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RESEARCH IN MALARIA - AN OUTLINE

Research in malaria has today assumed a degree of urgency perhaps not equalled at any time before. Malariology in all its aspects, including methods of control, underwent gradual expansion and change from the days of Sir Ronald Ross at the beginning of the present century, to the advent of DDT in the early 1940s, which led to the most spectacular reduction in the incidence of malaria in most parts of the world. India was in the forefront both in research in malariology as well as in the application of the relevant results of research for the control and subsequent eradication of the disease, on a nation-wide scale. The initial successful use of DDT on a limited scale was followed by its application in the National Malaria Control Programme (NMCP) in 1953 and later in the National Malaria Eradication Programme (NMEP) during 1958, and a near eradication of malaria in vast parts of the country was achieved by 1965. However, for reasons now well-known, reversal of the results started and in 1976 nearly seven million parasite-positive cases were detected in the country. The over-confidence in the ability of the two major control measures *viz.* indoor residual spraying, and radical treatment of malaria cases detected through active and passive surveillance operations, generated such a degree of complacency amongst the workers that there was near cessation of research in malariology. However, the disturbing new dimensions which malaria has now acquired, such as the resistance of vectors to conventional insecticides and the emergence of strains of *P. falciparum* resistant to 4-aminoquinolines, have considerably added to the gravity and urgency of the problem. The

situation calls for the use of the most effective scientific and technological tools. Recent advances in molecular biology, immunology and chemotherapy offer new opportunities for the development of such tools.

The situation today is that the scientists of the country not only have to make up for the quiescence of the past two decades or more but also to forge ahead in research in this field with newer ideas and innovations. Malaria cannot be allowed to confront India as one of its foremost public health problems as before and to handicap the country's over-all national development in various spheres. To combat the disease now and to overcome it completely in the near future it would be necessary to mobilize and utilize all the available talents and resources to the maximum. Research in all aspects, basic and applied, is of utmost importance and no time can be lost in determining the lines of research and embarking on the same.

The Government has made in-depth studies on the positive causes for the reversal and has now taken steps to reorganise the malaria control activities on the basis of a modified plan. The organisational as well as the technical aspects which needed attention have been looked into. A new order of priorities, commitments and administrative responsibilities has been defined. While these measures can be expected to halt a further deterioration of the situation, it has been amply clear that the programme is in dire need of additional and newer data on many aspects

in order that it may be tailored to deal with the new dimensions of the problem.

The information required covers a wide range of subjects, including those related to basic knowledge on the malaria parasites, the vectors, the drugs, control methods, socio-economic impacts and financial and logistic constraints.

In view of the obvious financial and manpower limitations, only the most relevant aspects can be studied. It becomes imperative that specific problems worthy of being selected for investigation should be identified for purposeful action with the least possible delay.

Eight Task Forces and the Expert Committee on Malaria met from 28th February to 3rd March, 1977, at the Council and deliberated on the problems which needed immediate attention. The deliberations and recommendations of the Task Forces and the Expert Committee, which form the basis on which the overall research policy of the Council has been developed, are highlighted in the following pages:

1. RESEARCH ON OPERATIONAL ASPECTS OF MALARIA CONTROL:

Operational research studies are of considerable importance since efforts in this area may be expected to yield rich dividends towards the effective control of the disease.

1.1 Mapping and monitoring *P. falciparum* resistant areas:

Between complete sensitivity and complete resistance there is gradation in the response of *P. falciparum* to chloroquine, ranging from a loss of effect demonstrable only by occasional recrudescence to a level of resistance at which the drug apparently has no effect on severe infection. In cases where the parasite is sensitive (S) to the drug there is clearance of asexual parasitaemia within seven days of initiation of treatment, without subsequent recrudescence. In R_1 low degree of resistance there is clearance of asexual parasitaemia followed by recrudescence. When the drug resistance is moderate of R_2 type, with chloroquine administration, marked reduction in asexual parasitaemia takes place but no clearance. In case the degree of resistance is of high R_3 type there is no significant reduction of asexual parasitaemia after the start of treatment. It is useful to detect R_1 type of

resistance, as it happens to be the beginning of drug resistance in parasites.

At least in two States in India the problem of chloroquine resistant *P. falciparum* has already been detected. The other areas with chloroquine resistant *P. falciparum* strains are still to be identified and demarcated.

1.1.1 *In vivo* and *in vitro* tests for determination of chloroquine resistance in *P. falciparum*:

Considering the results of the *in vivo* tests as obtained by the WHO, difficulties are likely to be encountered for classifying the strains into R_1 , R_2 and R_3 levels of resistance. It is agreed that while R_2 and R_3 types of resistance or susceptibility of the strain can be established easily, major difficulty may crop up in identifying strains with R_1 degree of resistance. In case active transmission of the disease is taking place subsequent parasitaemia may be due to reinfection. Detection of R_1 type of resistance is of great practical importance to the programme because this indicates the beginning of resistance. This may be established by an *in vivo* test, by interrupting malaria transmission through insecticide application or by carrying out the studies during the non-transmission season. *In vitro* test recommended by the WHO is also suitable for this purpose. The latter will furnish quick information and also has a high degree of reliability.

Considering the fact that it would take some time before *in vitro* testing could be taken up widely, the *in vivo* field tests recommended by the WHO should be put into immediate use. The NMEP machinery may be utilised for collection of blood slides, administration of drugs and examination of slides collected before and after treatment.

1.1.2 Monitoring Teams:

Monitoring teams should be deployed in three types of areas (i) where drug resistance in *P. falciparum* has already been identified or strongly suspected e.g. districts on the southern bank of Brahmaputra in Assam, Meghalaya, Tripura, Mizoram, Manipur, Nagaland and Arunachal Pradesh; (ii) states which are in the neighbourhood of the above areas e.g. districts on the northern bank of Brahmaputra in Assam and north Bengal and (iii) other parts of India (a) where *P. falciparum* incidence is high, (b) where persistent transmission of *P.*

falciparum is prevalent and (c) from where reports about lack of response to chloroquine are being received.

1.2 Studies for evaluating the efficacy of various drug schedules in *P. falciparum* cases resistant to chloroquine:

Studies should be undertaken to find out the shortest and most effective course of drug combination that could be used for the cure of chloroquine resistant *P. falciparum* cases. A great deal of work has already been done in this country by the Armed Forces, who have evaluated the various regimen of drugs. Their results have shown that:

- (i) Quinine plus tetracycline is the best combination;
- (ii) The next best is quinine plus pyrimethamine plus sulphadiazine;
- (iii) The addition of chloroquine to the above two schedules makes the combinations more potent.

The treatment schedule however, is very long *i.e.* about two weeks. There is, therefore, need for shorter courses of treatment, to be employed in the field. The following drug combinations have therefore, been suggested for field trial:

- (i) Quinine sulphate/hydrochloride plus minocycline for seven days;
- (ii) Single day course-Pyrimethamine plus sulphadoxine or sulphalene or dapsone (all in single dose);
- (iii) Quinine hydrochloride/sulphate plus sulphadoxine or sulphalene plus pyrimethamine for seven days;
- (iv) Quinine hydrochloride/sulphate plus tetracycline for seven days; and
- (v) Quinine hydrochloride/sulphate plus doxycycline for seven days.

(Seriously ill patients should not be included in the study).

1.3 Investigations on the epidemiological and entomological aspects of malaria:

It is essential to undertake a comprehensive study on all aspects of malaria in some

representative and problem areas of the country, in different types of epidemiological situations in order to study contemporary epidemiology. Hence, it would be useful to carry out investigations in one full district in each selected area where a medium sized town also exists. Different types of areas are:

- (a) Hyperendemic forested foothill areas of North East India *e.g.* Mikir Hills.
- (b) Hyperendemic hilly forested areas with tribal population *e.g.* Dandakaranya area.
- (c) Hyperendemic semi-forested sub-hilly areas *e.g.* Thana Palghar - Bulsar complex.
- (d) Mesoendemic areas of Gangetic plain of West Bengal.
- (e) Epidemic irrigated belt areas like Mathura - Meerut - Kurukshetra - Karnal.
- (f) Hypo-meso-endemic areas with tendency for epidemics in Deccan plateau *e.g.* Gulbarga.

In the above index areas after obtaining baseline data on the epidemiological and entomological aspects of malaria, the studies which can be initiated are as follows:

- (i) Effectiveness of integrated malaria control measures;
- (ii) Dynamics of *P. falciparum* malaria transmission;
- (iii) Studies to improve the operational efficiency of the ongoing programme;
- (iv) Studies to devise methods of assessment of various components of the malaria control programme including malariometric indices to be used by the programme;
- (v) Value of radical treatment in different localities;
- (vi) Studies on mass chemotherapy in selected areas to find out its impact on transmission;
- (vii) Studies to find out alternative approaches to reduce transmission *e.g.*

application of selective antilarval measures during the pre-transmission season with usual surveillance or with mass chemotherapeutic measures carried out concomitantly;

- (viii) Control of malaria by adoption of larvicidal measures in the rural areas throughout the year;
- (ix) Malaria control by the application of space spray (pyrethrum spray) in some double insecticide resistant vector areas;
- (x) Comparative study of the effectiveness of DDT suspension and DDT emulsion;
- (xi) Control of malaria by improving the water management practices; and
- (xii) Identification and the use of larvivorous fish for the control of mosquitoes.

1.4 Integrated malaria control programme with involvement of the community through part-time village health workers:

It is felt that part-time village workers, selected by the community itself (partly supported) and trained in primary health care and basic skills linked to the infrastructure of the health services at the grass-roots level and supervised through multipurpose supervisors and health workers, may be utilised for case detection, drug distribution, administration of presumptive and radical treatment, slide collection, spraying and supervising of spraying and antilarval and environmental sanitation measures.

Some states like Maharashtra have experience of such projects and it is felt that controlled experiments can be undertaken in such areas. The size of the experimental area may vary from a few community development blocks to a district, depending on the availability of finances.

1.5 Studies in forecasting of epidemics of malaria:

Studies may be carried out to forecast malaria epidemics in the north western states of the country. This can help the organisation to take precautionary measures for the prevention of the epidemics. A Research Cell may collect and analyse relevant epidemiologi-

cal and other data and attempt to forecast epidemics of malaria. Considerable information is available on the subject from the state of Punjab in undivided India and the same can be made use of after modification, keeping the present situation in view.

1.6 Studies on the use of antilarval operations in rural areas of arid and semi-arid zones of *A. culicifacies* malaria:

It is known that breeding grounds of *A. culicifacies* in arid and semi-arid zones are extremely limited during the summer months (March through May). Transmission starts when there is a good build-up of the vector density. Hence it is reasonable to expect that suppression of *A. culicifacies* breeding during summer period may retard eventual build-up and delay the onset of malaria transmission. Antilarval measures may be undertaken in some pilot project areas from March to May by either using Paris green or organophosphorus compounds. This can also be supplemented by chemotherapy e.g. single dose treatment with 600 mg chloroquine in predominantly *P. falciparum* areas, or chloroquine 600 mg plus primaquine 45 mg in predominantly *P. vivax* areas. Results of such an investigation can provide useful information for malaria control in similar areas in other parts of the country.

2. ECOLOGY AND CONTROL OF VECTORS OF MALARIA:

India is a vast country with several marked zoecological regions with different patterns of prevalence and behaviour of the vectors. It has long been recognised that the knowledge of the vectors and their behaviour in one region would not be applicable to other regions and the control measures would have to be developed with the needs of a particular locality in view. The variations are so many that the total picture of the vectors is rather complex and would need investigations in several types of situations. However, for practical reasons it is essential to select a few representative areas for intense study supplemented by additional, limited investigations in other areas.

2.1 Field studies on the bionomics of vector mosquitoes:

The specific studies to be undertaken in selected representative areas may include the composition of the anopheline fauna, their distribution, seasonal and relative densities, determination of the vectors by dissection,

and epidemiological studies keeping in view the fact that there may be major and subsidiary vectors.

Information and data may be obtained on: (i) seasonal prevalence and density; (ii) breeding habits and habitats; (iii) adult resting places; (iv) feeding habits and preferences *i.e.* degree of anthropophily, and time and place of biting (endophagy or exophagy), degree of contact with man *etc.*; (v) mating behaviour, gonotrophic cycles; (vi) dispersal; (vii) longevity and survival under natural conditions; (viii) susceptibility to insecticides; (ix) laboratory colonization; and (x) ecology of male mosquitoes with particular reference to feeding behaviour.

These studies should be conducted taking into consideration the socio-economic conditions in the rural or urban milieu, including housing, presence of domestic animals and agricultural practices which have a bearing on the problem. The degree of malaria prevalence in these areas should be periodically assessed so that the entomological studies yield meaningful results.

For this purpose, the following areas have been considered suitable for initiating studies:

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| (i) Tamil Nadu
(Salem area) | Problem of persistent malaria, with <i>A. culicifacies</i> , <i>A. stephensi</i> as known vectors and <i>A. subpictus</i> and <i>A. varuna</i> as suspected vectors. |
| (ii) Madhya Pradesh
(Ratlam) | Persistent transmission area with <i>A. culicifacies</i> as known vector. |
| (iii) West Bengal and Assam | Persistent transmission with <i>A. philippinensis</i> , <i>A. annularis</i> and <i>A. balabacensis</i> . |
| (iv) Maharashtra and Karnataka
(Gulbarga, Bijapur, Nira Canal Zone) | Epidemic areas and irrigation malaria with <i>A. culicifacies</i> , <i>A. fluviatilis</i> and <i>A. stephensi</i> as known vectors. |
| (v) Andamans | Problem connected with <i>A. sundaicus</i> and <i>A. balabacensis</i> . |

Well-equipped and adequately manned field stations should be established in these areas on a long-term basis, at least for an initial period of five years.

2.2 Species complexes and sibling species in Anopheles:

DDT spray has brought to light the variable behaviour of vector species indicating the existence of sibling species. In eastern India, *A. philippinensis* populations were known to transmit malaria only in Deltaic Bengal and not in Assam. Recently it has been established that *A. philippinensis* is a vector of major importance in Assam and not in Bengal. The species is further confused with *A. nivepes* known to be present in Assam. Similarly, *A. leucosphyrus* complex is represented by *A. balabacensis* and *A. elegans* and their role in the transmission of malaria over the range remains to be determined. *A. maculatus* which is known to be associated in transmission of malaria in Assam, exists as a polymorphic species and requires a comprehensive study to establish the precise role of its members. *Anopheles subpictus*, *A. tessellatus* and *A. sundaicus* are other species, which need detailed study.

2.3 Field studies in insecticide resistance in vector mosquitoes:

While the entomological staff under the new set up in the states, will continue to monitor the status of insecticide resistance in vector mosquitoes as a part of their routine activities, the field studies on resistance particularly on such sophisticated aspects as genetic analysis of the mosquito population, would have to be undertaken. Such studies would help in understanding the totality of the resistance problems and give leads for development of methods to overcome or mitigate them. Suitable technical facilities should be built into the regional field stations.

2.4 Biochemistry and genetics of insecticide resistance:

Research on the mechanism of resistance and the methods of overcoming it needs to be taken up on a priority basis. It is recognised that this would involve the development of expertise of a high order in biochemistry and genetics. Obviously such studies cannot be undertaken in many places but may be concentrated in a couple of properly equipped and well staffed institutions. It is proposed

that Cells for the study of these aspects be developed which will also be able to cytotoxonomically analyse the genetic basis of resistance.

2.5 Experiments in the use of space sprays:

In the pre-DDT era, very intense studies had been undertaken on the use of indoor space sprays with pyrethrum extract. Very promising results had been obtained in controlling malaria transmitted by *A. culicifacies* and studies on other vectors, particularly *A. fluviatilis* and *A. minimus* had been initiated. With the advent of DDT and its spectacular success, the use of pyrethrum was abandoned in public health practice. However, pyrethrum space sprays can offer a very practical solution in many types of malaria situations, particularly in localities with a short duration of transmission or in *A. culicifacies* transmitted malaria. It is considered essential to re-test the utility and applicability of this method in suitable rural situations. It is proposed to initiate a few carefully controlled studies on the mechanism and effectiveness of this method.

2.6 Biological control of mosquitoes:

While principles of source reduction by simple engineering methods such as filling, drainage, channelling, use of larvicides etc. are fairly well known, they are not applicable in many types of situations, particularly where the total elimination of stagnant water is not practicable because of technical, economic or social reasons. In many such situations, use of biological control methods has been known to be useful. Extensive programme of biological control has not hitherto been possible because of inadequate knowledge of these agents, their potentialities and hazards. The need for research in this field has now been recognised and it has become a major activity of the Vector Control Research Centre, Pondicherry. It is proposed that these studies should be intensified with carefully controlled field studies. The use of biological control agents i.e. fish, nematodes, fungi, bacteria etc. should be explored. It is, however, recognised that biological control can be effective only when used in conjunction with other methods.

2.7 Integrated control:

The control of insect pests or vectors of diseases by an integrated control method which includes improvement of the environment,

source reduction, chemical and biological methods, all used in a coordinated manner has been often postulated but so far, not well demonstrated. As the cumulative effect of these methods will be substantial and may bring about a permanent improvement, it should be demonstrated in a few localities. The work can be entrusted to the regional field stations being set up in index areas or to the local authorities in a few urban areas.

2.8 Testing of new insecticides:

While it is appreciated that the synthesis of new insecticides requires extensive facilities which are not available, it is essential that the country should have at least well equipped laboratories to test not only indigenous insecticides, but also insecticides of promise referred to it by other institutes or organisations.

2.9 Antilarval measures in rural areas:

Antilarval measures in rural areas have generally been regarded as economically not justifiable though sometimes technically feasible. In the changed context of the country's economic policies for rural development, it is essential to initiate measures or methods of control of the vectors in suitable rural and semi-urban areas. A few areas should be selected where methods of larval control using Paris green, minor engineering methods and water management (such as intermittent irrigation) can be tested. This can be undertaken as a part of the activities of the special field stations.

2.10 Bedbugs:

Though bedbugs are not directly related in anyway with the transmission of malaria, they have assumed a negative force working against the success of the malaria control programme, because of the refusal of householders to have DDT sprayed in their houses under an impression that the increase in bedbugs is due to DDT. Studies on the ecology and control of bedbugs should be undertaken to find methods of overcoming this problem.

3. CHEMOTHERAPY OF MALARIA.

Use of antimalarial drugs plays a vital role in the treatment and control of malaria. Chemotherapy, therefore, is to be considered in its various aspects.

3.1 Production of antimalarials:

With the proper and effective use of known drugs a considerable measure of success can be achieved in the control of malaria. Effective steps should be taken to ensure the availability of the drugs in order of priority viz. Chloroquine (300 tonnes); Primaquine (4 tonnes); Quinine (8 tonnes); Pyrimethamine (3.5 tonnes); Sulphadoxine (5 tonnes); and Tetracycline (5 tonnes).

The present production of chloroquine is only 30 tonnes which is very inadequate and though a number of private pharmaceutical concerns have been licenced, it will take a long time before the required quantity of chloroquine is manufactured by them. It is, therefore, necessary that a large quantity of this drug should be manufactured in the public sector.

Although the production of quinine in the country is around 16 tonnes, most of it is exported and not enough is available for distribution within the country. The Government must take steps to ensure availability of at least eight tonnes of quinine to the National Malaria Eradication Programme and only the balance may be exported.

Sulphadoxine is useful for the treatment of chloroquine resistant cases of *P. falciparum* and hence steps should be taken for its production in the country.

3.2 Studies on the effectiveness of anti-malarials:

For the effective treatment of malaria careful selection of the drugs to be used, is imperative and the regimen would depend upon the epidemiological and other logistic requirements in different areas. It is necessary to carry out field trials using different dosage schedules for different areas with drugs given alone and in combination, particularly with primaquine and fansidar in different parts of the country.

Efforts made by the Walter Reed Army Research and Development Agency have led to the development of some new drugs. Mefloquine, one of the new drugs, appears particularly promising. This drug should be obtained directly from the source or through some international agency and a field trial carried out in the country, particularly to assess its value as a single dose treatment for cases of both *P. falciparum* and *P. vivax* malaria.

A trial should also be undertaken with chloroquine and pyrimethamine in all fever cases for radical cure, with and without insecticidal coverage.

A trial should be carried out in *P. falciparum* resistant areas with one intramuscular injection of cycloguanil embonate and acedapsone.

3.3 Development of repository forms of drugs:

Long acting drugs would be of great advantage in a predominantly rural country like India. The production of repository forms of primaquine which is the most effective weapon for radical cure of malaria in India, needs to be encouraged. Development of repository forms of quinine should also be investigated.

3.4 Development of newer drugs:

In the development of new drugs major emphasis should be given to (i) drugs which are both casual prophylactic and curative and effective against exo-erythrocytic (EE) stage; and (ii) Gametocytocidal drugs active against resistant forms, preferably those which act by mechanism different from those of the known ones.

3.5 Screening of drugs:

At a few centres in the country complete models for screening new antimalarial drugs, including sporozoite induced infection should be established. This should include models in (i) rodents against *P. berghei*; (ii) chicks against *P. gallinaceum*; (iii) monkeys against *P. knowlesi* and *P. cynomolgi*; and (iv) against *P. falciparum* and *P. vivax* in owl monkeys.

3.6 Studies on the establishment of human malaria infection in animals:

Steps should be taken to import *Macaca fascicularis* monkeys from Malaysia and *Aotus trivigatus* (owl monkey) from Latin America and establish their colonies at a few laboratories in the country.

While attempts should be made to import *Aotus* monkeys from Columbia, simultaneous efforts should be made to establish human infection in some of the monkeys available in the country, particularly those which are similar to *Aotus* monkeys.

3.7 Models for *in vitro* screening of anti-malarials:

Models for *in vitro* screening should be established, which should include continuous culture of *P. falciparum* and *P. vivax* in human erythrocytes, culture of E.E. stages of *P. gallinaceum* in embryonic chick and of *P. berghei* in mouse liver cells.

4. DRUG RESISTANCE IN MALARIA PARASITE:

The problem of drug resistance in malaria parasite involves mainly the failure on the part of the parasite to respond to normal doses of the antimalarial drugs. This excludes other aspects such as immunity and natural non-susceptibility of different species, strains or phases of malaria parasite to drugs. With the finding of *P. falciparum* strains resistant to chloroquine, the matter of drug resistance has acquired significance. Therefore, the following studies may be taken up:

4.1 Monitoring of drug resistance:

It would be necessary to identify and demarcate areas in which chloroquine resistant strains of *P. falciparum* prevail, in order to assess the extent of drug resistant in our country. This would also help in evolving a programme for continuous monitoring of drug resistance.

4.2 Problem of drug absorption, distribution and metabolism in various conditions:

It is necessary to undertake pharmacological studies on different antimalarial drugs in relation to conditions like anaemia, malnutrition, malabsorption, liver disease, hypoproteinaemia etc. The studies may be carried out in volunteers or in malaria patients. It should include aspects like drug distribution between the plasma and the erythrocyte infected with malaria parasite, bio-physical characteristics of drug distribution and other related fields.

4.3 Biochemistry of malaria parasite:

Indepth studies may be carried out on the biochemistry of malaria parasite particularly with a view to assess the extent of differences, if any, between the sensitive and the resistant strains of the parasite. The biochemistry of the parasite as well as the mode of action of the various antimalarials and the

mechanism of development of drug resistance in the parasite should be studied.

For some of the above studies it would be necessary to look into the possibility of developing *in vivo* and *in vitro* models

5. SEROLOGY OF MALARIA:

The purpose of serological tests should be to identify the infecting species, differentiate between present and past infections and between primary infections and reinfections and indicate the immune status of the host. A test that fulfils these criteria should be not only a sensitive diagnostic method but also a valuable tool for the study of epidemiology of malaria. Further, the test should be simple enough to be carried out with minimal equipment under peripheral field conditions.

5.1 Serological tests for malaria:

Several tests are being tried but none satisfies all the above criteria. As such it may be desirable to concentrate on a few of them, viz., passive haemagglutination test, indirect immunofluorescent antibody test, enzyme-linked immunosorbent assays and counter immunoelectrophoresis.

Antigens commonly used in the above tests are primarily erythrocytic schizonts, extracts of schizonts, or soluble antigens recovered from sera of heavily infected individuals.

For a serological test to be sensitive and specific it is essential to have purified antigens. With such antigens, tests can be standardized and reference reagents made available. As far as malaria is concerned no such antigen is available so far.

In order to develop a reliable serological test the following investigations are necessary:

- (i) Purification of specific stages of the malarial parasite populations and the determination of the serological properties of the antigens derived from them;
- (ii) Further development of the four tests viz., passive haemagglutination test, indirect immunofluorescent antibody test, enzyme-linked immunosorbent

assay and counter immunoelectrophoresis and their evaluation, particularly when specific antigens become available;

(iii) Active search for new animal hosts of human malaria parasites and development of improved methods of cultivation to obtain sufficient quantities of antigens; and

(iv) The prevalence of antibodies to sporozoites in sera from populations living in highly endemic areas when adequate supplies of sporozoites of human malaria become available.

6. CULTIVATION OF MALARIA PARASITES IN VITRO:

Malaria parasites have three distinct cycles of multiplication *viz.*, the sporogonic cycle in the mosquito and the exoerythrocytic and erythrocytic cycles in the vertebrate host. Considerable work has been done on the *in vitro* cultivation of the different stages of avian, rodent, simian and human malaria parasites. Recently it has been possible to cultivate the erythrocytic stages of *P. falciparum* in continuous culture.

6.1 Studies on the cultivation of malaria parasite:

It has been demonstrated that effective immunity could be produced in experimental animals by the inoculation of sporozoites, exoerythrocytic or erythrocytic forms. As such the *in vitro* cultivation of all or any one of these stages of malaria parasite would help in the ultimate development of a vaccine against malaria.

(i) Sporogonic cycles:

Attempts at *in vitro* cultivation of the sporogonic cycle have not been very successful so far, either in vertebrate or invertebrate tissue cultures.

However, with a view to obtain sporozoites in a sufficient quantity, insect tissue cultures should be inoculated with gametocytes for securing viable ookinetes *in vitro*. Aseptically reared mosquitoes fed on gametocyte carriers should be used as a source of ookinetes or early oocysts to be placed in culture.

(ii) Exoerythrocytic cycle:

In most mammalian malarias and particularly in that due to *P. falciparum*, the exoerythrocytic cycle in the liver is a self limiting one but the corresponding cycle in avian malaria is not self limiting. Therefore, by modifying the *in vitro* culture conditions continuous propagation of the exoerythrocytic stage of *P. falciparum* in tissue culture may be possible. This should be investigated.

With several species of avian malaria it has been shown that the exoerythrocytic cycle can be carried on indefinitely and that large numbers of parasites can be produced in tissue culture. If the exoerythrocytic stages of avian malaria were cross-immunogenic against human malaria it may lead to a practical method of vaccine production. As such it is necessary to study the species specificity of the immunity produced by exoerythrocytic stages.

Attempts should be made to establish *in vitro* tissue cultures of mammalian malarial parasites. Liver cell cultures should be exposed to a small number of sporozoites.

(iii) Erythrocytic stages:

Recently it has been possible to grow the erythrocytic stages of the parasite *in vitro*, offering hope for continuous propagation and ultimately for quantity production.

In view of the species specificity of the immunity produced by erythrocytic stages, attempts should be made to cultivate the erythrocytic stages of *P. falciparum* in a suspension of human or Aotus red monkey cells. Where *P. falciparum* is not available, *P. coatneyi* with a similar 48 hours cycle and sequestration of schizonts, could serve as a model. Attempts should be made to induce gametocyte formation in cultures of erythrocytic forms.

(iv) Cell fusion techniques of genetic material:

The possibility of producing malarial antigens using cell fusion techniques or transfer of genetic material should be kept in mind.

It is obvious that the *in vitro* cultivation of malaria parasites is still an open field in spite of the large amount of basic

work already carried out in this direction. Any further progress, *viz.*, the preparation of a pure antigen for serological tests, *in vitro* testing of anti-malarials, production of a potent vaccine as well as the understanding of the basic aspects of the disease, will depend to a large extent on the successful *in vitro* cultivation of the parasite.

7. IMMUNOLOGICAL ASPECTS OF MALARIA:

For the ultimate development of malaria vaccine, extensive studies need to be undertaken on immunology of malaria. Similarly for the formation of effective treatment of the disease, investigations are necessary on the pathology in the various organs affected.

7.1 Immunoprophylaxis against malaria:

Recent work has revealed that there is increased immunoglobulin synthesis in the population as a result of malaria infection. Antibody specific to malaria may comprise only a third of this immunoglobulin, of which less than five per cent is functional. Nevertheless, studies with rodents and monkeys have demonstrated that immune serum exerted protective activity against malaria infection. The presence of enhancing antibody is also indicated in recent studies with immune sera.

T cells, largely as helper cells, seem to be intimately involved in conferring resistance to malaria infection. Apart from B and T cells, macrophages have a critical role in limiting the infection. Macrophages, in addition to their participation in processing malaria antigens seem to exert antibody-dependent cytotoxicity to parasites.

In the light of these observations there is an urgent need to study the immune response to malaria infection and the role of humoral and cell mediated immunity in malaria.

7.1.1 Studies on immunization against malaria:

There is considerable information available on the possibility of inducing immunity to malaria infection using different parasite stages and their antigens. These include sporozoites, asexual stages in the blood, sexual stages and non-living parasite antigens.

(i) Sporozoites:

It has been demonstrated that irradiated

sporozoites induce a near complete protection against malaria with *P. berghei* infection. But the results were less dramatic with *P. cynomolgi*. Studies with human volunteers and *P. falciparum* indicate that this method offers considerable promise.

It is found that while sporozoite immunized animals are fully protected against challenge with sporozoite stage, they remain susceptible to the erythrocytic stages of the same plasmodial strain. Also, immunization with sporozoites of any given rodent malarial species produced a broad range of protection against challenge with sporozoites of all rodent malaria species and subspecies.

In these studies various methods were used for treating sporozoites for immunization *i.e.* ultrasonication, freezing and thawing, heat inactivation, trituration and irradiation by X-rays or γ rays. Only irradiated sporozoites produced protective immunity.

The important investigations which could be undertaken with sporozoites are: (a) purification of sporozoite preparations; (b) characterization of the protective antigen; (c) development of methods to enhance and prolong the duration of sporozoites induced protection through the use of adjuvants; and (d) study of sporozoite induced immunity in a simian malaria model that resembles *P. falciparum* infection in man.

(ii) Asexual stages:

The effect of immunization with irradiated parasitized erythrocytes and parasitized erythrocytes in adjuvants has been tried and a significant increase in resistance to challenge has been reported.

Immunization of monkeys with merozoites of *P. knowlesi* resulted in very good protection of the animals against the homologous strain. With the recent advances in the *in vitro* cultivation of the parasite, this approach appears to be most promising in developing a vaccine.

The important lines of work which should be undertaken with merozoites are: (a) the protective value of irradiated or formulized, freeze dried preparations of merozoites in an acceptable adjuvant; (b) sporozoite challenge of merozoite vaccinated animals; (c) the effect of merozoite immunization in established chronic malarial infections; and (d)

the possibility of the occurrence of cross-immunization between merozoites of simian and other plasmodial species and the human parasite *P. falciparum*.

(iii) Sexual stages:

Recently successful attempts in immunization against the sexual stages of *P. gallinaceum* using radiation attenuated gametes have been reported. This line of study may be pursued further.

(iv) Non-living parasite antigens:

Several non-living parasite antigens have been tried with varying success. These include *P. berghei* parasites passed through Hughes press in rats, purified antigens of *P. knowlesi* in monkeys, and purified antigens of *P. cynomolgi* isolated from infected liver of rats against homologous strains. These important studies need to be confirmed and extended.

An analysis of the results of the above immunization procedures reveals considerable species, strain and stage specificity in the protection conferred. Further, the protection is, in general, not total with any of these methods. There is obviously great need for extensive research in this area which could eventually lead to the most promising method of immunization, keeping in view various factors such as availability of the material, feasibility of the technique and protean manifestations of the parasite.

7.2 Immunopathology of malaria:

Information on the immunopathology of *P. vivax* and *P. falciparum* infection is meagre. Hence, it is stressed that studies to be carried out in this regard should highlight the role of different types (I to V) of hypersensitive reactions in the causation of renal and cerebral lesions with special emphasis on type III hypersensitive reaction.

7.2.1 Immunopathological studies:

- (i) Clinico-immunopathological studies of cases of malaria with renal manifestations. Collaborative studies are

to be undertaken at selected institutions using a common protocol;

- (ii) Experimental immunological study of brain and other viscera in a monkey model; and
- (iii) Immunological studies of the brain and other viscera in fatal cases of malaria. These studies could be carried out in institutions where satisfactory autopsy facilities exist.

7.2.2 Immunosuppressive activity of malaria:

- (i) Immunosuppressive effect of malaria on a monkey model using tetanus toxoid; and
- (ii) A pilot study of immunosuppressive effect of malaria in a sample population.

It would be seen that the research studies to be undertaken as a support to the National Malaria Eradication Programme, are of considerable practical importance and are as much result oriented as possible. Studies on the operational aspects of malaria control have been designed with a view to get solutions to the problems encountered in the actual implementation of the programme. The emphasis all through is to find methods of tackling the disease, under varying conditions of its epidemiology and spread. It is envisaged to identify and demarcate areas of chloroquine resistant *P. falciparum* and to find out the most effective and at the same time shortest drug regimen to cure cases of this strain. For the formulation of a sound strategy for control of malaria, its diagnosis, epidemiology, prevention and treatment, attention has necessarily to be paid to basic studies on ecology of vector mosquitoes, serology of malaria, cultivation of parasite and development of newer drugs and insecticides. It is expected that with determined efforts in research in the field of malaria, it would be possible to meet the challenge adequately, by a multi-disciplinary approach.

ICMR NEWS

A Seminar on 'Immunisation against poliomyelitis' was held at the Indian Council of Medical Research, New Delhi on 28th March, 1977. The

participants included virologists, microbiologists, pediatricians, maternal and child health experts, epidemiologists, health

administrators and those involved in the production and quality control of vaccines. The current status regarding epidemiology, incidence, age distribution, control and prevention of poliomyelitis were reviewed and recommendation made with regard to surveillance of poliomyelitis, virological and serological studies and immunisation against poliomyelitis.

A symposium on 'Hospital' infections with special reference to *Pseudomonas aeruginosa* was held at the Indian Council of Medical Research, New Delhi on 31st March, 1977. There were 20 participants from all over the country. The various aspects highlighted at the symposium were the biology and epidemiology of *Pseudomonas aeruginosa* and the incidence and control of hospital infections due to *Pseudomonas aeruginosa*.

Dr. M.S. Bamji was appointed as Deputy Director at the National Institute of Nutrition, Hyderabad, w.e.f. 1st April, 1977.

Dr. F.M. Rodrigues and Dr. S.N. Ghosh, were appointed as Deputy Directors at the Virus

Research Centre, Poona, w.e.f. 1st April, 1977.

Dr. C. Gopalan, Director-General, Indian Council of Medical Research, chaired the third session of the WHO Regional Advisory Committee on Medical Research held at Colombo, Sri Lanka, between 4th and 7th April, 1977.

Dr. C. Gopalan, participated in the International Symposium on 'The Diet of Man - Needs and Wants', organised by the Rank Prize Funds at Bath, UK, between 17th and 22nd April, 1977.

Dr. R.A. Vaidya, Senior Research Officer, Institute for Research in Reproduction, Bombay, participated in the Ninth World Congress of Fertility and Sterility held at Miami Beach, Florida, between 12th and 16th April, 1977 and in the International Symposium on 'Human Ovulation' held at Detroit, Michigan, USA from 19th to 21st April, 1977.

Dr. K. Krishnaswamy, Assistant Director, National Institute of Nutrition, Hyderabad, proceeded on a one year WHO fellowship to the G.D.R., to work in the field of Drug Metabolism in Malnutrition.

ICMR AIDED SYMPOSIA/SEMINARS/WORKSHOPS

A symposium on 'Carcinoma breast and its Comprehensive Approach' was held in connection with the 30th Annual Congress of Radiology at Chandigarh, between 1st and 4th January, 1977.

A symposium on 'Atherosclerosis' was organised during the 3rd Annual Conference of the Association of Biochemists of India at the Medical College, Baroda, between 7th and 9th January, 1977.

A symposium on 'Nutrition and Functional Performance' was organised by the Nutrition Society of India, at the National Institute of Nutrition, Hyderabad on 28th January, 1977.

The Indian Council of Medical Research National Workshop on 'Newer perspectives in microbiological investigations of streptococcal infections and their sequelae', was held at the Lady Hardinge Medical College, New Delhi between 21st and 26th February, 1977.

An International Symposium on 'Venom and Toxins' was organised by the Haffkine Institute, Bombay between 4th and 6th March, 1977.

As part of the fifth All India Conference of Physical Medicine and Rehabilitation, symposia on various aspects of Rehabilitation Medicine were organised at the Medical Rehabilitation Centre, Patna between 20th and 22nd March, 1977.

A workshop and a symposium on 'Hospital infections with special reference to *Pseudomonas aeruginosa*' were organised by the Department of Microbiology, All India Institute of Medical Sciences, New Delhi between 23rd and 31st March, 1977.

A symposium on 'Filariasis', organised by the Department of Microbiology, All India Institute of Medical Sciences, is being held on 4th June, 1977, at the Indian Council of Medical Research, New Delhi.

A study group on 'Human tumors in tissues with European cell tissue and organ culture' is being organised at the Tata Management Training Centre, Poona, between 16th and 18th August, 1977.

A workshop on 'Electron Microscopy for Medical Postgraduates and Teachers' is being organised at the K.G. Medical College, Lucknow, in September/October, 1977.

A symposium on 'Relationship of pathological states and metabolism of drugs in infants and children' is being held at the Postgraduate Institute of Medical Education and

Research, Chandigarh, on 20th and 21st October, 1977.

An International Symposium on 'Biomolecular structure, conformation, function and evolution' is being organised at the University of Madras, between 4th and 7th January, 1978.

PROGRAMME OF SCIENTIFIC LECTURES AT THE INDIAN COUNCIL OF MEDICAL RESEARCH, NEW DELHI

Date	Topic	Speaker
1.4.1977	Epidemiology of Streptococcal infections in north India	Dr. K.B. Sharma, New Delhi.
15.4.1977	Functional significance of growth retardation (Basanti Devi Amir Chand Prize Oration, 1977)	Dr. S.G. Srikantia, Hyderabad.
29.4.1977	Brain catecholamines in hypertension and cardiac arrhythmia	Dr. K.P. Bhargava, Lucknow.

COUNCIL'S TRAINING PROGRAMMES FOR 1977

Leprosy:

At the Central Jalma Institute for Leprosy, Agra.

Training course in Leprosy for medical officers: (December, 1977).

Virology:

At the Virus Research Centre, Poona.

- (1) Diploma course in Virology of Poona University: (July, 1977).
- (2) *Training Course for Technicians for a period of one year.
- (3) Short-term course in Tissue Culture: (June 13 to 26, 1977).

Amoebiasis:

At the School of Tropical Medicine, Calcutta.

Technicians Training Course in Amoebiasis: (October 1 to December 31, 1977).

Reproductive Biology:

At the Institute for Research in Reproduction, Bombay.

- (1) Course on Immunological Techniques: (February, 1977).
- (2) Course in Hormone Assays: (March, 1, 1977 to April 30, 1977).
- (3) Course in Endocrinology and Fertility Control: (September, 1977).
- (4) Course on Human Cytogenetics: (September, 1977).
- (5) Course in Reproductive Biology and Fertility Control: (November, 1977 to February, 1978).

Nutrition and Endocrinology:

At the National Institute of Nutrition, Hyderabad.

- (1) Annual certificate course in Endocrinological Techniques and their Application: (August, 1977 to September, 1977).
- (2) Annual certificate course in Human Nutrition for Teachers in Agricultural Universities: (August, 1977 to October, 1977).
- (3) M.Sc. (Applied Nutrition) course: (June, 1977 to February, 1978).
- (4) Annual certificate course in Nutrition: (December, 1977 to February, 1978).

Haematology:

At the Blood Group Reference Centre, Bombay.

- (1) A certificate course in Blood Banking Technology and Basic Haematology for medical officers: (February 1, 1977 to March 31, 1977).
- (2) Training course in Blood Banking Methodology for Technicians: (May 2 to May 14, 1977).

Occupational Health:

At the National Institute of Occupational Health, Ahmedabad.

- (1) *ICMR/WHO training course in Industrial Toxicology.
- (2) *Refresher course in Occupational Health for E.S.I. medical staff.
- (3) *Training course on Chemical Hazards in Chemical Industry organised jointly by the NIOH and the National Safety Council.

Clinical Pharmacology:

At the Department of Pharmacology, Seth G.S. Medical College, Bombay.

Training course in Clinical Pharmacology: (January 15 to February 28, 1977).

Biostatistics:

At the National Institute of Nutrition, Hyderabad.

Annual Training course in Biostatistics for Statisticians: (November, 1977).

Laboratory Animal Technology:

- (1) At the Virus Research Centre, Poona.

Short-term course in Breeding and Maintenance of Laboratory Mouse Colonies: (March, 1977).

- (2) At the National Institute of Nutrition, Hyderabad.

Laboratory Animal Technicians Training Course: (August, 1977 to September, 1977)

COURSES FOR TALENT SEARCH SCHEME FELLOWS:

Immunological Techniques:

At the Department of Biochemistry, All India Institute of Medical Sciences, New Delhi: (January 24, 1977 to February 5, 1977).

Clinical Chemistry and Instrumentation:

At the National Institute of Nutrition, Hyderabad: (February 28, 1977 to March 12, 1977).

Use of Radioisotopes in Medicine:

At the National Institute of Nutrition, Hyderabad: (June 20-30, 1977).

Cell-Biology and Cyto-Genetics:

At the Indian Institute of Science, Bangalore: (June 29, 1977 to July 12, 1977).

*Haematological Techniques:

At the Department of Haematology, Postgraduate Institute of Medical Education and Research, Chandigarh.

*Principles and Methods in Medical Statistics:

At the Tuberculosis Chemotherapy Centre,
Madras.

*Clinical Pharmacology:

At the Department of Pharmacology, Seth
G.S. Medical College, Bombay.

*Dates to be announced later

ICMR PUBLICATIONS

TECHNICAL REPORTS

	Price Rs.
Research in Medical Education (1970)	4.00
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Nutrition for mother and child by P.S. Venkatachalam and L.M. Rebellow (1962), 2nd Edition 1971	1.50
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Nardostachys Jatamansi - A Chemical, Pharmacological and Clinical Appraisal by R.B. Arora (1965)	4.50
Dietary Allowances for Indians by C. Gopalan and B.S. Narasinga Rao (1968) Reprinted in 1974	4.00

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