REGIONAL MEDICAL RESEARCH CENTRE

BHUBANESWAR

ANNUAL REPORT

1989

REGIONAL MEDICAL RESEARCH CENTRE

(INDIAN COUNCIL OF MEDICAL RESEARCH)

NANDANKANAN ROAD
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ANNUAL REPORT

(1.4.1989 to 31.12.1989)

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ACKNOWLEDGMENT

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

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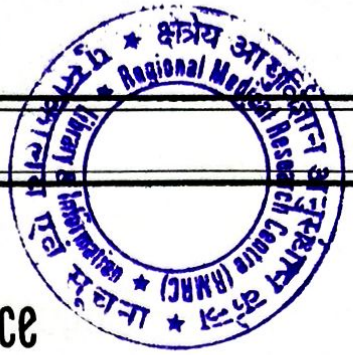

Preface

The annual report is a brief account of the achievements, of the sincere efforts and of trials and triumphs. It gives the sum total of the activities at this centre. Since it is decided to publish the annual report for each calendar year, this report for 1989 covers the events from **1st April 1989** to **31st December 1989.**

During these nine months the ongoing research programme was continued to achieve the objectives. These are reported under the broad headings like filariasis, malaria etc. Some of the earlier projects have come to an end and are listed under projects completed. A few new proposals are given shape to be taken up in the coming year. The Scientific Advisory Committee (SAC) during its meeting on 26.6.1989 gave the guidelines for continuing research activities, some of which have been implemented. Other recommendations could not be translated into action for difficulties beyond our control. One of the main impediments is financial restraint and the other is the ban on recruitment of staff, due to a 10% cut in the budget for 1989-90. However, a few new equipments have been procured and the research activities are sustained. The TDR grant for Phase-II clinical trial of Ivermectin was received and the project has just started. On the recommendation of the SAC the council is considering the proposal to convert the work on Sickle Cell disease into an intramural project.

The laboratory-cum-administrative building in the RMRC Campus is almost ready for use but has not yet been handed over to this centre by the State Public Works Department. The campus has been further developed by plantation of flowering trees and laying of grassy lawns. The centre has steadily grown all these years. It is now fairly well organised to take up the regional health problems one by one and move from the area of infectious diseases to other non-communicable diseases. Very recently Brig. M.S. Das (Retd) has joined this centre as an Emeritus Scientist (ICMR) to work on neurological aspects of Leprosy. The horizon of activities at this centre is gradually expanding.

On the eve of completion of my tenure I would like to bid good-bye to my colleagues & staff members at this centre. I am thankful to all of them for their kind co-operation and good will, inspite of my shortcomings. I am sure of the bright future for this centre under the care of the Director General and the Chief of the E.C.D. division of the council. It is the spirit of team work and a sense of belonging which will continue to inspire the scientists and other staff working at this centre to achieve the goal i.e. to promote the health of the nation. The members of the Scientific Advisory Committee will continue to guide the academic activities and help the scientists in their endeavour.

Prof. L. N. Mohapatra

Ongoing Projects

1. FILARIASIS :

F.1. Immunological studies on Filariasis

Scientist : Dr. Manoj K. Das
Technical staff : Mr. M. K. Beuria
Ms. A. Mishra
Starting date : July 1985

The project is an attempt to characterize the immunological properties of filarial parasites, especially the infective larval (L_3) state of *W. bancrofti*.

Progress :

1. The determination of antibodies specific to L_3 stage should indicate the intensity of filarial exposure in endemic regions. Based on this concept, L_3 antibody level was measured by ELISA. A soluble mixture of L_3 homogenates freed from immunogenic *Culex* components was used as the source of antigen. Results obtained so far is summarized as follows :—

i) L_3—specific IgE antibody response was highly stimulated in normal people (n=40) living in filaria endemic areas (Puri district) in comparison to those (n=22) in non-endemic regions (Keonjhar district). Such marked difference was however not noticed with IgM or IgG isotypes. IgE level in filaria infected people (asymptomatic carrier and chronic filariasis) though elevated was marginally higher compared to endemic normals. L_3—specific IgE level due to less cross-reactivity appears to be a superior indicator in determining exposure to filariasis. Analysis of more sera is being performed to substantiate the above conclusion.

ii) The variation in L_3 antibody level with increase in age was determined in endemic normal population. IgG and IgE levels were found to be increased significantly ($P < 0.01$) in adult group (mean age 51.0 $\pm$ 7.20 yrs,) compared to children (mean age 8.60 $\pm$ 3.40 yrs, n=15 for each group). The difference in IgM though enhanced in adults was less marked. Such acquisition of L_3 antibodies with increase in age may suggest repeated exposure to filariasis. As a part of IgE-mediated responses, immediate

hypersensitivity reactions (ITH) to filarial antigens was investigated in mice. Immunomodulators like Alum, and Liposomes enhanced ITH and this is reflected also in passive cutaneous anaphylaxis (PCA) titre. Interestingly DEC treatment to mice suppressed completely the filaria—antigen induced ITH which was however regained later. This model is utilized further in deciphering the mechanism of DEC induced suppression, that is known to occur also in man. Work is in progress to get defined antigens from *Setaria digitata* which we have demonstrated to have antigenic sharing with L_3 of *W. bancrofti*.

2. IgG subclass distribution of L_3 antibody response in different categories of filariasis (n=15 for each group) was measured. The salient features are as follows :—

i) Cases of Tropical Pulmonary Eosinophilia (TPE) exhibited the highest response in each class and endemic normals the lowest. IgG4 antibodies were highly elevated in carriers compared to cases of elephantiasis.

ii) Asymptomatic carriers and endemic normals have similar IgGI level, but this value is higher in chronic filariasis.

iii) For both IgG2 and IgG3 classes, carriers and clinical cases have nearly similar values.

3. To determine the presence of circulating L_3 antigens by ELISA, the enzyme horseradish peroxidase has been conjugated to - globulin fraction of rabbit anti-L_3 antisera. The detection of phosphorylcholine (PC) determinant by a mouse monoclonal HOPC-8 (gift of Dr. C.P.J. Glaudemans NIH) is also developed. These two systems are being compared for their efficacies.

F.2. Studies on antibodies with reactivity to Diethylcarbamazine.

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

Initially it was proposed to raise antibodies to DEC with a view to develop an immunoassay for measurement of the drug in body fluids. The anti-DEC antibodies raised

were however found to crossreact with surface of microfilariae of *W. bancrofti*. This was a unique instance of antigenic mimicry between the anti-filarial drug and filarial parasite (details of the above findings were reported earlier and published in Medical Science Research and in Immunology Letters—vide RMRC annual report 1987-88). Further studies were undertaken to characterize the reactivity of anti-DEC with other filarial parasites and to use it as a tool for developing an immunoprophylactic agent for blocking the transmission of microfilariae from vertebrate to mosquito hosts.

Progress :

1. Anti-DEC antibodies reacted strongly with *W. bancrofti* microfilaria as shown by immunoperoxidase assay and immunofluorescent assay.
2. Antibodies with reactivity to DEC could be detected in Bancroftian filariasis sera by ELISA using MPCA-BSA. The DEC antibodies in filarial sera were found to be primarily antifilarial antibodies cross-reacting with DEC (Clinical and Experimental Immunology—see under publications). A more direct assay for DEC antibodies (in human filarial sera) using C^{14}-labelled DEC is to be undertaken in the near future.
3. Apart from methyl piperazine carboxylic acid, MPCA prepared by acid hydrolysis of DEC, two other homologues of piperazine namely piperidine carboxylic acid (PCA) and piperdine propionic acid (PPA) are also being used, in an attempt to study the cross-reactivity of anti-DEC anti-bodies to these haptens.
4. Attempts are also underway to substitute BSA with other more effective carrier molecules such as tetanus toxoid, Diphtheria toxoid, Keyhole limpet haemocyanin etc.
5. Since antibodies to DEC cross react with as yet an unidentified filarial antigens, C-DNA library of *Brugia malayi* and *O. volvulus* have been procured with a view to use rabbit raised anti-DEC antibodies as a probe to identify the cross reacting filarial antigen(s). This will offer unlimited supply of the protective filarial antigen for further use.

F.3. Detection and characterization of anti-sheath antibodies in Bancroftian filariasis.

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

Progress :

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

Progress :

1. By performing immunoperoxidase assay and immunofluorescence assay anti-sheath antibodies were detected in filariasis sera and an inverse relationship was shown between peripheral microfilaraemia and the presence of antibodies to microfilarial sheath. Evidence indicating the involvement of carbohydrate determinants on mf sheath in inducing anti-sheath antibodies in Bancroftian filariasis was also produced (vide RMRC annual reports 1987-88 p.18 and 1988-89 p.13-14).
2. The studies were extended to investigate the species specificity of sheath antigens. Sera of Bancroftian filariasis and Brugian filariasis were tested for anti-sheath antibodies using sheathed microfilariae of *W. bancrofti* and *Setaria digitata*.
3. While 95.8% of microfilaraemic bancroftian filariasis sera did not have anti-sheath antibodies, 70.9% of amicrofilaraemic sera showed anti-sheath antibodies against mf of *W. bancrofti*. In contrast to this 95.2% of microfilaraemic and 92.8% amicrofilaraemic bancroftian filariasis sera had demonstrable anti-sheath antibodies to mf of *Setaria digitata*.
4. Less than 10% of non-endemic normal sera (European and American controls) had demonstrable antibodies to mf sheath of *W. bancrofti* or *Setaria digitata*.
5. About 43.75% of Bancroftian filariasis sera had demonstrable antibodies to mf sheath of both *W. bancrofti* and *S. digitata*, while only 6.25% of them had no detectable antibodies against both the above species of filarial parasites. These initial studies indicate that sheath antigens are far less cross-reactive in comparison to other somatic antigens which have been shown to be widely cross-reactive. Further studies are in progress.

F.4. *In vitro* culture of microfilariae.

Scientist	:	Dr. N. M. Pattnaik
Technical staff	:	Nil
Starting date	:	September 1985

The project has been de-emphasised due to lack of manpower and consequent difficulty in getting a continuous supply of microfilariae.

The culture is being used as a source of antigens Excretory-Secretory (E-S) for routine use only. Characterisation of the product has not been possible because of dilution problems. These products from *Setaria spp.* showing varying degree of cross-reactivity with human serum antibodies are being studied as a more readily available replacement.

F.5. Characterisation of plant microfilaricidals.

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

Technical staff : Sri Dasarathi Das

Starting date : February 1987

Having established the existence of an *in vitro* microfilaricidal activity in several plant extracts which are used as traditional drugs, their characterisation is currently underway.

In some plants both the rbc-lytic and mf-cidal activities are found but appear to be due to two different components as shown by the dialysable nature of the former (Table-1). The high molecular weight component of the Shakhotak bark extract showed only mf-cidal activity.

Table 1

Distribution of rbc-lytic and mf-cidal activities in some plant extracts, Effect of dialysis

Drug	Whole Extract			Dialysed Extract		
	A_{280}	Activity/ml		A_{280}	Activity/ml	
		rbc-lytic	mf-cidal		rbc-lytic	mf-cidal
Echinacea	7.1	45	10	1.32	5	2.5
Hydrocotile	3.4	10	5	0.95	3	2.5
Belladonne	4.8	7.5	5	1.21	2.5	2.5
Shakhotak	60	0	400	45	0	300

Since the rates of lysis and mf-killing were not linear with respect to time and concentration, operational definitions of these activities were used as follows. One unit of

lytic activity was defined as that releasing A_{540} of 0.1 in 3 hrs. at 37°C. Mf-cidal activity was defined in terms of the inverse of time taken for complete killing (t_{100}) and the amount giving a $1/t_{100}$ of 1 h^{-1} was defined as one unit. While these are adequate for the present purpose more rigorous probit/logit analysis will be used for kinetic analysis when computation facilities become available.

The Shakhotak activity was best extracted in aqueous buffers at 25-40°C while ethanol failed to extract the same. Representative extractability figures being 35 O.D. and 150 activity U in water and 3 O.D. and 60 activity U. in ethanol, respectively. Starting with the whole aqueous extract partial purification has been achieved through a combination of TCA precipitation and ion exchange chromatography (Table-2).

Table 2

Purification of mf-cidal activity from Shakhotak extract

		Total O.D.	Specific Activity (Units/OD)	Recovery (%)	Purification (fold)
A)	Whole extract	720	6.7	100	—
	DEAE-Eluate				
	I	54	14.7	25	2
	II	35	11.3		
B)	Whole extract	60	2.1	100	—
	TCA ppt	10.5	9.1	80	4.5

The whole extract and the partially purified fractions showed a broad absorption peak between 270 and 340 nm with steeply rising absorbance below 250 nm. The fractions were routinely quantitated in terms of absorbance at 280 nm. However, the contribution of proteins to this absorption is yet to be established. The activity is lost on exposure to pH extremes (<4 and >10). It is also lost, although rather slowly, at temperatures over 80°C. Thus the active component appears to be a moderately heat stable glycoprotein with a molecular weight of over 100,000.

The rate of mf killing shows a sigmoidal dependence on both time of incubation and amount of the extract (Fig). The probit transforms of the same (Fig. 1) yield straight lines as would be expected of a dose and exposure dependent killing. The lag period, however, does not vary when the mf is incubated with the extract at 4°C prior to incubation at 37°C indicating the absence of any passive binding/uptake.

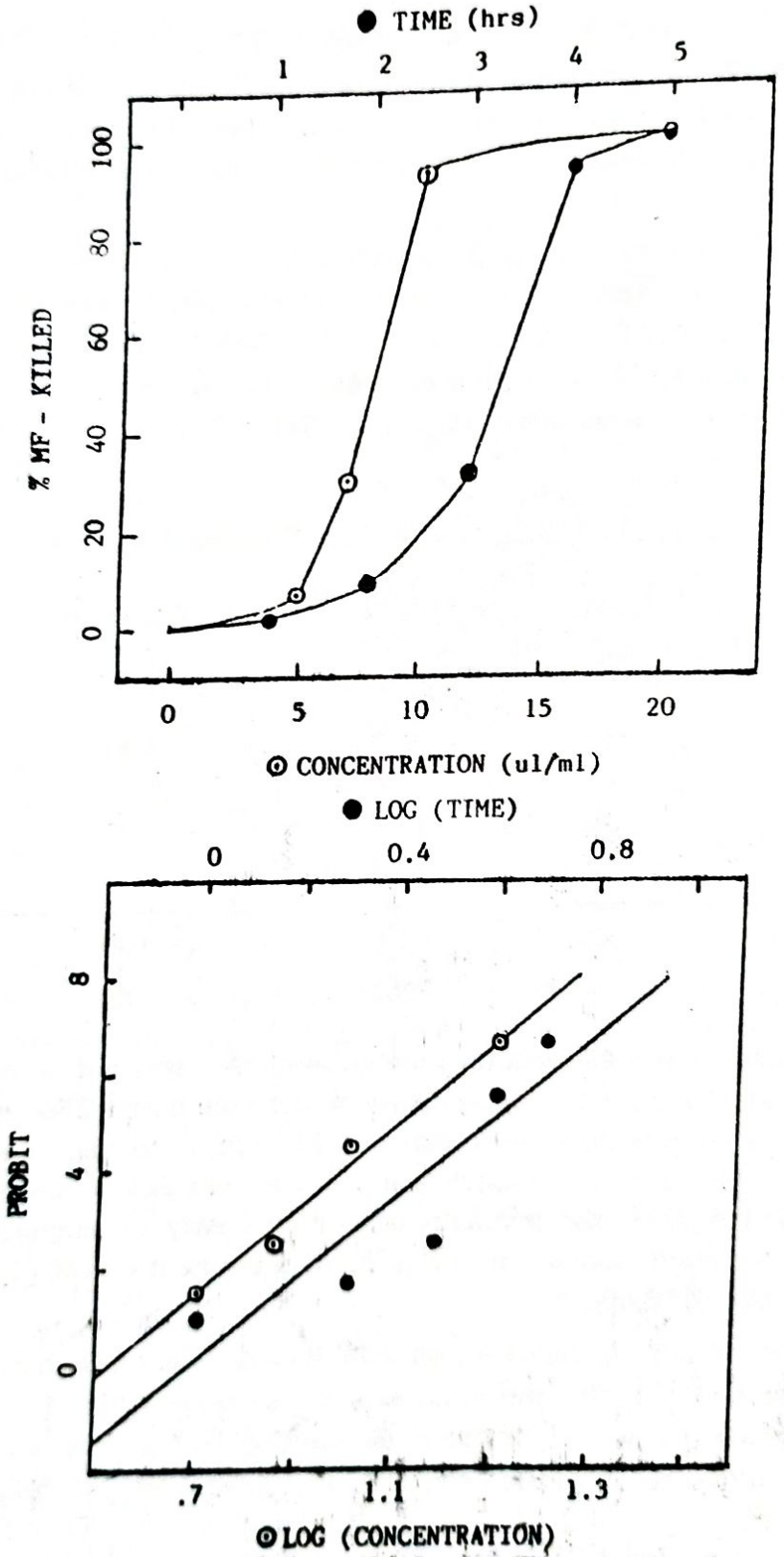

Fig.1 Concentration and time dependence of activity.

F.6. Role of Insect Haemolymph in microfilarial development in culture.

Scientist	:	Dr. Nikhil Mohan Pattnaik
Technical Staff	:	Mr. Pramod Kumar Rana
Starting date	:	July 1987

Microfilaricidal component that had been shown to occur in the haemolymph of several insect species is being studied in detail currently.

The distribution of the microfilaricidal/red blood cell eytic activity is found not to be uniform but shows a bimodal pattern in the adult tussar moths (Fig. 2). About half the insects exhibit this activity in their haemolymph in appreciable quantity. The activity in larvae were generally lower. The stability of this activity is variable with a half life of less than one month whether stored frozen or at 4°C. The loss of activity at higher temperatures is rapid with $t_{1/2}$ around ten minutes at 60°C. The heat inactivation profiles were different,

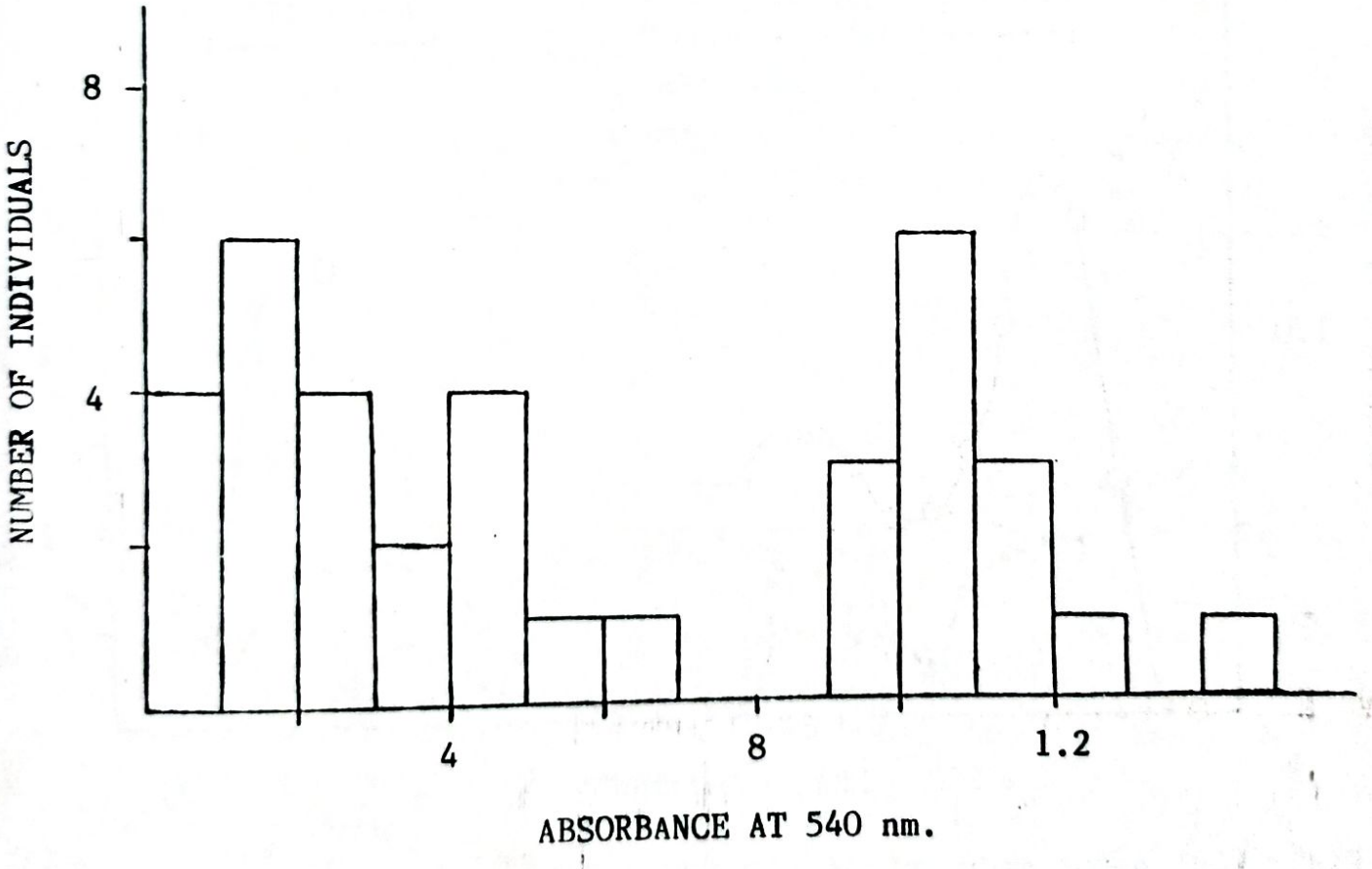

Fig.2 Distribution of rbc lytic activity in the adult tussar haemolymph

however, for the various fractions. The activity in low molecular weight form and larval haemolymph being more stable than that in the high molecular weight form and adult haemolymph.

Attempts to generate this activity *in vivo* by sensitising the larvae with injections of bacteria or yeast have not been successful. Nor has the proteolytic treatment of the collected haemolymph *in vitro*.

As noted earlier, the adult tussar haemolymph shows multiple activity peaks on gel permeation (Fig. 3). The two lipoprotein containing peaks (I & II) account for nearly 50% of the activity while the third (III) for the rest, the last active fraction eluting at the

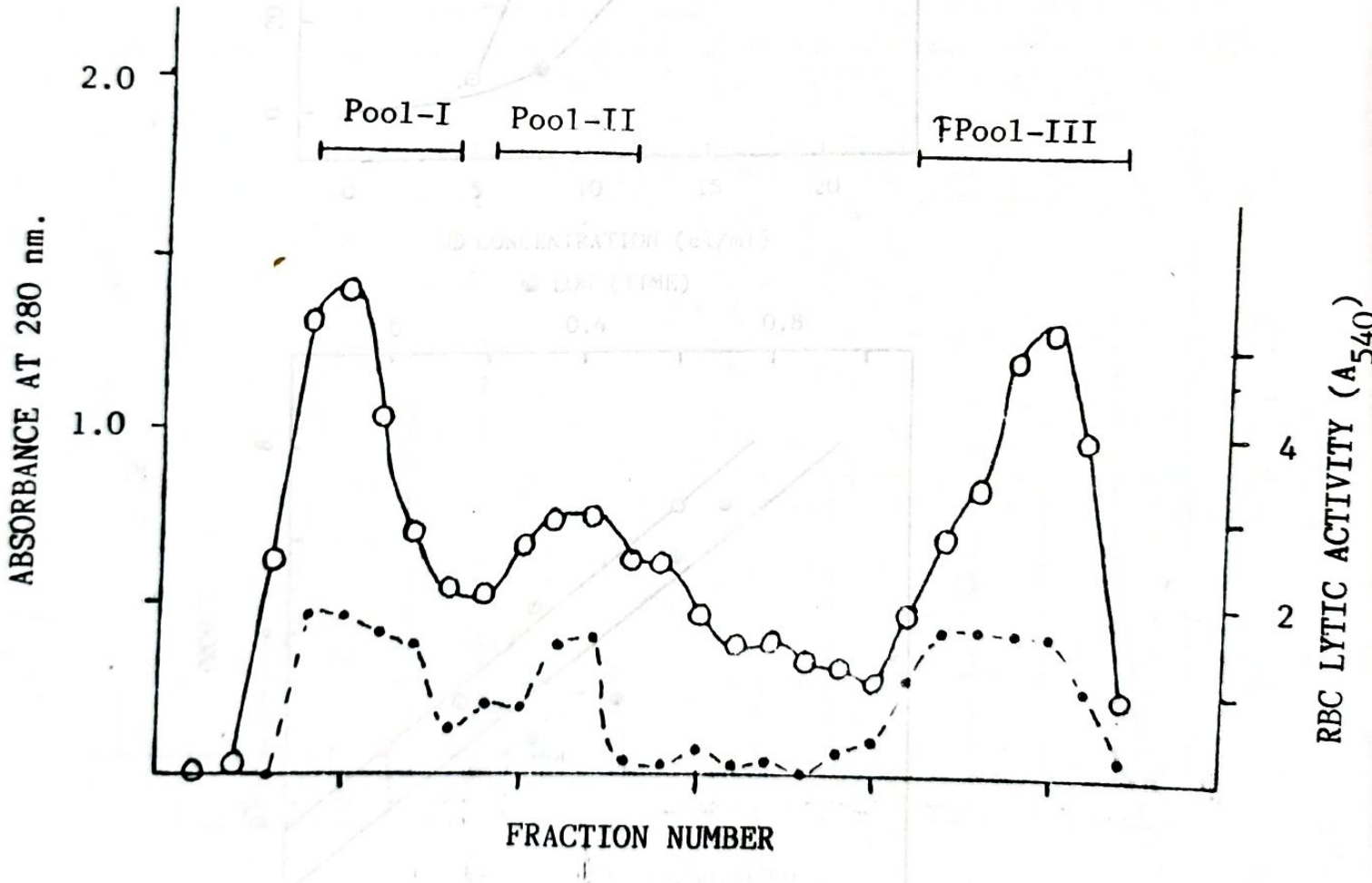

Fig.3 Gel permeation chromatography of adult haemolymph on Sepharose 6B. -o- Absorbance at 280 nm. -o- rbc lytic activity (A_{540}) (Pool-I 19%, Pool-II 22.5%, Pool-III 58.5%).

included volume is similar to the larval form. On rechromatography the three active adult fractions eluted at their corresponding positions indicating that the activity profile in adult haemolymph does not represent an association equilibrium. Disruption of lipoprotein structure with organic solvents did not yield clear results regarding the possible non-covalent association of the active component as the activity was destroyed in the process.

At present efforts are being made to probe the integral/associated nature of the activity in the high molecular weight fraction. Tyrosine metabolites of similar molecular weight are also being examined as the possible source of this activity.

F. 7 Public Health Perspectives of Filariasis and Evaluation of Alternate Medicines.

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

Technical Staff : Sri Dasarathi Das
Sri Pramod Kumar Rana
Sri Harisankar Nayak

Starting date : February 1986

This ongoing project aims at collection of samples for laboratory work as well as utilising the efforts for evolving a suitable control measure using the available medicines. During this year manpower constrains, particularly the lack of medical personnel, did not permit any new interventions to be initiated. Only general assessment of the study populations in Mendasal, Jamujhari and Beldal was done to identify the active filarial cases (Table 3). Follow up of microfilaremia was done only to a limited extent and found to remain unchanged in Jamujhari and Beldal.

Table 3

Filarial disease status of the study populations.

Population	Treatment	Active cases/Total followed up					
		Pre-treatment	%	Post-treatment	(%)	% decrease	Medicine / Placebo
1. Mendhasal	Placebo	62/155	(40)	37/102	(36.3)	9.3	1
2. Mendhasal	DEC 1.0g	44/223	(19.7)	15/98	(15.3)	22.4	2.7
3. Jamujhari	DEC 3.0g	72/286	(25.2)	22/159	(13.8)	45.0	4.8
4. Beldal	Placebo	43/190	(22.6)	27/180	(15.0)	33.7	1
5. Beldal	Homoeopa-thic Medicine	41/190	(21.6)	16/180	(8.9)	58.9	1.8

F.8 Characterization of filarial antigens in bancroftian filariasis.

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

Technical Staff : Mr. Keshar K. Mohanty (CSIR Research Fellow)

Starting date : October 1988

Progress :

One of the objectives of this work is to detect, quantitate and characterize the parasite antigen(s) from human sera (bancroftian filariasis), using antibodies raised in rabbits against parasite antigens and diethylcarbamazine (DEC). The following necessary reagents have been prepared towards realising this objective.

1. Antibodies in rabbits against soluble antigens of *Setaria digitata* adult worms. The high titered serum gives three precipitin lines (in gel diffusion) against the parasite antigen.
2. Immuno-affinity purified parasite specific antibodies. Rabbit anti-setaria hyper immune serum was passed through a column of *S. digitata* antigen coupled to CNBr activated Sepharose 4B. Specificity of the affinity purified antibodies has been verified by an ELISA.
3. IgG fraction of Rabbit anti DEC antisera, using a Protein A-sepharose column.
4. Anti DEC antibody (IgG)—alkaline phosphatase conjugate. The conjugate was prepared by the glutaraldehyde method and it works optimally at 1:200 dilution.

F.9 Chemotherapy of Filariasis : Comparative study on efficacy of DEC in different dosage schedule.

Scientist : Dr. S. K. Kar

Technical staff : Ms. J. Mania
Mr. P. K. Kar
Mr. S. C. Rout

Starting date : January 1988

Subjects presenting with grade II and grade III filarial lymphoedema and/or hydrocele were screened from an area (Khurda) endemic for bancroftian filariasis.Age and sex matched controls were taken from subjects showing similar clinical manifestations.

The regimens of DEC were 10 mg/kg wt/day and 6 mg/kg wt/day for 3 weeks for study and control group respectively (annual report 1989 p.5). Periodic assessment of clinical status, blood microfilaria, filaria specific antibody (IgG/IgM), CIC, and biochemical tests (SGOT, SGPT, Alkaline phosphatase, serum cholesterol) are being carried out. The tissue tonometry, limb circumference are measured every month.

Previous pilot chemotherapy study (annual report 1988 p.7) on filarial lymphoedema had shown beneficial results with long term high doses of DEC in reversing lymphoedematous state. A total of 125 cases (grade II and III lymphoedema and hydrocele) and 21 control subjects are studied in controlled fashion in the field (annual report 1989). The periodic evaluation of study cases show reduction of limb size as assessed by limb circumference measurements and tissue tonometry with associated relief of pain and heavyness of the limb. This was particularly observed in 23 out of 28 subjects with grade II lymphoedema of lower limbs. The rate of reduction of limb size in grade III cases is slower. Blood samples collected periodically from the cases and controls are analysed for estimation of circulating filaria specific antibody, antigen and immune complexes. The liver function tests are within normal limits in all cases studied. It was observed that subsequent attacks of lymphangitis was associated with increase in level of circulating immune complexes. The reduction of limb circumference in both grade II and III lymphoedema and hydrocele (reduction of thickness of skin and firmness and size of testecles) are observed. The study needs to be continued for a long period in order to assess the effect of therapy on reversibility of lymphoedema and restoration of normal state of the limb with associated immune changes in the host.

F.10 Evaluation of cardiopulmonary involvement in Tropical Pulmonary Eosinophilia and response to DEC.

Scientist	:	Dr. S. K. Kar
Technical staff	:	Ms. J. Mania
		Mr. K. Dhal
		Mr. R. N. Nayak
Starting date	:	June 1988

This study was organised in Unit III dispensary, Bhubaneswar where suspected cases of TPE are being referred to the clinic twice a week.

Till now 52 cases of both the sexes have been studied with a mean age of 31.3 $\pm$ 11.2. The subjects having absolute eosinophil count more than 3000/cu mm are included in the study after detailed clinical examination. Majority presented with symptoms of cough

& wheezing. However 11 out of 52 (21.15%) did not have any respiratory symptom or sign but had peripheral blood eosinophil of more than 3000 per cu mm of blood. Cases having helminthic infestations were treated with antihelminthic drugs.

After the treatment with DEC the mean eosinophil percentage (47.9 ± 21.1) and absolute eosinophil count (10,624 ± 8,290) reduced significantly to 22.1 ± 14.2 and 3,141 ± 2,146 ($p < 0.01$) respectively.

Significant reduction of filaria-specific anti-bodies of IgG and IgE class and increase in the circulating immune complex level was observed. However, there is no significant change in the antigenemia status after the initial treatment with DEC.

The above immune alterations were more pronounced in cases presenting with marked eosinophilia ($E\% > 50$) in their peripheral circulation. The spirometric values (respiratory function tests) and ECG are recorded besides clinical examination in each follow up assessments.

Justification for continuation :

Long term follow up of patients after the DEC therapy is needed to study the reversibility of episodic attack and to correlate with blood eosinophil level and immunological status of these patients. Besides, various dosage schedule of DEC can be assessed in controlled fashion during follow up period.

F.11 Long term follow-up of asymptomatic microfilaraemics in an endemic area with reference to their peripheral eosinophils and antibody level.

Scientist	:	Dr. S. K. Kar
Technical staff	:	Ms. J. Mania
		Mr. P. K. Kar
		Mr. T. Moharana
Starting date	:	November 1985

Progress :

Eighty subjects out of 114 asymptomatic microfilaria carriers co-operated for the fourth year follow-up studies. Out of them 9 cases developed early clinical manifestations of filarial disease. Majority 67 (83.75%) subjects continued their microfilaraemic status. The circulating antigenemia was detected in 73 (91.25%) cases. Filaria specific antibody of

IgG class was higher in the persons converted to disease as compared to other asymptomatic cases. Four subjects became amicrofilaremic without developing any marked clinical manifestations. In these cases the antibody titre was higher than the cases presenting with clinical filarial signs.

Justification for continuation :

The above study will highlight the changes in host immune responses as observed during conversion of carrier state to amicrofilaraemic or disease state.

F.12 Studies of Ivermectin in the treatment of Bancroftian Filariasis in Orissa, India.

Scientist	:	Dr. S. K. Kar
Technical Staff	:	Ms. J. Mania
		Mr. P. K. Kar
Starting date	:	June 1989

This study was approved by the council and aided by WHO (TDR). The study aims to determine the optimal dosage of Ivermectin effective for clearing *W. bancrofti* with fewest associated side reactions in patients from Puri district, Orissa. Asymptomatic microfilaria carriers were screened as per the criteria of the study from village around Khurda, Puri District for the trial. The fund for the study was received in Nov.'89. The initial hospital based study was initiated in last week of January using 10 observation beds arranged in Unit IX hospital, Bhubaneswar with permission from local health authorities of the state. The study has got the ethical committee clearance and the patients are admitted to the trial obtaining their consent. Ivermectin is given to the carriers in 20, 50, 100 or 200 ug/kg, single dosage to patients in double blind fashion and the effects on microfilarial clearance and side reactions if any are observed. Two SRFs (Medical), trained staff nurses and attendants were engaged for the trial during hospital based study.

The study will take approximately one year for completion.

2. MALARIA :

M.I. Studies on naturally occurring antibodies with ∝-galactosyl activity in *P. falciparum* malaria.

Scientists	:	Dr. B. Ravindran
		Dr. M. K. Das
Technical staff	:	Mr. Ashok K. Satapathy
		Mr. Ramakrishna Das
		Mr. Prakash K. Sahoo
Starting date	:	March 1986

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

Progress :

(a) The development by us of a cell-ELISA for quantification of anti-gal, the increase in their titres in human *P. falciparum* infections and sub-class typing of anti-gal titres in human sera were reported earlier and the details were published in Immunology Letters (vide Annual Report 1988-89).

(b) Further studies were undertaken to affinity purify anti-gal using a column of the absorbent Gal 1-3 Gal B 1-4 Glu Nac-Syncorb. The eluate were found to contain IgG, IgM and IgA isotypes with affinity to ∝-linked galactose but not to β-linked galactose on rabbit erythrocyte monolayers.

(c) However, more interestingly the anti-gal were found to have multiple reactivity—affinity purified anti-gal (using the above mentioned ligend) was found to have reactivity towards various phospholipids (phosphatidyl choline, phosphatidyl serine, phosphatidyl

inositol, phosphatidyl ethanolamine) double stranded and single stranded DNA and keyhole limpet haemocyamin. This cross-reactivity could be significantly inhibited by preincubation of affinity purified anti-gal with melibiose. Further studies are in progress.

M.2 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
Ms. T. Hussain
Starting date : March 1986

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

Progress :

a) Serum autoantibodies with specificity to N-acetyl neuraminic acid reacting with the surface of normal mouse as well as *P. berghei* infected mouse erythrocytes were reported by us earlier (vide annual report 1988-89).

b) These autoantibodies reacted with the surface of splenic lymphocytes also. However when the titres of the autoantibodies (by ELISA) were compared with lymphocytotoxic antibodies (by cytotoxicity assay) there was no positive correlation.

c) The studies were extended to *P. chabaudi* infected mice also. Unlike *P. berghei* which causes a lethal infection in mice *P. chabaudi* causes a self-limiting infection and the animals completely recover in about 15 days. Autoantibodies with specificity to N-acetyl neuraminic acid increase 5-6 fold during the recovering phase of *P. chabaudi* infection. Further, the antibody reactivity of normal mouse sera to the surface of *P. chabaudi* infected erythrocytes indicating that host neo-antigens are expressed on entry of parasites.

M.3 Induction of cell mediated immunity in malaria by immunomodulators.

Scientists : Dr. M. K. Das
Dr. B. Ravindran
Technical Staff : Mr. M. K. Beuria
Mr. H. S. Naik
Starting date : June 1986

Cellular immune response (CMI) are known to be depressed in malaria possibly due to a defect in macrophage processing of antigens. The present project is to utilize immunomodulators in enhancing CMI in malaria.

Progress :

We earlier showed that *P. berghei* antigens mixed with adjuvant (Alum & saponin) induced appreciable DTH and production of MIF in infected (Balb/c) mice. Recently we have used dextran sulfate (DS), which is known to be a strong adjuvant for CMI and a liver blocking agent, on the course of *P. berghei* (NK 65 strain) infection. Infected mice (n=6) received DS (40 ug/kg/b.wt) at 48 hr intervals from the first day of infection and parasitaemia was monitored. The observations are :

1. The latent period (the time between inoculation and the rise of growth of parasites in peripheral blood) was about 5 day in the control mice compared to 12 day in DS-treated group.

2. Mice in the control group were dead by 18 day post-infection (range 13-18 day) but DS-treated mice survived longer (range 17-30 day). 50% mortality was noted in control and DS-treated mice on day 14 and on day 17 respectively.

The *in vitro* toxicity of DS on parasitized erythrocytes was ruled out. The exact mechanism by which DS was effective in limiting parasitaemia remains unknown at present.

Dr. S. S. S. Mohapatra, Research Officer of this centre is working as one of the members of VCRC team of Scientists on various studies on malaria, being located at Jeypore. All the studies are being designed and carried out under the guidance of the Director, VCRC, Pondicherry.

3. CLINICAL PATHOLOGY :

3.1. Study of Erythrocytic Glucose-6-Phosphate dehydrogenase (G6PD) deficiency and its variants in a malaria endemic area with tribal population.

Scientist : Dr. G. P. Chhotray

Technical Staff : Mr. M. R. Ranjit

Mr. H. K. Khuntia

Mr. B. Sethi

Starting date : April 1986

A pilot study has been conducted to find out the prevalence of G-6-PD deficiency in the Banspal block of Keonjhar district where 13.14% of tribals were found to be G-6-PD deficient as compared to 3.69% in nontribals (vide annual report 1988-89 page 19). The study was further continued with a view to concentrate on the variants. More male 97 cases (77 tribals & 20 nontribals) were included in the study during this period. Apart from a detailed clinical examination their place of origin, ethenic group, language of parents etc. were noted as per protocol.

10-20 ml of venous blood was collected from 14 persons having severe G-6-PD deficiency and 5 non-deficient persons of the same area (to serve as control) and was transported to RMRC laboratory in ACD anticoaglunat at 4°C within 12 hrs of collection. The haemolysate was prepared and G-6-PD enzyme assay was carried out. The enzyme activity varied from (1.14—2.34 IU/g of Hb) in deficient samples as compared to 7.4—9.69 IU/g of Hb in control cases. The Km for G-6-PD was calculated which ranged from 40-47 um in deficient cases as against 57-66 um in non-deficient cases. Electrophoretic characterisation of G-6-PD enzyme was done by submerged agarose gel electrophoresis using G-6-PD non deficient persons blood as control.

From these data collected so far it appears that the most common deficient samples have similar properties with a G-6-PD activity of about 10-20% of normal and B-like electrophoretic mobility.

DNA extraction was carried out and the purity of the DNA extracted was established by running in a gel with control (in Prof. Luzzatto's laboratory at U.K.).

Efforts are being made to characterise its variants and to establish whether they are all the same mutation or not by probing at the molecular level in collaboration with Prof. Lucio Luzzatto of Royal Post Graduate Medical School, London.

Justification for continuation :

The above research proposal was recommended by the Scientific Advisory Committee for a collaborative study with Prof. Lucio Luzzatto, Royal Postgraduate Medical School, Hammersmith Hospital, Haematology Division, London W OHS. It was also approved in the Health Ministry's Screening Committee Meeting held on 23.3.88 and has been forwarded by the ICMR headquarters.

4. ENTOMOLOGY

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

Scientist : Dr. A. P. Das

Technical Staff : Sri R. K. Hazra

Sri H. K. Tripathy

Sri G. D. Mansingh

Starting date : September 1988

Progress :

4.1.1 Distribution of *B. malayi* :

Brugia malayi infection was not known in India till Korke reported it in Balasore district of Orissa state in 1929. Earlier workers reported that *B. malayi* infection had a localised distribution in the districts of Orissa namely Balasore, Bolangir and Puri districts. Subsequently, filaria surveys carried out in 1979-80 in areas of Orissa reported to be endemic for *B. malayi* four to five decades ago, reveals complete disappearance of *B. malayi* infection as well as *Mansonioides* mosquitoes. Recently, however, this centre has detected *B. malayi* infection in Balasore district and some interior areas of Orissa. Since systematic studies on prevalence of *B. malayi* and *Mansonioides* in Orissa were lacking, the present work was undertaken to report the present status of *B. malayi* infection and its distribution in Orissa. Extensive surveys were carried out in three districts viz. Balasore, Bolangir and Puri, which were known to be endemic for *B. malayi* infection earlier. Table 4 represents the area wise collection and examination of blood smears. Out of the blood smears collected from 858 persons; 686, 135 and 37 were from Puri, Balasore and Bolangir districts respectively. As would appear from table 4, *B. malayi* infection is still existing in Puri and Balasore districts whereas none of the slides so far collected from Bolangir district was positive for *B. malayi*. Prevalence of filariasis was 11.8% and 5.18% in Puri and Balasore districts respectively. Infection due to *B. malayi* was 4.8% and 4.44% in Puri and Balasore districts. From table 4, it is seen that the percentage of *B. malayi* infection in total positive cases was 40.7% and 85.7% in Puri and Balasore districts respectively.

Table 5 compares the incidence of *B. malayi* during 1928-29, and 1988-89.

Table 4

Prevalence of *B. malayi* in Orissa

Sl. No.	Name of the area (village)	B.S.C./ B.S.E.	No. Positive	B.m.	W.b.	Mix	Remarks
	Puri District						
1.	Kadua	123/123	25	12	13	0	
2.	Bhatapur	71/71	10	3	7	0	
3.	Bira Ramachandrapur	295/295	24	11	12	1	
4.	Ahmedpur	16/16	0	0	0	0	
5.	Bira Gobindapur	181/181	22	7	11	4	
	Total	686/686	81	33	43	5	
	Balasore District						
1.	Soro area	18/18	0	0	0	0	
2.	Talanagar	40/40	4	4	0	0	
3.	Mirzapur (Chandipur)	77/77	3	2	0	1	
	Total	135/135	7	6	0	1	
	Bolangir District						
1.	Patnagarh (Tikapali)	37/37	2	0	2	0	
	Grand Total	858/858	90	39	45	6	

Table 5

A Comparison in the Incidence of *B. malayi* Infection in Balasore District During 1928-29 and 1988-89.

	1928-29 (Korke, 1929)		1988-89 (Present Authors)	
No. Examined	1450		135	
No. Positive	234	(16.13%)	7	(5.18%)
W. bancrofti	113	(7.79%)	0	(0)
B. malayi	121	(8.3 %)	6	(4.44%)
Mixed	0		1	(0.74%)

4.1.2 Biting rhythm of two species of *Mansonioides* in Orissa.

Though transmission of *Brugia malayi* depends upon man vector contact, no report on the biting rhythm of its vectors exists in the state. Therefore the present work was undertaken to report the biting rhythm of *Ma. annulefera* and *Ma. uniformis* the two important vectors of *B malayi* in the state. All night collection of mosquitoes was done in Puri district. Mosquitoes were collected on human and cattle baits throughout the night i.e. from dusk to dawn. The collection was made hourly with a frequency of ten minutes interval. It is observed from Table 6 that both the species of *Mansonioides* i.e. *Ma. annulifera* and *Ma. uniformis* bite throughout the night. Peak biting activity of *Ma. annulifera* was between 01.00 to 04.00 hour and that of *Ma. uniformis* was 18.00 to 19.00 hour. Average number of mosquitoes belonging to *Ma. annulifera* and *Ma. uniformis* feeding on man and cattle per night was 53.1 and 27.4 respectively. An average of 28.6 and 24.5 *Ma. annulifera* were collected on man and cattle respectively per night while those of *Ma. uniformis* were 12.3 and 15.1. *Ma. annulifera* feeds more on man than *Ma. uniformis.* The study reports that biting rhythm of *Ma. annulifera* and *Ma. uniformis* is not similar i.e. when biting activity of one species reaches the peak, the same of the other species comes down and vice versa.

Entomological data have been collected from localities of Puri and Balasore districts. *Mansonioides* were the dominant mosquitoes. Per man-hour density of *Ma. annulifera* and *Ma. uniformis* are presented below. (Table 7)

Table 7

Month and Year		Per man hour density of	
		Ma. annulifera	*Ma. uniformis*
April	1989	8	15.2
May	1989	3	5.5
June	1989	3.5	3.16
July	1989	15.3	28.5
August	1989	22.8	11.25
September	1989	12.3	6.1
October	1989	5.2	2.6
November	1989	2.5	1.6
December	1989	3.5	4

Table 6

Man and Cattle Biting Rate of *Mansonia uniformis* and *Mansonia annulifera* in Biragobindapur, Puri District, Orissa

Time	*Ma. uniformis*						*Ma. annulifera*					
	Human bait		Cattle bait		Total		Human bait		Cattle bait		Total	
	Biting rate	%Biting	Biting rate	%Biting	Biting rate	%Biting	Biting rate	%Biting	Biting rate	%Biting	Biting rate	%Biting
06—07 PM	3.6	29.3	1.1	7.2	4.7	16.8	1.0	3.5	1.3	5.3	2.3	4.3
07—08 PM	1.6	13.0	2.0	13.2	3.6	13.1	1.0	3.5	1.6	6.5	2.6	4.9
08—09 PM	0.8	6.5	1.3	8.6	2.1	7.7	1.8	6.3	1.3	5.3	3.1	5.9
09—10 PM	1.6	13.0	2.1	13.9	3.7	13.1	1.8	6.3	1.0	4.0	2.8	5.2
10—11 PM	1.6	13.0	1.7	11.2	3.3	11.7	1.8	6.3	1.0	4.0	2.8	5.2
11—12 PM	0.3	2.4	2.0	13.2	2.3	8.4	2.0	6.9	1.3	5.3	3.3	6.2
12—01 AM	0.2	1.6	1.0	6.6	1.2	4.4	2.2	7.7	2.6	10.6	4.8	9.0
01—02 AM	1.6	13.0	0.6	3.9	2.2	8.0	5.0	17.5	3.6	14.6	8.6	16.2
02—03 AM	0.6	4.9	1.0	6.6	1.6	5.9	4.0	13.9	3.6	14.6	7.6	14.3
03—04 AM	0.2	1.6	—	—	0.2	0.7	3.4	11.9	5.6	22.9	9.0	16.9
04—05 AM	0.2	1.6	2.3	14.8	2.5	9.0	1.6	5.6	3.6	14.7	5.2	9.8
05—06 AM	—	—	—	—	0.3	1.0	3.0	10.5	1.0	4.0	4.0	7.5
TOTAL	12.3		15.1		27.4		28.6		24.5		53.1	

The progress of work is slow due to non-availability of staff (post sanctioned are lying vacant). The routine work is seriously affected after the resignation of the Research Assistant (who joined CSIR as Research Officer) and the sudden death of an Insect Collector.

4.2 Influence of two aphid extracts and five Juvenoids on some biochemical composition during the post-embryonic development of *Cx. quinquefasciatus*

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

Technical staff : Mr. M. R. Ranjit

Starting date : January 1987

In the present study an attempt has been made to investigate the comparative effect of natural juvenile hormones extracted from two local common aphids viz. *Aphis craccivora* (TAEac) and *Aphid gosypii* (TAEag) and five Juvenoids viz., methoprene, Neporex, OMS 3007, OMS 3019 and DPE 28 having different functional groups, on the total glycogen, lipid and protein content and activities of some enzymes viz. alkaline phosphatase, (ALP) Acid phosphatase (ACP), Glucose-6-phosphate dehydrogenase (G-6-PD) and Acetyl choline esterase (AchE). Test concentrations of median lethal dose (LD_{50}) and maximal lethal dose (LD_{90}) were prepared for individual compounds by adding appropriate volumes of stock solutions (1 μg/10/μl) in 1 litre of water kept in 12" x 12" x 3" white enamel tray. Separate controls with acetone and another set containing plain water were taken simultaneously for comparison with the treated ones. Eggs were released into the individual trays and kept at 27°C and 75-85% RH for the study.

4.2.1. Effect on total Glycogen content

Treatment of the mosquito eggs by the aphid extracts and Juvenoids have invariably resulted in a lower glycogen content of the 4th instar larvae and pharate adults in comparison to the untreated and control groups. In our study the Juvenoid OMS 3007 has been found to be most effective amongst the TAEac, TAEag, Methoprene, Neprex, OMC 3019 and DPE-28 in reducing the total glycogen content of the fourth instar larvae as well male and female pharate adults.

4.2.2. Effect on Lipid metabolism

The importance of lipid in insect development and metabolism is well known especially when great changes occur such as those before and during metamorphosis, diapuse and starvation. Comparative effects of aphid extracts (TAEac and TAEag) and the

juvenoids (Methoprene, Neporex, OMS 3007, OMS 3019 and DPE-28) on the lipid metabolism on the fourth instar larvae and pharate adults (male and female) are reported here. No statistically significant change of total lipid accumulation in 4th instar larvae and pharate male and female adults was observed in comparison to the untreated and control groups. However, the study is on the way to find out any change in the classes of lipids accumulated by the influence of aphid extracts and juvenoids.

4.2.3. Effect on some enzyme activity and protein content

The effect of aphid extracts and the juvenoids on the activities of Alkaline phosphatase, Acid phosphatase, Glucose-6-phosphate dehydrogenase and Acetyl choline esterase of the 4th instar larvae and pharate adults (male and female) were studied after treating the eggs of the *Cx. quinquefasciatus*. It is found that ALP activity has been significantly increased and G-6-PD activity has been significantly decreased while the activities of ACP and AchE remained unchanged. The total protein content has also been decreased significantly in comparison to the untreated and treated groups.

4.3. Laboratory biology and colonization of mosquitoes

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

Aedes aegypti

Culex quinquefasciatus

Culex tritaeriorhynchus

Mansonia uniformis

The laboratory colonized mosquitoes are used for various studies.

Colony of black headed Liverpool strain of *Aedes aegypti* failed to continue due to high temperature in May/June, 1989 and non-availability of A.C. facilities.

5. MYCOBACTERIAL GENETICS

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

Progress :

Because of inadequate supporting staff (one JRF who was earlier associated with this work has obtained a CSIR fellowship and has opted to work on filariasis), not much has been done other than periodical maintenance and marker-verification of the mycobacterial mutants. A few additional experiments have been done to supplement the data on mutants. Briefly, the results are :

1. In cross feeding experiments, the results were negative (and hence unilluminating) with six methionine (SM24, SM25, SM26, SM27, SM39), six purine-(SM3, SM19, SM21, SM38, SM40, SM44) and three pyrimidine-(SM33, SM34, SM37) auxotrophs.

2. The minimum inhibitory concentration of silver nitrate was found to be 64 ug/ml for *M. smegmatis, M. fortuitum* and *M. vaccae* and that of mercuric chloride was 64 ug/ml for *M. smegmatis* and *M. fortuitum,* and 32 ug/ml for *M. vaccae*. The auxotrophic mutants derived from these three species exhibited MIC values similar to the parent-strains.

6. BIOSTATISTICS

Mr. Anil Kumar S.R.O.

Statistical consultation is provided to scientific workers. Attempts are being made to incorporate the essential facilities for computing A project was initiated in July 1989 to assess the trend of mortality and morbidity level in Orissa and data, secondary in nature, have been collected from Govt. sources. Two documents are being prepared on this data.

a) District level Abridge life table for Orissa in rural-urban areas.

b) Health situation in Tribal dominated districts of Orissa.

Projects Completed

1. Characterization of immune response in man elicited by mosquitoes

Scientists : Dr. Manoj K. Das
Dr. A. P. Dash

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

Starting date : April 1986

We reported earlier the presence of antibodies to female *Culex quinquefasciatus* in normal and filariae-infected population (RMRC Annual Report 1987-88, 88-89). The antibody level in the two groups did not differ significantly. Antigenic extract from salivary gland was found to be superior regarding reproducibility in ELISA compared to whole-body extracts. The age-specific prevalence of anti-*Culex* IgG and IgE antibodies in healthy human population living in filariae-endemic regions was determined. The sera from 75 people collected by finger-prick method were arranged in 5 groups for comparison—group A for children ($<$ 5 yrs), group B for 10-16 yrs old and group C to E adults (18-70 yrs).

1. From the table-8 it is clear that there was a progressive increase in the titer of antibodies with the increase in age.

2. The titre of IgG antibodies (but not of IgE) increases considerably in group B (mean age 12.85 $\pm$ 1.34 yrs) compared to that in group A (mean age 4.10 $\pm$ 1.17 yrs). This increase in IgG titer continued albeit marginally in group C and D (mean age 29.42 $\pm$ 5.89 and 43.45 $\pm$ 3.95 yrs).

3. In contrary to IgG, IgE titer did not differ significantly between group A and B, but it showed considerably enhancement in group C and others.

4. It may be inferred that the maturation of IgE titer occurred at later age in group C (29.42 yrs) whereas IgG titer attained maximum at earlier age.

Table 8

Titer of *Culex quinquefasciatus* specific antibodies in human sera

Group	Age (yrs)	n	Titer	
			IgG	IgE
A	1- 5	10	337 ± 155.20	180 ± 133.23
B	10-16	21	1100 ± 660.87*	300 ± 272.34
C	18-40	21	1500 ± 620.67*	850 ± 354.90*
D	41-50	13	1610 ± 633.20*	790.9 ± 308.07*
E	51-70	10	1170 ± 457.16*	810 ± 170.29*

*Compared to Gr.A, $P < 0.01$.

2. Sciatica-like syndrome in Filariasis

Scientist : Dr. S. K. Kar

Technical staff : Ms. J. Mania
Mr. T. Moharana
Mr. R. N. Nayak
Mr. K. Dhal

Starting date : October 1987

A variety of syndromes like tenosynovitis, lateral nerve palsy, dermatosis have been suggested as manifestations of filariasis. During survey in endemic areas it was observed that a segment of population had complaints of neuralgic pain originating at a focus approximately one and-a-half to two inches lateral to midline of L_3-L_4 vertebra level radiating downwards along sciatic nerve distribution. These symptoms were of waxing and waning type persisting for 2 to 3 years. During acute episodes the individual becomes incapacitated. Palpable tender nodules were detected at the above referred sites. Few of these patients were microfilaraemic and had other associated filarial lesions. Subjects having similar pain manifesting in hand had palpable tender nodules in the same side interscapular region. Treatment with DEC resulted in disappearance of pain and reduction of size of the nodule. Hence, the study was undertaken to evaluate the association of the filarial infection with these lesions and to assess the effect of DEC therapy in these patients.

AR-38

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

Seventeen out nof 78 (21.79%) subjects were microfilaraemic. The mean eosinophil percentage was foud to be 11.1 $\pm$ 6.1. The subjects positive for filaria specific antibody were 47 (60.25%), 25 (32.05%) and 19 (24.36%) for IgG, IgM and IgA class respectively. The mean circulating immune complex level (as determined by PEG precipitation) in these cases was higher (0.121 $\pm$ 0.058) than that of endemic normals (0.098 $\pm$ 0.001). Fifty nine subjects (75.64%) were positive for filaria specific antigen.

Tender subcutaneous nodule was detected (1.5—2.0 cm in diameter) in all the subjects approximately 2" lateral to mid ventral line at L_3-L_5 level. In few cases the upper limb was affected where the tender nodules were observed at interscapular area on the same side.

The X-ray examination of spine (antero-posterior and lateral view) centering around L_{3-4} done in 10 patients did not reveal any bony abnormality so as to cause the neuralgic pain. Neurological examinations did not reveal any neurological deficit which could be attributed to cause unilateral limb pain.

Ultrasound scanning carried out in these 10 cases demonstrated nodules at L_{3-5} level defining its size and position. The nodules were subcutaneous and situated above the muscle plane.

Aspiration cytology carried out in 7 cases from nodules showed few lymphocytes and eosinophils not specifying any definite aetiology. Biopsy of the nodule in one patient revealed that the nodules are in chain in lumbar area appeared to be enlarged lumbar lymph nodes.

In order to assess the effect of DEC in these patients, 6 out of 10 cases (whose ultrasound was carried out) were given DEC and rest 4 placebo (Vit C tab). DEC was administered in dose of 6 mg/kg wt/day for 3 weeks every month for 4 months. The cases and controls were evaluated periodically by clinical and blood examination and by repeat ultra sound scan after 4th month of therapy.

The repeat scan demonstrated significant reduction of size of these nodules in 6 study cases whereas in rest 4 cases (with placebo) no change in size was observed.

Besides, the cases were relieved of pain and associated symptoms following treatment which was not observed in 4 cases taken as controls.

Above parasitological, immunological and clinical evidences and response to DEC therapy in these cases suggest that painful syndrome resembling sciatica may be a result of the filarial infection. However, histopathological study of the nodules in a number of these cases may confirm the pathogenesis of this syndrome.

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

Scientist	:	Dr. S. K. Kar
Technical Staff	:	Ms. J. Mania
		Mr. T. Moharana
		Mr. S. C. Rout
		Mr. K. Dhal
Starting date	:	March 1986

Few reports have shown the relationship of a form of monoarthritis to filarial infection. Although it appears that filarial infection may manifest in various forms in susceptible individuals, this particular feature needs to be established. In the present study, an attempt to find out the association of filarial infection to a form of nonspecific arthritis prevalent in filariae endemic areas in Puri district was made. Nintythree study cases were selected after screening the endemic population by detailed clinical and parasitological examination. The study cases had manifestation of arthritis (with or without other filarial lesions) not pertaining to any specific aetiology as seen by clinical examination (Rhumatoid, tubercular, osteoarthritis, sepsis, syphilis or drug induced). Seven subjects (taken as controls) manifesting arthritis of known aetiology (osteoarthritis and rhumatoid arthritis) selected at Khurda hospital were also studied for comparison. The blood samples from study cases and controls were assessed for presence of filarial parasite, filaria specific antigen and antibody and circulating immune complexes. Besides synovial fluid taken from knee joint from 7 study cases and 2 controls presenting with effusion were subjected to above immunological tests.

The age and sex, associated filarial lesions and microfilaraemic status of study cases are presented (Table 9).

The positive filaria specific antibody titre of IgG, IgM and IgA carried out by ELISA using mf-ES antigen was detected in 47 (50.5%), 29 (31.2%) and 19 (20.4%) cases respectively. Sixtyfour out of 93 (68.8%) study cases had filaria specific antigen as detected

by using Fs-IgG in ELISA. The mean circulating immune complexes level in study cases was higher (0.129 ± 0.071) than that of 58 endemic normals (0.098 ± 0.01) taken from same village.

Table 9

Subjects with Arthritis and Associated Filarial Lesion and Microfilaraemic Status

Age groups in years	Nos of subjects with arthritis			Nos of cases associated with filarial disease	Nos of cases positive for M.F.	Nos with monoar-thritis	Nos with effusion in joints
	M	F	Total				
0—19	8	5	13	3	5	6	2
20—39	9	8	17	5	7	13	7
40+	30	33	63	11	5	38	29
Total	47	46	93	19	17	57	38

Evidence for presence of C-reactive protein in sera was obtained from 9 out of 12 study cases examined.

Synovial fluid collected from 7 study cases manifesting with effusion revealed high antibody titre (IgG) against mf E-S antigen in all the cases. Out of 5 control patients presenting with effusion of knee (due to other causes) only one had positive antibody titre. Circulating filarial antigen was detected in 4 out of 7 synovial fluid samples taken from study patients whereas none of the control samples had detectable circulating filarial antigen.

Treatment with DEC accompanied by relief of pain and other signs of effusion.

It was observed that the host immune responses against filarial infection was higher in study cases as compared to the controls. The association of microfilaraemia, clinical filarial disease and good response of DEC therapy as observed in study cases also corroborate the relationship of arthritis to filarial infection. However, immunohistochemical and histopathological studies of synovial membrane of these patients may confirm this association and add to the knowledge of pathogenesis of this manifestation of filariasis.

New Projects Proposed for 1990

1. Enzymes of Filarial parasites : Anti-oxidant and Proteinases

Scientist : Dr. Manoj K. Das

Recently, considerable evidence has accrued to implicate the action of oxidants (free radicals) generated by phagocytic cells as a major component in parasitic killing. It has been shown that the effect of DEC, used in therapy and control of filariasis, is mediated by a mechanism that involves the participation of free radicals. However there is the possibility that the presence of anti-oxidant enzymes in the parasites could scavenge the free radicals. Such enzymes are superoxide dismutase, catalase and Glutathione peroxidase. Glutathione-S-transferase, a detoxifying enzyme of xenobiotic compounds, is also related to this class.

Another class of enzymes in helminths is the proteinases. These enzymes which could arise as secreted materials from the parasites have been proposed to have physiological roles in the disease process. Some of these are potent immunogens and have been used as protective antigen, immunodiagnostic marker. Our results (unpublished) from this centre showed for example that anti-collagenase antibody level is higher in filarial sera compared to normal individuals.

The present project aims to detect the enzymatic activity, the comparative levels in various stages of filarial parasites, and their antigenic properties.

2. Induction of Eosinophilia in animals with filarial parasites

Scientist : Dr. Manoj K. Das

Increased production of Eosinophils is a constant feature of helminthic infections. There has been a growing body of evidence to implicate eosinophil as an effector cells in the killing of microfilariae. The present project would aim to establish eosinophilia in experimental animals with filarial parasites and its products. The model so set-up would be helpful in the studies involving eosinophil functions and in characterizing parasite-derived chemoattractants.

3. Methods for the large-scale production of infective-larvae of *W. bancrofti*

Scientists : Dr. Manoj K. Das
Dr. A. P. Dash

Availability of L_3 stage of *W. bancrofti* in large numbers is a serious obstacle for undertaking crucial studies on the immunology of the parasite. The lack of an experimental host as a laboratory model for *W. bancrofti* makes it a formidable task for L_3 isolation in large numbers. The present methods of isolating L_3 are from infected *Culex quinquefasciatus* from the field in endemic regions; from feeding the mosquitoes on microfilaraemic carriers and by membrane-feeding technique. Problems often associated with these methods are inefficiency and inconvenience. Attempts will be made to isolate L_3 by membrane-feeding technique and by intra-abdominal injection of *W. bancrofti* if in the *Culex* (through micro-manipulator). The last method though labor intensive if proved successful should be highly efficient and reproducible.

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

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

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

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

5. Immunologic status of the patients with sickle cell anaemia

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

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

The above mentioned project has already been approved by ICMR expert committee and necessary financial grant has been sanctioned. This is to be started from this year.

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

Scientist : Dr. A. P. Dash

Scientific background and justification :

The present knowledge on the taxonomic aspects of Indian mosquitoes is more than 50 years old. Orissa is highly endemic for both the mosquito-borne diseases, filariasis and malaria. There has not been any systematic study on mosquito fauna of Orissa and consequently our knowledge on mosquito fauna of the state is poor. In view of the importance of the two mosquito-borne diseases there is an urgent need to study the mosquito fauna, their habitat, behaviourial status and vectorial capacity. The study would provide information on (i) changes that have taken place in the mosquito fauna over the years (ii) ecological succession of mosquito species (iii) basic knowledge on morphometry (iv) reveal naturally occurring variation within each taxon and (v) information on age composition, susceptibility status etc. The study would also help in the control/containment of filariasis and malaria in the following manner :

a) Stratification of region as per the relative abundance would help in designing the control measures so that efforts are directed on the target species.

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

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

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

This project was recommended and forwarded to the ICMR, on 25.10.1984 as advised by the Director, RMRC, Bhubaneswar. The same was again sent to the ICMR in six copies vide letter No : 394 dt. 11.2.85 of the RMRC (as advised by the ICMR by telex dt. 19.1.85). Again a reminder was sent to ICMR vide RMRC letter dt. 13.8 86 for communicating the decision. Since no action was taken by the ICMR in this regard, the detailed proposal was submitted in the 3rd Scientific Advisory Committee meeting on 7th July 1988. The SAC unanimously recommended the project strongly and also recommended to strengthen the entomology department. The SAC advised to start this project immediately after recruitment of four insect collectors and one Technical Officer, already sanctioned for the centre. The recruitment of these sanctioned staff is still awaited due to

want of funds and unfortunately the project could not be started till now for obvious reasons.

In the 4th SAC meeting on 26.6.89 it was felt that the project is an important one for the fact that the information gathered will be of great help. The SAC unanimously advised to start the work as soon as the staff sanctioned is recruited. Unfortunately the recruitment of staff is still awaited; posts are lying vaccant because of the ban though interview for some posts are already over since October 89. The project will start as soon as the vaccant posts are filled up, in the next financial year.

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

Scientist : Dr. A. P. Dash

Orissa is one state where not much concentrated efforts have been put in for demonstrating filariasis control through integrated methods. Since it is the function of the RMRC to carry out research on diseases and their control and since Orissa state is highly endemic for bancroftian filariasis, the first Scientific Advisory Committee met on 24.1.1987 advised to take up a control project. A detailed proposal was submitted and the second Scientific Advisory Committee which met on 4.9.1987 accepted the objectives and feasibility of the proposal. As advised by the SAC, the local authorities (NAC) of the area selected were contacted who assured to render all sorts of co-operation for integrated vector control. The Director, RMRC forwarded the proposal to the Govt. of Orissa and Govt. has given approval on 15.3.87. The proposal was submitted to the ICMR for funding. The 3rd SAC was held on 7.7.1988 & in matters arising out of the minutes, there was a discussion on the action taken in the matter as recommended by the second SAC. The Director informed the members that though the preliminary work in connection with this project had been done by the centre, the work could not be started because of lack of sanction for the staff and budget required for this purpose. All the members felt that this work should not be undertaken in a haphazard manner. It was decided by the SAC that this project should be submitted to the WHO for funding as a TDR project.

As advised and recommended by the last SAC, this project alongwith few other projects were submitted to ICMR for onward transmission to WHO. This project was forwarded to ICMR in proper proforma by the Director (vide his letter No. : DIR/RMRC/88/264 dt. 27.7.88). After several correspondence during Oct./Nov. 1988, finally a letter was received from ICMR that this project could be started as an intramural project since it could

not be forwarded to WHO. Again this issue was raised in the 4th SAC met on 26.6.89. This project was strongly recommended by the members unanimously and it was suggested to obtain TDR funding of this project. Though the Director was advised to write to the D.G. for getting the approval of the Health Ministry and submission of this project to the WHO for funding, the project is still in the cold storage.

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

Scientist : Dr. A. P. Dash

As advised and recommended by the SAC, this project was also submitted to ICMR for onward transmission for TDR funding, since *B. sphaericus* has to be obtained from WHO. This project was sent to ICMR by the Director with the above project. In this connection, Dr. B. Dobrokhotov, Secretary, Steering Committee, Biological Control of Vectors, WHO, Geneva visited RMRC on 31.10.88, made a field survey in relation to this project, discussed with the local authorities in the field and also with the State Govt. high officials including the Hon'ble Minister of Health, Science, Technology and Industry about starting of this project in Orissa. The work was to be started after the visit of Dr. Dobrokhotov and sanction of funds from WHO (as per minutes of the meeting with the Govt. on 28.10.88). After several correspondence the IMCR sent a reply to start it as an intramural project since it was not forwarded to WHO. Dr. Rishikesh, Chief of the Biological Control of Vectors, WHO, visited RMRC in April '89. He has offered his co-operation in this matter. This project was rediscussed in the 4th SAC met on 26.6.89. The SAC unanimously recommended strongly that this work could be taken up by the RMRC and D.G. should get the programme started through the Health Ministry for TDR funding.

9. Health Survey of Aluminium Company workers in Orissa with special reference to fluoride exposure

(submitted through proper channel to Department of Science, Technology & Environment, Govt. of Orissa)

Scientist : Dr. G. P. Chhotray

Aims & Objectives :

a) Biological assessment of fluoride explosure amongst the workers of the aluminium company.

b) To assess the clinical effect of occupational exposure to fluoride.

10. Assessment and characterisation of Vit. A deficiency and xeropthalmia in tribal children of Orissa

Scientists : Dr. G. P. Chhotray
Mr. Anil Kumar

Introduction :

Vit. A or Retinol is a fat soluble substance found in liver (fish liver, poultry meat dairy products) Carotenes—the potential precursors present in greenleafy vegetables, red palm oil, yellow fruits etc. are converted to retinol in the wall of the gut.

Vit A deficiency and xeropthalmia are among the most widespread and serious nutritional disorder that affect the mankind. Vit A deficiency is a systemic disease affecting epithelial structures in a variety of organs. The eye being the most obvious one. Keratinising Metaplasia of the respiratory and intestinal epithelia is thought to be responsible for the pulmonary and gastrointestinal symptoms in the affected children. But the classical clinical expression is xeropthalmia or "dry eye". Xerophthalmia is the general term applied to all the occular manifestations of impaired Vit A metabolism from night blindness to complete corneal destruction. In India it has recently been estimated that among 92 million children between 1-5 years, 7.4 million have noncorneal and 0.22 million have corneal xeropthalmia. It is estimated that 52,500 children become blind and between 10,000 and 132,000 become partially blind in India every year. The prevalence of Vit A deficiency is high in all states except northern states. The rate is even higher in remote tribals and rural areas as has been reported.

Aims & Objectives :

a) To find out the prevalence and severity of Vit A deficiency & xeropthalmia amongst the tribal children at risk group (0-12 yrs) by clinical examination such as occular signs, night blindness, conjunctinal xerosis, Bitot's spot etc. and extraoccular effect such as growth, anorexia etc.

b) Assessment of Vit A level (total body reserve of Vit A) by determining the Vit A (Retinol) and Retinol blinding protein (RBP) levels in plasma and liver.

c) To assess its relationship with the various socio-economic status and dietary habits in the community.

11. A chronological study of the histopathology of Malayan Filariasis in cats of Orissa

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

Technical staff : Mr. M. R. Ranjit
Mr. H. K. Khuntia
Mr. B. N. Sethi

Introduction :

Lymphatic filariasis in humans caused by *Wuchereria bancrofti* and *Brugia malayi* is a major health problem in the tropics. There is no satisfactory laboratory mammalian host for *W. bancrofti* although it will develop in *Presbytis cristatus*. Whilst rodents such as Jirds, Hamsters, and rats can be infected with Brugia sapp, their responses to an artificial infection are different from the responses in human beings to such parasites. *Brugia pahangi* occurs naturally as a parasite of cats in South East Asia. It's adults parasitise the lymphatics and microfilaria circulate in the blood. The human form of *B. malayi* can be transmitted to the cats by the bite of mansonoid mosquitoes. The period of full development of the adult filaria in this animal varies from 81 to 96 days before microfilariae appear in the blood. The adult recovered from the cat correspond to the description of *B. malayi* in man. It has been reported that malayi like microfilariae have been found in the cats of Orissa (Manson Bahar et al. 1982). *B. malayi*, the causative agent for human lymphatic filariasis has been found to be prevalent in some geographic areas of Orissa. Studies in Malaysia have demonstrated that the zoonotic nature of sub-periodic *B. malayi* in endemic areas where the prevalence rate in man was accompanied by a corresponding increase in the rate in cats. So there is an urgent need to study the existance of *B. malayi* amongst the cat population of Orissa.

Aims & Objectives :

a) To examine the prevalence rate of *B. malayi* amongst the cat population in the endemic pockets of malayan filariasis in Orissa.

b) If the cats show evidence of infection with *B. malayi* they will be taken as animal model to study the pathogenesis of lymphatic filariasis caused by *B. Malayi*.

c) To study the histopathological changes (macroscopic & microscopic) in various tissues and organs in the cats naturally infected with *B. malayi*.

12. An assessment of availability, utilization and impact of Health Services in rural Orissa

Scientist : Mr. Anil Kumar
Technical Staff : To be appointed
Duration of the Project : 2 yrs

Good Health is crucial to socio-economic development of any population. So for a society, to achieve her developmental goal, it is important to see that all the individuals are healthy and live in healthy environment.

Health situation can be assessed through the mortality statistics as an outcome of existing factors. In India, the mortality situation has been slowly improving in rural areas but in urban areas, it has improved impressively. Some of the mortality indicators such as infant and child mortality rate upto age 5 indicate about the health situaton. In rural India, infant (before age 1) mortality rate (IMR) in 1980-81, was 124/1000 new born and in urban around 65/1000 new born. In rural India, about another 75 die before reaching 5th birthday, thus 20% of the infant born die before age 5. These estimates for rural Orissa are 171/1000 and additional 75 so about 25% die before age 5. This sorry state of health in rural areas certainly have an adverse impact of household economy and social status. Since health of the people is the Govt. responsibility, it is important to assess the availability and utilization of health services available and its impact on their health. It would also be important to know as how people perceive the utility of these available services.

Review of mortality situation in Rural Orissa :

There is very limited information available about mortality rate at district level. The accuracy of these estimates is also questionable. The latest estimate available from the Registrar General of India, using indirect techniques for census data, 1981 suggest that the risk of death to a new born dying before age 1 year (ql) was highest in Phulbani (211/1000) followed by Cuttack 207/1000 and lowest in Mayurbhanj i.e. 105/1000. If we classify districts in groups (a) above the state level of ql = 171/1000 (b) below 171 the following scene emerged.

Group (a)		Group (b)	
	ql		ql
1. Phulbani	(211)	1. Ganjam	(162)
2. Cuttack	(205)	2. Koraput	(136)
3. Keonjhar	(196)	3. Sundargarh	(132)

4. Balasore	(195)	4. Sambalpur	(126)
5. Balangir	(191)	5. Mayurbhanj	(195)
6. Dhenkanal	(180)		
7. Kalahandi	(177)		
8. Puri	(175)		

$\bar{x} + SD_{n-1} = 191.3 \pm 13.1$ % Population

$\bar{x} \pm SD_{n-1} = 132.2 \pm 20.5$ % Population

Almost similar scenerio emerged while comparing q5, risk of death before age 5. In this situation, it becomes necessary to know the factors responsible for the difference.

Selection of study area :

Basing the classification on ql, the well known index of mortality, two districts from Group (a) Cuttack and Puri and one district from Group (b) Mayurbhanj would be selected. A sample of 1000 household each would be drawn using two stage stratified sampling scheme with population proportion weights.

Methodology :

A survey protocol would be prepared, tested and then used for collecting information. This protocol would enquire about the levels knowledge and awareness about the services available and their utilization by different groups including MCH services. This would also enquire about the living conditions and household economy besides socio-demographic characteristics and sickness.

Staff requirement :

a) Temporary on consolidated salary for one year.

1) Investigators : 4 (Four) Salary Rs. 1200/- PM

2) Supervisor : 1 (one) Salary Rs. 1500/- PM

b) Regular : For the divn.

1) Research Assistant : 1 (one)

2) Computer Assistant : 1 (one)

13. Studies on Cerebral Malaria

Principal Investigator : Dr. B. Ravindran

Co-investigators : Dr. G. P. Chhotray

Dr. P. C. Das

Dr. T. K. Bose

The study proposes to investigate (i) the role of various cytokines such as Tumour necrosis factor-α, interferon α and γ, interleukin-1, interleukin-2, interleukin-4 and interleukin-6 and other autoantibodies in the pathogenesis of human cerebral malaria (ii) a suitable therapeutic regimen along with quinine for cerebral malaria. It is proposed to submit this project for grant from the South-East Asia Regional Office of the World Health Organization.

14. Social mobilization for the control of filariasis

Scientists : Dr. N. M. Pattnaik

Dr. V. R. Subramanyam

This project was approved by the SAC. The initial ground work was to be done with RMRC funding (in the form of short term fellowships etc). Due to constraints of man power it has not been possible to initiate this study so far.

Library and Information facilities

Asst. Librarian : Mr. Banamber Sahoo, B.Sc., MLISc.

The Regional Medical Research Centre library has now the services of a professional hand as an Asst. Librarian, since September 1989. The Library & Information Centre has a number of scientific and technical publications and is equipped with reprographic facilities like xerox and microfilm reader. As in the preceding year, the library continued to receive 62 journals on subscription and through exchange from Indian & abroad.

The library possesses :

Books	—	748
Bound periodicals	—	673
Reports	—	244
Other mixed publications	—	100
Reprints	—	1219
Total no. of journals :		
Subscription (foreign)	—	42
Subscription (India)	—	15
Graties/Exchange (foreign & India)	—	5
Circulation of Books, Journals	—	583
Xerox facilities offered		
(official)	—	6273
(personal)	—	4966

The library facilities are extended to Scientists, Research Assistants, Research Fellows & Laboratory staff. The Library & Information Centre of RMRC aims to provide weekly current awareness service (CAS) and to compile a "Union periodical list" of all ICMR libraries and important medical libraries in India.

Sickle Cell Research Centre
V. S. S. MEDICAL COLLEGE, BURLA

Co-investigator	:	Prof B. C. Kar
Scientist	:	Dr. G. S. Bhattacharyya
Technical staff	:	Mr. B. P. Dash

1. Ongoing Research Projects

a) Study on Natural history of Sickle Cell Disease in India.

b) Study on Glucose metabolism in Hb.S containing red blood cells.

c) G6PD deficiency in Sickle Cell Disease.

d) Effect of Hb.F on sickling of RBC.

e) Anaemic crisis in sickle cell disease.

f) Characterisation of sickle cell disease patient clinically & haematologically.

2. Projects Completed

a) Immunoglobulins in sickle cell disease.

b) Insulin and Glucose metabolism in sickle cell.

c) Relationship between non Haem Iron, Lipid membrane peroxidation product and vit E, Serum Zinc in sickle cell disease of paediatric age group.

3. Proposed New Projects

a) **Development of objective criteria for definition of crisis in sickle cell disease**

Scientists	:	Dr. G. S. Bhattacharyya
		Mr. Anil Kumar
		Prof. B. C. Kar

Aims & Objective

i) Development of scoring system for assessment of severity of crisis following statistical analysis of laboratory parameters.

b) **Study of some aspects of coagulation in sickle cell disease**

Scientists : Dr. G. S. Bhattacharyya
Dr. G. P. Chhotray
Prof. B. C. Kar

Technical staff : Mr. B. P. Dash

Aims & Objectives

i) Ascertaining coagulation changes in sickle cell disease in clinically steady state and during crisis.

ii) Effect of Disprin on duration of crisis & incidence of crisis attacks.

c) **Incidence of 'α' thalassaemia and it's interaction with Hb.S and other Haemoglobinopathy**

Scientists : Dr. G. S. Bhattacharyya
Dr. G. P. Chhotray
Prof. B. C. Kar

Technical staff : Mr. B. P. Dash

Aims & Objectives

i) Detection of Hb Part in cord blood in cases of thalassaemia, sickle cell disease, sickle cell trait and in normal cases.

ii) DNA analysis for study of RFLP after DNA digestion to ascertain the exact type of 'α' thalassaemia and study the clinical variables in relation to 'α' thalassaemia type.

iii) To analyse the effect of 'α' thalassaemia on clinical & haematological parameters.

d) **Study of Jaundice on sickle cell disease**

Scientists : Prof. B. C. Kar
Dr. G. S. Bhattacharyya

Technical staff : Mr. B. P. Dash

e) **Study of Red cell morphology in sickle cell disease**

Scientists : Prof B. C. Kar
Dr. G. S. Bhattacharyya

Technical staff : Mr. B. P. Dash

4. Papers published/accepted for publication

—Nil—

5. Scientific conferences attended & papers presented

a) Annual Conference of Indian Society of Haematology and Blood Transfusion, 1,2,3, Dec. 89.

i) Prevalence of Sickle Cell Disease and Trait in Western Orissa—B. P. Dash and others.

ii) Clinical profile of Sickle cell disease in India—Dr. G. S. Bhattacharyya and others.

iii) Hb-F and Sickle Cell Disease—Dr. B. C. Kar and others.

iv) Insulin secretion and Glucose metabolism in Sickle Cell Disease—Dr. G. S. Bhattacharyya and others.

v) Relationship between Non-Heme Iron, Lipid Membrane Peroxidation product and Vit-E, Serum Zinc in S.C.D. of Paediatric age group—Dr. G. S. Bhattacharyya and others.

b) Annual Conference of O & G Society, Rourkela.

i) Pregnancy & Sickle cell disease—Dr. B. C. Kar.

c) Annual Conference of Association of Physicians of India, Orissa State Branch at Rourkela.

i) Alpha-Thalassaemia and Sickle Cell Disease—Dr. B. C. Kar.

d) Joint Annual Conference of the Association of Physicians of India, Jabalpur.

i) *'Netaji Oration'* on 'Sickle Cell Disease—the Indian Picture' by Dr. B. C. Kar.

ii) Paper—Interaction of Hb F and SS Disease—Dr. B. C. Kar and others.

6. Awards Received

Dr. B. C. Kar was awarded 'NETAJI ORATION' by the Association of Physicians of India and he delivered the oration at Jabalpur on 20.01.90. The topic was *"Sickle Cell Disease—the Indian scene"*.

Dr. B. Ravindran, SRO—Recipient of Shakuntala Amir Chand Prize 1989.

7. Works done in the Centre since 01.04.89 to 31.12.89 :

a) Number of new cases of sickle cell disease detected and included in the series 178. Thus total number of cases being studied and followed up till 31.12.89 is 750.

b) Attendance in the centre during the period 3319 screening, detailed clinical examination, pathological investigations, advise and follow up of all these patients have been done.

c) No further population survey has been taken up as it was decided to suspend the survey due to lack of hands.

d) Field visits to study important cases of clinical interest are being conducted as and when necessary.

Publications

1. Partial characterization of *M. fortuitum* and *M. smegmatis* auxotrophs by syntrophism using *Bacillus subtilis*.

   V. R. Subramanyam & K. K. Mohanty. *Journal of General Microbiology* (1989) 135, 2651-2654.

2. Inducibility and stability of auxotrophic mutations in *M. fortuitum*, *M. smegmatis* and *M. vaccae*.

   V. R. Subramanyam, B. B. Pal & K. K. Mohanty. *Letters in Applied Microbiology* (1989) 8, 161-164.

Publications

A. **Papers :**

1. Study of medical mycology in India—an overview. L. N. Mohapatra.
   Indian J. Med. Res. **89**, 351-361 (1989)

2. Prevalence of winter forms of *Anopheles annularis* in Orissa. A. P. Dash, M. S. Bendle and M. Das.
   J. Com. Dis. **20** (4) 287-292

3. Antibodies in human filariasis sera react with diethylcarbamazine.
   B. Ravindran, A. K. Satapathy, T. Hussain & N. M. Pattnaik.
   Clinical and experimental Immunology **78** (2), 219-223 (1989)

4. Chloroquine sensitivity of *P. falciparum* in Koraput district, Orissa.
   S. S. S. Mohapatra, L. K. Das and S. P. Pani
   Indian J. of Malariology, Vol. 26, p. 33-39 (1989)

5. Detection of *Plasmodium ovale* in Koraput district, Orissa State.
   P. Jambulingam, S. S. S. Mohapatra, L. K. Das, P. K. Das and P. K. Rajagopalan
   Indian J. Med. Res. pp. 115-116 (March 1989)

6. Malaria and other common ailments among Upper Bonda tribals in Koraput district, Orissa.
   L. K. Das, S. S. S. Mohapatra, P. Jambulingam, K. Gunasekaran, S. P. Pani and P. K. Das.
   Indian J. Med. Res. pp. 334-339 (September 1989)

B. **Papers submitted for publication :**

1. Prevalence of erythrocytic G-6-PD deficiency in a malarial endemic tribal population of Orissa.
   G. P. Chhotray and M. R. Ranjit, Indian J. Med. Res.

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

SCIENTIFIC CONFERENCES/WORKSHOPS/SEMINARS ATTENDED

Name of the Scientist	Scientific conferences/workshops/seminars attended with date	Papers presented/ Lecture delivered
Dr. L. N. Mohapatra	IAPM Orissa Chapter meeting : 13.5.89 S.C.B. Medical College, Cuttack	Inaugural address on Pathology and Microbiology
	Workshop on AIDS : S.C.B. Medical College, Cuttack, 25.5.89	Inaugural address on HIV Infection & its detection.
	Summer School Orissa Veterinary College, Bhubaneswar, 19.6.89	Recent advances in fungal diseases.
	Summer School Orissa Veterinary College, Bhubaneswar, 20.6.89	Diagnosis of mycotic diseases in man and animals.
	WHO NIHFW Workshop S.C.B. Medical College, Cuttack, 1.8.89	Inaugural address on population & family planning
	Regional Research Laboratory (CSIR) Bhubaneswar, Foundation day, 26.9.89	Chief guest & talk on Science in India
	NMEP—WHO Workshop on Severe and Complicated Malaria, I.G.H. Hospital, Rourkela, 21.12.89	Inaugural address on Cerebral Malaria
Dr. A. P. Dash	6th Annual Conference of National Environmental Science Academy, Vellore, Dec. 29-31, 1989	Distribution of *B. malayi* in Orissa
Dr. N. M. Pattnaik	Workshop on Computers in Biology, Indian Institute of Chemical Biology, Calcutta, November 1989	—
Dr. V.R. Subramanyam	13th National Congress of Ind. Assoc. Med. Microbiologists; SMS Medical College, Jaipur, Nov. 89	Estimation of reverse mutation frequency of *Mycobacterium* spp auxotrophs by a statistical method.

Name of the Scientist	Scientific conferences/workshops/seminars attended with date	Papers presented/ Lecture delivered
	Indo-US Workshop on major advances in tuberculosis research. TRC, Madras, Dec. 1989	Isolation and Characterization of auxotrophic mutants of *Mycobacterium*, first step towards a genetic approach to the genus.
Dr. B. Ravindran	7th International Congress of Immunology at Berlin, July 30th—August 5, 1989	Antigenic mimicry between filarial parasites and anti-filarial drug diethylcarbamazine.
Dr. G. P. Chhotray	XVII Annual Conference of IAPM (Orissa Chapter) Bhubaneswar, 11th November, 1989	Asymptomatic microhaematuria in a filarial endemic area.
	38th Annual Conference of IAPM, LTM Medical College, Bombay, 9-12 Dec. 1989	-do-
Mr. M. K. Beuria	58th Annual Meeting of the Society of Biological Chemists (India), Izat Nagar, Oct. 1989	Induction and Regulation of Immediate hypersensitive response to filarial antigens.
Mr. Dasarathi Das	-do-	Effect of Homoeopathic Drugs on Microfilaria *in vitro*.
Mr. P. K. Rana	-do-	Microfilaricidal Activity in Insect Haemolymph
Ms. K. K. Mohanty	16th Annual Conference of Indian Immunology Society and Symposium on Tropical Immunology.	Circulating 'DEC like' filarial antigens in bancroftian filariasis detection and quantitation by a sandwich ELISA.
Mr. A. K. Satapathy	XVI Annual Conference of the Indian Immunology Society at MGIMS, Sevagram, 12-14th Oct. 1989.	*Plasmodium berghei* malaria in mouse autoantibodies with specificity to N-Acetyl Neuraminic acid react with lymphocytes.

Name of the Scientist	Scientific conferences/workshops/seminars attended with date	Papers presented/ Lecture delivered
Mr. P. K. Kar	XVI Annual Conference of Indian Immunology Society "Symposium on Tropical Immunology" Wardha, Oct. 12-14, 1989.	Study of Immune status in filarial fever.
Mr. M. R. Ranjit	6th Annual Conference of National Environmental Science Academy, Vellore, Dec. 29-31, 1989.	Effect of aphid extracts on the total glycogen content during the growth of *Culex quinquefasciatus.*
Mr. R. K. Hazra	-do-	Biting rhythm of two species of *Mansonioides.*

OTHER ACTIVITIES

Dr. L. N. Mohapatra	Went on a short term WHO assignment to Myanman, 29.9.89 to 28.10.89.
	All India Radio, Cuttack, 7.7.89—Talk on AIDS and it's prevention (in Oriya).
Dr. Shantanu K. Kar	Awarded WHO fellowship to attend the training programme in USA and UK from 25th September '89 to 25th November '89 on Epidemiological studies on Filariasis and Immunological studies in helminthic diseases. Received training on application of nuclear magnetic imaging response and lymph scientigraphic techniques in filarial lymphoedema observed in Ferret model and in humans at Health Sciences Centre, University of Arizone (USA) and Immunological studies in filariasis at London School of Hygiene and Tropical Medicine, London.
Dr. A. P. Dash	WHO Course on Logistics and Material Managements. National Institute of Health & F. W., New Delhi. Dec. 2-15, 1989.
	Acted as a referee of the Indian Journal of Medical Research. He has also been an examiner of various Universities.
Dr. N. M. Pattnaik	Editing since its launching in August 1989 a Science appreciation magazine in Oriya—"BIGYAN TARANGA"—aimed at School students and teachers. This is a part of the People's Science Activity of Dr. Pattnaik and co-workers.
Mr. G. P. Pattnaik	WHO Course on Logistics and Material Managements. National Institute of Health & F.W., New Delhi. Dec. 2-15, 1989.
Dr. S.S.S. Mohapatra	All India Radio, Jeypore, 5.12.89—Gave a talk on "Ye Anchalare Malaria Rogara Bhayabhayata" (in Oriya).
Mr. M. K. Beuria	Selected and attended the "Laboratory course on Human Leucocyte Antigens" at Institute of Immuno Haematology, Parel, Bombay. 6th-17th March 1989.

Mr. A. K. Satapathy	Selected and attended the International Union of Immunological Societies— WHO advanced immunology course held at AIIMS, 14-25 Nov. 1989.
Mr. Prakash K. Sahoo	Selected and attended the 'X' Laboratory Animal Supervisor Training Programme at LAISC, ICMR—DBT Centre, National Institute of Nutrition, Hyderabad. 1st Sept to 30th Nov. '89.
Mr. L. Satyanarayana Rao	Selected and attended the 36th Programme on Human Relations in Organisations. Institute of Secretariat Training & Management, New Delhi from 7-11th Aug. '89.

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07.04.89	Prof. M. S. Kanungo Emeritus Scientist Jawaharlal Nehru Fellow B.H.U. Varanasi
17.04.89	Dr. D. P. Rastogi Director, Central Council for Research in Homeopathy New Delhi
25.04.89	Dr. N. Rishikesh Chief, Development of Vector Control Technology Division of Vector Biology and Control WHO, Geneva.
	Dr. P. K. Rajagopalan Director Vector Control Research Centre, Pondicherry.
12.06.89	Sri S. N. Dhar Sr. Projects Officer British Council Division.
03.08.89	Prof. S. Bhatnagar NIHFW New Delhi.
08.08.89	Dr. R. Prabhakar Director, TRC Madras.
06.09.89	Dr. Shanta Devi Asst. Director, TRC Madras.
11.10.89	Dr. S. P. Tripathy Addl. D. G. ICMR, New Delhi.

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