SUMMARY
I. CHEMOTHERAPEUTIC STUDIES

(A) Malaria Parasites Used in Chemotherapeutic and Drug Resistance Studies

For experimental chemotherapeutic studies, choice of model plays very important role. Therefore, it is necessary to select a host in which the virulence of parasite should be high and mortality rate should be 100%.

On the basis of above objectives two type of malaria parasites were used in our study. Rodent malaria parasite P. yoelii nigeriensis produces a peak parasitaemia level ranging from 62.5 - 80.0 % in Swiss mice and the mean survival time of the infected mice was found to be short (5.5 - 7.8 days).

For the studies on simian malaria, P. knowlesi infection has been used in rhesus monkey as host. In rhesus monkey, the parasitaemia of P. knowlesi reaches very high level, and it was observed that infection was always fatal.

(B) Chemotherapeutic Studies with P. yoelii nigeriensis

It has been reported earlier that P. yoelii nigeriensis has certain degree of natural resistance to chloroquine, amodiaquine, mefloquine and quinine. Reports emanating from several regions i.e. South America, South East Asia, South India and Africa show that resistance of P. falciparum to standard antimalarial drugs had been established and this is a serious problem in the control of malaria. In
view of natural resistance to several antimalarials, *P. yoelii nigeriensis* has a great value as a screening model for chemotherapeutic studies and development of new drugs.

1. **Long acting nature of antimalarials and sulfa drugs:**

   Studies carried out have shown that 4 x M.E.D. dose of acedapsone, which, if administered 9 days before infection, i.e. only day -9, suppressed parasitaemia for 21 days. On the other hand, other antimalarials, i.e. pyrimethamine, metakelfine, dapsone and sulphanilamide administered at 8 x M.E.D. dose level on day 0 suppressed the parasitaemia till day 4. Administration of these drugs on -3 and -9 days did not suppress day 4-7 parasitaemia and their excretion was quick. The results indicate that acedaposone is most long acting and no mortality was observed in acedapsone treated group. It was found that excretion of sulphadiazine and other sulfa drugs from the Swiss mice was relatively raised and these drugs, if administered on day -3 or -9, failed to suppress parasitaemia.

2. **Efficacy of single dose treatment:**

   Efficacy of several antimalarial drugs has been tested against *P. yoelii nigeriensis* (normal). In single dose treatment, the M.E.D.'s of various antimalarial drugs which clear the parasitaemia from day 4-7 were found to be as follows
sulphadiazine, 320 mg/kg; sulphanilamide, 640 mg/kg; sulphadoxine, 40 mg/kg; DDS, 80 mg/kg; DADDS, 20 mg/kg; fansidar (2.5 mg sulphadoxine + 0.125 mg/kg pyrimethamine), triple combination of sulphadoxine/pyrimethamine/mefloquine (MED = 1 mg/kg sulphadoxine + 0.125 mg/kg pyrimethamine + 0.5 mg/kg mefloquine), metakelfine (MED = sulphamethoxypyridazine 12.5 mg/kg + pyrimethamine 6.25 mg/kg). In a single dose therapy, mefloquine and pyrimethamine were found to show low protection. It was observed that mefloquine at 640 mg/kg and pyrimethamine at 10-80 mg/kg dose level suppressed parasitaemia till day 4 only. The maximum tolerated dose of pyrimethamine was found to be 80 mg/kg, while higher doses of 160-640 mg/kg were toxic.

Mean survival time - Among sulphonamides, extension of mean survival time (M.S.T.) with sulphadiazine was found to be not more than twice as compared to untreated control at any dose level. However, other drugs i.e. fansidar, metakelfine, sulphanilamide, sulphadoxine, DDS, DADDS and triple combination of dapsone and acedapsone with pyrimethamine + mefloquine when tested at M.E.D. doses, showed extension of mean survival time to more than double as compared to the corresponding controls.

3. Challenge and Rechallenge of Mice Which Survived After Treatment

The protection of mice which survived after single dose treatment was evaluated by challenge and rechallenge ex-
periment after 22-70 days. Challenge studies carried out showed that protection of sulphadiazine and sulphanilamide treated mice was found to be poor at low dose treatment, and it was observed that the mice which survived after single high dose (640 mg/kg) therapy with sulphanilamide were protected on challenge, whereas sulphadoxine and dapsone treated mice were also protected after challenge and rechallenge with P. yoelii nigeriensis showed best protection in DADDS treated mice. It was observed in present study that the mice cured with acedapsone (DADDS) showed maximum protection after two challenge.

4. Efficacy of new antimalarial compounds in single dose Treatment

Activity of compounds No. 1, 2, 4, 5, S3 received from Chemistry Department of Lucknow University and compound Nos. 42/183, 80/695, 82/142, 42/183, 83/495, 83/494, 80/693, 83/498, 83/496, 83/38, 82/143 and 82/628 have been tested. No parasitaemia clearance was found in any compound. The extension of survival time of compound No. 83/495 was found to be double as compared to control at highest 640 mg/kg dose. Rest of the compounds were found to be inactive or of low activity.
5. Blood Schizontocidal Activity of New Antimalarial Compounds

Using standard 4 day test 3 compounds No. S8, S4 and S12 were tested against *P. yoelii nigeriensis* in Swiss mice host. M.E.D. of these compounds was found to be as follows. S8 = 2 mg/kg, S4 = 1 mg/kg (at toxic level), S12 = 1 mg/kg, and extension of mean survival time was found to be double at M.E.D. dose of compound S8 and S12. Extension of mean survival time of compound S4 at 1 mg/kg dose level was found to be double as compared to control.

(C) Chemotherapeutic Response of Antimalarial Drugs Against Normal W₁ Strain of *P. knowlesi*

Activity of chloroquine, amodiaquine, quinine and sulphadiazine was studied against drug sensitive W₁ *P. knowlesi*. Curative dose of chloroquine and sulphadiazine was found to be 5 mg/kg x 3 days and 500 mg/kg x 3 days respectively. Treatment with amodiaquine and quinine at 5 mg/kg x 3 days and 100 mg/kg x 3 days showed recrudescence of parasitaemia.

II. DRUG RESISTANCE

(A) Selection of Sulphadoxine/Pyrimethamine/Mefloquine Resistance strain of *P. yoelii nigeriensis*

In the present study, a strain of *P. yoelii nigeriensis* resistant to triple combination of sulphadoxine + pyrimethamine + mefloquine was selected in 64 serial passages over a period of 464 days and the strain was found to be
resistant at a dose of 1.25 mg/kg sulphadoxine + 0.125 mg/kg pyrimethyamine + 0.5 mg/kg mefloquine x 4 days, thus showing 16-fold resistance on the M.E.D. basis, the stability of the strain has remained unaltered after cryopreservation of the strain.

(B) Cross Chemotherapeutic Studies

Cross chemotherapeutic response of several antimalarials was studied with *P. yoelii nigeriensis* resistant to fansidar + mefloquine combination. M.E.D. of several antimalarials found out in 4 day test are as follows:

- DDS, 4 mg/kg; acedapsone, 0.5 mg/kg; dapsone + pyrimethamine, 0.5 mg/kg DDS + 0.125 mg/kg pyrimethamine; acedapsone + pyrimethamine, 0.125 mg/kg acedapsone + 0.625 mg/kg pyrimethamine;
- DDS + pyrimethamine + mefloquine combination, 0.25 mg/kg dapsone + 0.625 mg/kg pyrimethamine + 1 mg/kg mefloquine; acedapsone + pyrimethamine + mefloquine combination, 0.625 mg/kg acedapsone + 0.78 mg/kg pyrimethamine + 0.125 mg/kg mefloquine; Mepacrine, 64 mg/kg; sulphadoxine, 4 mg/kg; pyrimethamine 1.5 mg/kg; fansidar, 1.25 mg/kg sulphadoxine + 0.625 mg/kg pyrimethamine; Mefloquine, 128 mg/kg Metakelfine, 2.6 mg/kg sulphamethoxypyridazine + 0.125 mg/kg pyrimethamine; sulphadiazine, 12.5 mg/kg; sulphanilamide, 100 mg/kg; amodiaquine, 256 mg/kg. Studies carried out showed that above strain of *P. yoelii nigeriensis* (resistant fansidar...
+ mefloquine) was found to be highly resistant to chloroquine and quine at maximum tolerance dose.

On the basis of M.E.D. resistance to mefloquine, amodiaquine had increased to 16 fold. There was also increase in M.E.D. of fansidar, mepacrine, sulphadoxine, sulphadiazine and dapsone to 17-fold, 8-fold, 6-fold, 312-fold, and 2-fold respectively.

Strain of P. yoelii nigeriensis after exposure to fansidar + mefloquine combination has maintained sensitivity to pyrimethamine, acedapsone, sulphanilamide and combination of dapsone, acedapsone with pyrimethamine + mefloquine, metakelfine.

Our study strongly suggests that fansidar cannot protect the emergence of mefloquine resistance if both the drugs are used in combination and there is need for caution to use mefloquine + fansidar combination in fields against multiple resistant P. falciparum malaria.

Our study also suggests the possible use of either a combination of dapsone and acedapsone with pyrimethamine + mefloquine, or metakelfin alone in fields for the ultimate control of multiple resistant falciparum malaria.

(C) Resistance to Chloroquine and Amodiaquine Against P. knowlesi

In present study exposure of W₁ stabilate of P. knowlesi to subcurative treatment in successive passage
was tried to select stabilates, which should be resistant to chloroquine and amodiaquine. Treatment was given at both low parasitaemia as well as high parasitaemia and recrudescence after various dosages of both chloroquine and amodiaquine was occasionally observed. However, when these parasites were subinoculated in healthy monkeys, the resistance of *P. knowlesi* to high doses of chloroquine or amodiaquine was not demonstrable.

III. Immune Status of Monkeys after Curative or Subcurative Treatment

In order to see immune status of rhesus monkeys, 6 monkeys treated with subcurative doses of chloroquine and amodiaquine and 19 monkeys which had been given curative treatment with chloroquine, were challenged with *P. knowlesi*. The results have shown poor protection of monkeys after curative treatment with chloroquine, whereas after subcurative therapy 33% of the monkeys were found to be protected. It shows that small dosages of drug given for long period could stimulate indirectly the host's defence mechanism against *P. knowlesi* infection.