REGIONAL MEDICAL RESEARCH CENTRE

BHUBANESWAR

ANNUAL REPORT

1987-88

REGIONAL MEDICAL RESEARCH CENTRE

(INDIAN COUNCIL OF MEDICAL RESEARCH)

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ANNUAL REPORT
(1.4.87 to 31.3.88)

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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 in time.

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Preface

The RMRC, Bhubaneswar has addressed itself to regional health problems since its inception. Studies on human lymphatic filariasis are continued with a multi-disciplinary approach including the entomological aspects. The work on control of malaria vectors in the Koraput district has been taken over by the V.C.R.C. The parasitological aspects and drug resistance of *P. falciparum* the main aetiologic agent of malaria in that area are constantly being monitored by the RMRC research officer posted there, under the Director, V.C.R.C. Other important regional health problems like, G-6-PD deficiency, intestinal protozoal & helminthic infections etc. have also received attention during the year under report. A separate unit established at the V.S.S. Medical College, Burla to study the Sickle Cell disease in a comprehensive manner has started functioning, though the full complement of senior staff could not be recruited as yet.

As reported in the previous year, the work done in the field studies as well as in the laboratory is presented under "projects in hand" which are to continue in the next year and "projects completed" with results and conclusions. A few new proposals are also listed under "proposed projects" giving in brief the plan of work.

The RMRC Campus which has now expanded with addition of ten acres of land requires to be developed. The construction of the main laboratory building is in progress and will, hopefully, be over by the end of the current year. A set of garages have been constructed in this campus for the vehicles. A separate water supply line to the campus has been laid, though it is yet to be commissioned. The plan for the construction of a laboratory animal house in the campus is awaiting sanction of the council.

The year under report has been eventful. Most of the scientists attended the national scientific conferences and Dr. Shantanu K. Kar attended the International Conference of Lymphology at Vienna. Several scientific staff from this centre actively participated in the "Bharat Vigyan Jatha" programme to promote the development of scientific temper in the country. Dr. Santosh K. Kar submitted his resignation which was accepted by the D.G. w.e.f. 1/8/87 (FN). Dr. S. Jayachandran has joined this centre on 23rd Feb. 88 as a Deputy Director on transfer from V.C.R.C., Pondicherry. The Administrative Officer, Sri P. Prabhakar Rao was transferred to the Epidemiology Unit at Tiruvellur (under the TRC, Madras). Recruitment to several senior scientists posts was held up because of the ban imposed. It is hoped that these vacant posts will be filled up soon to boost the scientific activities at this centre.

The S.A.C. of the centre met on 4th September 1987 under the Chairmanship of Dr. R. G. Roy. All members attended the meeting where the scientists presented their work. The members gave valuable suggestions and guidance for the benefit of the research staff. Suggestions were also made to strengthen some sections where expertise is available and take up field demonstration work as pilot projects to prove the success of the strategy adopted by the centre for control of communicable diseases, filariasis in particular. The visit by the team under the auspices of the British Council was exploratory in nature for close collaboration between this centre and overseas development programme of United Kingdom.

Bhubaneswar
April 15, 1988

(Prof. L. N. Mohapatra)
Director

Global estimates for lymphatic filariasis :

(WHO 1984)

Number of persons living in countries where the disease is endemic :		*2677 million*
Number of persons living in endemic areas where transmission is known to occur and who are "at risk" of infection.		*905 million*
Number of persons infected with :		
Wuchereria bancrofti	*81.6 million*	
Brugia malayi and B. timori	*8.6 million*	
Total number of persons infected		*90.2 million*

Projects in Hand on 31.3.1988

A : FILARIASIS

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

Scientist : Dr. Shantanu Kumar Kar

Technical staff : Miss Jayanti Mania
Mr. K. Dhal
Mr. T. Moharana
Mr. R. N. Nayak
Mr. S. C. Rout

Starting date : November 1985

Progress :

a) Only 95 subjects out of 114 asymptomatic microfilaria carriers co-operated during 2nd year follow-up study for the clinical assessment, measurement of antibody titre (Filaria specific IgG, IgM, IgA) by ELISA, circulating immune complex (PEG-precipitation method) and peripheral eosinophil level.

b) The majority; i e. 88 (92.6%) carriers showed continuity in their microfilaraemic status and only 3 subjects developed early manifestation of filarial disease.

c) No significant alteration in their mean eosinophil level and circulating immune complex level were observed.

d) The filaria specific antibody titre of the carriers during second year of the follow up study revealed that 40 (42.1%), 34 (35.8%) and 17 (17.9%) subjects were positive for IgG, IgM & IgA respectively.

Justification for continuation :

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

A-2. Study of arthritis with relation to filariasis in filarial endemic area.

Scientist : Dr. Shantanu Kumar Kar

Technical staff : Miss Jayanti Mania
Mr. K. Dhal
Mr. T. Moharana
Mr. R. N. Nayak
Mr. S. C. Rout

Starting date : March 1986

Progress :

a) Screening of the population of villages endemic for filariasis revealed 90 individuals with features of arthritis not pertaining to any specific aetiology, as seen by clinical examination (Rheumatoid, tuberculosis, syphilis, osteoarthritis, sepsis).

b) Out of 90 study subjects with features of arthritis, 13 (14.4%) were found to be microfilaraemic.

c) The mean circulating immune complex level in blood (By PEG-precipitation method) in study group was higher (0.124 ± 0.077) than that of 58 endemic normals (0.098 ± 0.01) tested for the same.

d) The C-reactive protein was tested in 12 patients out of which 3 showed positive results. Not a single control serum in 10 endemic normals was found to be positive.

e) The positive filaria specific antibody titre for IgG, IgM and IgA were demonstrated in 46 (51.1%), 27 (30.0%) and 16 (17.8%) subjects in study group and 37(63.7%), 36 (62.0%) and 21 (36.0%) subjects in endemic normals respectively.

Justification for continuation :

To carry out synovial membrane biopsy for study of immune complex deposits (by immunoperoxidase and immunofluorescence method) and histopathological study for evaluating the filarial aetiology. Approximately 25 cases of arthritis of various known aetiology (from Hospitals) will be studied for comparison. Few reports have shown the association of filariasis with arthritis in endemic areas, where filarial aetiology is not proved. Hence attempts need to be made to examine cases of nonspecific arthritis prevalent in endemic areas and to study in depth its association with filarial aetiology.

A-3. Clinico-pathological study of lymphatic nodules in human filariasis.

Scientists	:	Dr. Shantanu Kumar Kar
		Dr. G. P. Chhotray
Technical staff	:	Miss Jayanti Mania
		Mr. K. Dhal
		Mr. T. Moharana
		Mr. R. N. Nayak
		Mr. S. C. Rout
Starting date	:	March 1986

Progress :

a) Lymphatic nodules were detected in 183 subjects in filarial endemic population. They were studied for clinical manifestation, microfilaraemic status, peripheral eosinophil level, filaria specific antibody titre (IgG, IgM & IgA) by ELISA and circulating immune complex level (by PEG-precipitation).

b) Age and sex matched 48 subjects with chronic filariasis (as controls) were also tested similarly.

c) Out of 183 subjects, 60 (32. 8%) cases with nodules were found to be microfilaraemic.

d) The mean circulating immune complex level was lower (0.094 ± 0.059) in study group as compared to that of 48 chronic cases of filariasis (0.119 ± 0.072), tested for the same.

e) The positive antibody titre for IgG, IgM and IgA were demonstrated in 76 (41.5%), 43 (23.5%) and 32 (17.5%) study subjects respectively. The sera of 48 chronic cases of filariasis, showed positive antibody titres in 35 (72%), 13 (27%) and 5 (10%) cases for IgG, IgM and IgA respectively.

Justification for continuation :

Biopsy of the nodules from the subjects will be taken after facilities are available at this centre and the material will be subjected to histopathological examination. It is relevant to know whether the group of individuals manifesting with nodules in filariasis is different from endemic normals or the groups with obstructive pathology in regard to their immune responses.

A-4. Sciatica like syndrome in Filariasis.

Scientist	:	Dr. Shantanu Kumar Kar
Technical staff	:	Miss Jayanti Mania
		Mr. K. Dhal
		Mr. R.N. Nayak
		Mr. T. Moharana
		Mr. S.C. Rout
Collaborator	:	Prof. R.N. Sahu
		Dept. of Neurology
		S.C.B. Medical College
		Cuttack
Starting date	:	October 1987

Progress :

a) Seventy patients with neuralgic pain pertaining to lower limb and/or upper limb for six months or more were detected in filaria endemic population and subjected to detailed clinical, parasitological & immunological examination.

b) The mean eosinophil percent was found to be 10.8 $\pm$ 5.3.

c) Fifteen (21.4%) out of seventy study subjects were microfilaraemic.

d) The study of subjects showing positive filaria specific antibody titre (IgG, IgM & IgA by ELISA) were 35 (50%), 15 (21.4%) & 11 (15.7%) respectively as compared to that of 58 endemic normals, i.e. 37 (63 7%), 36 (62.0%) and 21 (36.0%) positive for the above antibody response.

e) The mean circulating immune complex level (by PEG-precipitation) of study subjects was higher (0.119 $\pm$ 0.051) than that of endemic normals (0.098 $\pm$ 0.01).

f) Sera from these subjects are stored for detection of circulating antigen.

g) Tender subcutaneous nodules (1.5-2.0 cm dia) were detected in all these subjects approximately 2'' lateral to mid vertebral line at L_{3-4} level. In few cases where upper limb was affected, such tender nodules were observed at interscapular area on the same side.

Justification for continuation :

The above nodules will be detected in ultrasound and re-examined for its regression or dis appearance after DEC therapy. Detailed neurological investigation (EMG, Nerve conduction, Nerve biopsy) will be carried out in few of these subjects in collaboration with Dept. of Neurology, S.C.B. Medical College. It is relevant to understand this atypical manifestation and to ascertain the filarial aetiology; to study whether nerve is involved in the pathogenesis of filariasis.

A-5. Chemotherapy of Filariasis : Comparative study on efficacy of DEC in different dosage schedule.

Scientist	:	Dr. Shantanu Kumar Kar
Technical staff	:	Miss Jayanti Mania
		Mr. K. Dhal
		Mr. T. Moharana
		Mr. R. N. Nayak
		Mr. S.C. Rout
Starting date	:	January 1988

The pilot chemotherapy study undertaken in a small group of cases of grade II & III filarial lymphoedema revealed that long term "high dose" DEC administration along with conservative physiotherapy have beneficial effect in reducing filarial lymphoedema of long duration. Out of the various dosage schedule of DEC (6 mg/kg body wt/day, 8 mg/kg, 10 mg/kg, 12 mg/kg body wt administered intermittently for 4 months) with or without steroid, it was observed that 10 mg/kg body wt dosage of DEC intermittently for above period with conservative physiotherapy has shown better result in reversing lymphoedematous state. Although the beneficial role of above DEC regimen was apparent in reducing the size of hydrocele (reduction of size of enlarged testecle, and thickened scrotal skin) this could not be evaluated systematically. Hence to evaluate the response of DEC regimen & the additive effect of conservative physiotherapy in a large number of cases with grade II & III lymphoedema and hydrocele, the above study was initiated.

Progress :

a) The subjects for the study were selected from an endemic village (Taraboi) after clinical and parasitological examinations.

b) The subjects with grade II & III lymphoedema and hydroceles were recorded.

c) The subjects are grouped according to the grade of lymphoedema or presence of hydrocele. Age sex matched subjects with similar manifestations of lymphoedema and/or hydrocele were taken as controls.

The result of previous pilot chemotherapy study carried out in 81 cases of grade II & III lymphoedema and 24 controls (age sex matched) after 4 months of therapy is presented below :

a) The study group received DEC 10 mg/kg body wt/day for 3 weeks every month for 4 months along with conservative therapy and controls (with grade II & III lymphoedema) received 6mg/kg body wt/day for 3 weeks repeated every third month of DEC + conservative therapy.

b) Periodic evaluation at intake phase and subsequently, every month was carried out for :

   i) Limb circumference
   ii) Tissue compressability
   iii) Filaria specific IgG, titre
   iv) Circulating immune complex determination
   v) Liver function tests like serum protein, albumin, SGOT, SGPT, ALK Phos, cholesterol to assess any liver toxicity
   vi) Detailed clinical evaluation in both study and control cases.

c) Significant reduction of limb circumference; was observed in study cases as compared to that of controls after 4 months of therapy (Table No. 1).

d) Initial fall and subsequent rise of antibody titre was observed in significant ($P < 0.05$) percentage of study cases. The reversal of above pattern was observed in circulating immune complex level possibly due to release of antigen into circulation and binding of antibodies to form complexes (Figure 1).

e) The liver function test values of both study and control cases were within normal range as observed during periodic blood examination while continuing chemotherapy.

f) The patient reported relief of heaviness of limb, reduction of pain and oedema. The side reactions were within tolerable limits and did not warrant stoppage of therapy.

g) The pilot chemotherapy study has shown significant improvement in lymphoedematous state without much side reactions or apparent damage to the host. It can be postulated that long term DEC therapy may have beneficial role in reversing lymphoedematous state.

Table 1

The effect of therapy on lower limb circumference/Tissue resistance during the period of treatment. All values (CM) are the average differences of affected limb over normal limb (n=81)

Duration of Therapy (months)		0	1	2	3	4
Upper Part of Leg	Circumference	5.115	3.365	2.596	2.058	1.907*
	Tonometry	0.807	0.638	0.636	0.633	0.632**
Lower Part of Leg	Circumference	0.963	6.611	5.759	5.463	5.352*
	Tonometry	0.890	0.889	0.827	0.709	0.667**
Foot	Circumference	4.222	3.241	2.736	2.204	2.130*
	Tonometry	1.170	0.941	0.888	0.867	0.785**

* Significant ($p < 0.01$, t test)

** Not Significant ($p > 0.05$)

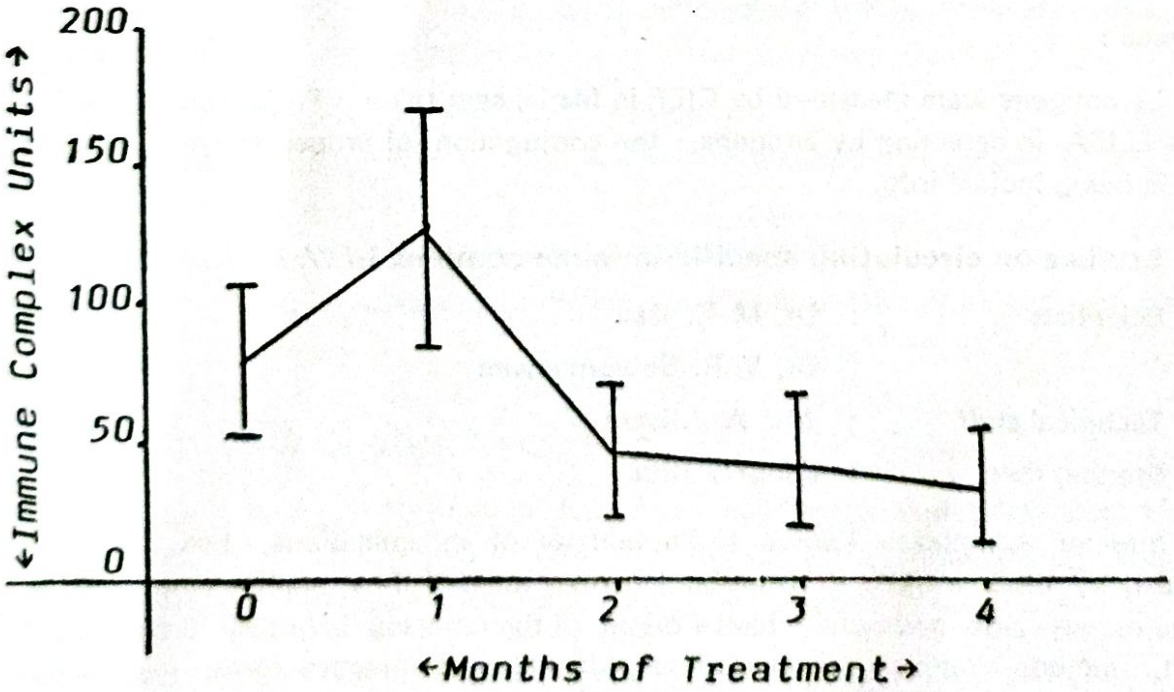


Fig. 1 : Circulating Immune Complex Level (Average Units ± 1 S. E.) Before and During Chemotherapy (n=81).

Justification for continuation :

The DEC regimen and conservative physiotherapy will be used in cases and controls in various dosage in a double blind fashion so as to facilitate the proper evaluation. The periodic evaluation of limb circumference, tissue tonometry, skin thickness, immunological status and drug toxicity will be carried out.

The above study taken up in large number of cases in controlled fashion will highlight the efficacy of the prolonged high dose DEC and the conservative therapy in treatment of chronic manifestations of filariasis.

A-6. Development of antigen detection assays in human filariasis.

Scientist : Dr. M. K. Das
Technical staff : Mr. M. K. Beuria
Starting date : July 1985

Detection of parasite specific antigen in the blood of the host is regarded as an useful indicator of active infection. In this project, attempts are made to measure microfilarial or L_3 antigens of *Wuchereria bancrofti* by various immunological technique. like CIEP or ELISA.

Progress :

L_3 antigens were measured by CIEP in filarial sera (vide "Project completed" No. 2). To use ELISA in detecting L_3 antigens, the conjugation of proper enzymes witn anti-L_3 serum is being looked into.

A-7. Studies on circulating specific immune complex in *W. bancrofti* filariasis.

Scientists : Dr. M. K. Das
Dr. V. R. Subramanyam
Technical staff : Ms. A. Mishra
Starting date : January 1986

Immune complexes, known to be initiator of inflammation, has been measured previously by other workers in filariasis. However most of these studies employed methods that are non-specific and hence filarial origin of the complex is largely unknown. In this project, immune complex specific to microfilarial and infective larval (L_3) antigens of *W. bancrofti* is being quantitated. Attempts will also be made to measure immune complex

specific to a defined antigen namely phosphorlycholine (PC). Recently PC antigens are detected in the circulation of filarial patients and also are secreted in culture media from the parasites *in vitro*. No studies have been reported so far to measure PC-containing complex. A detailed study of the immune complex correlating with disease status will be conducted.

Progress :

Hyperimmune anti-L_3 serum was prepared in rabbits. A mouse myeloma protein HOPC-8 (IgA), specific to PC determinant, was a gift from Dr. C. P. J. Glaudemans, NIH, USA. These two reagents are used to quantitate the specific circulating immune complexes (vide "projects completed" No. 3). IgE-specific complexes are being measured at present.

A-8. Culture of *W. bancrofti* microfilariae.

Scientist	:	Dr. N. M. Pattnaik
Technical staff	:	Mr. Dasarathi Das
		Mr. Prasant K. Kar
		Mr. Pramod K. Rana
		Mr. Hari Shankar Nayak
Starting date	:	September 1985

Incidental to the main objectives, i.e. development of the microfilariae (mf) in culture into infective larvae, the other aspects are :

i) Purification and viable cryopreservation of the mf.
ii) Maintenance in serum-free medium for the production of excretory—secretory (E-S) material.
iii) Screening for growth promoters and microfilaricidals.
iv) Preparation of mf antigens of various types and their use for production of antibodies.

Progress :

a) **Purification and preservation :**

Consequent to the availability of Nuclepore membranes with pore sizes of 3 to 14 μ a method has been standardised where viable mf are routinely purified nearly free of cell debris and in quantitative yields. The method is suitable for sterile operations.

The method of choice for cryopreservation has been through the use of serum as a preservative for the first time. Briefly, purified mf are incubated in a suitable medium (see

below) at 37°C for about 30 min. and equal volume of autologous serum is added. Aliquotes of the suspension are kept in vials which are then allowed to freeze slowly in well insulated styrofoam containers in a 80°C deep freeze. After about 24 hr. the vials are transferred to liquid nitrogen for long term storage. Upon rapid thawing and warming to 37°C viable mf are recovered. These then are used as such or washed free of serum if necessary.

b) **Maintenance :**

Available animal cell culture media were screened and Libovitz L-15 medium was found to be most suitable closely followed by Medium 199. For routine maintenance at 37°C for 3 to 4 days and for other short term work L-15 is now used.

Grace's insect tissue culture medium mixed with L-15 or 199 helps in keeping the mf viable longer in culture. Attempts are on for preparing a composite medium after identifying the essential ingredients in these media.

c) **Development :**

Three categories of nutrient media are now being tried for compatibility with mf viability :

i) Bacteriological media

ii) animal cell culture media

iii) insect tissue culture media

The bacteriological nutrients including broths, malt extract, tryptone, peptone, casitone and yeast extract were ineffective in keeping the mf alive. Even in combination with animal cell culture media the mf could not survive beyond 24 h. In malt extract and tryptone the mf were rapidly killed suggesting the presence of a toxic component.

The best results so far have been obtained with a 1 : 1 mixture of L-15 and Grace's media as well as of 199 and Grace's. In both the cases the mf remain viable for 10 to 12 days at 30°C and show morphological changes characteristic of development into first stage larvae. The frequency or the extent of these changes, however, were not consistent or pronounced.

Use of growth supplements, e.g. serum, along with the above basal medium is being tried out at present.

The protein-free culture supernatant, however, continues to be a rich source of E-S material for use in the study of immune response (see reports of Dr. M.K. Das and Dr. B. Ravindran).

d) **Antigen :**

Purified mf has been the major source of antigens for our immunochemical studies including the studies with anti-sheath and anti-DEC antibodies (see report of Dr. B. Ravindran). Besides mechanical homogenisation of mf two selective extraction methods are being used for the preparation of soluble antigens. The latter two are :

i) extraction of the mf with hot (80°C) 0.1% triton X-100 for 10 min and

ii) solubilisation of the mf with hot (80°C) 1N NaOH for 10 min.

While the triton extraction does not disrupt the mf structure the NaOH treatment leads to the total dissolution of the cuticle. Both, however, show reactivity against specific antibodies and are useful reagents for ELISA tests.

Biochemical characterization of these antigens are currently under way. Preliminary results of SDS-PAGE analysis by silver staining show bands corresponding to :

i) a very high Mw

ii) serum albumin and

iii) Mw of about 14,000

The first, presumed to be collagen with reference to standards and digestion with collagenase, predominates in the triton and NaOH preparations along with a minor albumin like band. In E-S preparations, however, the 14,000 Mw band appears consistently along with the collagen like band but the albumin like band is variable.

e) **Antibodies :**

Polyclonal anti-mf antibodies have thus far been raised in rabbits using mechanically homogenised or NaOH solubilised mf as antigens. An attempt is under way to produce mouse monoclonal antibodies using live mf (and their metabolic products in the peritonium) as well as NaOH solubilised mf through the hybridoma technique. Myeloma line SP 2/0 has been adapted to this laboratory and mice immunised in preparation for the fusion.

Justification for continuation :

To date no headway has been made elsewhere on the development of mf in culture. In view of the importance of the problem, difficulties notwithstanding, efforts must continue. These efforts, in any way, produce results and materials useful to many other projects and should therefore, continue.

A-9. Characterisation of some plant microfilaricidals.

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

This work is an off-shoot of the epidemiological study in collaboration with the Clinical Research Unit (Homoeopathy), Puri, when certain Homoeopathic drugs were tested for their action towards microfilariae in culture. These drugs as mother tinctures are aqueous-alcoholic extracts of (i) plants, (ii) animal products or, (iii) minerals. After removing the alcohol the residues are taken up in aqueous buffers and incubated (37°C) with cultures of mf in serum-free medium at various concentrations. The motility of the mf is monitored at various time points to judge the microfilaricidal properties. Similarly, lysis of red blood cells by these drugs are monitored by measuring the released haemoglobin spectrophotometrically.

Progress

Of the twenty drugs in the three categories above, three plant products *Hydrocotile asiatica* (Hydrocotile) *Rhus toxicodendron* (Rhus tox) and Alfalfa showed strong mf-killing and rbc-lytic properties. Similarly two drugs of animal origin-*Apis mellifeca* and *Blatta orientalis* were very effective in both.

The hydrocotile and Rhus tox extracts were chosen for further characterisation in the first instance. By differential extraction of the dried residues with organic solvents of increasing polarity (Hexane-chloroform-methanol) various fractions were prepared. The least polar fraction showed the maximum killing and lytic effect in both the drugs. This was confirmed by elution from preparative thin layer chromatograms. At present fractionation in a preparative scale using silicic acid columns is under way as a prelude to chemical characterisation.

Justification for continuation :

This is in keeping with the global search for effective antifilarials/microfilaricidals and would also be useful in understanding the mechanisms of action of these traditional remedies.

A-10. Role of Insect Haemolymph in microfilarial development in culture.

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

In view of the development of mf in the mosquito body it is natural to expect that the insects haemolymph killed the mf rapidly in culture. These were also lytic for rbc. This was reminiscent of toxic peptides reported in other insect haemolymphs, e.g. secropins, which have been postulated to be rudiments of an immune system. It was, therefore, of interest to isolate and characterise the putative toxic component of the haemolymph (cockroach) to study (i) its role and mode of action and (ii) the ability of the "detoxified" haemolymph to support mf growth.

The whole haemolymph when analysed on SDS-PAGE shows two major protein bands corresponding to about 90,000 and 200,000 Mw besides several minor ones. On gel permeation column using sephadex G-100 the toxic components elutes with the major protein peak at void volume.

Justification for continuation :

This work will provide an insight into the defence machanism in insects and may possibly yield a factor for mf growth.

A-11. Public Health perspective of filariasis & Evaluation of alternate medicines.

Scientists : Dr. N.M. Pattnaik
Dr. V.R. Subramanyam
Technical staff : Sri Dasarathi Das
Sri Prasanta K. Kar
Sri Pramod K. Rana
Sri Hari Sankar Nayak
Starting date : February 1986

While a lot of effort goes into searching for newer diagnostic methods and drugs, practically no attempt is made to use the available antifilarials on a wide scale or to evaluate various traditional lines of treatment already in use. The field work, therefore, is designed to study some of these problems in addition to collecting materials for

laboratory investigations, in particular (i) assessing the work of a filaria clinic at one of the primary health centres and (ii) evaluating the efficacy of Homoeopathic treatment for filariasis.

Progress :

It has been noted that the PHC filaria survey team manages to collect night blood smears from about fifty percent of the study population but only 30% of those found to be microfilariae positive take DEC. This hardly makes any impact on the transmission of the disease. Major reasons for not taking the medicine appears to be it not being distributed in the village itself besides the side reactions associated with this drug.

The villages of Beleswarpatna and Beldal near Puri were surveyed for filariasis (Table 2) and Homoeopathic treatment provided by the "Clinical Research Unit (Homoeopathy) for Filariasis and Malaria", Puri, in the village Beldal. The villagers of Beleswarpatna did not accept this treatment because of personal preferences and thus acted as the control group. The microfilariae carriers (both asymptomatic and clinical cases) were specially targeted for this study and were followed up periodically over two years. Results on microfilariae densities presented in Table 3 indicate the absence of any effect of the drugs used towards clearing the circulating mf. A small sample of the clinical cases suggests a similar lack of impact on the treatment in controlling the acute attacks. A clear picture is expected to emerge when all the clinical cases are assessed during follow up.

Justification for continuation :

The cases need to be monitored for a longer period before a definite clinical assessment can be made.

A-12. Detection and characterization of anti-sheath antibodies in Bancroftian Filariasis.

Scientists	:	Dr. B. Ravindran
		Dr. N.M. Pattnaik
		Dr. M.K. Das
		Dr. V.R. Subramanyam
Technical staff	:	Mr. Ashok K. Satpathy
		Miss. T. Hussain
		Mr. Prakash K. Sahoo
Starting date	:	October 1986

Table 2

Profile of filariasis in the study populations (Feb/March 1986)

	Numbers (Percentages)	
	Beleswarpatna	Beldal
Examined	353	383
Mf carrires	51 (14.5)	58 (15.1)
Clinical cases	141 (40)	168 (43.9)
Mf carriers with disease	25 (7.1)	31 (8.1)

Table 3

Dynamics of microfilaremia (Feb/March 1986 to March 1988)

Population		Microfilaria density (No. of carriers examined)					
		Feb-March 1986	Sept. 1986	Jan. 1987	May 1987	Dec. 1987	March 1988
Beleswarpatna (Control untreated)		53.5 (54)	—	—	—	28.5 (48)	—
Beldal	Total	55.5	42.8	33.4	31.5	—	20.2
	Placebo	(56)	(38)	(50)	(42)		(44)
	Medicine	35.5	26.2	16	7.6	—	9.7
		(23)	(13)	(20)	(14)		(17)
		68	51.5	45	43.4	—	26.8
		(33)	(25)	(30)	(28)		(27)

Antibodies to the microfilarial sheath have been clearly shown to play an important role in the immune opsonization and elimination of circulating microfilaria in human and animal filariasis. Their role in Bancroftian Filariasis however has not been studied in detail. The present investigation was initiated to quantify the various immunoglobulin isotypes in the different clinical spectra of Bancroftian Filariasis and further to analyse the specific determinants on the sheath that are recognized by anti-sheath antibodies.

Progress :

a) Using a polyspecific anti-globulin reagent anti-sheath antibodies were detected by an indirect immunofluorescent assay and immunoperoxidase assay. An inverse relationship was demonstrated between microfilaraemia and presence of anti-sheath antibodies (for details see RMRC Annual Report 1986-87)

b) Since nearly 80% of chronic filariasis cases and endemic normals had demonstrable anti-sheath antibodies, further analysis for specific detection of IgG and IgM antibodies to microfilarial sheath was undertaken. (The detailed results are shown in Table 4).

Table 4

Distribution of IgG and IgM Antisheath antibodies in Chronic Filariasis and Endemic Normals*

Clinical status	Number (%) Positive for					
	Both IgG & IgM	IgG	IgM	IgG only	IgG or IgM	IgM only
Chronic Filariasis n=33	10 (33.3)	21 (63.6)	10 (33.3)	11 (33%)	21 (63.6)	-O-
Endemic Normals n=44	11 (25)	20 (45.4)	13 (29.5)	9 (20%)	22 (50)	2 (4%)

* Tested at a serum dilution of 1 : 16 by Indirect Immunoperoxidase assay.

c) IgE antibodies to microfilarial sheath could be detected by indirect immunoflurescent assay in about 25-33% of Chronic Filariasis cases and endemic normals. About 5% of asymptomatic carriers had demonstrable anti-sheath antibodies of IgE class.

Part of the results on anti-sheath antibodies have been published in the Journal of Tropical Medicine and Hygiene. Further studies are in progress to detect and characterize various IgG sub-class with reactivity to microfilarial sheath.

A-13. Studies on antibodies with reactivity to Diethylcarbamazine.

Scientists	:	Dr. B. Ravindran
		Dr. N. M. Pattnaik
Technical staff	:	Mr. Ashok K. Satapathy
		Miss T. Hussain
		Mr. Prakash K. Sahoo
Starting date	:	July 1985

To raise antibodies to DEC that could be used for developing an immuno-assay for measurement of the drug in body fluids and to use it as a tool in investigations on the mode of action of DEC against filarial parasites.

Progress :

a) DEC hydrolysis product, 4-methyl piperazine-1-carboxylic acid (MPCA) was coupled to BSA to raise antibodies to DEC. Details are given in RMRC Annual Report 1986-87 and published in Medical Science Research (see under publications).

b) Further it was observed that anti-DEC raised in rabbits would cross-react with the microfilarial sheath of *Wuchereria bancrofti*. Table 5 shows the anti-DEC and anti-mf titres of the various rabbit sera. Anti-DEC was found to react only with microfilarial sheath and not to the cuticle. There was no reaction between anti-DEC and the microfilarial surface of two animal filarial parasites *Dirofilaria immitis* and *Litomosoides carinii*. Preliminary observations however indicate that anti-DEC would react with microfilariae of *Brugia malayi*. Part of the above observations have been published in Immunology Letters (see under publications).

c) Since anti-DEC reactivity to microfilariae indicated a possible similarity or overlap of antigenic determinants between the drug and the parasite, it was decided to look for antibodies with reactivity to DEC in different clinical spectra of Filariasis. About 46% of endemic normals, 43.7% of chronic filariasis cases, 60% of cases of tropical pulmonary

eosinophilia and 26% of asymptomatic microfilariae carriers had significantly elevated titres of anti-DEC as shown by ELISA. Predominant anti-DEC antibody activity was found in the IgG class only with very little or no activity in IgM and IgA class antibodies.

Further work is in progress to affinity purify anti-DEC antibodies in human filarial sera for characterization.

Table 5

Antibody titres in immunized rabbit sera.[a]

Rabbit No.	Material used[b]	Batch	Anti-DEC titre[c]	Anti-mf titre[c]	
			ELISA	IFA	IPA
R-45	MPCA-BSA	I	6400	8	NT
R-46	MPCA-BSA	I	12800	64	64
R-305	MPCA-BSA	I	12800	16	8
R-901	MPCA-BSA	I	25600	8	8
R-546	MPCA-BSA	II	25600	32	32
R-718	MPCA-liposomes		<50	NR	NR
R-719	MPCA-liposomes		3200	4	4
R-715	PQ-BSA		<50	NR	NR
R-716	PQ-BSA		<50	NR	NR

a Sera collected between 30-40 days after immunization were used for the assays.

b All the animals except R-718 received the respective conjugates mixed with CFA.

c Reciprocal of titre.

NT=not tested; NR=non-reactive.

B. MALARIA

B-1. Induction of cell mediated immunity in malaria by Immunomodulators.

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

Technical staff : Mr. M. K. Beuria

Starting date : June 1986

Role of cell mediated immunity in malaria is poorly understood, although it is known that non-antibody dependent immunity has a protective role in malaria infection. Various immunomodulators are being assessed in order to check their efficacy in inducing CMI to malarial antigens. Experimental protocol involves measurements of DTH, MIF and products of activated macrophages.

Progress :

DTH responses to *Plasmodium berghei* antigens as induced by the adjuvants were measured in Balb/c mice ("Project completed" No. 4). The other parameters like MIF etc. are being investigated.

B-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. Prakash K. Sahoo

Starting date : March 1986

Anti-erythrocytic autoantibodies in sera of malaria infected patients have been demonstrated, although their precise specificities are not known. Autoantibodies with a distinct α-galactosyl specificity (anti-gal) that constitutes nearly 1% of the circulating IgG in every human serum has been described. They have been incriminated in the physiological process of opsonization and elimination of senescent erythrocytes from circulation. Carbohydrate residues particularly terminal α-linked galactose determinants have been identified in the asexual blood stage antigens of *P. falciparum*. Hence an attempt is being made in the present study to investigate the status of anti-gal antibodies in human sera vis-a-vis exposure to *P. falciparum* infection.

Progress :

a) A cell-ELISA was developed by us for quantification of anti-gal titres in human sera (for details see RMRC Annual Report, 1986-87).

b) Significantly elevated levels of anti-gal were seen in 38.2% of *P. falciparum* endemic sera and in 51.2% of sera collected from patients with acute *P. falciparum* infections. Patients who had cerebral complications had anti-gal titres comparable to non-endemic control samples.

c) Qualitatively there was difference between infected and control sera samples. IgG 3 was the predominant antibody with anti-gal activity in *P. falciparum* infected patients while IgG 2 was the predominant sub-type in control samples. These results clearly indicate a role for IgG 3 anti-gal antibodies in *P. falciparum* malaria.

The details of the above investigation have been communicated for publication.

B-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
Starting date	:	March 1986

Malarial parasites by virtue of their intracellular localization in erythrocytes interact with macrophages in many organs. Parasitized erythrocytes have been shown to express a number of carbohydrate determinants on their surface. Endogenous lectins and specific antibodies play an important role in the interaction between parasitized erythrocytes and macrophages. The present study is aimed at analysing the factors involved in such interactions.

Progress :

a) A cell-ELISA was developed initially to quantify opsonizing antibodies (to *P. berghei* infected erythrocytes) in the mouse sera.

b) Antibodies to the surface of parasitized erythrocytes were found to be elevated 5-6 fold during the peak parasitaemic phase.

c) Antibodies to the surface of normal mouse erythrocytes and human O group erythrocytes were also elevated in infected mice sera.

d) Cross-absorption studies revealed that the above antibodies were recognising a common determinant present on normal and parasitized mouse erythrocytes and on human O group erythrocytes.

e) Most of the antibody was found to be IgG in nature while IgM and IgA class also contributed to the activity.

f) The surface determinant on the parasitized erythrocyte was found to be resistant to heat, sodium periodate and lipase treatment but were sensitive to trypsin, chymotrypsin and neuraminidase treatment. The antibody activity could be competitively inhibited by preincubation of the sera with N-Acetyl neuraminic acid. These results indicated the specificity of the autoantibody to sialic acid determinants present on parasitized and normal mouse erythrocytes.

Further studies are in progress to affinity purify the autoantibody and to study its role in phagocytosis of *P. berghei* infected erythrocytes by macrophages.

B-4. Studies on malaria & its control.

Scientist : Dr. S.S.S. Mahapatra

Starting date : November 1986

It is under the Vector Control Research Centre through the Officer-in-Charge, VCRC Field Station, Jeypore. The studies are continuing on (i) the causes of persistance of malaria in this area (ii) the prevalence of malaria and other diseases among the Bonda tribes (iii) the prevalence and incidence of malaria in Malkangiri (iv) the drug sensitivity status of *P. falciparum.*

C. CLINICAL PATHOLOGY

C-1. A study on erythrocytic G-6-PD deficiency in a malarial endemic tribal population of Orissa.

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

Progress :

a) Three hundred fifteen persons were examined for Glucose-6-phosphate dehydrogenase deficiency in the Banspal PHC area situated about 28 kms from Keonjhar district headquarters which is predominantly inhabited by tribal population and is highly endemic for malaria.

b) Various haematological investigations were performed (Table 6) showing rationale of investigation.

c) The study population included 315 persons, 252 males and 63 females of the age group ranging from 5-60 years. 188 were Scheduled tribe people (Mainly Kolha & Bhuyan), 52 were Scheduled caste people (Mainly Patra & Dasa) and 75 were non-scheduled caste people (Khandayat and Brahmin etc).

d) The overall incidence of G-6-PD deficiency was found to be 28.88 percent (91 cases). (Table 7).

e) Intermediate G-6-PD deficiency was detected in 57 (18.09%) of cases and severe G-6-PD deficiency in 34 (10.79%) of cases.

f) The over-all incidence of G-6-PD deficiency in the Scheduled tribe population was found to be 34.04% which is more than the incidence in Scheduled caste population i.e. 25% and Non-Scheduled caste population 18.66%.

g) Out of the 315 samples studied malarial parasite (all *P. falciparum*) was present only in 48 smears.

h) Of the 91 G-6-PD deficiency cases studied malaria parasite was positive only in 12 (13.18%) of cases (Table 8).

Table 6

RATIONALE OF INVESTIGATION

Sample	Anticoagulant Used for Preservation	Tests Performed	Method	Reference
Blood Collected by Venepuncture in Disposible Syringe	EDTA (1.5mg/1 ml)	a) Hb Estimation	Cynomethaemoglobin Method	Wintrobe—1981
		b) TLC	Visual Method	Dacie—1980
		c) PCV	Wintrobe's Method	Dacie—1980
		d) Osmotic Fragility	Saline Concentration Method	Whitby & Brilton —1969
		e) TRBC	Visual Method	Dacie—1980
	ACD (1 ml/5 ml)	a) Haemolysate Preparation	—	Wintrobe—1981
		b) Starch-Agarose Gel Electrophoresis	—	Dash & Dash et al—1976
		c) Mr Test for G-6-PD Deficiency	—	Brewer et al—1962
		d) Hbf Estimation	Alkali Denaturation Method	Singer, K. et al—1951
	Direct	a) Sickling Test	Sodium Metabisulphite Method	Dacie—1980
		b) Differential Count	—	Wintrobo—1981
		c) Malaria Parasite	From this smear by Wright's stain 3% Giemsa stain	—

Table 7

Incidence of G-6-PD Deficiency in Studied Population

Caste	Cases Studied			No of Cases of G-6-PD Deficiency			No of Cases of G-6-PD Detected		Incidence in %
	Male	Female	Total	Male	Female	Total	Moderate	Severe	
Scheduled Tribe	157	31	188	52	12	64	40 (21.28)	24 (12.76)	34.04
Scheduled Caste	38	14	52	10	3	13	7 (13.46)	6 (11.54)	25.00
Higher Caste	57	18	75	8	6	14	10	4	18.66
Total :	252	63	315	70	21	91	57 (18.09)	34 (10.79)	28.88

The total population in the study area =67,237 out of which Scheduled Tribe population= 47,664, Scheduled Caste population=2,698 & Higher Caste population=16,865.

By applying the test of significance for difference of proportions (Large samples) It is found that :

1. G-6-PD deficiency is significantly more in Scheduled Tribe in comparison with Higher Caste at $0.02 > p > 0.01$ but is not significantly more in Scheduled Tribe in comparision with Scheduled Caste.

2. G-6-PD deficiency is significantly more in Scheduled Tribe in comparison with non tribes (i.e. Higher Caste and Scheduled Caste) at $0.02 > p > 0.01$.

Abbreviations : G-6-PD=Glucose 6 phosphate dehydrogenase

The numbers in parentheses indicates percentage.

Table 8

Correlation of Malaria with G-6-PD Deficiency in the Studied Population

	Malaria +ve	Malaria-ve	Total
G-6-PD Positive	12	79	91
	(13.18)	(86.82)	
G-6-PD Negative	36	188	224
	(16.07)	(83.92)	
Total :	48	267	315
	(15.23)	(84.76)	

By applying $\times^2$ —test of significance it is found that malaria & G-6-PD are independent ($p > 0.5$).
The numbers in paraentheses indicate percentage.

i) Not a single case of Haemoglobinopathy or Sickle Cell disease, was detected amongst the studied population.

Justification for continuation :

Since the incidence detected is quite high the following further detail studies are indicated, such as :

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

ii) Characterisation of G-6-PD variants : Efforts are being made for a collaborative study with Prof. Dr. Lucio Luzzatto of Haematology Dept., Royal Post-graduate Medical School, Hammersmith Hospital, London who has offered his help for characterising G-6-PD deficiency variants by analysing at DNA level.

C-2. Unexplained asymptomatic micro-haematuria in a Filarial endemic population of Orissa.

Scientist : Dr. G. P. Chhotray
Technical staff : Mr. M. R. Ranjit
Mr. H. K. Khuntia
Starting date : October 1987

Progress :

a) Two hundred persons, 132 male & 68 females in the age group of 30 to 70 years were examined at Pipili Hospital, about 20 km away from Bhubaneswar which is a filarial endemic area, to find out the prevalence of unexplained asymptomatic microhaematuria in urine.

b) Of the 200 persons whose morning midstream urine samples were examined, 31 persons were endemic normals, 47 people had acute filarial symptoms, 92 persons had chronic filarial manifestations and 30 patients had only hydrocele.

c) Detail clinical examination was performed in all these persons (recording of Blood Pressure, history of any urinary sign & symptoms, per-rectal examination in case of males, Gynaecological/Obstetric history in case of females, sign & symptoms of filarial disease etc. were recorded).

d) The midstream morning urine samples of these patients were collected in sterile plastic bottles supplied to them earlier and both macroscopic & microscopic examination was performed at the RMRC laboratory within 3 hrs of collection. A special stain sudan III was used to detect the urinary lipids. Estimation of total urinary lipids was also performed. The results are as follows :

e) Of the 200 persons examined 66 persons (33%) had asymptomatic microscopic heamaturia. The microscopic heamaturia was further graded into four grades according to the no. of cells seen in the average high power field when the sediment from a centrifuged specimen was examined microscopically.

Microhaematuria grade I — 1-8 erythrocytes/HPF
grade II — 8-30 erythrocytes/HPF
grade III — 30-3/4th of the field
grade IV — entire field is packed with erythrocytes.

f) The incidence of microhamaturia according to age & sex is shown in Table 9. It has been observed that 66 cases (33.03%) of the studied population had asymptomatic microhaematuria in urine, of which 35 (53.03%) were male and 31 (46.97%) were female .

g) The incidence of asymptomatic microhaematuria in the various groups of the studied population is depicted in Table 10. It is evident from Table 10 that the incidence of microhaematuria of different grades is more frequent in chronic filarial disease, 39 cases (42.39%) in comparis on to endemic normals, 3 cases (9.67%), acute filariasis 12 cases

Table 9

Incidence of Microhaematuria according to Age & Sex

Age in Years	Total No. (%)	Male No. (%)	Female No. (%)
<30	24 (12.00)	12 (50.00)	12 (50.00)
31—40	14 (7.00)	6 (42.85)	8 (57.14)
41—50	11 (5.50)	5 (45.45)	6 (54.54)
51—60	10 (5.00)	8 (80.00)	2 (20.00)
61—70	6 (3.00)	4 (66.6)	2 (33.33)
70 or more	1 (0.5)	0 (0.00)	1(100.00)
Total	66 (33.00)	35 (53.03)	31 (46.96)

Youngest : 10 years
Oldest : 75 years
Mean : 35.65 years

Table 10

Incidence of Microhaematuria & Filarial Diseases

Persons Studied	Total M/F	Microhaematuria				
		Total M/F	Grade I No. (%)	Grade II No. (%)	Grade III No. (%)	Grade IV No. (%)
Endemic Normal	31 (20/11)	3 (2/1)	3 (9.67)	0	0	0
Acute Filariasis	47 (29/18)	12 (6/6)	8 (17.02)	3 (6.38)	1 (2.12)	0
Chronic Filariasis	92 (44/48)	39 (15/24)	37 (40.2)	1 (1.09)	1 (1.09)	0
Only Hydrocele	30 (29/1)	12 (12/0)	11 (36.6)	0	0	1 (3.3)

Grade I = 1-8 RBC/HPF
Grade II = 8-30 RBC/HPF
Grade III = 30-3/4th of HPF
Grade IV = HPF full of RBC

(25.5%) and only hydrocele 12 cases (40.0%). Various grades of microhaematuria has been observed in acute filariasis, chronic filariasis & hydrocele cases only, whereas in endemic normals 3 persons (9.67%) had grade I microhaematuria.

h) Only 37 cases (18.5%) out of 200 urine samples examined revealed presence of lipids. 14 persons (21.2%) had lipids in urine along with microhaematuria out the 66 cases of asymptomatic microhaematuria detected.

i) The total estimation of urinary lipids was done in these cases by using modified gravimetric method (mean value 3.92 μg/10ml).

j) Only 2 cases of frank chyluria (1%) was detected in the study population.

k) None of the cases of asymptomatic microscopic haematuria detected had Proteinuria, granular or hyaline casts or significant amount of puscells in urine to suspect any pathologic lesion of the urinary tract.

Justification for continuation :

Coastal Orissa is an endemic home for lymphatic filariasis. It's prevalence is found to be more than 20%. Chyluria (lymph in urine) and haematuria (RBC in urine) are important manifestations of lymphatic filariasis caused by *W. bancrofti* and *B. malayi*. Although the prevalence of filariasis is very high yet frank chyluria and haematuria cases are infrequent. Their incidence is not exactly known. It is believed that frank chyluria and haematuria occur in a very late stage of the filarial disease although asymptomatic microhaematuria of various grades and lipids in urine persist from the early onset of the disease and gradually progress to cause frank haematuria and chyluria.

This warrants a detailed study in the filarial endemic area. Although we lack in detailed urological investigation facilities like intravenous pyelography, cystoscopy, urethroscopy and retrograde pyelography etc. to exclude other possible associated causes of microhaematuria, yet a pathologic significance of asymptomatic microscopic haematuria can be established and correlated with the filarial disease process.

C-3. Histopathological study of genital lesions in bancroftian filariasis.

Scientist : Dr. G.P. Chhotray

Technical staff : Mr. M.R. Ranjit

Mr. H.K. Khuntia

Starting date : December 1987

AR-36

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.

In order to study the histopathological changes occuring in the scrotal tissue and to establish the cause of hydrocele as filarial origin the work has been envisaged.

Progress :

In a study conducted at Pipili, an endemic area for bancroftian filariasis, 30 cases presented with only hydrocele without any other chronic filarial manifestation, out of the 200 persons examined so far. All of them were negative for microfilaria in blood.

27 of them in the age group of 20 to 53 years were operated upon at Pipili hospital and scrotal tissue (skin, subcutaneous tissue, hydrocele sac & tunica) and hydrocele fluid were collected. The tissueswere preserved in 10% formaldelyde and transported to RMRC laboratory.

Paraffin sections were prepared and stained with Haematoxyline & Eosine stain, & Masson's trichrome stain. Immuno Peroxidase staining was also done by using rabbit anti-body to L_3 of *W. bancrofti* in order to detect presence of filarial antigen in the tissue evoking pathological changes.

The hydroceles were unilateral to bilateral of varying duration (2 to 20 yrs) and of varying sizes.

Gross Examination :

Most of the scrotal tissue examined had thick, corrugated skin and verrucous changes of epidermis over moist homogeneous white tissue.

Microscopically H & E stain :

In most of the micro-sections there were interlacing bundles of smooth muscles interspersed with loose connective tissue, some of them containing dilated lymphatics, cluster of inflammatory cells, perivascular cuffing and disorganisation of muscle layer.

The hydrocele sac and tunica were grossly thickened and fibrosed. Microscopically there was increased collagenous tissue infiltrated with chronic inflammatory cells like plasma cells and lymphocytes. Varying degree of fibrosis was also marked.

Masson's trichrome stain revealed increased amount of collagen fibers depending upon the duration of hydrocele.

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

Justification for continuation :

To study the histopathological changes occuring in various tissues in bancroftian filariasis.

Efforts are being made for a collaborative study with Institute of Pathology. (ICMR), Delhi.

Filariasis is of great public health importance in India. The disease is prevalent in all the States except those of Western and some far Eastern regions. Heavily infected areas are Uttar Pradesh, Bihar, Andhra Pradesh, Orissa, Tamil Nadu and Kerala. About 236 million people are estimated to be living in known filarious zones, of which 62 million live in urban areas and 174 million in rural areas. About 18 million persons are estimated to harbour microfilariae and 14 million persons with filarial disease. Of the three types of filaria infection found in India : (a) nocturnally periodic W. bancrofti is widely prevalent, it constitutes 97.8 per cent of cases. (b) B. malayi has a localised distribution, especially in the rural areas. The largest single endemic tract of B. malayi infection is along the coast of central part of Kerala covering Quilon, Alleppey, Kottayam, Ernaculum and Trichur districts. Small pockets of this infection are known to exist in Andhra Pradesh, Assam, Orissa, Madhya Pradesh and West Bengal. (c) The diurnal subperiodic W. bancrofti is found exclusively in Nicobar group of Islands, covering about 20,000 population.

D : ENTOMOLOGY :

D-1. Biology of Culicine mosquitoes in relation to the development of filaria parasites.

Scientist : Dr. A.P. Dash

Technical staff : Ms. N. Tripathy
Mr. R.K. Hazra
Mr. H.K. Tripathy
Mr. G.D. Mansingh

Starting date : October 1986

Progress :

a) **Identification of larval instars in two species of *Culex* (Diptera : Culicidae) :**

Studies on different instars and changes which the larva undergoes in the course of its growth are necessary in larval biology. Two species of *Culex* i.e. *Culex quinquefasciatus* Say and *Culex tritaeniorhynchus* Giles are widely distributed in Orissa State. Determination of larval instars in natural habitats is necessary for evaluating control programme. So far a key for the identification of larval instars of Indian culicines is not available. An attempt has been made to describe the morphological details of the larvae of *C. quinquefasciatus* and *C. tritaeniorhynchus* and to develop a key for the identification of different larval instars of these species on the basis of length and breadth of head capsule, thorax, abdomen, siphon and length of antenna. A pictorial key has been developed for determination of the above species at different larval instars using number of frontal, antennal, abdominal and cratal hair alongwith tuft and pecten teeth of siphon and siphonal index.

b) **Application of Dyar's rule to the development of two species of *Culex* (Diptera : Culicidae).**

In 1890, Dyar studied 28 species of Lepidoptera and concluded that the rate of growth of the head capsule in successive nymphal instars was in regular geometric progression. It is known as Dyar's rule. He suggested that the quotient of increase in width of the head at each moult (usually about 1.4) is constant for a given species and that this rule also applies to many other parts of the body. When the logarith mic values of the measurements of any character of insects are plotted against the number of instars, a straight line is generally obtained. Since then few studies were initiated on this aspect. An attempt was made to determine whether Dyar's rule can be applied to the growth of the

head capsule and other body parts of *C. quinquefasciatus* and *C. tritaeniorhynchus* and whether range of measurements of different characters can be used as a tool for instar identification of these species.

The measurements of different morphological characters i.e., length and width of head, thorax, abdomen, siphon tube, length of antenna of different instars of *C. quinquefasciatus* and *C. tritaeniorhynchus* follow the Dyar's rule. The average Dyar's factors of the above characters are 1.54, 1.58, 1.61, 1.66, 1.56, 1.67, 1.49, 1.49 and 1.58 in *C. quinquefasciatus* and 1.5, 1.52, 1.61, 1.62, 1.61, 1.52, 1.65, 1.5 and 1.7 in *C. tritaeniorhynchus*. The range of measurements of above characters can be used as tools to determine different larval instars of both the species.

c) **Laboratory rearing of *Mansonia annulifera* (Theobald) using three indigenous plants**

Literature on laboratory colonization of *Mansonia* mosquitoes is limited due to their peculiar mode of larval behaviour. Various aquatic plants have been tried in the laboratory for growing *Mansonia* larvae. Successful rearing of these mosquitoes on water plants depends upon survival of plant in the water medium. Different species of *Mansonia* has preference for particular plants. An attempt has been made to find out locally available suitable plants for the laboratory rearing of *Mansonia annulifera* (Theobald).

Mansonia annulifera (Theobald) was reared in the laboratory using three indigenous aquatic plants viz., *Pistia stratiotes* Linn., *Eichornia crassipes* Solms. and *Marsilea quadrifoliata* Linn. *P. stratiotes* and *E. crassipes* started to rot on day 5 and 10. *M. quadrifoliata* proved to be the most tolerant one and survived indefinitely. The larval survival percentage on *P. stratiotes, E. crassipes* and *M. quadrifoliata* was 31.7 ± 3.5, 25.8 ± 4.4 and 38.9 ± 2.5. Percentage of adult emergence of these plants was 19.4 ± 3.4, 15.9 ± 3.3 and 32.8 ± 1.9 respectively. The most common and preferred plant *M. quadrifoliata* was useful for mass rearing of *M. annulifera*.

The study is in progress and the project has been accepted and registered for the Ph.D. degree under Utkal University.

D-2. Effect of aphid extracts on the development of mosquitoes.

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

Progress :

a) Effect of *Aphis craccivora* aphids on the development of *Culex quinquefasciatus*.

Juvenoids, the synthetic bioanalogues of Juvenile hormones have been under trial for controlling mosquito populations. In recent years, attention is being paid to naturally occuring juvenile hormones in animals and plants. In the present study an attempt is made to demonstrate the effect of local common aphid *Aphis craccivora* Koch on the development of the common filarial vector *C. quinquefasciatus*.

As would appear from the Table 11 there is a remarkable difference between the average duration of larval and pupal periods of treated and control mosquitoes. The growth index of treated mosquitoes is lower as compared to control and untreated individuals. The larvae and pupae either die at their respective stages or during moulting, since pupae and

TABLE 11

Effect of *Aphis carccivora* extracts on the development of *Culex quinquefasciatus*

Test concentration	Average larval period in days	%Larval mortality	Average pupal period in days	%pupal mortality	%of total mortality	%Adult emergence (a)	Average Dev. period (b)	Growth index (a/b)
Untreated	10	0	2	0	0	100	12	8.33
Control	10	0	2	0	0	100	12	8.33
200 ppm	11	20	3	20	40	60	14	4.28
400 ppm	11	50	3	10	60	40	14	2.85
600 ppm	12	70	3	10	80	20	15	1.33

adults fail to emerge during moulting. The treatment with 200, 400 and 600 ppm extract results in 40, 60 and 80% mortality respectively. The prolongation of the larval and pupal period is due to the presence of juvenile hormone in the extract. The delayed and abortive ecdysis leads to larval-pupal intermediate stage in the above species due to the juvenilizing effect.

Analysis of the growth index at different concentrations gives interesting results. The growth index remains constant in the control and the untreated group while it decreases according to the increasing concentrations of aphid extract in the experimental populations. However, the study is further being carried out to establish the exact apterising and juvenilising effect of the aphid extracts on the developing stages of mosquitoes.

D-3. Laboratory Biology and colonization of mosquitoes.

Scientist : Dr. A.P. Dash
Technical staff : Mr. R.K. Hazra
Mr. H.K. Tripathy
Mr. G.D. Mansingh
Starting date : April 1987

To establish cyclic colonies of the important species of mosquitoes like :

Aedes aegypti
Anopheles annularis
Armegeres theobaldi
Culex quinquefasciatus
C. tritaeniorhynchus

To use the above species for bioassay studies etc.

Progress

Cyclic colonies of the above five species are maintained in the laboratory. Dog biscuit and yeast powder are given as food to the larvae and blood is given to adults. The mosquitoes are used for various studies, which include developmental studies of *W. bancrofti* in mosquitoes and susceptibility status of mosquitoes to different concentrations of insecticides, etc.

D-4. 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

The project is aimed to characterize mosquito antigens, that will help in understanding vector biology.

Progress

Earlier the presence of natural antibodies in man specific to *Culex quinquefasciatus* was demonstrated by us (RMRC Annual Report 1986-87). Antibody types as checked by ELISA now showed that quantity of IgM mosquito specific antibodies is negligible. The mean IgG levels in normal and filariae-infected people are 0.14 $\pm$ 0.05 and 0.26 + 0.11 respectively (n = 26 in each case), not appreciably different. It was shown that mixtures of *Culex* antigens could induce (immediate hypersensitive) type-1 immunological reactions on skin testing (~1.0 ug dose) in man. These antigens will be purified in future in order to characterize immunochemically.

The specificity of rabbit anti-*Culex* serum was checked against other members of the order, diptera. The reactivity was as follows : *C. quinquefasciatus* > *C. vishnui* > *Anopheles subpictus* > *Aedes vittatus* and negligibly with *Drosophila melanogaster* (gift from Dr. K. Krishna, TIFR, Bombay).

"Quite recently a new measure has been proposed which, in the opinion of many, promises to rank with house sanitation and preventive inoculation as a means of saving life on a large scale...The new sanitary policy to which I refer is that which aims at a reduction of disease bearing insects, especially those which are the disseminating agents of malaria, yellow fever and filariasis..."

Sir Ronald Ross (1905)

E. MYCOBACTERIA

E-1. Mycobacterial Genetics :

Isolation of Mycobacterial mutants.

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

Progress :

Several mutants have been obtained by NTG-treatment of *M. smegmatis* and *M. fortuitum*. Though a large number of mutants were found during initial screening experiments, all but a few proved to be unstable. The 'stable' mutants have been characterised in some detail and the markers are listed in Table 12.

Justification for continuation :

This is an ongoing project with the objective of isolating and characterising a large number of mycobacterial mutants for use in gene transfer experiments. When a gene transfer system is standardised for mycobacteria, it would be of great advantage to have a large number of marker strains.

E-2. Characterisation of Mycobacterial auxotrophs by syntrophism.

Scientist : Dr. V. R. Subramanyam
Technical staff : Ms. K. K. Mohanty
Starting date : April 1987

Progress :

Auxotrophic mutants have an indispensible role in studies on biosynthetic pathways in bacteria. Blockage by mutation in many pathways results in accumulation of metabolic intermediate preceding the block. Identifying the accumulated intermediate can throw light on the site of block in the pathway. Syntrophism (cross feeding) is a simple and convenient means of obtaining information on the affected sites in a pathway based on the observation that on a minimal medium with limiting amounts of the required nutrient, mutants blocked at a particular step in the biosynthetic pathway can, by virtue of accumulating the intermediate preceding the block, cross feed mutants blocked at an earlier stage in the pathway.

Table 12

Mycobacterial mutants

Strain	Colonies screened	Mutants obtained	Markers
M. fortuitum (NIHJ 1615)	178	SM2	*arg-1*
		SM3	*pur-1*
SM2	1914	SM29	*arg-1 leu-2 his-1*
SM3	4129	SM24	*pur-1 met-1*
		SM25	*pur-1 met-2*
		SM26	*pur-1 met-3*
		SM27	*pur-1 met-4*
		SM28	*pur-1 met-5*
SM24	600	Nil	
M. smegmatis (NIHJ 1628)	903	SM10	*lys-1*
		SM13	*leu-1*
		SM19	*pur-2*
		SM20	*lys-2*
		SM21	*pur-3*
		SM22	*arg-2*
SM10	1370	SM33	*leu-1 cyt-1 arg-3*
		SM34	*leu-1 cyt-2*
		SM38	*lys-1 pur-4*
		SM39	*lys-1 met-6*
		SM40	*lys-1 pur-5*
		SM42	*lys-1 phe-1*
		SM44	*lys-1 pur-6*
SM13	711	SM37	*leu-1 cyt-3*

Both interspecies and intergeneric cross feeding was seen between mutants of *M. fortuitum*, *M. smegmatis* and *B. subtilis*, auxotrophic for arginine, histidine, lysine and phenylalanine. There was no cross feeding amongst leucine auxotrophs.

Based on the cross feeding experiments, the mycobacterial mutants could be arranged sequentially according to the possible site of mutation in the biosynthetic pathways (Table 13). Corroboration for this has been obtained at least for the arginine auxotrophs by checking for their growth on basal medium supplemented with ornithine or citrulline, which are intermediates in arginine biosynthesis. SM22 grows equally well on ornithine, citrulline or arginine, SM33 grows on citrulline or arginine, but not on ornithine; and SM2 and 1A510 grow only on arginine but not on ornithine or citrulline.

Table 13

Possible site of mutation in the biosynthetic pathways of *Mycobacterium* auxotrophs, based on cross feeding data.

Biosynthesis of	Mutants*
Arginine	SM22→SM33→SM2 & 1A510**
Histidine	1A10→SM29
Lysine	SM10→SM20→1A8
Phenylalanine	1A96→SM42

*Relative positions from left to right indicate early to later steps in biosynthesis. Strains with prefix SM are mycobacterial mutants derived in our laboratory (see Table 1) those with prefix 1A are *Bacillus subtilis* mutants obtained from the *Bacillus* Genetic Stock Centre, Ohio State University, U.S.A. 1A8 : *trpC2 argD120 lys-11* IA10 : *trpC2 hisA1 thr-5;* 1A96 : *trpC2 pheA1;* 1A510 : *leuA8 arg15 thrA recE4* $r^{-}m^{-}$ *stp.*

From the results of cross feeding experiments, the following could be surmised—

Since SM42 cross feeds 1A96 which lacks prephenate dehydratase (the enzyme catalysing the penultimate step in the biosynthesis of phenylalanine), SM2 is blocked at the very final step in phenylalanine biosynthesis.

In the pathway of arginine biosynthesis, SM2 is blocked in the final step of arginine formation, either at the argininosuccinate synthetase or at the argininosuccinase level; SM33 and SM22 are suspected to be mutants at two different loci preceding that of SM2.

In histidine biosynthesis, SM29 is expected to be blocked down-stream of *hisA*.

In lysine biosynthesis, SM10 and SM20 are blocked at two different points (SM10 at an earlier step than SM20) preceding the final step of L-lysine formation from *meso*-diaminopimelate.

Justification for continuation :

In *Mycobacterium* information on the biosynthesis of several amino acids is either scanty (e.g. lysine) or non-existing (e.g. arginine, histidine etc.). Syntrophism is a simple way of obtaining information on the biosynthetic pathways. We are probably the first to have characterised mycobacterial mutants based on syntrophism.

E-3. Spheroplast formation in *Mycobacterium*

Scientist	:	Dr. V. R. Subramanyam
Technical staff	:	Ms. K. K. Mohanty
Starting date	:	May 1987

Progress :

It has been reported in literature that glycine-sensitised mycobacterial cells, on treatment with lysozyme and lipase are converted into spheroplasts. We have been experimenting with this procedure but have only had a limited success and the results have been inconsistent. We are now trying out alternative procedures for making spheroplasts.

Justification for continuation :

Fusion of cell wall-deficient bacteria (spheroplasts/protoplasts) is a highly potential technic and has been used successfully for obtaining a variety of recombinants. Its application to mycobacteria has also been reported. Since the conventional modes of gene transfer (transduction, transformation, conjugation) have not been very fruitful when applied to mycobacteria, genetic recombination via spheroplast fusion offers exciting possibilities as a gene transfer system in mycobacteria.

F. AIDS :

F-1. Sero surveillance for human immunodeficiency virus (HIV) infection.

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

Progress :

Regional Medical Research Centre, Bhubaneswar has been designated as a surveillance centre for HIV infection. We are geared to perform ELISA test for anti HIV antibody in suspected sera. So far, a total of 121 sera have been received and tested. One hundred and eight samples (Indian) were sent from the Department of Microbiology, S. C. B. Medical College, Cuttack and 13 (foreigners) from other academic institutions of Orissa. All were negative for anti-HIV antibodies by ELISA. Pertinent details of the samples are given in Tables 14 & 15.

Justification for continuation :

Infection with HIV can cause the dreaded Acquired Immuno Deficiency Syndrome (AIDS). Constant sero surveillance is essential for identifying virus—carriers and for monitoring the spread of the virus.

Table 14

Sera screened for anti-HIV antibody

Age Group	Male	Female	Total
< 20	39	5	44
21-30	30	6	36
31-40	16	5	21
> 41	15	3	18
Not known	2	—	2
Total	102	19	121

Table 15

Individuals at high risk tested for anti-HIV antibodies

Category	Sex	No.
Homosexuals	M	10
	F	—
Heterosexually promiscuous	M	24
	F	6
Corrective Institution inmates	M	23
	F	—
Recipients of blood/blood products	M	1
	F	1
Total		65

REMEMBER

- *The causative agent of AIDS is present in the white blood cells, therefore, the virus is present in highest concentrations in blood and semen.*
- *Though the virus is present in white blood cells, found in saliva, tears and other body fluids, transmission via tears and saliva does not occur. Probably there are few white blood cells in these fluids.*
- *The disease is not transmitted by casual contact, air, mosquitoes, water, food or living together.*
- *The virus is easily inactivated by heat, alcohol and glutaraldehyde etc.*
- *Health care professional who observe safety precautions as observed for hepatitis B infection are not at increased risk for AIDS.*
- *Practising good infection control is the key to protecting yourself and others from contracting AIDS.*

NICD, Delhi, 1987

SICKLE CELL RESEARCH CENTRE

V. S. S. MEDICAL COLLEGE, BURLA

Report For The Period 01.04.87 To 31.03.88

1.	Communication received regarding the sanction of the project	Sept. 1986
2.	Release of funds by I.C.M.R.	Feb. 1987
3.	**Staff**	**Joining date**
i)	R.A. (Chemistry)	27.03.1987
	(Presently the post is lying vacant)	(Relieved on 31.03.88)
ii)	Attendant	30.03.1987
iii)	Driver	April 1987
iv)	Senior Steno	06.04.1987
v)	R.A. (Bio-Science)	15.04.1987
vi)	R.O. (Anthropology)	29.04.1987
vii)	SRO (Medicine) (vacant)	Not yet filled up
viii)	SRO (Pathology) (Vacant)	Not yet filled up
	(advertised once, no suitable candidates were found)	

4. Availability of Space & Setting up of the Laboratory :

After the receipt of the sanction of the Project, Govt. of Orissa, Directorate of Medical Education and Training, Superintendent, V.S.S. Medical College Hospital, PHED and GED were approached for making the necessary laboratory available. With much persuation, at last the space was made available in August 1987 and the Research Centre was inaugurated on 17.08.87. Although the PWD and PHED work for the Laboratory is complete, electrical wiring etc. are yet to be taken up, pending sanction of funds by the DMET which is expected this month.

Progress :

a) Sickle Cell Clinic is held on Monday, Wednesday and Friday from 10 A.M. to 4 P.M. when patients from outside and referred from other departments turn up for clinical examination, necessary laboratory investigation, diagnosis and advice. On other days when

some patients come from distant places they are also examined. Investigation report, diagnosis and advice etc. are given to the patients who have attended earlier.

i) **Sickle Cell Clinic :**

Total no. of patients/suspected patients	... 948
No. of new Sickle Cell Disease patients discovered and recorded	... 205
No. of Sickle Cell Trait cases detected	... 464
Total no. of Sickle Cell disease patients examined, recorded and followed up. (including previously recorded cases).	... 382

The patients are usually asked to turn up for follow up at intervals of 1-2-3 months depending upon the condition of the patient. Patient compliance is very satisfactory.

b) In addition to the Sickle Cell Clinic effort has been made to collect population data for the district of Sambalpur and to plan out the survey by subjecting the population data to Random Sampling with the help of the Research Officer (Anthropology).

c) **Clinical :—** For the suspected cases & their relatives who attend the Sickle Cell Clinic either directly or being referred by doctors in the periphery or doctors in charge of various wards of the Medical College Hospital; the following screening tests are done in each case while recording their name and address etc.

i) Blood Sickling Test.

ii) Solubility Test.

iii) Hb Electrophoresis by CAM (Alkaline).

Based on the results of this screening test if an individual is found to have Sickle Cell trait, he is given necessary advice, especially marriage counselling and future precautions in a suitably printed card. In case of Sickle Cell disease the patient is examined thoroughly clinically and a case sheet is prepared in the printed proforma recording the details of family history etc. Clinical examinations include fundoscopy and a search for conjuctival vascular changes. Individual case sheets thus prepared are numbered serially and maintained in separate cover files. Their parents, siblings, children and other blood relations if available (either immediately or when they come in future on our request) are subjected to similar screening tests to know their genotype and to detect clinically unsuspected cases. This helps in determining the actual genotype of the patients.

In each patient the following laboratory investigations are carried out.

a) Repeat electrophoresis in Agar Gel medium (acid) to detect any other abnormal haemoglobin present.

b) Haemoglobin estimation.

c) Determination of PCV and MCHC.

d) Reticulocyte Count.

e) Differential W.B.C. Count.

f) Peripheral blood smear examination.

g) Irreversible Sickle Cell Count.

h) Haemoglobin F estimation.

i) Haemoglobin A_2 estimation.

j) Serum Bilirubin estimation (Conjugated & total)

k) Serum alkaline phosphatase.

l) SGPT and SGOT estimation in deeply jaundiced patients.

m) Urine urobilinogen estimation and detection of bile pigment.

n) Test for G-6-PD deficiency by Methylene Blue reduction test as and when necessary.

When a case is found to be Sickle Cell Thalassaemia, Osmotic fragility test is also carried out. At the end of the investigation each patient is finally given necessary advice in a card printed for the purpose with instruction for check up on a specified date.

During follow up visits by the patients a thorough clinical examination and haematological assessment are done with Hb estimation, PCV, MCHC, and reticulocyte count. Other investigations are repeated depending on the necessity. The follow up findings and clinical notes are recorded in their respective case sheets and they are given necessary advice.

d) Population Survey :

After procuring the population data of Sambalpur district in different blocks from the Collectorate of Sambalpur it was decided to start with Sambalpur (Dhankauda) Block. A list of villages with population of each village of the block according to the 1981 census was procured. From the list a few villages were selected applying random sampling tables so as to represent 10% of the population of the block. A few preliminary visits were made to the selected village to make a list of different families, recording their Caste/Community and

the actual number of members in the house hold. From this list all the families with blood relations to each other (clan) are grouped together in respect of each caste/community. Again applying the random sampling tables families are selected from each such group so as to represent at least 10% of the actual population of the group. When such families were earmarked, in subsequent visits to the village, members of the family were tested. A proforma in respect of each family is made out recording the data in respect of each available member of the family. Besides certain Socio-economic data a few anthropometric findings are noted and a few vital clinical data are also recorded after a thorough clinical examination. Blood samples are collected from such individuals for :-

1. Sickling Test.
2. Hb Electrophoresis (CAM) and
3. Haemoglobin estimation.

Whenever a case of Sickle Cell trait is detected, a card with necessary genetic and marriage counselling is handed over during subsequent visits. If a Sickle Cell Disease patient is discovered from the screening he/she is dealt with in the usual manner as per the Sickle Cell Disease patients in the Clinic.

Several such visits are made to the individual village till the target families have been covered. After finishing the work in that village the procedure is repeated for the next selected village.

The following table gives the population survey upto 31st March '88 and the result there-of.

Name of village	Total Population	No. of persons tested	No. where S. gene detected.
Village Kainsir	1686	143 (others were not available inspite of repeated visits)	17
Village Kalamati, (Proper Kalamati, Bhoitikira & Kudapada)	394	117	14
	2080	260	31

Amongst the above 31 persons, all were Sickle Cell trait cases and there was no case of Sickle Cell Disease. Caste wise break up of all the people surveyed and the finding is presented in the following table.

Sl. No.	Caste	No. of members studied	No. 'S' were present	No. of SCD.
1.	Oram (ST)	7	—	—
2.	Khadia (ST)	6	1	—
3.	Munda (ST)	16	—	—
4.	Kandha (ST)	8	—	—
5.	Saora (ST)	20	—	—
6.	Pap (ST)	8	—	—
7.	Gond (ST)	20	7	—
8.	Bhuyan (ST)	3	—	—
9.	Ghasi (SC)	9	3	—
10.	Ganda (SC)	22	—	—
11.	Keuta (OBC)	8	—	—
12.	Karana	10	—	—
13.	Khandayat	2	—	—
14.	Bhandari (OBC)	5	—	—
15.	Kumbhar (OBC)	4	—	—
16.	Gauda	41	9	—
17.	Kulita	38	8	—
18.	Kurmi	3	—	—
19.	Mirdha (ST)	1	—	—
20.	Brahmin	20	—	—
21.	Suda	3	3	—
22.	Sundhi (OBC)	5	—	—
23.	Teli (OBC)	1	—	—
	TOTAL :	260	31	0

Projects Completed

1. Quantitation of antibodies specific to infective larvae of *W. bancrofti* in filariasis.

Scientist : Dr. M.K. Das.
Period of study : November 1986-June 1987

Results and Discussion :

a) A total of 110 sera representing various stages of filarial infection was checked by ELISA for L_3 -specific antibody level using L_3 antigen.

b) Taking the antibody level of endemic normals as the reference point (mean + 2. S.D.), the following rate of increased titre (positivity) was calculated—70% in TPE, 40% in clinical filariasis, 28% in asymptomatic carrier and 8% in endemic normals.

c) The low positivity rate in endemic normals as observed here is in contrast to the results of other workers. One possible reason could be that L_3 antigens used were freed by immunoadsorption from the contaminating *Culex* components which we showed to be immunogenic in man (RMRC, Annual Report 1986-87, p.33).

d) L_3 antibody assay suggested that it is possible to differentiate endemic normals from the infected population. The superiority of L_3 antigens compared to mf as diagnostic reagent was also noted by IHA. (RMRC Annual Report 1986-87, p.31).

2. Detection of L_3 antigens in filarial sera by counterimmuneelectrophoresis.

Scientist : Dr. M.K. Das
Technical staff : Mr. M.K. Beuria
Period of study : January 1986-July 1987

Results :

Rabbit hyperimmune antiserum specific to infective larvae (L_3) of *W. bancrofti* was used in CIEP to detect the presence of L_3 antigens.

		Presence of L_3 antigens
Group	*n*	*no. (%) Positive*
Non-endemic normal	6	0
Endemic normal	26	2 (7.69)
Asymptomatic carrier	25	18 (72.00)
Clinical filariasis	18	8 (44.44)
T P E	24	14 (58.33)

As noted above, highest percentage of positive sera belonged to microfilariaemic carriers (72.0%) and least in endemic normals (8%). It is interesting to note that as much as 60% of TPE patients who lack free microfilariae do contain parasitic antigens in their sera. Data reported here suggest that L_3 antigen assay could be useful in determining the active infection caused by *W. bancrofti*. Work is continuing at present to make it ELISA based for higher sensitivity.

3. Assay of Phosphorylcholine (PC) and L_3 antigen specific circulating immune complexes (CIC) in Filariasis.

Scientist : Dr. M.K. Das
Technical staff : Ms. A. Mishra
Period of study : November 1987-February 1988

Progress :

PC and L_3 specific CIC level was measured by HOPC-8 (murine monoclonal antibody) and rabbit anti-L_3 serum by enzyme immuno assays. Using the IC level in endemic population as the reference point, the number of positive samples in each category was calculated. IgM and IgG specific CIC were evaluated.

PC containing CIC levels

Group	No. tested	Positive No. (%)	
		IgM-CIC	IgG-CIC
Endemic normal	23	1 (4.34)	0
Asymptomatic carrier	24	3 (12.5)	2 (8.33)
Clinical	28	7 (25.92)	5 (20.83)
T P E	31	14 (45.16)	12 (38.71)
		L_3 specific CIC levels	
Endemic normal	26	1 (3.84)	1 (3.84)
Asymptomatic carrier	19	2 (10.52)	2 (10.52)
Clinical	26	5 (19.23)	9 (34.61)
T P E	20	14 (70.0)	18 (90.0)

Following observations could be made from the above data.

a) In PC-CIC, IgM antibodies are more prevalent than IgG types.

b) TPE are shown to have highest level of filaria specific immune-complexes among all other category of filariasis. This is in conformity with the PEG-assay (RMRC Annual Report 1986-87 p. 32).

c) PC containing CIC assay may not have as much specificity and sensitivity as that of L_3 antigens in filariasis.

4. Enhancement of DTH responses to *Plasmodium berghei* antigens by the adjuvants.

Scientists : Dr. M.K. Das
Dr. B. Ravindran
Technical staff : Mr. M. K. Beuria
Period of study : January 1987-November 1987

Results :

DTH response was measured in Balb/c mice to soluble *P. berghei* antigens as specific increase in foot-pad thickness.

Results are shown in the table given below.

Treatments	DTH response: Mean difference in foot pad thickness (mm ± S.D.)	% Increase
Saponin	0.323 ± 0.15	22.8
Alum	0.845 ± 0.37	51.24
PC Liposomes	0.360 ± 0.22	22.24
DPPC Liposomes	0.59 ± 0.05	36.41
Saline	0.093 ± 0.05	5.7

P. berghei antigens in saline do not exhibit DTH response. However when mixed with the adjuvants, it showed good responses. Alum helped in inducing the highest DTH response followed by synthetic liposomes.

It was also demonstrated that *P. berghei* antigens adsorbed in alum could induce DTH in infected mice (7 to 14 days of infection).

5. Production and characterization of rabbit antibodies to Primaquine.

Scientists : Dr. B. Ravindran
Dr. M. K. Das
Technical staff : Mr. Ashok K. Satapathy
Period of study : July 1985-December 1987

Immunoassays for quantitative estimation of the antimalarial drug Primaquine does not exist, since antibodies with specificity to the drug has not been raised so far. The project was undertaken with a view to make Primaquine 'antigenic' in rabbits so that antisera may be prepared for development of specific and sensitive immunoassays that would be applied in the field.

Progress and conclusions :

Primaquine was coupled to bovine serum albumin (BSA) using glutaraldehyde (G) and also by carbodiimide reaction (C). The resultant conjugate PQ-BSA was used to raise antisera in rabbits. The anti-PQ activity was quantified by ELISA using a heterocarrier system, tetanus toxoid coupled to PQ (PQ-TT) as antigen in the test system. Brief results are presented in Table 16. Inhibition ELISA using a conjugate of PQ and alkaline phosphatase demonstrated that nanogram quantities of PQ can be detected in samples. The test also however indicated a possible cross-reaction of anti-PQ antibodies with chloroquine, the most commonly used antimalarial drug.

The detailed results of the work is to be published in Medical Science Research (see under publications.)

Table 16

Anti-primaquine titre in normal and immunized rabbit sera

Rabbit no.	Conjugate	ELISA[a]
R-13	PQ-BSA (G)	2000
R-14	PQ-BSA (G)	1000
R-715	PQ-BSA (C)	4000
R-716	PQ-BSA (C)	1000
R-13 (H)[b]	PQ-BSA (G)	32000
NRS I	—	< 100
NRS II	—	< 100

a Reciprocal of titre; sera collected 30 days post immunization were tested.
b R-13 (H), hyperimmune serum.

6. Prevalence of the intestinal parasites amongst school going children in a sub urban area.

Scientist : Dr. G. P. Chhotray
Technical staff : Mr. M. R. Ranjit
Mr. H. K. Khuntia
Period of study : August, 1986-July, 1987

a) The prevalence of intestinal helminthic and protozoal infection was studied over a period of one year amongst 297 school children varying in age from 5 to 20 years in a sub-urban village Gangapada situated about 22 km away from Bhubaneswar.

b) Stool samples were collected in plastic cups and were examined in the R. M. R. C. laboratory by Formol-ether concentration technique (WHO manual 1980).

c) The over all prevalence of various parasitic infection at the first examination was 64.64 percent, 34.34 percent had helminthic infections and 14.47 percent had mixed (protozoal and Helminthic infection).

d) Round worm infestation was the commonest helminthic infection (8.08 percent).

e) Giardiasis was the commonest protozoal infection (22.22 percent).

f) Effective anti helminthic/anti protozoal treatment such as :

i) Mebendazole : One tablet (100 mg) twice daily for three consecutive days was administered.

ii) Metronidazole 200 mg three times daily for 7 days was given for giardiasis & *Entamoeba histolytica* infection.

Students having no parasitic infection whatsoever after repeated stool examination were not given any treatment.

g) Six months after the administration of effective antihelminthic/antiprotozoal treatment repeat stool examination of all the student (irrespective of who had the treatment or who did not have the treatment) revealed that the overall prevalence rate had dropped considerably to 4.14 percent, protozoal to 10.77 percent & combined infection to 0.33 percent.

h) During 3rd round of study (after one year) the overall prevalence of various parasitic infections was found to be 25.92 percent; 5.72 percent had helminthic infection, 17.50 percent had protozoal infection and 2.69 percent had combined infection.

(Table 17 figure 2)

i) It was observed that with the increase of age the protozoal infection declined ($F=10.04$, $df=2$, $p < 0.05$) because of health consciousness and improved personal hygiene amongst the elder higher class students but there was no significant correlation between helminthic infection and different age group. ($F=1.13$, $df=2$, $p < 0.05$).

j) Although most of the children defecate in the open field the prevalence of hookworm infection is comparatively low (2.69 percent) which is due to the topographic situation

of the village in a elevated area surrounded by vast fields with porous latterite soil. The terrian is not very conducive for the development of the infective filariform larvae. The area being vast, people seldom use any particular patch of land for repeated defecation.

k) Analysis of the data reveals that there was a significant reduction in the intestinal parasitic infection in the children after effective treatment. ($F=6.94$, $df=2$, $p<0.05$). This suggest that in a sub-urban community intermittent administration of proper treatment could effectively control intestinal parasitic infection and break the infection cycle. The reinfection rate could be further reduced by taking adequate sanitary measures.

l) With the administration of antihelminthic treatment it is observed that (Table 17, figure 2) there is a significant reduction of parasitic infections in all age groups from 5 to 20 years but there is a slight increase of these parasitic infections at the end of one year of treatment. This is due to the reinfection.

m) The reinfection rate was found to be 6.25 percent in the present study which may be due to non-intervention in the sanitary measures, poor personal hygeine and toilet habits, and other factors responsible for infection with intestinal parasite. These factors are difficult to cope with without overall socio-economic improvement and full cooperation from the community.

In conclusion the high prevalence rate of intestinal helminthic and protozoal infection appears to be due to promiscuous defecation, lack of personal hygeine, health education and absence of safe drinking water.

7. Effect of deworming treatment on the nutritional status of the school going children in a sub-urban community.

Scientist : Dr. G. P. Chhotray
Technical staff : Mr. M. R. Ranjit
Mr. H. K. Khuntia
Period of study : August 1986 to up-to-date

a) While conducting the study of the prevalence of intestinal parasitism amongst the school going children in a sub-urban area, efforts were made to assess the nutritional status of the same children within the age group 5—20 years at the beginning of the study and subsequently 6 months and one year after the effective antihelminthis/anti protozoal treatment was administered.

Table 17

Age wise distribution of different Intestinal Parasitic Infection

Age Group in Years	Cases Studied	1st Round Study				2nd Round Study				3rd Round Study			
		Only Protozoal Infection	Only Helminthic Infection	Protozoal + Helminthic Infection	Total	Only Protozoal Infection	Only Helminthic Infection	Protozoal + Helminthic Infection	Total	Only Protozoal Infection	Only Helminthic Infection	Protozoal + Helminthic Infection	Total
05 - <10	72	33	7	9	49	13	2	1	16	22	3	4	29
		(45.8)	(9.7)	(12.5)	(68.05)	(18.05)	(2.77)	(1.38)	(22.2)	(30.55)	(4.16)	(5.55)	(40.27)
10 - <15	191	67	30	28	125	19	6	0	25	27	10	2	39
		(35.07)	(15.70)	(14.65)	(65.4)	(9.9)	(3.1)	—	(13.08)	(14.1)	(5.2)	(0.10)	(20.4)
15 - <20	34	2	10	6	18	0	1	0	1	3	4	2	9
		(5.88)	(29.4)	(17.6)	(52.94)	—	(2.94)	—	(2.94)	(8.82)	(11.76)	(5.88)	(26.47)
Total	297	102	47	43	192	32	9	1	42	52	17	8	77
		(34.34)	(15.82)	(14.47)	(64.64)	(10.77)	(3.30)	(0.33)	(14.14)	(17.50)	(5.72)	(2.69)	(25.92)

Figures in parantheses indicates the percentage.

b) **Recording of Anthropometric data :**

The height, weight, age and mid-arm circumference of individual students were measured by means of standard clinical scale and recorded initially before examining the faeces. Subsequently the above measurements were recorded six month and one year after administering the anti parasitic treatment. The anthropometric data were collected, tabulated and the nutritional status of the children was estimated by using the following methods.

c) **Calculation of various Anthropometric data :**

The individual age, height and weight were recorded after six monthsand one year of administering the effective treatment and tabulated for 3 indicators of the nutritional status recommended by WHO, weight/age, height/age and weight for height. The pattern of distribution of these three indices to reference population was shown by S. D. score distribution.

$$\text{S.D. Score} = \frac{\text{Individual value} - \text{Median value of the reference population}}{\text{SD Score value of the reference population}}$$

(The reference population used as per the NCHS reference population data).

d) Taking wt/age as a criterion it was observed that in the first round of study (before treatment) 88.8% of the children were within the normal limit of nutritional status (percentage above median — 2 S D) and 11.11% were below normal (percentage below median — 2 S D). After six months of administering the effective antihelminthic/ anti protozoal treatment it was found that 93.60% of children were normal and 6.93% were below normal. Similarly one year after it was seen that 93.2% were normal and 6.73% were below normal.

Analysing the data statistically it has been observed that there is no significant improvement in the nutritional status of the children six months and one year after the administration of effective treatment as compared to the initial data obtained before the treatment ($X^2 = 0.6247$, $p < 0.05$).

e) Similarly taking ht/age as a criterion it has been observed that 79.8%, 80.5% and 80.8% of the children were within normal limit and 20.2%, 19.5% and 19.2% of the students were below normal, of nutritional status in the 1st, 2nd and 3rd round of study respectively.

The statistical analysis of the data revealed that there is no significant difference in 2nd and 3rd round of study (six month and one year after administering the effective treatment) as compared to the initial observations ($X^2 = 0.0377$, $p < 0.05$).

f) Considering wt/ht as a criterion it's been observed that 66.7%, 71.7%, 72.8% of the children were within normal limit and 30.3%, 28.31% and 27.16% were below normal limit of nutritional status in the 1st, 2nd and 3rd round of study respectively.

Statistically it is concluded that there is no significant improvement in the nutritional status of the children after the effective treatment when compared with the initial observations ($X^2 = 0.61$, $p < 0.05$).

8. Present status of *Anopheles sundaicus* in Orissa.

Scientist : Dr. A. P. Dash
Technical staff : Mr. R. K. Hazra
Mr. G. D. Mansingh
Period of study : October 1986-September 1987

Results :

Orissa, especially the Chilika lake area, was the home for the notorious malaria vector *Anopheles sundaicus*. In the past, role of this species as a vector in Orissa and Bengal was well known. In recent years it has become very scarce and has almost withdrawn from many of the areas of distribution. Great changes are sometimes noticed as a result of natural trends extending over years. Due to various developmental activities in Orissa, lot of ecological changes have taken place and the present status of this vector needs a reappraisal. Though it is apprehended that *A. sundaicus* has disappeared from Orissa and Andhra Pradesh where it was present till early 1950s, systematic studies are lacking to prove the disappearance or presence of this species. Therefore, the study is undertaken to know the present status of this species in Orissa particularly in areas where it was present in the earlier days.

Extensive and intensive surveys were carried out in the Chilika lake area (22 villages including the islands in the lake), Paradeep (4 villages), Puri and other coastal areas of Orissa to study the present status of *A. sundaicus* in the state. Morning, evening and night collections of indoor resting mosquitoes, window trap collection, larval collection and rearing of larvae have been carried out during October and November, 1986; February, April, July and September, 1987; covering all the seasons. Mosquitoes were identified and age-composition, susceptibility status, anthropophilic index of few species (available in good numbers) have been carried out.

During the survey as many as 22 species of mosquitoes (14 anophelines, 1 *Aedes*, 1 *Armegeres*, 4 *Culex* and 2 *Mansonia*) were encountered. The prevalence of different

Table 18

Prevalence of Mosquito Density in Chilika Area

Sl. No.	Name of the species	Oct. 1986	Nov. 1986	Feb. 1987	April 1987	July 1987	Sept. 1987
		PMHD	PMHD	PMHD	PMHD	PMHD	PMHD
1.	*Anopheles aconitus*	—	2.3	0.36	0.4	2.0	—
2.	*Anopheles annularis*	5.3	2.82	1.45	0.4	17.0	15.7
3.	*Anopheles barbirostris*	0.08	0.7	—	—	—	—
4.	*Anopheles culicifacies*	0.52	3.76	0.72	1.3	3.0	1.7
5.	*Anopheles 'hyrcanus'* group	6.26	21.88	1.09	1.3	—	0.4
6.	*Anopheles jeyporiensis*	—	0.23	—	—	—	—
7.	*Anopheles karwari*	—	0.15	—	—	—	—
8.	*Anopheles pallidus*	—	—	—	—	—	0.6
9.	*Anopheles philippinensis*	—	0.23	—	—	—	—
10.	*Anopheles ramsayi*	—	0.7	—	—	—	—
11.	*Anopheles subpictus*	6.8	7.2	51.9	15.5	37.0	81.7
12.	*Anopheles tessellatus*	—	0.15	—	—	—	—
13.	*Anopheles vagus*	2.52	8.4	5.0	—	52.0	1.3
14.	*Anopheles varuna*	—	0.46	0.36	—	3.0	—
15.	*Aedes aegypti*	—	0.23	—	—	—	—
16.	*Armegeres theobaldi*	0.08	0.23	—	—	—	—
17.	*Culex epidesmus*	—	0.47	—	—	—	—
18.	*Culex gelidus*	3.47	0.47	—	—	—	—
19.	*Culex quinquefasciatus*	9.47	17.8	2.9	0.4	5.0	40.0
20.	*Culex 'vishnui'* group	14.78	5.88	1.09	3.5	—	4.2
21.	*Mansonia annulifera*	0.08	11.5	—	4.8	—	—
22.	*Mansonia uniformis*	—	—	2.54	5.3	—	—
	TOTAL	49.4	85.7	66.5	33.3	119	145.6
	TEMP (°C)	30.3	28.4	29.8	36.26	34.44	33.92
	R.H. (%)	72.9	67.4	58	65.9	79.2	70.5

species in the Chilika lake area is tabulated in table 18. Out of the 22 species encountered, *An. subpictus* was found to be the dominant one followed by C. *quinquefasciatus*, constituting 31.5 and 15.5% of the total mosquitoes respectively. *An. 'hyrcanus'* group consisted of 10.8% while the percentages of *An. annularis* was 7.5% of the total collection.

Data on malaria incidence (table 19) indicates that the API in the villages surveyed varies from 0.95 to 1.91. The SPR is low as would appear from the PHC records. Most of the malaria positive cases were found in bonded labourers/*'Dadan'* labourers who are working in North eastern states and comes back with infection and fever.

Anthropophilic index of *An. annularis, An. subpictus* and *An. vagus* was found to be 4.3, 3.7 and 6.8% respectively (table 20).

Table 19

Malaria position in villages around Chilika (source : PHC data)

Year	B.S.C. / B.S.E.	Positives			SPR
		Total	Dadan	p.f.	
1985	2046 / 2046	48	27	14	2.34
1986	1729 / 1729	60	52	25	3.47
1987	1015 / 1015	56	48	18	5.51
Mean	1596 / 1596	54	42	19	3.8

Table 20

Results of precipitin tests of some mosquitoes of Chilika lake

Species	Number of mosquitoes				Anthropo-pilic index
	Tested	Pos. for human	Pos. for cattle	No reaction	
An. annularis	91	4	85	2	4.3%
An. subpictus	176	12	162	2	6.8%
An. vagus	27	1	26	0	3.7%

Age composition of wild caught female mosquitoes of Chilika lake area are presented in table (table 21), which indicates that 11.1% of *An. culicifacies* and 3.3% of *An. annularis* had three dilatations in the ovariole.

Table-21

Age composition of wild caught female mosquitoes of Chilika lake area.

Species	Total no. dissected	No. of mosquitoes having ovarian dilatations				
		0	1	2	3	4
An. annularis	114	60	12	40	2	0
An. culicifacies	54	12	24	12	6	0
An. subpictus	211	23	155	33	0	0
An. vagus	76	33	35	8	0	0

None of the mosquitoes dissected was positive for gut and gland.

The susceptibility tests of *An. annularis* and *An. subpictus* to DDT 4% and malathion 5% shows mortality of *An. annularis* as 20 and 100% in DDT and malathion respectively while it is 80 and 100% in DDT and malathion respectively in case of *An. subpictus.*

During the survey not a single larva or adult of *An. sundaicus* was encountered.

The records of malaria incidence in the area as presented in table 19 is interesting. The mean SPR is low (3.8). Most of the malaria positive cases during 1985 to 1987 were found to be among the bonded labourers who used to work in northeastern states and come back when they suffer from fever. So only few of the malaria cases are locally transmitted in the villages around Chilika lake area under survey. Among the known malaria vectors, *An. annularis* and *An. culicifacies* were found. The density of *An. culicifacies* was negligible for malaria transmission. The density of *An. annularis* was significantly higher than *An. culicifacies.* Earlier *An. annularis* was established as a vector in coastal Orissa. Probably the local transmission was due to this species and not *An. sundaicus.*

Summary and conclusion :

A total of 22 species of mosquitoes were encountered including *An. subpictus, Cu. quinquefasciatus, An. "hyrcanus"* group and *An. annularis* which dominated the

collection in order of merit. Anthropophilic index, age-composition and susceptibility status of most of the above species have been studied. Not a single larva or adult of *An. sundaicus* was found and the species seems to have disappeared from the area.

9. Comparative vector studies in Kumarabasta.

Scientist : Dr. A. P. Dash

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

Period of study : May 1986-April 1987

Results :

The R.M.R.C., Bhubaneswar was carrying out clinical studies on filariasis in the village Kumarabasta of Puri district having a population of 2020. The tribal population

Table 22

Permanhour densities of mosquitoes in the tribal and non-tribal areas of Kumarabasta.

Month and Year	Non-Tribal			Tribal		
	Total mosq.	Anophe-lines	*Culex quinque-fasciatus*	Total Mosq.	Anophe-lines	*Culex quinque-fasciatus*
June '86	14.4	0.4	13.0	18.5	3.0	6.5
July ' 6	41.12	6.0	33.0	15.6	4.0	9.2
August '86	65.8	26.7	29.2	25.6	17.6	4.0
Sept. '86	30.76	9.0	21.6	50.2	30.2	0.5
Oct. '86	21.7	3.42	12.5	12.0	1.3	4.0
Nov. '86	24.0	4.8	13.3	12.0	11.0	4.0
Dec. '86	42.2	2.2	40.0	24.0	5.3	13.3
Jan. '87	25.3	0	25.0	22.0	10.0	10.0
Feb. '87	33.0	0.5	32.0	0.2	0	0
April '87	12.0	0.57	10.28	5.14	0.57	3.4

(about 320) is isolated from the main village and was reported to have less filarial incidence. Weekly mosquito collections were made from these areas following statistical random sampling methods. The permanhour densities of *Culex quinquefasciatus*, the filariasis vector and other mosquitoes are set in table (table 22) which indicates high density of the vector in the non-tribal area than the tribal area.

The non-tribal area has a number of sources for the breeding of *C. quinquefasciatus*.

Summary and Conclusion :

The density of the filariasis vector *C. quinquefasciatus* in the non-tribal area due to the availability of lot of suitable breeding places is probably the cause of high incidence of filariasis.

10. Creating Scientific Awareness among people (All India People's Science Festival, 1987).

Scientist : Dr. N. M. Pattnaik

Technical staff : Mr. Dasarathi Das
Sri Prasant K. Kar
Sri Pramod K. Rana
and several other RMRC & non-RMRC personnel.

Background :

While much has been made of the progress of Science & Technology in our country it is very clear that majority of the population are left untouched by it. Despite the size of the S & T manpower in India there is a singular absence of a scientific temperament in general. Even among the "Scientists" and "educated" a proper scientific outlook is sadly lacking. The present research activities have generally failed to attain any intellectual height nor have these been oriented to our people's immediate needs. Thus voices of concern have been raised by many at all levels to stop this mutual alienation between science/scientists and the people at large.

The Project :

We have been working for sometime in individual capacities in popularising science and helping to make school science teaching more meaningful. Since there are many small groups all over the country doing this, a need was felt to have a co-ordinated programme throughout the country to highlight these activities.

Thus under the sponsorship of the Department of Science and Technology, Government of India, several voluntary organisations came together to organise a "Bharat Jan Vigyan Jatha" or the "All India People's Science Festival" between September and November 1987. Apex bodies like the UGC, National Laboratories etc. readily extended their co-operation.

The programme involved intensive interaction among the scientists/educationalists/ social activists and common people/students etc. Using a variety of media such as posters, slides, lectures, and even performing arts ideas concerning Science, Health, Education, Technology, Superstitions etc. were analysed, debated and critically assessed. The aim was to inculcate in the common man the scientific seed of questioning and to make the "Scientists" realise the tenuousness of their position away from the society.

During the programme twentyfive local groups were formed around Orissa involving Schools, Colleges and Community groups which actively conducted the festival. The staff of RMRC—many in personal capacities—played a focal role in the Orissa programme.

Future :

Activities of this group continues—in the individuals' spare time and with their personal resources. A proposal is being formalised to involve RMRC institutionally as we feel this to be very much a part of any scientific endeavour.

11. Human immuno deficiency virus antibody reactivity in filariasis and malaria sera.

Scientist : Dr. V. R. Subramanyam
Technical staff : Mr. P. K. Sahoo
Period of study : December 87-March 88

Summary and Conclusions :

Several workers have reported an intriguing association between seropositivity for human T-lymphotropic virus (HTLV) antibodies and certain parasitic infections. Such a study has not so far been reported from India. We tested for antibodies against the respective parasite antigens and for anti-HTLV-III (=HIV) antibodies in 118 sera from Puri district (endemic for Bancroftian filariasis) and 119 sera from Keonjhar district (endemic for falciparum malaria). Indirect haemagglutination test was used for detecting antibodies against *Wuchereria bancrofti* microfilariae somatic antigen and ELISA was used for antibodies against solubilized *Plasmodium falciparum* schizont infected erythrocytes. Anti-HTLV-III antibodies were assayed in all sera using commercially available Wellcozyme anti-HTLV-III

ELISA kit (Wellcome Diagnostics, England). To economise on reagents, anti HTLV-III antibodies alone were assayed in pools of sera. Each pool contained not more than 5 sera.

The sera tested had high titers of antibody against the homologous parasite antigen, as expected in endemic areas (table 23). However, all sera were negative for anti-HTLV-III antibodies.

The correlation between high malaria and filarial antibody titers and retrovirus antibody reactivity in certain geographic areas (Africa & Gato Islands of Japan) and the lack of such correlation in certain other areas needs explanation.

Table 23

Particulars of the sera tested for antiparasite antibodies and anti-HTLV-III antibodies.

Category	No.	Titer of antibody against	
		Microfilariae*	*Pl. falciparum***
FILARIASIS (Bancroftian)			
Endemic normal	48	73.8	NT
Asymptomatic mf carrier	28	57.0	NT
Acute	9	64.0	NT
Chronic	33	91.6	NT
MALARIA			
Endemic normal	61	NT	0.27
Pl. falciparum	54	NT	0.43
Pl. vivax	4	NT	0.48

* Geometric mean of positive values

**Arithmetic mean of A_{492} values

NT not tested

ALL SERA WERE NEGATIVE FOR ANTI-HTLV-III ANTIBODIES

12. Chloroquine Sensitivity of *P. falciparum*

Scientist : Dr. S. S. S. Mahapatra
Period of study : July, 1986-March, 1988

Results :

Chloroquine sensitivity study of *P. falciparum* by *invivo* method were carried out in the Mathili PHC during the period March-April, 1987 and in the Dasamantpur and Borigumma PHCs in June, 1987. In Mathili PHC the extended test showed that all strains were sensitive. In Dasamantpur PHC the standard test showed 93.75% sensitive and 6.25% resistance at R_{II} level. (table 24).

In the previous year, the similar tests done in three PHCs had shown sensitive result in Borigumma, R_I level resistance (11.4%) in Lamptaput and R_I (5.8%) & R_{II} (8.8%) resistance in Malkangiri PHC (Table 25).

These studies have indicated that chloroquine resistance of *P. falciparum* exists in localised pockets in different areas of Koraput District.

Malaria : One Hundred Years After Laveran

On November the 6th 1880 Charles Louis Alphonse Laveran, a French Army doctor stationed in Algeria examined under a microscope, under a dry lens with a magnifying power of about 400 times, a preparation of fresh blood from a soldier with intermittent fever. Looking at some pigmented bodies inside the red blood cells he noticed at the periphery, motile, flagella-like projections. Laveran concluded that they were the parasites of the well known intermittent fever which was given the name "malaria" or "paludism". Laveran's discovery was first met with a great deal of disbelief but soon the Italian workers confirmed it and described the cyclical development of malaria parasites and their three main species. The transmission of the infection remained unknown and conjectural until 1897 when Ronald Ross in India discovered the presence of the infection in a mosquito and thus prepared the basis for the control of this ancient disease. A series of historical milestones in our knowledge of the parasitology, epidemiology, entomology, prevention and treatment of malaria mark the past one hundred years.

L.C. Bruce-Chwatt,
Wellcome Museum of Medical Science,
London, U.K.

Table 24

Result of Invivo Chloroquine sensitivity study in *P. falciparum* in Mathili, Boriguma and Dasmantpur PHCs of Koraput

Sl. No.	Name of the PHC	Period of test	Type of test	D_0	D_1	D_2	D_3	D_4	D_5	D_6	D_7	D_{14}	D_{21}	D_{28}	Remarks
1.	Mathili (with 1500 mg. base of Chloroquine)	March & April '87	28 days	40/40	27/40	12/38	4/37	0/37	0/37	0/37	0/37	0/37	0/37	1*/37	All' S'
2.	Dasmantpur & Borigumma (with 1500 mg. base of Chloroquine)	June '87	7 days	20/20	17/20	9/19	3/16	1/16	1/16	1/16	1**/16				=15 (93.75%) R_{11}=1 (6.25%)

* Reinfection on D_{28} ** Treated with Metakelfin and Primaquine

Table 25

Result of *invivo* (28 Days) Chloroquine Sensitivity Test in *P. falciparum* in Koraput District

P.H.C. Area	Period of test	D0	D1	D2	D3	D4	D5	D6	D7	D14	D21	D28	Remarks
Borigumma (with 1500 mg base of Chloroquine)	July '86	33/33	25/33	9/33	0/33	0/33	0/33	0/33	0/33	0/33	0/33	0/33	All 'S'
Lamtaput (with 1500 mg base of Chloroquine)	Sept. & Oct. 1986	33/38	21/38	7/37	3/36	3/35	1/35	0/35	0/35	5/35	4/35	4/35	S=29 (82.9%) R_I =4 (11.4%) (delayed type) Reinfection=2 Discontinued=3
Malkangiri (with 1500 mg base of Chloroquine)	Oct. & Nov. 1986	38/38	30/38	21/38	11/38	8/38	6/38	4/37	4/36	4/36	6/34	5/34	S=28 (82.4%) R_I =2 (5.8%) (delayed type) R_{II} =3 (8.8%) of w which one blood smear collection was discontinued from D14 Reinfection=2 Discontinued=3

Numerator=No. of cases found Positive, Denominator=No. of cases examined

Note : Possibility of reinfection in these cases cannot be totally ruled out since the transmission is perennial.

New Projects Proposed for 1988-89

1. Evaluation of cardio-pulmonary involvement in Tropical Pulmonary Eosinophilia and response to DEC.

Scientist : Dr. Shantanu Kumar Kar

Tropical pulmonary eosinophilia is known to be a hyper sensitive reaction to microfilarial antigen manifesting with intermittent episodes of wheezing, cough and systemic allergic reactions. Both restrictive and obstructive pulmonary changes and sometimes cardiac abnormalities like ECG changes, in form of Cor.-pulmonale, right ventricular hypertrophy are reported. Though DEC is commonly used in treatment of TPE (6 mg/kg/day for 2 weeks), reversal of symptoms are frequently observed in many patients. Hence it is planned to study the following aspects :-

i) Clinical manifestation and course of T.P.E. with reference to pulmonary function and cardiac involvement.

ii) Response of T.P.E. to DEC therapy (6 mg/kg/day for 2 weeks). This will be evaluated by clinical examination, ECG and pulmonary function tests and immunological studies.

This study will be undertaken with referred cases of TPE (from various hospitals of Bhubaneswar) at Unit III Dispensary, Bhubaneswar.

The study will high-light the course of the disease, its response to DEC, and associated immunological alterations in the patients.

2. Immune responses of patients with clinically defined bancroftian filariasis.

Scientist : Dr. Shantanu Kumar Kar

The human lymphatic filariasis is a chronic infection that evokes host immune responses which have been implicated in pathogenesis of various clinical manifestations of the disease. The pattern of host immune responses to filarial parasite is critical enough to determine the range of clinical pathology seen among people in the endemic region. Although characteristic clusters of immune responses are observed in different groups of patients, work done recently indicates that even among patients with overt clinical filariasis, there is a wide spectrum of cell mediated responsiveness. The endemic normals as a group appear to be heterogeneous and need to be defined by clinical and parasitological methods. The groups of patients manifesting with filarial fevers, adenolymphangitis with or without

microfilaraemia, have been little studied as they blend into a larger group of overt lymphatic obstruction. Among patients with lymphoedema and elephantiasis few manifest with recurrent attacks of adenolymphangitis while others show progress of lymphoedema uneventfully. Hence it is felt that host immune responses to the parasite or its products could be further delineated in well defined clinical groups of patients. Evaluation of immune responses including detection of specific antigen in sera by western blotting technique in these groups of patients may show better correlation and understanding of the pathogenesis. This includes evaluation of different class and sub-class level of antibodies, detection of specific circulating immune complexes and circulating antigen in clinically defined groups of cases The above study will be taken up in collaboration with Dr. D. A. Denham, London School of Hygiene and Tropical Medicine, London for which a detailed proposal has been submitted to the council.

3. 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 ICMR has also agreed to sanction additional funds required to carry out this project. But the work is yet to commence because some of the senior research staff are yet to be recruited at Burla to assist in the project work.

4. Demonstration of control of Bancroftian filariasis by various methods including integrated vector control technologies in a compact area.

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 R. M. R. C. to carryout research on diseases and their control and since Orissa state is highly endemic for bancroftian filariasis, the first Scientific Advisory Committee on 24-1-1987, advised to take up a control project. A detailed proposal was submitted to the second Scientific Advisory Committee which met on 4-9-1987 and 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. They have agreed to render all co-operation for integrated vector control. The Director, R. M. R. C. submitted the proposal to the Govt. of Orissa and the Govt. has given approval on 15-3-88. Financial and administrative approval for the project from ICMR are awaited.

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

Scientist : Dr. A.P. Dash

Scientific background and justification :

Our present knowledge on the taxonomic aspects of Indian mosquitoes is more than 50 years old. Orissa is highly endemic for both the mosquito borne diseases, filariasis and malaria. There has not been any systematic study on mosquito fauna of Orissa and consequently our knowledge on mosquito fauna of the state is poor. In view of the importance of the two mosquito borne diseases there is an urgent need to study the mosquito fauna, their habitat, behaviourial pattern and vectorial capacity in the state (anophelines, *Mansonoids & Culex)*. 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) information on age composition, susceptibility status etc. The study would also help in the control/containment of filariasis and malaria in the following manner :

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

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

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

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

(The proposal has been submitted to the ICMR headquarters for approval and funding.)

6. Feasibility of control of *Culex quinquefasciatus* using indigenous agents.

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

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

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

7. Study of Erythrocytic Glucose-6-Phosphate Dehydrogenase Deficiency and it's variants in malaria endemic tribal area in Orissa.

Principal Investigator : Dr. G.P. Chhotray

Co-Investigator : Prof. Lucio Luzzatto
Royal Postgraduate Medical School,
Haematology Division
Hammersmith Hospital
London W12 OHS

Objectives :

i) a) Quantitative estimation of G-6-PD enzyme in a malaria endemic tribal area in Orissa.

b) Electrophoretic and kinetic characterization of G-6-PD variants.

c) Further characterization of G-6-PD variants at the DNA level.

ii) Summary of the proposed research (up to 150 words) indicating overall aims of the research, importance of the objectives and application of the work in the context of national priorities of medical research.

Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency is a wide spread heritable X-linked metabolic disorder of RBC which effects approximately 100 million people world wide. G-6-PD has been recognised as an important clinical and genetic entity. More than 180 red cell variants have been reported from all over the world (Beutler, 1982 & Fence, *et al* 1982). These variants differ from normal and from each other in one or more physical and kinetic characteristics. The severity of the clinical manifestation also depend on these variants. All the variants in a population may not have clinical importance but the genetic study is highly valuable to find the ethnic origin of the population.

The incidence of G-6-PD deficiency in various tribal population of India ranges from 4.0—17.4% (Rao 1979 & Jain *et al* 1981). Orissa has a high concentration of tribal population constituting 23.1% of the State's total population and 12% of the country's tribal population. In a pilot study conducted by the RMRC, Bhubaneswar in the tribal areas revealed a high incidence of G-6-PD deficiency amongst the scheduled caste and scheduled tribe population in comparison to the non-scheduled caste/tribe population residing in the same area. This warrants an indepth study of G-6-PD deficiency and its variants in this part of the country, which has not been carried out earlier. This area is also an endemic focus of malaria.

iii) Preliminary work already done by the investigator on this problem :

Three hundred and fifteen persons were examined for Glucose-6-phosphate dehydrogenase deficiency in the Banspal PHC area situated about 28 kms from Keonjhar (district headquarters) Orissa, which is predominantly inhabited by tribal population and is also highly endemic for malaria. The study population included 188 scheduled tribe people (mainly Kolha & Bhuyan), 52 scheduled caste people (mainly Patra & Dasa) and 75 non-scheduled caste people (Khandayat, Brahmin etc). The overall G-6-PD deficiency in this group was 28.88% of which 19.09% showed intermediate degree of deficiency and 10.79% showed severe G-6-PD deficiency. The incidence of G-6-PD deficiency in the scheduled tribe population was found to be 34.04% which is more than the incidence in scheduled caste population (25.0%) and non-scheduled caste population (18.66%). There was no significant correlation of G-6-PD deficiency with haemoglobinopathy and malaria as found in the present study. This warrants a detailed indepth study on the above subject in the tribal belt of the country.

iv) It is proposed to have a collaborative study with Prof. Lucio Luzzato of Royal Postgraduate Medical School, Hematology Division, Hammersmith Hospital, London W12 OHS.

8. Investigation of bacteria and algae in vector habitats for cloning the delta endotoxin gene.

Scientist : Dr. S. Jayachandran

The sporeforming *Bacilli, Bacillus thuringiensis* subsp. *israelensis* (Bti) and *B. sphaericus* produced endotoxin that kills the larvae of Black fly and mosquitoes. Though many workers have successfully cloned the toxin gene from *Bti* and *B. sphaericus* into *E. coli* and *Bacillus* sp. as yet no information has been generated in identifying microorganisms living in the mosquito breeding habitats that could be used in cloning the toxin gene. Since *Bti* and *B. sphaericus* are known to persist only for a short duration after application in mosquito breeding habitats, it is imperative that field surveys are conducted for isolating and identifying microbes that are more competitive and better adapted for various ecological regimes of the Indian subcontinent with a capacity to persist for longer durations. It is important that the microbes selected for cloning the toxin gene fulfil the following criteria. a) They are well adapted to the ecological conditions where mosquitoes breed, b) They have simple nutritional requirements, c) They are unicellular and have the ability to immobilise the transferred genes and d) Their cell walls are digestable by mosquito gut enzymes.

The present investigation proposes to :

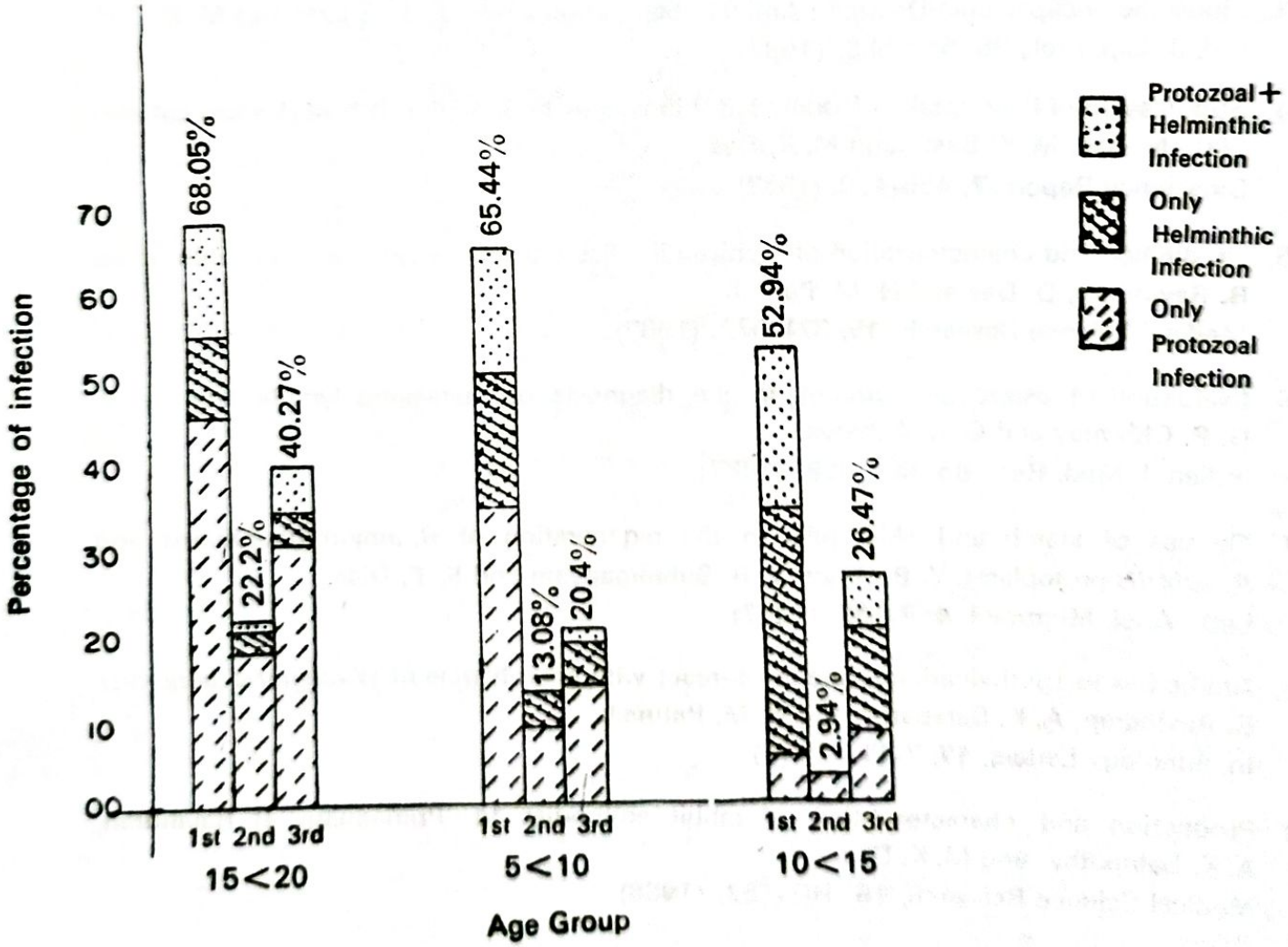
i) Isolate bacteria and algae from vector habitats and gut contents of larvae.

ii) Determine their nutritional requirements.

iii) Assess the feasibility of using them to increase their persistance in mosquito breeding habitats.

iv) Study their genetic pattern, antibiotic sensitivity and ability to immobilise transferred genes.

Figure 2 (Page 53 & 54)



Distribution of Different Intestinal Parasitic Infection in Various Age Groups

Papers published

1. Epidemiological study on Lymphatic Filariasis in an Endemic Zone of Orissa. Shantanu Kumar Kar.
J. Com. Dis., **18** (4) : 312, (1986).

2. A study of the antigen, antibody and immune complex levels in *Wuchereria bancrofti* filariasis with reference to clinical status. M. K. Das, V. R. Subramanyam, B. Ravindran and N. M. Pattnaik.
J. Trop. Med. Hyg., **90**, 135-141, (1987).

3. Liposome encapsulated Dextran : Antigen distribution study. A. K. Sarkar and M. K. Das.
Ind. J. Exp. Biol., **25**, 587-588, (1987).

4. Suppression of liver uptake of orally fed Liposomes by injection (IP) of dextran sulfate-500. N. Das, M. K. Basu and M. K. Das.
Bioscience Report, **7**, 465-470. (1987).

5. Production and characterization of rabbit antibodies with reactivity to Diethylcarbamazine. B. Ravindran, D. Das and N. M. Pattnaik.
Medical Science Research, **15**, 371-372, (1987).

6. Evaluation of aspiration cytology in the diagnosis of metastatic lymphodemopathy. G. P. Chhotray and G. S. Acharya.
Indian J. Med. Res., **85**, 685-688, (1987).

7. The use of starch and skim milk in the regeneration of *B. amyloliquefaciens* and *B. subtilis* protoplasts. Y. B. Bhatt, V. R. Subramanyam and F. F. Dias.
Lett., Appl. Microbiol. **4**, 77-80, (1987).

8. Antibodies to Diethylcarbamazine cross-react with microfilariae of *Wuchereria bancrofti*. B. Ravindran, A. K. Satapathy and N. M. Pattnaik.
Immunology Letters, **17**, 7-11, (1988).

9. Production and characterization of rabbit antibodies to Primaquine. B. Ravindran, A. K. Satapathy and M. K. Das.
Medical Science Research, **16**, 161-162, (1988).

10. A simple technique for membrane feeding of mosquitoes. A. P. Dash
J. Zool. Soc. India, **38** : 107-109 (1988).

Papers submitted/accepted for publication :

1. Pilot Chemotherapy study on Filarial Lymphoedema'' Experta Medica. S. K. Kar, J. Mania and S. R. Nayak.
   International Congress Series, ELSEVIER Publication.
2. Detection of infectivelarval antigens in bancroftian filariasis. M. K. Das, M. K. Beuria, A. P. Dash and N. M. Pattnaik.
3. Quantitation of antibodies to infective larvae in *Wuchereria bancrofti* infection. M. K. Das, A. P. Dash, B. Ravindran, N. M. Pattnaik and V. R. Subramanyam.
4. Naturally occuring anti-galactosyl antibodies in human *Plasmodium falciparum* infections—a possible role for auto-antibodies in malaria. B. Ravindran, A. K. Satapathy and M. K. Das.
   Clinical and Experimental Immunology.
5. Malaria among Bonda tribals, in Koraput district, Orissa, India—A preliminary report. Lalit Kumar Das, S. S. S. Mohapatra, P. Jambulingam, K. Gunasekaran, S. P. Pani and P. K. Das.
   Transactions of the Royal Society of Tropical Medicine and Hygiene.
6. Effects of Aphid extracts on the development of mosquitoes. M. R. Ranjit, A. P. Dash and G. P. Chhotray.
   Proceedings of second symposium on Vectors and Vector borne diseases.
7. Erythrocytic G-6-PD deficiency in a malarial endemic tribal population of Orissa. G. P. Chhotray and M. R. Ranjit. Indian J. Med. Res.
8. The prevalence of Intestinal Parasites amongst school going children in a sub urban community. G. P. Chhotray and M. R. Ranjit. Ann. Trop. Med. Parasitol.
9. Diagnosis of metastatic squamous cell carcinoma by Fine needel Aspiration Cytology. G. P. Chhotray and G. S. Acharya. Acta Cytologica.
10. Effects of Anti Protozoal/Anti helminthic treatment on the Nutritional status of school going children. G. P. Chhotray and M. R. Ranjit. WHO Bulletin.
11. Bionomics and vectorial capacity of mosquitoes in Puri district, Orissa. A. P. Dash, N. Tripathy and R. K. Hazra. Proc. 2nd symp. on Vector and Vector borne diseases.
12. Identification of larval instars in two species of *Culex* (Diptera : Culicidae). N. Tripathy and A. P. Dash. Ibid.

13. Laboratory rearing of *Mansonia annulifera* (Theobald) using three ind igenous plants. N. Tripathy and A. P. Dash.
Ibid.

14. Characterization of *Mycobacterium fortuitum* and *M. smegmatis* auxotrophs by syntrophism, using *Bacillus subtilis*. V. R. Subramanyam and K. K. Mohanty.

15. Cryptosporidiosis in children of Eastern India. V. R. Subramanyam, R. L. Broadhead, B. B. Pal, J. B. Pati and G. Mohanty.

16. Absence of HTLV-III antibody reactivity in filariasis and malaria sera. V. R. Subramanyam.

Chapter written for Books :

on "Rhinosporidiosis"

by L. N. Mohapatra.

Second Edition. Eds : Kenneth S. Warren, M. D.
Adel A. F. Mahmoud. M. D.

Mc. Graw-Hill Book Company, New York.

SCIENTIFIC CONFERENCES/WORKSHOPS/SEMINARS ATTENDED

Name of the scientist	Scientific conferences/workshops/seminars attended with date	Papers presented/ Lecture delivered
Dr. L.N. Mohapatra	Indian Association of Pathologists & Microbiologists. XV All Orissa Chapter meet at Keonjhar. 15th November 1987.	Chief guest. Addressed on "Role of Pathology and the Pathologists in modern medicine".
	Indian Society of Anaesthetists. XVIII Annual Conference, Orissa State Branch. 21st November 1987, Bhubaneswar.	Guest of Honour. Addressed to gathering on "resuscitation".
	Indian Association of Preventive and Social Medicine : XVII National Conference 27th November 1987, Cuttack. XXXVI Annual Conference.	Key note address & Chaired two Scientific sessions.
	Indian Association of Pathologists & Microbiologists. 10th-13th Dec. 1987, Hyderabad.	On Emerging "Opportunistic infections" (mycotic) (symposium, 12.12.87).
	Annual Day, Science Society, Khallikote College, Berhampur 27th February 1988	Chief guest. Addressed on "Science Society and development".
Dr. Shantanu K. Kar	XIth International Congress on Lymphology Vienna, Austria, 24-27th September 1987.	Pilot chemotherapy study on filarial lymphoedema. Panel discussion on "Pathophysiology and management of filarial lymphoedema".
	East Zone and Orissa State Branch Association of Physicians of India. 15-16th November 1987, Cuttack.	Chemotherapy of lymphatic filariasis.
	43rd Annual Conference of the Association of Physicians of India. 21-25th January 1988, Pune.	Effect of long term DEC treatment in conjuction with conservative physiotherapy in filarial lymphoedema.

Name of the scientist	Scientific conferences/workshops/seminars attended with date	Papers presented/ Lecture delivered
	Mid term Conference of Association of physicians of India Orissa State Branch 27th March 1988, Bhubaneswar.	Atypical features of lymphatic filariasis.
Dr. M.K. Das	Third National Symposium on Bio-organic Chemistry. RRL/CCMB (CSIR) 9-11th July 1987, Hyderabad	Lecture on "Presence and characterization of antibodies to mosquito antigens in man".
	XIV Annual Conference of Indian Immunology Society and symposium on "Immune deficiencies" 12-14th October, 1987, Srinagar.	Immunoassay for Tropical Pulmonary Eosinophilia.
	Dept. of Biochemistry, Delhi University. 19th October 1987, Delhi.	Lecture on "Immuno diagnostic studies in human filariasis".
Dr. A.P. Dash	Second symposium on vectors and vector borne Diseases. 8-10th February 1988, Trivandrum.	a) Identification of larval instars in two species of *Culex*. b) Laboratory rearing of *Mansonia annulifera* using three indigenous plants. c) Effect of aphid extracts on the development of *Culex quinquefasciatus*. d) Bionomics and vectorial capacity of mosquitoes in Puri district, Orissa.
	Fauna of Orissa and its conservation. 12-13th December 1987, Bhubaneswar.	Culicine fauna of Orissa, India.

Name of the scientist	Scientific conferences/Workshops/seminars attended with date	Papers presented/ Lecture delivered
Dr. B. Ravindran	Non-isotopic immunoassays. April 1987, New Delhi.	—
	Indo-US advanced course on "T. lymphocyte biology" 15th December 1987, New Delhi.	—
	Annual conference of the Indian Immunology Society. October 1987, Srinagar	Antibodies to DEC cross-react with microfilariae of *Wuchereria bancrofti*.
Dr. G.P. Chhotray	XV Annual all Orissa chapter meet of Indian Association of Pathologists & Microbiologists. 23-24th November 1987, Keonjhar	Erythrocytic G-6-PD deficiency in a malaria endemic tribal population of Orissa.
	XXXVI annual conference of IAPM. 10-13th December 1987, Hyderabad.	Erythrocytic G-6-PD deficiency in a malaria endemic tribal population of Orissa.

Other important academic activities :

The technical staff in the clinical division of this centre had undergone a short training course on immunological methods (western blotting and immunofluorescence technique) by Dr. D.A. Denham and his team, London School of Tropical Medicine from 7th-16th December 1987.

Dr. Shantanu K. Kar A.D. was elected as a Fellow of the Royal Society of Tropical Medicine and Hygiene on 17th March 1988.

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e) Mr. S. N. Dhar
Project Officer
British Council Division

6. 07.12.87 : Dr. D. A. Denham
Reader in Medical Helminthology
London School of Hygiene & Trop. Med.

7. 28.12.87 : Dr. J. S. Pillai
Associate Professor of Microbiology
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8. 09.03.88 : Dr. B. Patnaik
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Accounts Officer	Sri A. K. Mohapatra, B.A., LL.B.

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