I. Chemotherapeutic studies with asexual erythrocytic stages of *P. berghei*:

a) Infection of *P. berghei* in different hosts: A comparative study of the course of infection of *P. berghei* in four rodent hosts viz: Swiss mice, albino rats, *Mastomys natalensis* and golden hamsters has shown that after a standard inoculum of 10 million parasitized cells, the untreated (control) animals show cent per cent mortality. The death of different batches of mice and rats occurred in 6-10 days, whereas, *Mastomys natalensis* survived for 11.2-14.8 days and the golden hamster survived longest (14.7-18.5 days). Out of the four hosts tested, highest parasitaemia (47.8-62.5%) was reached in the case of *Mastomys natalensis*. *P. berghei* infection was 100% fatal for 20-25 gm rats, 62.5-75% for 50 gm rats; 16-33% for 100 gm rats and there was no mortality in 150 gm rats showing thereby that there was age resistance in this species. In weanling rats (20-25 gm), there was increase of patency with decrease of inoculum of the parasites, but the infection was fatal with all the doses of parasite inocula used (0.625x10⁶-100x10⁶ parasites). In view of the fatal course of parasitaemia in weanling rats even with low inocula, they were found to be suitable host for selection of drug resistant strains of *P. berghei*. The weanling rats behaved as non-immune hosts, which favoured the survival of parasites during selection pressure of drugs.

b) Chemotherapeutic studies with antimalarial drugs:

The activity of the eight known and a new antimalarial drug (mefloquine) has been compared using three criteria namely: (i) determination of minimum effective dose (MED) (ii) determination of mean survival time and (iii) determination of ED₅₀ and ED₉₀ values.
Minimum effective dose: The MEE of the standard antimalarial drugs was determined in the four rodent hosts and similar values were obtained in all the hosts. The MEE values obtained for various drugs used in this study were as follows: Chloroquine, 8.0 mg/kg; amodiaquine, 8.0 mg/kg; mepacrine 10.0 mg/kg; primaquine, 17.5 mg/kg; pyrimethamine, 1.0 mg/kg; dapsone, 4.0 mg/kg; sulphadiazine, 0.2 mg/kg (0.4 mg/kg in rats); sulphanilamide, 0.4 mg/kg and mefloquine, 4.0 mg/kg.

Mean survival time: Extension of survival time in the treated mice to twice that of the corresponding controls, was observed after 4 day treatment with antimalarial drugs at following dose levels: chloroquine, 4 mg/kg; amodiaquine 4 mg/kg; mepacrine 5 mg/kg; primaquine, 8.5 mg/kg; pyrimethamine, 1.0 mg/kg; dapsone 4.0 mg/kg; sulphadiazine, 0.2 mg/kg; sulphanilamide, 100 mg/kg and mefloquine, 2.0 mg/kg.

ED_{50} and ED_{90} values: The ED_{50} and ED_{90} values of various antimalarials in mice, as determined by log dose analysis of day 4 parasitaemia were as follows: Chloroquine, 1.83 and 3.65 mg/kg; amodiaquine, 1.67 and 4.11 mg/kg; mepacrine, 2.55 and 6.37 mg/kg; primaquine, 2.21 and 6.04 mg/kg; pyrimethamine, 0.20 and 0.45 mg/kg; dapsone, 0.45 and 1.54 mg/kg, sulphadiazine, 0.045 and 0.11 mg/kg; sulphanilamide, 15.20 and 37.30 mg/kg and mefloquine, 0.60 and 3.11 mg/kg respectively.

With most of the antimalarials the MEE values, which represent the complete clearance of parasites till day 7 were nearly two fold to the corresponding ED_{90} values. Similarly the MEE values of five drugs namely, chloroquine, amodiaquine, mepacrine, primaquine and mefloquine were found to be twice the dose required for extension of survival time to double that of control while in the case of pyrimethamine, dapsone, sulphadiazine and sulphanilamide, the same dose level as the MEE was required to extend
the survival time to twice that of control. Furthermore, it has been observed that with drugs like chloroquine, amodiaquine, primaquine and mefloquine, the MED values were able to produce clearance of parasitaemia for more than three weeks in the treated animals, while with other drugs like dapsone, pyrimethamine, sulphasalazine and sulphanilamide similar results were obtained with 2 x MED doses.

c). Chemotherapeutic studies with antibiotics:

Chemotherapeutic studies have also been carried out with some of the antibiotics using the criteria of mean survival time and determination of \( \text{ED}_{50}/\text{ED}_{90} \) values. Results with both these tests have revealed that minocycline and doxycycline possess antimalarial activity at significantly lower dose levels as compared to other antibiotics as demeclocycline, tetracycline, oxytetracycline, erythromycin and chloramphenicol. The respective \( \text{ED}_{50} \) and \( \text{ED}_{90} \) values for various antibiotics obtained in this study were: doxycycline, 9.41 and 93.70 mg/kg; minocycline, 1.57 and 13.21 mg/kg; demeclocycline, 32.55 and 140.3 mg/kg; tetracycline, 57.68 and 302.0 mg/kg; oxytetracycline, 48.08 and 297.3 mg/kg; erythromycin, 62.81 and 375.0 mg/kg and chloramphenicol, 57.76 and 516.4 mg/kg. Chemotherapeutic studies with antibiotics clearly show that these are not curative in action and possess slow suppressive plasmodicidal activity.

The data obtained from the chemotherapeutic studies with drugs and antibiotics forms a base-line for subsequent screening tests. It is concluded from these results that during primary screening of potential compounds for antimalarial activity, the criterion of extension in the survival time can be useful for preliminary selection of potential compounds. However, it does not indicate the total clearance of parasitaemia. MED on the other hand indicates the clearance of parasitaemia till day 7. The criterion of extension in survival time to 21 days, along with the absence of parasitaemia on day 21 is the most
reliable test for detecting strong blood schizontocidal activity in the test compounds. Determination of ED$_{50}$ and ED$_{90}$ values by the log dose analysis of day 4 parasitaemia provides the most suitable and quick method for determining the relative efficacy of test compounds and the standard drugs.

II. Studies on chemoprophylaxis with *P. gallinaceum*- chick model:

A test model comprising of *P. gallinaceum* - *Aedes aegypti* - chick has been standardized for evaluating the causal prophylactic activity of potential compounds. Maintenance of infected mosquitoes for 10-12 days at 76-80°F and 70- 80% relative humidity has been found to be ideal for the maturation of sporozoites. Feeding of 5 or more mosquitoes per normal chick ensures the cent per cent infectivity. Further, it has been observed that infected mosquitoes are able to transmit the infection for as long as 30 days after the blood meal. These findings are of great epidemiological importance. This model has been found to be suitable for detecting the activity of drugs like pyrimethamine, however, the activity of primaquine could not be recorded. The new drug mefloquine has also been found to possess no activity against the tissue stages of *P. gallinaceum* upto a dose of 45 mg/kg/day. Out of the 8 antibiotics tested for the causal prophylactic activity, minocycline and doxycycline were found to be active at 15 mg/kg as compared to chlortetracycline, demeclocycline, tetracycline and oxytetracycline which showed complete activity only at 135 mg/kg. Lower doses of these antibiotics were able to show a significant prolongation of the prepatent period in treated chicks.
III. Drug resistance in rodent malaria:

Strains of *P. berghei* resistant to pyrimethamine, chloroquine, primaquine and mefloquine were selected during the course of present study. Weanling rats (20-25 g) which behave as non-immune host, have been employed for the first time in this study for the selection of resistant strains. The method of 'interrupted subcurative therapy' was employed which implies that the parasites were treated with subcurative doses of drug, interruptedly, depending on the rate of increase in parasitaemia.

a). Pyrimethamine resistant strain:– The pyrimethamine resistant strain was selected in 43 serial passages and the strain was resistant to maximum tolerated daily dose of 133 mg/kg, thus showing 133 fold resistance on MED basis and more than 295 fold resistance at ED₉₀ level. The resistant strain selected in weanling rats was also resistant to treatment at daily dose of 132 mg/kg/day in other rodent hosts such as mice, *Mastomys* and hamsters. The stability of pyrimethamine resistance remained unaltered during drug free maintenance of the parasites for 56 days and also after cryopreservation of the strain for 160 days. Cross sensitivity studies with the resistant line show that the parasites remain sensitive to treatment with chloroquine, amodiaquine, mepacrine, primaquine and mefloquine. A two fold decrease in sensitivity to sulphadiazine and sulphanilamide was recorded on MED basis. All the antibiotics tested were found to be active against the resistant parasites at dose levels comparable to that of the normal strain. Minocycline and doxycycline were effective in suppression of parasitaemia at low dose level.

b). Chloroquine resistant strain:– This strain was selected in 42 serial passages and the parasites were resistant to treatment with daily dose of 248 mg/kg base. The strain showed 31 fold resistance at MED level and more than 66 fold resistance at ED₉₀ level. The chloroquine
resistant strain was also resistant to daily dose of 248 mg/kg in mice and *Mastomys* while in hamsters the strain was resistant to only 16 mg/kg/day. The parasites of the resistant strain maintained high level resistance even after drug free maintenance for 220 days and also after cryopreservation for 244 days. The cross sensitivity studies in rats showed high degree of cross resistance to amodiaquine (31 fold) and mepacrine (24 fold) while there was no change in sensitivity to primaquine, pyrimethamine, dapsone, sulphadiazine, sulphanilamide and mefloquine when compared on the basis of MED. In mice, the strain showed nearly 12, 4 and 2 fold cross resistance to amodiaquine, mepacrine and primaquine respectively on the basis of both MED and ED$_{90}$ level. The sensitivity of the resistant parasites to antibiotics showed that except for two fold decrease in sensitivity to minocycline and demeclocycline, there was no significant variation in the values for other antibiotics.

c) **Primaquine resistant strain** :- The primaquine resistant strain was selected in only 10 serial passages and the strain showed 4 fold resistance on MED basis and nearly 12 fold resistance at ED$_{90}$ level. The level of resistance could not be enhanced from daily dose of 70 mg/kg since higher doses were found to be toxic for the host. Primaquine resistant strain was found to be resistant at the same dose level of 70 mg/kg in all the four hosts. The stability of resistance was maintained after drug free passages for 72 days and after cryopreservation for 44 days. The cross-sensitivity studies with the resistant population revealed that the parasites were sensitive to treatment at normal dose level, effective for the normal (sensitive) strain, with other drugs like chloroquine, amodiaquine, mepacrine, pyrimethamine, dapsone, sulphadiazine, sulphanilamide and mefloquine. Except for slight change in sensitivity to minocycline and demeclocycline, the sensitivity to other antibiotics also remained unaltered.
d) **Mefloquine resistant strain**: The mefloquine resistant strain was selected in only 10 serial passages and the parasites were resistant to daily dose of 243.2 mg/kg thus showing nearly 61 fold resistance on MED basis. The level of resistance in mice and hamsters was checked upto 128 mg/kg/day. The mefloquine resistance was not stable when parasites were kept in absence of drug pressure and the resistance was lost in 6–7 serial passages over a period of 30–40 days. However, the resistance was found to be stable when checked after cryopreservation for 56 days. The cross sensitivity studies carried out in rats showed that mefloquine resistant strain remains sensitive to other antimalarials as chloroquine, amodiaquine, mepacrine, pyrimethamine, dapsone and sulphadiazine at normal dose levels excepting primaquine to which it showed 2 fold resistance.