh. In all suspected cases and relations of new cases haemoglobin electrophoresis and sickling are done routinely. In all newly detected patients a complete clinical examination and battery haematological investigations are done in the initial stage and recorded in the permanent case records while giving them necessary advice. The Sickle Cell Trait cases are given necessary diagnosis including marriage and genetic counselling. Besides the above number of thallassaemia cases have also been investigated here when referred for the purpose.

## 7.2 Detection of new cases of Sickle Cell Disease in the Sickle Cell clinic and their clinico pathological study.

During the year since 1.4.88 a total number of 241 new cases have been detected. But complete clinico pathological and haematological study has been done in 190 cases.

## 7.3 Survey for the incidence of Sickle Cell Trait and Sickle Cell Disease in the general population in Western Orissa.

After 1.4.88, survey for the detection of Sickle Cell Gene in the village KALAMATI, BASANTAPUR and KANKHINDA has been completed. 883 number of persons have been tested representing 6784 number of persons of the villages and 137 persons were found to have the gene out of whom 8 were patients.

## 7.4 Immunoglobins in Sickle Cell Disease.

This is a new project which has been taken up during the year and IgG, IgM and IgA has been estimated in 25 cases of Sickle Cell disease, 3 normal persons and 5 cases of Sickle Cell Trait to serve as control. Further study in this regard is being taken up during 89-90 in collaboration with R.M.R.C., Bhubaneswar.

# Projects Completed

## 1. Clinicopathological study of lymphatic nodules in human filariasis.

Scientist : Dr. S. K. Kar
Technical staff : Miss J. Mania
Mr. T. Moharana
Mr. R. N. Nayak
Mr. K. Dhal

The occurence of lymphatic nodules in clinical spectrum of Bancroftian and Brugian filariasis was reported in 1986 from the RMRC. The nodule formation in extremities with clinical filarial disease revealed granulomatous reaction around the adult worm *(W. bancrofti)* in few cases studied. One of the possible mechanisms postulated for such nodule formation was host immune responses to the parasitic antigen. Hence this study was initiated to assess the prevalence of cases presenting with nodules and study their humoral immune responses to the parasitic antigen.

**Result and Discussion :**

Out of 2965 endemic subjects examined, 194 (6.5%) had lymphatic nodules with or without clinical sign of filariasis. Nodules were observed in extremities, particularly in mid humoral region of upper arm. Seventy three subjects had more than one nodule (1.5-2.5 cm size) in the same region. The history of downward shifting of the nodule was elicited from 17 (8.8%) subjects. Development of lymphatic nodules was preceded by lymphangitis of the affected limb. After a few days the lymphangitis subsides, but the nodule persists for a long time.

Sera collected from 53 study cases revealed 16 (30.2%) subjects with positive filaria specific antibody and 25 (47.2%) with circulating filarial antigen. The mean circulating immune complex level as estimated by P.E.G. precipitation assay was $0.089 \pm 0.061$.

The clinical status of the cases presenting with nodule was assessed (Table 9). It was observed that out of 194 cases presenting with lymphatic nodules 42 (21.6%) were asymptomatic microfilaria carriers, 34 (17.5%) had acute filarial disease, 97 (50%) had associated chronic filarial disease. There were 21 (10.8%) cases among the endemic

normals without any filarial manifestations at the period of assessment. It may be noted that 12 out of 21 endemic normals initially presented with nodules became microfilaraemic as detected after one year follow-up.

Table 9

Clinical Status of Patients Presenting with Lymphatic Nodules

| Age Group | Total Population Covered | Subjects with Lymphatic Nodules | | | Clinical Status of Cases with Nodules | | | | | | Total No. of Cases with Nodules and Filarial Diseases |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Male | Female | Total | AMC | | AFD | | CFD | | |
| | | | | | No. | % | No. | % | No. | % | |
| 5-19 | 1354 | 46 | 19 | 65 (33.5%) | 21 | 50 | 19 | 55.9 | 18 | 18.6 | 58 |
| 20-44 | 1026 | 70 | 15 | 85 (43.8%) | 16 | 38 | 7 | 20.6 | 51 | 52.6 | 74 |
| 45+ | 585 | 23 | 21 | 44 (22.6%) | 5 | 11.9 | 8 | 23.5 | 28 | 28.9 | 41 |
| Total | 2965 | 139 | 55 | 194** | 42 | | 34 | | 97 | | 173 |

AMC = Asymptomatic Microfilaria Carrier
AFD = Acute Filarial Disease
CFD = Chronic Filarial Disease
** 21 cases with nodule were from endemic normals.

The filaria specific antibody titre, circulating filarial antigen and immune complex status of study cases were compared with that of control sera of endemic normals.

No significant difference was observed in immune status of subjects with nodules associated with various clinical manifestation and that of cases with chronic filarial disease (table 10). The humoral immune response of patients presenting with lymphatic nodules are comparable to that of various clinical stages of filarial infection. This shows that although lymphatic nodules are manifested in a few endemic subjects, it is an outcome of filarial infection. Filarial infection should be considered as differential diagnosis in a patient presenting with lymphatic nodules particularly if he comes from an endemic area. Study of

histopathology of large number of these nodules could have revealed the changes in these nodules at various stages of its development correlating with the immune status.

Table 10

Comparison of Immune Status of Patients with Lymphatic Nodules vs Endemic Normals and Chronic Filarial Disease

| Cases | Total sample collected | mf status | | Ab | | Circulating Ag | | Mean CIC level ±SD |
|---|---|---|---|---|---|---|---|---|
| | | Nos. of positive | % | Nos. with +ve IgG | % | Nos. with +ve Ag | % | |
| Subjects with Nodules | 53 | 16 | 30.2 | 16 | 30.2* | 25 | 47.2* | 0.089* ±0.061 |
| Subjects with chronic filarial disease (without nodule) | 15 | 0 | 0 | 4 | 26.7 | 6 | 40.0 | 0.105 ±0.073 |

* Not significant

**2. Study of prevalence of filariasis in two ethnic groups in Kumarabasta village.**

Scientist : Dr. S. K. Kar

Technical staff : Miss J. Mania
Mr. K. Dhal

Epidemiological study on filariasis was carried out in an endemic village Kumarabasta with a population of 2020. This village is inhabited by caste Hindus (1692) and scheduled tribes (307). The scheduled tribes who are mostly labourers stay 1 km away from others but in the same territory. The study was initiated to compare the prevalence of clinical filarial disease, mf status and severity of clinical manifestation in both ethnic groups.

The population census was carried out in both the areas recording to their age, sex, residential status and occupation. The stratified (age and sex) sample of population was included in the study. The detailed clinical and parasitological survey was undertaken in study cases of both the areas and results are shown in table 11.

Table 11

Comparison of Clinical Status of Subjects of Kumarabasta
( Hindus vs Scheduled Tribes )

| Caste | Total Population | Population Examined | Nos. mf +ve | Mean mf Density/ 20 cmm Blood | Clinical Filarial Disease | | | | Nos. +ve for Fs Ab | Nos. +ve for Fs circulating Ag | Mean CIC level ±SD |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | AMC | Acute | Chronic | Total | | | |
| Hindu(n) | 1692 | 591 (34.9) | 90 (15.2) | 15.22 | 65 | 27 | 146 | 238 (40.3) | 12 (57) | 5 (24) | .126 0.086 |
| Scheduled Tribes (n) | 307 | 106 (34.5) | 23 (21.7) | 21.69 | 15 | 3 | 15** | 33 (31.1) | 4* (36) | 2* (28) | .131* 0.040 |
| Total | 1999 | 697 | 113 | | 80 | 30 | 161 | 271 | 16 | 7 | |

n = number
(%)

* Not significant
** Significant

It was observed that microfilaraemic status of both population was comparable and the difference was not statistically significant. Hence the filarial infection was equally prevalent and tribal population was equally susceptible to infection as the caste Hindu population. But significantly higher prevalence of chronic filarial disease was observed in cast Hindu population (40.3%) as compared to the tribals. Except 15 cases of soft lymphoedema no case of filarial elephantiasis with thickened skin was observed in the tribal population. The average acute lymphangitis attack rate (per year) was higher in general population (5.23) as compared to the tribals (2.1). Sera of chronic filarial disease cases from caste Hindu population (21) and tribals (11) were tested for filaria specific antibody titre and circulating filarial antigen. No significant difference in the humoral antibody response was elicited.

Since microfilaria rate in both groups is high and comparable, absence of repeated mosquito bites in tribals cannot be postulated as the cause of nonprogression of early lymphoedema to elephantiasis. Both the population are within the mosquito flight range which is more than 2 km in radius. Both the groups are inhabitants of that area over the same period and present data indicate that there is continued transmission of infection. It is possible that the lymphatics in tribals may be structurally different or their occupation might be directly or indirectly contributing to better lymph circulation mechanism. However

study of a large number of tribals and other associated epidemiological factors, besides lymph scintigram studies in lymphoedema cases of both population groups might explain the low prevalence of chronic filarial lesion in the tribals.

### 3. Immune response of patients with filarial fever

Scientist : Dr. S. K. Kar

One of the most compelling issues of filariasis is the aetiology of filarial fever. Present study evaluates any altered immune response or role of bacterial infection in 58 cases presenting with filarial fever in their natural course in an endemic village.

a) Sera were collected before, during and after 1 month of the adenolymphangitic attack from study cases. This was carried out during detailed screening and follow up of the endemic population.

b) The blood samples of study cases were tested for DC, TLC and presence of microfilaria. Immunological tests like filaria specific IgG titre (ELISA, using mf-ES Ag) circulating filarial antigen and circulating immune complexes level were carried out in serial samples obtained from study cases.

c) Significant rise of total leukocytes or polymorphs was not marked during the fever.

d) There was an increased eosinophil response (mean % = 14.4 ± 8.4) after one month of fever. There was rise in mean microfilarial density (24.6 per 20 cub mm blood) during the fever.

e) Sera of 41 (70.7%) subjects showed reduction of IgG level during the fever, while the mean circulating immune complex level was increased (108.7 ± 83.6) during that period. The circulating filarial antigen was detected in higher percentage of subjects 37 (63.8%) during the fever episode as compared to samples collected prior to that.

f) This altered response probably indicates release of parasitic products during the fever and binding of antibodies to form immune-complexes. The study shows that there may be release of parasitic antigen/product which initiates febrile attack in filariasis. However, further evaluation of level of parasite-derived products may reveal if it has any direct role in pathogenesis of filarial fever.

### 4. Asymptomatic microhaematuria in a filaria endemic area.

Scientist : Dr. G. P. Chhotray

Technical staff : Mr. M. R. Ranjit
Mr. H. K. Khuntia

Starting date : October 1987

a) 320 presons, 213 males and 107 females, in the age group of 10 to 75 years were examined in a filaria endemic area near Bhubaneswar to find out the prevalence of asymptomatic microscopic haematuria.

b) Of the 320 persons whose morning midstream samples were examined, 71 were healthy persons, 77 persons had acute filarial symptoms, 122 persons had chronic filarial manifestations and 50 patients had only hydrocele.

c) Out of 320 persons on whom detail clinical examination was performed 79 persons (24.68%) had asymptomatic microscopic haematuria. The haematuria was further graded into four grades according to the no. of red blood cells seen in the average high power field when the sediments from a centrifuged specimen was examined microscopically.

d) The incidence of asymptomatic microhaematuria in the various groups of the study population is depicted in table 12. It is evident from the table that the incidence of microhaematuria of different grades is more frequent in chronic filarial disease, 47 (38.52%), as compared to endemic normals 3 (4.23%), Acute filarial disease 14 (18.18%), and only hydrocele cases 15 (30.0%).

e) Urinary lipid was found to be present (by Sudan III stain) in 72 cases (22.50%). 23 persons (29.11%) had lipids in urine along with microhaematuria.

f) The total estimation of urinary lipids was done in these cases by using modified gravimetric method (mean value 3.92 $\mu$g/10 ml).

g) Only 2 cases (0.62%) of frank chyluria was detected in the study population.

h) None of the cases of Asymptomatic microscopic haematuria detected had proteinurea, granular or hyaline casts or significant amount of puscells in urine to suspect any other pathologic lesion of the urinary tract. Only one person had a mild degree of Benign prostatic hyperplasia on per rectal examination.

i) 3 cases belonging to grade III & grade IV haematuria were subjected for special investigations like plain X-ray (abdomen), Intravenous pyelography & cystoscopy which did not reveal any abnormality in the genito-urinary system.

j) The serum sample from each person was collected and the antibody titre in different groups such as healthy normal (control), acute filariasis, chronic filariasis and those with only hydrocele, was measured by ELISA test using soluble mf-antigen and antihuman IgG, IgM, IgA HRP conjugate in 1/500 dilution and the results were compared with the antibody level in nontropical nonendemic sera (Fig. 1).

Table 12

Incidence of Asymptomatic-Microhaematuria in various Groups of Study Population

| Persons studied | Total (M/F) | Microhaematuria | | | | |
|---|---|---|---|---|---|---|
| | | Total (M/F) | Grade I 1-8 RBC/ HPF No. (%) | Grade II 8-30 RBC/ HPF No. (%) | Grade III 30-3/4th of field RBC/HPF No. (%) | Grade IV Full of RBC/HPF No. (%) |
| Endemic Normal | 71 (50/21) | 3 (2/1) (4.23%) | 3 (4.23) | 0 | 0 | 0 |
| Acute Filariasis | 77 (49/28) | 14 (8/6) (18.18%) | 10 (12.99) | 3 (3.90) | 1 (1.30) | 0 |
| Chronic Filariasis | 122 (64/58) | 47 (21/26) (38.52%) | 45 (36.89) | 1 (0.82) | 1 (0.82) | 0 |
| Only Hydrocele | 50 (50/0) | 15 (15/0) (30.00%) | 14 (28.00) | 0 | 0 | 1 (2.00) |
| Total | 320 (213/107) | 79 (46/33) (24.68%) | 72 (22.5) | 4 (1.25) | 2 (0.65) | 1 (0.31) |

M/F — (Male/Female)
HPF — High Power Field

k) It was observed that the antibody titre was low in patients having asymptomatic microscopic haematuria as compared to persons having no haematuria, although the antibody titre was high in all the groups studied as compared to the control group (non-endemic sera).

l) The total serum protein in all the groups studied with or without haematuria was raised when estimated by Biuret method.

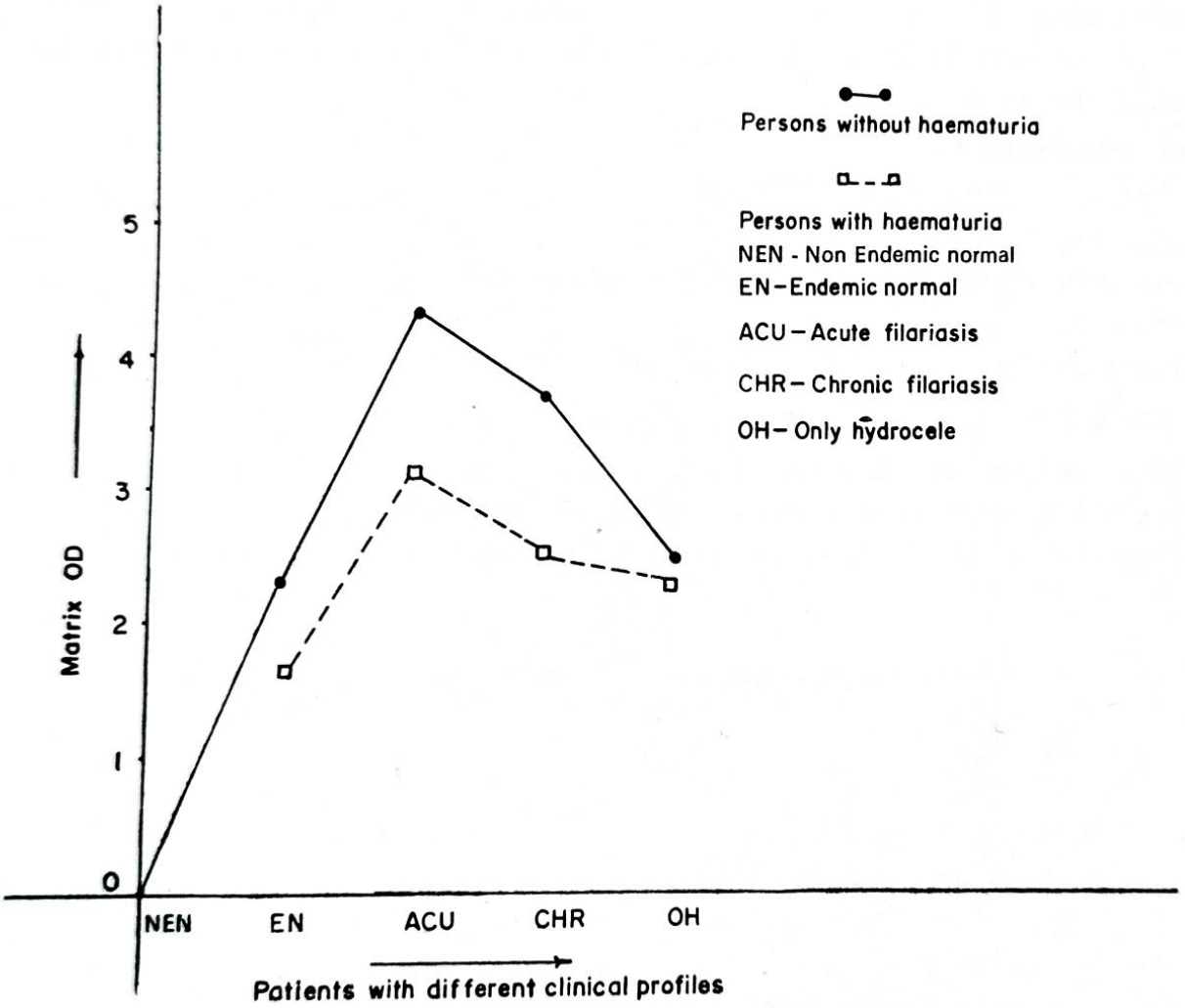
**5. Histopathological Study of genital lesions in bancroftian filariasis.**

Scientist : Dr. G. P. Chhotray

Technical staff : Mr. M. R. Ranjit
Mr. H. K. Khuntia

The pathology of genital involvement is limited almost exclusively to *W. bancrofti* infection. Genital lesions include funiculitis, epididymo-orchitis & hydrocele. Hydrocele is the most common genital manifestation of chronic bancroftian filariasis.

Fig.-1




In a study conducted at Pipili Primary Health Centre hospital 32 cases presented with hydrocele out of the 320 persons examined clinically. 2 of them had acute filarial manifestation with hydrocele, 3 of them had chronic filarial manifestations with hydrocele, 15 of them had no filarial manifestation and 12 of them had history of filarial attack in form of lymphangitis, funiculitis, fever etc. The hydroceles (unilateral or bilateral) were of varying duration (2-20 yrs) and of varying sizes.

27 of them in the age group of 20-53 yrs were operated upon at Pipili hospital and scrotal tissue (skin, subcutaneous tissue, hydrocele sac & tunica) were collected.

The tissues were preserved in 10% formal-dehyde and transported to RMRC laboratory. Hydrocele fluid from 12 patients was also collected and stored to measure the antibody titre.

Paraffin sections were prepared and stained with haematoxyline, Eosin and Masson's trichrome stain. Immunoperoxidase staining was also done by using rabbit antibody to $L_3$ of *W. bancrofti* in order to detect presence of filarial antigen in the tissue showing pathological changes.

**Gross examination**

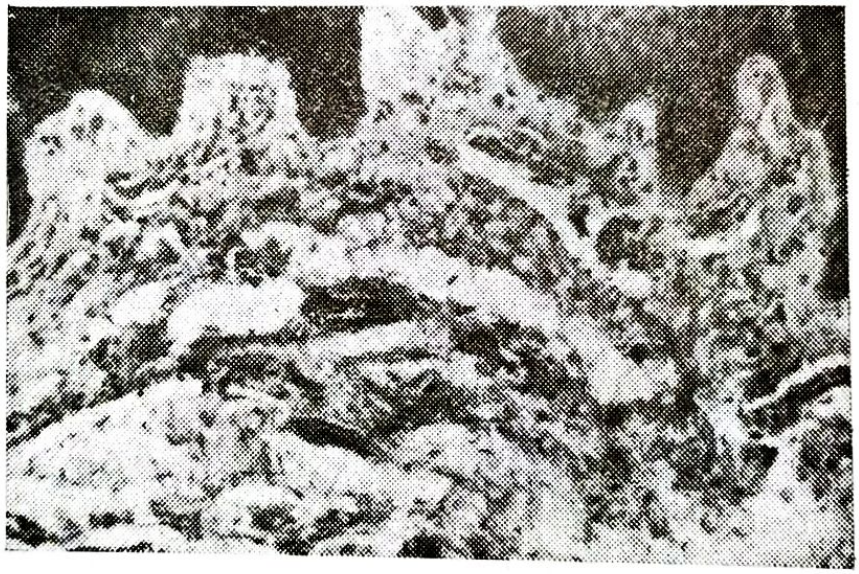
26 out of the 27 scrotal tissue examined had thick, corrugated skin & verrucose changes of epidermis over moist homogenous white tissue. In one case there was an abscess with discharging sinus tract. The hydrocele sac and tunica were grossly thickened and fibrosed.

**Microscopically**

a) **Skin & Subcutaneous tissue**

Microsections (H & E stain) revealed interlacing bundles of smooth muscles interspersed with loose connective tissue dilated lymphatics (in some cases), clusters of inflammatory cells, parivascular cuffing and disorganisation of muscle layer (Fig. 2).

Fig. 2

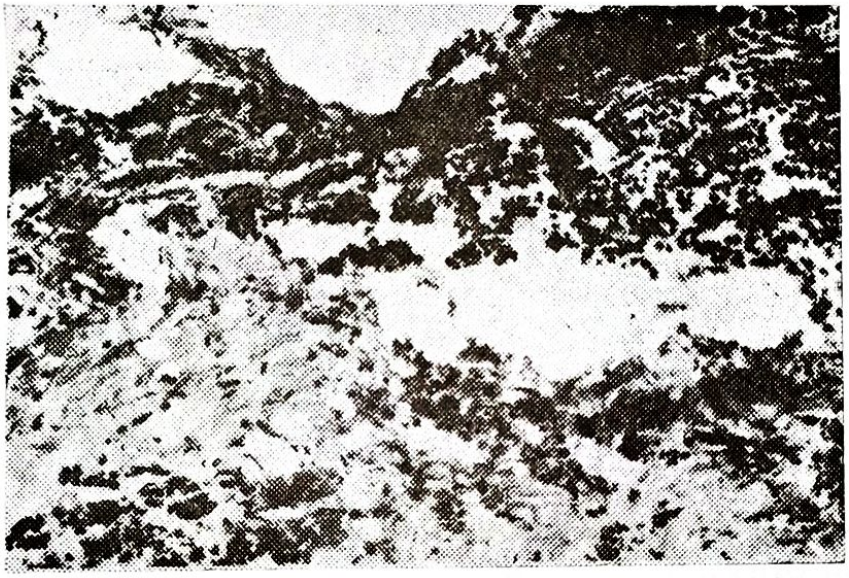


Skin & sub-cutaneous tissue (H & E stain 10 × 40 ×)
The microsection reveals skin, loose connective tissue with dilated lymphatics and cluster of inflammatory cells.

b) **Sac and tunica**

There was infiltration with chronic inflammatory cells like plasma cells & lymphocytes. Varying degree of fibrosis was also marked (fig 3). Masson's trichrome stain revealed increased amount of collagen fibers depending upon the duration of hydrocele.

Fig. 3



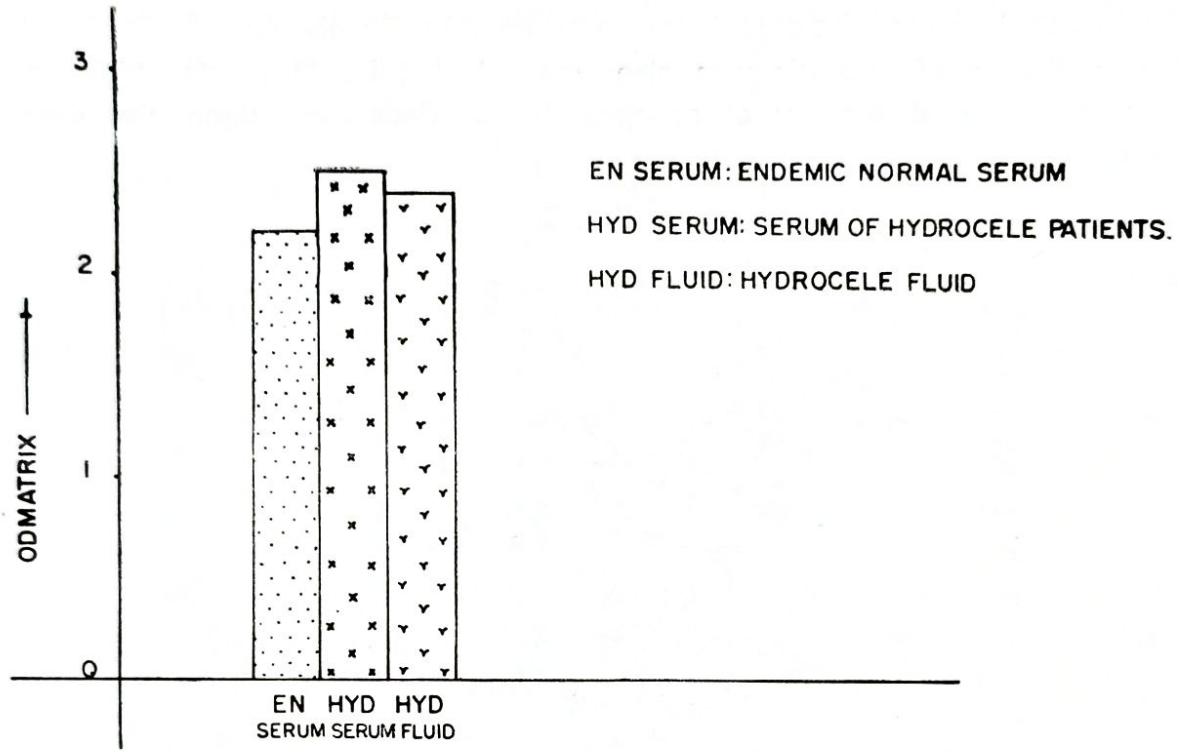
Sac & tunica (H & E stain 10 x 40 x)
Microsection showing infiltration with chronic inflammatory cells like lymphocytes & plasma cells.

c) Immunoperoxidase staining was also done by using rabbit antibody to $L_3$ of *W. bancrofti* which did not reveal presence of filarial antigen in the tissues examined.

d) The antifilarial antibody titre in the serum and hydrocele fluid of these patients was measured by ELISA using soluble mf-antigen and compared with that of the endemic normals. It was observed (fig 4) that the antibody titre in the serum of hydrocele patients is higher than that of endemic normals. The antibody level in hydrocele fluid was also high.

e) The hydrocele fluid was amber coloured and had plenty of lipids. Centrifuged deposits revealed vacuolated mesothelial cells. Microfilaria was not found in any of the samples.

Fig. 4




**6. Biology of Culicine mosquitoes in relation to the development of filarial parasite in Orissa.**

Scientists : Dr. A. P. Dash
Ms. Namita Tripathy

Period of study : October 1986 to August 1988

**Results :**

The detailed results have appeared in the previous Annual reports (1986-87 and 1987-88). The study carried out was on the development of *W. bancrofti* in *C. quinquefasciatus*. Laboratory-bred mosquitoes were fed on blood containing microfilaria (mf) in the laboratory by the membrane feeding technique and the detailed development of the mf in the mosquito species has been studied. The $L_1$, $L_2$ and $L_3$ appeared in the mosquito on the 2nd, 7th and 12th days respectively.

**Summary and conclusion :**

Bionomics and vectorial capacity of mosquitoes in Puri district, Orissa has been studied with reference to filariasis. Out of 18 species of mosquitoes encountered, *C. quinquefasciatus* was the dominant species followed by *C. tritaenorhynchus*. *C. quinquefasciatus* was found throughout the year with a peak in January. There is a significant relationship between *C. tritaeniorhynchus* and relative humidity. The anthropophilic index of *C. quinquefasciatus* and *C. tritaeniorhynchus* was 98 and 14% respectively. The gonotrophic cycle of *C. quinquefasciatus* was 96 hours at 25.5°C. The infection and infective rates of this species varied between 2.59 to 25 and 1.29 to 12.5% respectively, the latter being highest in January. This species is resistant to DDT. *Cardiofilaria* sp. was found to develop in *C. tritaeniorhynchus*. A key has been developed to identify the immature stages of both the species of mosquitoes and the Dyar's factor for all the stages have been determined. The development of *W. bancrofti* in *C. quinquefasciatus* has been studied and the distribution pattern of culicine mosquitoes in Orissa has been mapped.

**7. Study on outbreak of meningitis in Koraput district, Orissa.**

Scientists : Dr. S. K. Kar
Dr. G. P. Chhotray

Technical staff : Mr. M. R. Ranjit
Mr. H. K. Khuntia
Mr. T. Moharana

The team visited Laxmipur, Baipariguda, Khairput PHCs, Tikiri dispensary, Jeypore and Koraput civil hospitals to find out the possible aetiology of the outbreak of meningitis in the Koraput district. The available acute cases affected by the epidemic in the above mentioned PHCs, 56 convalescent cases who were discharged from the hospitals recently, epidemic records of various hospitals and dispensaries were examined to determine the course of the disease since onset.

The following observations were made by the team :

a) The epidemic started in February 1986 and affected 19 PHC of the district.

b) The incidence reached the peak during February 1988.

c) Males between 19-30 yrs of age belonging to Scheduled Castes/Tribes were more affected.

d) The observations made by the team confirmed the cases to be of bacterial meningitis because of the following features :—

i) acute onset with intense headache, vomiting, neck rigidity with loss of consciousness,

ii) positive kernig's sign and neck rigidity,

iii) examination of blood, total and differential WBC count revealed high leucocytosis with 70-90% neutrophils,

iv) lumbar puncture yielded turbid fluid with high pressure,

v) examination of CSF revealed large no. of neutrophils (mean count 700-3000/cumm),

vi) presence of Gram negative intracellular diplococci in the smears of CSF,

vii) table 13 shows the Ab titre in the serum and CSF of the affected persons.

Table 13

| Serial No. | Materials Tested | CIE | IHA | ELISA |
|---|---|---|---|---|
| 1. | SERUM | + | 1 : 8 | 0.41 (—) |
| 2. | SERUM | + | 1 : 32 | 0.63 (+) |
| 3. | SERUM | — | 1 : 2 | 0.45 (—) |
| 4. | SERUM | — | 1 : 16 | 0.60 (+) |
| 5. | SERUM | + | 1 : 2 | 0.64 (+) |
| 6. | SERUM | — | 1 : 8 | 0.58 (+) |
| 7. | SERUM | — | 1 : 8 | 0.53 (+) |
| 8. | CSF | — | 1 : 4 | 0.54 (+) |
| 9. | CSF | — | 1 : 4 | 1.08 (+) |
| 10. | CSF | — | 1 : 4 | 0.62 (+) |
| 11. | CSF | — | 1 : 16 | 0.41 (—) |

**Note :** CSF = cerebro spinal fluid
CIE = counter immuno electrophoresis
IHA = Indirect haemagglutination
ELISA = Enzyme linked immunosorbent assay.

This indicates the epidemic was due to the meningococcal meningitis spreading over a wide area in Koraput district. Since this form of meningitis occurs in sporadic form with slower development of herd immunity, this may continue for a few more years.

**8. Science Awareness Programme.**

Personnel : Dr. N. M. Pattnaik
Dr. V. R. Subramanyam
Sri Dasarathi Das
Sri Pramod K. Rana

Following the "Bharat Jan Vigyan Jatha" last year an "All India People's Science Network" was formed to further the spread of scientific temper and to catalyse a proper appreciation of scientific spirit among the general public and professionals alike.

This year the observance of a "Science Month" nationally under the auspices of the Network and "Srujanika" (a voluntary group formed by the scientists) undertook the programme in Orissa. The project was supported by the National Council for Science and Technology Communication, the Department of Science and Technology, Government of India.

The programme spanned from November 7, 1988 to March 3, 1989 and covered all of Orissa through slide/poster shows on "Nature, Science and Society" and "Fun with Science Activity Fairs". The birth centenaries of Prof. C. V. Raman and Pandit Jawaharlal Nehru, champions of scientific outlook, this year provided appropriate occasions and themes. It has generated several publications on popular themes including a magazine which will continue as a regular periodical.

## RESEARCH : SOME VIEW POINTS

*The ability to investigate systematically and truely all that comes under your observation in life.*

—Marcus Aurelius

*Scientific activity dedicated to discovery what makes grass green.*

—Russel Baker

*Not to find truth but to investigate and search after it.*

—Max Nordau

*The variety of teaching often tempts a man to forget he is a blockhead.*

—Lord Halifax

# New Projects Proposed for 1989-90

## 1. Studies of Ivermectin in the treatment of Bancroftian Filariasis in Orissa.

Scientist : Dr. S. K. Kar

The above study will be carried out at RMRC in collaboration with WHO/TDR with following objectives.

a) To determine the optimal dosage of Ivermectin effective for clearing *W. bancrofti* with fewest associated side reactions in patients from Puri district, Orissa.

b) To compare the efficiency, clinical safety and tolerability of Ivermectin with Diethycarbamazine-citrate (DEC-C) and placebo in patients with filarial infection caused by *W. bancrofti*.

**Summary :**

The present study has two parts—40 males from villages around Khurda, (Puri district) who are 18-50 years old and have microfilaraemia will be hospitalized for 10 days and given single oral dosage of Ivermectin (20, 50, 100 or 200 $\mu$g) per kg body wt. in double blind fashion. Microfilaraemia will be assessd at intervals for 6 months and the tolerability, safety and efficacy of each dosage will be compared in order to arrive at the optimal Ivermectin dose for treating the patients in the area. Since some reactions may be there following Ivermectin therapy, these have to be compared with that of DEC, in addition to the placebo required to observe back-ground reactivity. The duration of antimicrofilarial activity of Ivermectin has to be compared with DEC. Therefore double blind Ivermectin vs DEC and placebo is planned in the second phase of the study. Each part of the study will approximately take one year for completion.

## 2. Social mobilization for the control of filariasis.

Scientists : Dr. N. M. Pattnaik
Dr. V. R. Subramanyam

Although filariasis is a major disease of the world and in our country not enough efforts are being extended for its control, at least, in this region. While research emphasis is on newer diagnostic methods and drugs, few attempts are being made to find ways of implementing better the existing ones.

Based on our studies in this area we find that the problems of people's co-operation and acceptance can readily be overcome through community participation and education. The former is achievable by involving the local voluntary groups while the latter would be possible with the help of student volunteers and inter-personal communication. The already available shorter regimens of DEC, now under field trial in this region by us, will be used for reducing transmission while treating the clinical cases selectively.

We propose to extend our socio-economic survey over the entire Mendhasal PHC (Bhubaneswar block) and follow up through an awareness campaign. Mass therapy would then be started.

RMRC funding should be sufficient to meet the initial staff and operational requirements but an UNDP/WHO "Social and Economic Research" grant will be sought for continuation.

Staff requirements will be in the form of fellows, associates and a large number of student-fellows on short term assignment. These will include medical, science and humanities graduates as well as artists to develop communication materials. Emphasis has to be laid on flexible recruitment procedures since temperament and motivation rather than degrees and erudition are absolute requirements for this work. Moreover timely availability of men and material need not be emphasised.

### 3. Immunobiological studies on antibodies to Diethylcarbamazine—an approach for developing on immunoprophylactic agent against filarial parasites.

Scientists : Dr. B. Ravindran
Dr. N. M. Pattnaik

Our studies on cross-reactivity of antibodies to DEC with filarial parasites have indicated its possible use in *in vitro* elimination of microfilariae in infected hosts. It is proposed to investigate the role of different carriers and adjuvants for effective elicitation of anti-DEC antibodies in experimental animals. The effect of anti-DEC on the course of microfilaraemia in experimental animals will be studied, by active immunization and by passive transfer of affinity purified anti-DEC antibodies.

Details of the proposal will be submitted to the Department of Science and Technology, New Delhi for funding.

**4. Immunologic status of the patients with sickle cell anaemia.**

Scientists : Dr. G. P. Chhotray
Dr. B. Ravindran

Technical staff : Mr. M. R. Ranjit
Mr. H. K. Khuntia

The above mentioned project has already been approved by ICMR expert committee and the necessary financial grant has been requested for the project.

**Aims and Objectives of the project :**

a) To determine the proportion of *T. lymphocyte* and their functions in sickle cell anaemia.

b) To determine the proportion of *B. lymphocyte* and their function in sickle cell anaemia.

c) To study serum bactericidal & opsonising defect in sickle cell anaemia if any.

d) To quantify serum immunoglobulin IgG, IgM, IgA & $L_3$ by radial immunodiffusion method in sickle cell anaemia.

The project has been cleared by the ethical committee of this centre and the work has to be started this year.

**5. Studies on mosquitoes of Orissa with special reference to Filariasis and Malaria.**

Scientist : Dr. A. P. Dash

**Scientific background and justification :**

The 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 habitat, behaviourial status and vectorial capacity. 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 designing the control measures so that efforts are directed on the target species.

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 mosquitoes in the state and their susceptibility status which will help in adopting control measures.

This project was recommended and forwarded to the ICMR, on 25.10.1984, as advised by the Director, RMRC, Bhubaneswar. The same was again sent to the ICMR in six copies vide letter No. 394 dt. 11.2.85 of the RMRC (as advised by the ICMR by telex dt. 19.1.85). Again a reminder was sent to ICMR vide RMRC letter dt. 13.8.86 for communicating the decision. Since no action was taken by the ICMR in this regard, the detailed proposal was submitted in the 3rd Scientific Advisory Committee meeting on 7th July 1988. The SAC unanimously recommended the project strongly and also recommended to strengthen the entomology department (pages 4, 7 and 8 of the 3rd SAC minutes). The SAC advised to start this project immediately after recruitment of four insect collectors and one Technical Officer, already sanctioned for the centre. The recruitment of these sanctioned staff is still awaited due to want of funds and unfortunately the project could not be started in this year for obvious reasons, though the philosophy behind the project is highly laudable. It is hoped that this project will start in 1989-90 after recruitment of the above staff.

## 6. Control of Bancroftian filariasis in a selected area in Orissa state by integrated vector control, technologies chemotherapy and combination of both.

Scientist : Dr. A. P. Dash

Orissa is one state where not much concentrated efforts have been put in for demonstrating filariasis control through integrated methods. Since it is the function of the RMRC to carry out research on diseases and their control and since Orissa state is highly endemic for bancroftian filariasis, the first Scientific Advisory Committee met on 24.1.1987 advised to take up a control project. A detailed proposal was submitted and the second Scientific Advisory Committee which met on 4.9.1987 has accepted the objectives and feasibility of the proposal. As advised by the SAC, the local authorities (N. A. C.) of the area selected were contacted, who has assured to render all sorts of co-operation for integrated vector control. The Director, RMRC has moved the proposal to the Govt. of

Orissa and Govt. has given approval on 15.3 87. The proposal was submitted to the ICMR for funding. The 3rd SAC was held on 7.7.1988. Arising out of the minutes, there was discussion on the action taken in the matter as recommended by the second SAC. The Director informed the members that though the preliminary work in connection with this project had been done by the centre, the work could not be started because of lack of sanction for the staff and budget required for this purpose. He read out the letter received from the ICMR headquarters in this connection. All the members felt that this work should not be undertaken in a haphazard manner. It was decided by the SAC that this project should be submitted to the WHO for funding as a TDR project.

As advised and recommended by the last SAC, this project alongwith few other projects was submitted to ICMR for onward transmission to WHO. This project was forwarded to ICMR in proper proforma by the Director vide his letter No : DIR/RMRC/88/ 264 dt. 27.7.88. Though on the basis of the same SAC recommendations other projects were forwarded to the WHO, after the Joint Screening Committee in the last week of August 1988; this project was not listed at all for the said committee. After several correspondence during Oct./Nov. 1988, finally a letter was received from ICMR that this project could be started as an intra mural project since it could not be forwarded to WHO.

**7. Field evaluation of *B. sphaericus* formulations against mosquitoes.**

Scientist : Dr. A. P. Dash

As advised and recommended by the SAC, this project was also submitted to ICMR for onward transmission for TDR funding, since *B. sphaericus* has to be obtained from WHO. This project was sent to ICMR by the Director with the above project. In this connection, Dr. B. Dobrokhotov, Secretary, Steering Committee, Biological Control of Vectors, WHO, Geneva visited RMRC on 31.10.88, made a field survey in relation to this project, discussed with the local authorities in the field and also with the State Govt. high officials including the Hon'ble Minister of Health, Science, Technology and Industry about starting of this project in Orissa. The work was to be started after the visit of Dr. Dobrokhotov and sanction of funds from WHO (as per minutes of the meeting with the Govt. on 28.10.88). Unfortunately it was not listed in August 88 screening committee for forwarding to WHO and after several correspondence the ICMR sent a reply to start it as an intra-mural project since it was not forwarded to WHO like other projects as recommended by the RMRC, SAC. Dr. N. Rishikesh, Chief of the Biological Control of Vectors, WHO is visiting RMRC in this connection. It is hoped that the ICMR will reconsider it and forward the project to WHO in 1989-90.

## 8. Enzymology of mycobacterial mutants

Scientist : Dr. V. R. Subramanyam

Collaborator : Dr. P. R. Wheeler
(Department of Biochemistry,
University of Hull, U K.)

**Background information & justification**

Not much is known regarding the machinery for biosynthesis of amino acids and nucleotide bases in mycobacteria. It is reasonable to assume that the biosynthetic machinery in mycobacteria would be similar to that found in other well worked out bacteria like *Escherichia coli*. However, there must be some differences to explain certain unique features of mycobacteria (like for e.g. their slow growth rate). Hence it is pertinent to study the biosynthetic pathways in mycobacteria.

Recent findings that some mycobacterial enzymes are yeast-like (e.g. fatty acid synthase complex), while others are typically bacterial (e.g. B. lactamase) justify the relevance of studying mycobacterial enzymes.

The various auxotrophic mutants isolated in our laboratory and Dr. Wheeler's experience with mycobacterial metabolism would be very useful for the proposed study.

## 9. A clinical and microbiological study of diarrhoea in children.

Scientists : Dr. V. R. Subramanyam
Dr. L. N. Mohapatra

Collaborators : Dr. G. Mohanty (Paediatrician, Capital Hospital)
Dr. R. L. Broadhead (Paediatrician, Liverpool Sch. Trop. Med.)
Dr. C. A. Hart (Microbiologist, Liverpool Sch. Trop. Med).

**Background information and justification :**

Diarrhoeal diseases are among the ten major causes of death among children in developing countries. Among the research priorities of the W.H.O's "Diarrhoeal Disease Control Programme" are epidemiological studies to determine the clinical features and aetiology of diarrhoeal disease in different geographical areas.

Diarrhoea is a major problem encountered here, in Orissa, as in other parts of the country. But there has been no report of any systematic study conducted in Orissa for assessing the immensity of the problem or the prevalence and relative importance of different microbes in the aetiology of diarrhoeas.

Objectives of the proposed study are to seek information on—

a) the prevalence and seasonal variations in the occurrence of different microbes,

b) the pathogenic potential of the encountered microbes for causing diarrhoea,

c) the importance of socio-economic and nutritional status of the children *vis-a-vis* the incidence of diarrhoea.

d) the application of novel diagnostic tools like monoclonal antibodies and ELISA in rapid identification of the aetiologic agents,

e) the patterns of drug resistance in the bacterial isolates.

# Papers Published

1. Pilot Chemotherapy study on filarial lymphoedema. S. K. Kar, J. Mania and S. R. Nayak. *Progress in Lymphology*, Experta Medica International Series. Elsevier Publication p. 669, (1988).

2. Pathogenesis and Pathology of Filariasis. S. K. Kar. *Souvenir*, XXXVII Annual Conference of Indian Association of Pathologists and Microbiologists. p. 21, (1988), Calcutta.

3. Quantitation of antibodies to infective larvae in *Wuchereria bancrofti* filariasis. M. K. Das, A. P. Dash, B. Ravindran, N. M. Pattnaik and V. R. Subramanyam. *Acta Tropica* **45**, 387 (1988).

4. Detection of Infective larval antigen in bancroftian filariasis. M. K. Das, M. K. Beuria, A. P. Dash and N. N. Pattnaik. *Ind. J. Exp. Biol.*, **26**, 1003 (1988).

5. Immune complexes in *Wuchereria bancrofti* filariasis specific for phosphorylcholine and infective larval antigens. M. K. Das, V. R. Subramanyam, B. Ravindran and N. M. Pattnaik. *Med. Sci. Res.* **16**, 1055 (1988).

6. Bionomics and vectorial capacity of mosquitoes in Puri district, Orissa. A. P. Dash, N. Tripathy and R. K. Hazra. *Vectors and vector borne diseases*, **2** : 90-100 (1988).

7. Mosquito fauna of Chilika lake area. A. P. Dash, N. Tripathy and R. K. Hazra. *Proc. Nat. Symp. on Chilika*, pp. 58-62, (1988).

8. Prevalence of winter forms of *Anopheles annularis* in Orissa. A. P. Dash *et. al.* *J. Com. Dis.* **19** (4) : (1989) (in press).

9. Inducibility and stability of auxotrophic mutations in *Mycobacterium fortuitum*, *M. smegmatis* and *M. vaccae*. V. R. Subramanyam, B. B. Pal and K. K. Mohanty. *Lett. Appl. Microbiol* (1989) (in press).

10. Cryptosporidiosis in children of Eastern India. V. R. Subramanyam, R. L. Broadhead, B. B. Pal, J. B. Pati and G. Mohanty. *Annals Trop. Paed.* **9** (2), (1989).

11. Naturally occuring anti-galactosyl antibodies in human *P. falciparum* infections—a possible role for autoantibodies in Malaria. B. Ravindran, A. K. Satapathy and M. K. Das.
*Immunology Letters* **19**, 137-142, (1988).

12. Effect of aphid extract on the development of *Cx. quinquefasciatus* Say (Diptera : Culicidae). M. R. Ranjit, A. P. Dash and G. P. Chhotray.
*Vectors and vector borne diseases,* (1988), **2** : 186-188.

13. Oral application of insulin encapsulated liposomes. N. Das, M. K. Basu and M. K. Das. Biochem. International. **16**, 983 (1988).

14. Identification of larval instars in two species of *Culex*. N. Tripathy and A. P. Dash.
*Vectors and vector borne diseases,* **2** : 152-158, (1988).

15. Laboratory rearing of *Mansonia annulifera* (Theobald) using indigenous plants. N. Tripathy and A. P. Dash.
*Ibid,* **2** : 159-162, (1988).

16. Immune response of patients with filarial fever (Abstract). S. K. Kar and J Mania.
Souvenir, Joint Annual Conference of *Association of Physicians of India* p. 51 (1989).

## Papers submitted for publication

1. Incidence of erythrocytic G6-PD deficiencies in a malarial endemic tribal population of Orissa. G. P. Chhotray and M. R. Ranjit.
*Indian J. Med. Research.*

2. Prevalence of intestinal parasites amongst the school going children in a sub-urban community of Orissa. G. P. Chhotray and M. R. Ranjit.
*Indian J. Med. Research.*

3. Medical Mycology in India, an overview. L. N. Mohapatra.
*Indian J. Med. Research,* (Platinum Jubilee Year 1989).

# VISITORS TO REGIONAL MEDICAL RESEARCH CENTRE

## BHUBANESWAR

1. 31.10.88 : Dr. Boris Dobrokhotov
   Secretary, Steering Committee of the Scientific Working Group on Biological Control of Vectors
   W. H. O., Geneva.

2. 01.11.88 : Dr. P. Ranque
   Chief, Filariasis
   W. H. O., Geneva.

3. 01.11.88 : Dr. C. P. Ramachandran
   Secretary, Steering Committee on Filariasis
   W. H. O., Geneva.

4. 21-23.12.88 : Prof. R. Ananthanarayan
   Retd. Principal & Professor of Microbiology, Trivandrum, and Chairman of the Project Review Committee, I.C.M.R.

5. 27.02.89 : Dr. A. K. Chakravorty
   Prof. of Immunology & Cell Biology, Centre of Life Sciences University of North Bengal.

## SCIENTIFIC CONFERENCES/WORKSHOPS/SEMINARS ATTENDED

| Name of the Scientist | Scientific conferences/workshops/seminars attended with date | Papers presented/ Lecture delivered |
|---|---|---|
| Dr. L. N. Mohapatra | Ispat Hospital, Bhilai Steel Plant 13.4.88 14.4.88 | *Guest lectures* Opportunistic Infections. Immunity in Enteric fever. Infection control. (in hospital). |
| | State level seminar on "Review of our National achievements in the field of Health during last 40 years", Bhubaneswar 27.9.88 | Guest speaker (medical research) |
| | Continuing Medical Education, National Academy of Medical Sciences, Cuttack, 15.11.88 | Chaired the scientific session |
| | Seminar on Blood Banking, Cuttack, 25.11.88 | Lecture on Viral Hepatitis & AIDS. |
| | 4th Annual conference of the Association of Physiologists, Cuttack 10.12.88 | Guest speaker |
| | IAPM Annual Conference, Calcutta, 19.12.88 | Moderator on "Nosocimial infection" |
| | Annual meeting of IMA, Balasore, 12.3.89 | Guest lecture on "mycotic infections" |
| | Silver Jubilee Celebration, MKCG Medical College, Berhampur, 26.3.88 | Guest speaker on "medical education-the needs for a change" |
| Dr. M.K. Das | 57th Annual Conference of Society of Biological Chemists (India), CSIR Centre for Biochemicals, Delhi University October 9-12, 1988 | "Role of immunomodulators in cellular immune response to *Plasmodium berghei* antigens" |
| Dr. Shantanu K. Kar | XXXVIIth Annual Conference of the Indian Association of Pathologists and Microbiologists, Calcutta, December 18, 1988 | Pathogenesis and Pathology of filariasis |
| | XLIV Joint Annual conference of the Association Physicians of India, January 19-25, 1989. | "Immune response of patients with filarial fever. |

| Name of the Scientist | Scientific conferences/workshops/seminars attended with date | Papers Presented/ Lecture delivered |
|---|---|---|
| Dr. A.P. Dash | Symposium on 'A significant advance in Vector Control with special reference to malaria' New Delhi, November 21, 1988 | Discussion on vector control |
| | 'National Symposium on Entomology' Haffkine Institute, Bombay, March 25, 1989 | Guest lecture on ''Role of mosquitoes in transmission of filariasis in man and animals in India'' |
| Dr. N. M. Pattnaik | National symposium on the Development of Indigenous Drugs in India during the last 25 years, New Delhi, April 8-10, 1988. | ''Lessons for Traditional Medicinal Research from the Management of filariasis'' |
| | Society of Biological Chemists (India), 57th Annual Meeting, New Delhi, October 9-12, 1988 | ' Microfilaricidal Protein from Insect Haemolymph'' |
| Dr. B. Ravindran | Annual Conference of the Society of Biological Chemists, New Delhi, October 9-12, 1988 | (i) ''Antibodies with reactivity to Diethylcarbamazine in human filarial sera react with *W. bancrofti* microfilariae'' |
| | | (ii) ''*P. berghei* malaria in mouse—A role for autoantibodies with specificity to N-Acetyl neuraminic acid'' |
| Dr. V.R. Subramanyam | Association of Microbiologists of India, 29th Annual Conference at Hisar, February 9-11, 1988 | (i) Inter and intra-genus cross feeding for characterising auxotrophic mutants of mycobacteria |
| | | (ii) Species differences in susceptibility to NTG-mutagenesis of Mycobacterium. |

| Name of the Scientist | Scientific conferences/workshops/seminars attended with date | Papers presented/ Lecture delivered |
|---|---|---|
| Dr. G.P. Chhotray | Workshop on meningitis at Christian Hospital, Bisam Cuttack (organised by Orissa Voluntary Health Association). 9th October 1988 | Clinico-pathological aspects of meningitis. |
| | Annual conference of Orissa Chapter IAPM, Berhampur, 13th November 1988 | Prevalence of intestinal parasites amongst the school going children in a sub urban community of Orissa |
| | XXVIIth Annual IAPM Conference, Calcutta, December 17-20, 1988 | Effects of antihelminthic/antiprotozoal treatment on the nutritional status of school going children in a sub urban community of Orissa, India |
| | **Other activities :** | |
| Dr. A. P. Dash | Three popular scientific lectures on the National Science Day, in the All India Radio.<br>1. The world of mosquitoes<br>2. Mosquitoes & Filariasis<br>3. Mosquito coils | |
| Dr. N. M. Patnaik | Faculty member at the "Biochemical Education for Students and Teachers (BEST)" Workshop, Society of Biological Chemists (India), New Delhi, October 8-9, 1988. | |
| | Resource Person for the "Teaching Aids" workshop, at the "State Level Science Fair", State Council for Educational Research and Training (SCERT), Jajpur Road, January 17-18, 1989. | |
| | Resource person for the "Work Experience Camp on Science Toys/Teaching Aids" at the Eastern India Science Camp. National Council for Science Museums (NCSM) and State Council for Educational Research and Training (SCERT), Bhubaneswar, February 16-18,1989 | |

## Ph. D. Programme under Utkal University

| Sl. No. | Name of the Candidate | Topic (Thesis) | Name of the guide |
|---|---|---|---|
| 1. | Mr. Prasanta Kumar Kar | Antigenic presentation of stage specific antigens of *W. bancrofti* and *B. malayi* and their immune response in filariasis. | Dr. Shantanu K. Kar |
| 2. | Mr. Dasarathi Das | Microfilaricidals from plants : Biochemical studies on the active components of *Streblus asper*. | Dr. N. M. Pattnaik |
| 3. | Mr. Pramod Kumar Rana | Characterisation of insect haemolymph components with particular reference to their action on *W. bancrofti* microfilariae. | Dr. N. M. Pattnaik |
| 4. | Mr. A. K. Satapathy | Studies on Host-parasite interactions : role of carbohydrate determinants in mammalian Malaria. | Dr. B. Ravindran |
| 5. | Ms. Tahaziba Hussain | Immunobiological studies on antibodies to Diethylcarbamazine with reference to filariasis. | Dr. B. Ravindran |
| 6. | Mr. M. R. Ranjit | Effect of acetone extracts from aphids on the development of mosquitoes. | Dr. A. P. Dash |
| 7. | Ms. N. Tripathy | Biology of *Culex* mosquitoes in relation to the development of *W. bancrofti*. | Dr. A. P. Dash |
| 8. | Mr. R. K. Hazra | Mansonoid mosquitoes and *B. malayi* in Orissa. | Dr. A. P. Dash |

| Sl. No. | Name of the Candidate | Topic (Thesis) | Name of the guide |
|---|---|---|---|
| 9. | Ms. K. K. Mohanty | Characterization of filarial antigens in bancroftian filariasis. | Dr. V. R. Subramanyam |
| 10. | Mr. Mihir K. Beuria | Role of adjuvants in the immune response of filarial and malarial antigens. | Dr. M. K. Das |
| 11. | Ms. Anindita Mishra | Studies on the enhancement of immune response to parasitic antigens following oral administration of immunopotentiators. | Dr. M. K. Das |
| 12. | Dr. D. K. Panda | Immunobiological diagnosis of filariasis. | Dr. L. N. Mohapatra |

# SCIENTIFIC ADVISORY COMMITTEE

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AR-37

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| | R.O. | Dr. S. S. S. Mohapatra, M.B.B.S. |
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| **Pathology** | S.R.O. | Dr. G. P. Chhotray, M.D. |
| **Statistics** | S.R.O. | Mr. Anil Kumar, M.Sc., M. Phil., D.C.P. |

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