Chapter V
DISCUSSION
CHEMOTHERAPY

I. Studies with asexual erythrocytic stages of P. berghei

A. Standardization of model: The course of P. berghei infection in Swiss mice, albino rats, Mastomys natalensis and golden hamsters has been compared in the present study (Table 5). The infection was 100% fatal in all the hosts. The mean survival time after infection with a standard inoculum (1x10^7 parasites) was highest in case of golden hamsters (14.7-18.5 days) followed in decreasing order by Mastomys (11.2-14.8 days), rat (6.8-9.5 days) and mice (6.1-7.6 days). Comparative studies on 4 hosts have shown that maximum parasitaemia was reached in Mastomys (47-62%); it was lower in hamsters and rats and the mice showed the lowest peak parasitaemia. Further studies showed that the course of infection in rats was greatly influenced by the weight of the animal; the maximum parasitaemia and cent-per cent mortality was obtained only in the weanling rats (20-25 gm) while the older animals showed practically no mortality (Table 6). Galliard and Lapierre (1951) had reported that the mortality rate was 50% in rats of 50 gm. and less than 25% in rats above 120 gm. Singer et al. (1955) had reported the decrease in percent mortality in group of rats above 40 gms and the mortality rate was nil in rats above 120 gms. Hauf and Geiman (1952) found the infection to be fatal in albino rats of 60-100 gms with an inoculum of 60 million parasites per rat. Ramakrishnan et al. (1950) and Zuckerman et al. (1954) have also reported the decrease in the mortality rate and occasional chronicity in older rats. Observations on the increase in prepatent period in 25 gm. rats when inoculated with decreasing number of parasites, are also in agreement with earlier findings of Fabiani et al. (1951a) and Cantrell et al. (1970). In this study, the albino rats have been used for the first time, for selection of drug resistant
strains, because they were found to behave as completely non-immune host, which facilitated the selection of resistant population of parasites.

B. Schizontocidal activity of known and new antimalarial drugs and antibiotics against P.berghei.

1) Studies with antimalarial drugs: With a view to develop a rational system for screening the potential compounds for their antimalarial activity, a detailed assessment of the activities of antimalarial drugs has been carried out using P.berghei - mouse model. In addition a detailed comparison has been made of the different criteria employed by earlier workers for assessment of antimalarial activity, such as:

(i) Minimum effective dose (MED);
(ii) Mean survival time, and
(iii) ED$_{50}$ and ED$_{90}$ values.

(i) MED: The earlier workers have used different criteria to find out the MED of known drugs with the result that different MED values have been reported in various studies (Thurston, 1950; Hill, 1950; Schneider et al., 1952). Thurston (1950) employed treatment from day 0-3 and the dose which produced mean parasitaemia of less than 1% on day +4 was considered MED. Hill (1950) used a parasitaemia of less than 0.25% after 4 day treatment, as a criterion for MED, while Schneider et al. (1952) employed 5 day treatment and defined MED as the lowest dose showing clearance of parasitaemia for 3 consecutive days. In the present study, in order to determine the MED four day treatment schedule (day 0-3) has been used as suggested by WHO (1973) and Thurston (1950), and the dose producing clearance of parasites till day +7 has been found to be reliable index for comparing the antimalarial activities of known and new antimalarial drugs. The MED values obtained in present study are more or less in agreement with those of the
earlier reports of Thurston (1950), Hill (1950) and Schneider et al. (1952). However, slight variations observed in this study can be explained because of different criteria and dose schedule employed by the earlier workers for assessment of MED. It is important to mention here that the earlier workers employed mainly mice for chemotherapeutic studies, but efforts have been made for the first time in this study to determine the MED of the known antimalarial drugs, in four different hosts namely Swiss mice, albino rats, Mastomys natalensis and golden hamsters, with a view to find out if the host influences the MED of the antimalarials or not. The data presented in Table 9 clearly showed that MED of most of the drugs tested was not influenced by the host used for evaluation of drugs. Thus MED of chloroquine and amodiaquine in different hosts was 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, and sulphanilamide 100 mg/kg. Mefloquine, a new antimalarial, gave an MED of 4.0 mg/kg in all the hosts. Follow-up of the parasitaemia of treated animals beyond day 7 was also studied and it has been found that the MED doses produced total clearance of parasitaemia in case of chloroquine, amodiaquine, mefloquine and primaquine, while MED doses of other drugs such as mepacrine, pyrimethamine, dapsone, sulphadiazine and sulphanilamide did not produce clearance of parasitaemia till day +21, but on the other hand 2×MED doses were required to produce parasite clearance till day +21.

(ii) Mean survival time: Among the earlier workers (Jacobus 1967) employed the criteria of mean survival time after treatment with a single subcutaneous dose of drugs administered 72 hours after infection for evaluating the activity of antimalarial drugs. In the present study, the known drugs have been evaluated using the criterion of mean survival time after four day oral treatment from day 0-3. In general, it has been found that doses equivalent to MED or 2×MED were required to produce extension of
survival time to three weeks (i.e. day 22 from day 0 of infection). WHO (1973) have laid the criterion that the extension of survival time to double that of control group, should be considered as significant for demonstrating the antimalarial activity. It is significant to point out that in present study (Table 8), the dosage of antimalarials which have produced extension of survival time to 22 days were able to produce more or less complete clearance of the parasitaemia while lower doses which resulted in survival for double the time of the controls failed to clear the parasites.

(iii) $ED_{50}$ and $ED_{90}$ values: Peters (1965a) had determined the $ED_{50}$ and $ED_{90}$ values of a number of antimalarial drugs after four day subcutaneous treatment from day 0 to day +3. In present study, the $ED_{50}$ and $ED_{90}$ values were determined from day 4 parasitaemia after oral drug treatment. In general it has been found that $\frac{1}{2}xMED$ value produces 90% suppression in parasitaemia on day 4. The $ED_{50}$ and $ED_{90}$ values of chloroquine, mefarine and mefloquine obtained in present study are slightly lower than those reported by Peters (1965a) and Peters et al. (1977a). On the other hand $ED_{50}$ and $ED_{90}$ values of pyrimethamine, dapsone and primaquine recorded in this study are higher than those reported by Peters (1965a). The $ED_{90}$ value of sulphadiazine reported by Peters (1965a) is exceptionally high ($2.7 \text{ mg/kg}$) compared to that obtained in the present study ($0.11 \text{ mg/kg}$).

2). Studies with antibiotics: Although the therapeutic antimalarial activities of antibiotics have been known for nearly last 20 years, no systematic study has been carried out so far to evaluate the precise efficacy of the antibiotics, using standardized procedure of determining their $ED_{50}$ and $ED_{90}$ values or the survival time of the host after treatment, which would give a better comparison of their antimalarial activity. Since there has been a renewed interest for last couple of years in the antimalarial
activity of antibiotics for the control of highly drug resistant strains of human malaria (Willerson et al. 1972; Colwell et al., 1973), it was considered of particular interest to compare the efficacy of known and new antibiotics using the criteria of $ED_{50}/ED_{90}$ values as well as the mean survival time after 4 day treatment. Detailed studies carried out with the normal (sensitive) strain have shown that minocycline and doxycycline are active at relatively low doses as judged by the prolongation of survival time. The other antibiotics namely demeclocycline, tetracycline, oxytetracycline, erythromycin and chloramphenicol were found to exert some activity only at higher dose levels which can not be recommended for clinical use. The present study clearly establishes the efficacy of doxycycline and minocycline at low doses although these dose levels were not curative in action as shown by the persistence of parasitaemia.

A comprehensive account of the $ED_{50}$ and $ED_{90}$ values of antibiotics has been described for the first time which provides opportunity to compare their antimalarial activity. Using this criterion also, only minocycline and doxycycline were found to be active at low dose level. On the other hand, other antibiotics such as demeclocycline, tetracycline, oxytetracycline, erythromycin and chloramphenicol showed comparatively high $ED_{50}$ and $ED_{90}$ values. Only earlier reports of the $ED_{50}$ and $ED_{90}$ values of antibiotics are by Kaddu et al. (1974) for minocycline and Warhurst et al. (1976) for erythromycin, who reported higher $ED_{50}$ and $ED_{90}$ values than observed in this study. Inspite of the antimalarial activities shown in this study, the antibiotics being slow acting can not be used alone for treatment of acute cases of malaria.

The chemotherapeutic activities presented here provide a comparison of antimalarial drugs and antibiotics using the three criteria. This data would serve as a baseline for screening potential antimalarial compounds and
plant extracts in this Institute. The newly discovered quinolinemethanol compound, mefloquine, has shown highest antimalarial activity out of all the antimalarials tested and its blood schizontocidal activity is 2 fold to that of most commonly used drugs like chloroquine and amodiaquine.

C. Activity of drugs after single dose treatment: In the present study the activity of known and new antimalarial drugs was compared in relation to their prolonged effect after oral administration in a single dose. Thompson et al. (1963) have recorded protection to a challenge infection of P. berghei, lasting for 1 to 9 weeks after sub-cutaneous administration of single dose of CI-501 (Camolar) at 50-600 mg/kg. CI-501 in a single intramuscular injection at 50 mg/kg has also shown prolonged prophylactic protection for several months in sporozoite induced P. cynomolgi (Schmidt et al. 1963). Such a significant protection was not obtained with any of the drugs used in this study even when administered at very high dose level. Drugs like primaquine, sulphadiazine and dapsone were found to be quickly excreted as even the animals which were challenged after 2 hours of drug treatment were not protected. The effect of most of the drugs viz. mefloquine, chloroquine, amodiaquine, mepacrine, and pyrimethamine was found to last upto 24 hours. Thompson et al. (1965c) have recorded prolonged plasmodicidal action with some of the sulfones e.g., DADDS after single treatment at 100-400 mg/kg prevented or greatly suppressed P. berghei infection over a period of 6-14 weeks, and with P. cynomolgi the single dose at 50 mg/kg prevented patent infection for 63-268 days. Peters et al. (1977a) have observed that the activity of mefloquine in mice was last after 48 hours as determined by the appearance of parasites in treated animals infected 48 hours after subcutaneous administration of single dose of 60 mg/kg. In the present study also 100 and 200 mg/kg (orally) were unable to cure the infection when mice were challenged 48 hours later. However, long term suppression
of parasitaemia for periods upto 2-4 weeks with this drug has been reported in human volunteers infected with P. falciparum after a single dose of 1000-1500 mg drug administered orally. (Kieckmann et al. 1974; Trenholme et al. 1975). Studies along these lines for the development of long acting antimalarials would go a long way to tackle the problem of malaria.

II. Studies with tissue stages of P. gallinaceum.

a) Chemoprophylaxis with pyrimethamine: Pyrimethamine has been found to possess activity against the tissue stages in most of the plasmodium species. The results in the present study agree to the observations of Singh et al. (1952) who reported complete protection against chicks with daily dose of 0.3 mg/kg or more. Greenberg et al. (1953b) obtained complete prophylaxis against P. gallinaceum with pyrimethamine at 0.25 mg/kg. Schmidt and Genter (1953) observed extension in prepatent period of P. syneomolgi after treatment of the monkey with 0.075 to 20 mg/kg for 8 days, although complete causal prophylaxis was not obtained even with maximum tolerated dose of 20 mg/kg. Coatney et al. (1952) have found that a single dose of pyrimethamine produced long periods free of relapses from sporozoite induced chesson strain of P. vivax.

b) Chemoprophylaxis with primaquine: From the available literature it is observed that there is remarkable diversity on the efficacy of primaquine in treatment of malaria. Coatney et al. (1948) studied a large number of compounds of the 8-aminoquinoline series including pamaquine, none of these was capable of preventing gallinaceum malaria in chicks even at maximum dose level. Gingrich (1946) reported partial prophylactic action with nearly 30 such compounds in P. cathemerium- canary model and similar results were obtained by Coggeshall and Porter (1946) in P. lophurae- turkey model. Davey (1963) in the studies with
sporozoite induced *P. gallinaceum* found that pamaquine at 4 mg/kg/day for 6 days produced only a slight delay in survival time and no cure was attained. In the present study also no causal prophylactic activity was observed with primaquine up to 5 mg/kg/day. However, Singh et al. (1950) have found these compounds to be active against *P. gallinaceum* and complete protection was achieved with pamaquine at 1.25 mg/kg/day and with primaquine at 0.5 mg/kg/day. Hawking and Thurston (1952) have found that pamaquine at 10 mg for 5-7 days from day of infection completely protected monkeys from *P. cynomolgi*. Primaquine administered at 3 mg/kg/day prevented the development of pre-erythrocytic forms of *P. cynomolgi* when drug was given for 8 days beginning 24 hours before inoculation. Covell et al. (1955) have recommended that primaquine diphosphate at 15 mg for 14 days produced radical cure in *P. vivax* and *P. malariae* infections. This study clearly shows that *P. gallinaceum*-chick model is not suitable for evaluating the causal prophylactic activities of 8-aminoquinolines.

C). Chemoprophylaxis with mefloquine: Mefloquine has not been found to possess any causal prophylactic activity in this study as the treatment up to 45 mg/kg was not effective in the clearance of the tissue stages of *P. gallinaceum*. Treatment with 15 and 45 mg/kg prevented the appearance of patent infection. Similar results were obtained by Peters et al. (1977a) who found that mefloquine at 30 or 100 mg/kg (sub-cutaneous) had a marked residual action in mice against sporozoite induced *P. Y. nigeriensis* infection but it had no action on the maturation of pre-erythrocytic schizonts. Schmidt (1973) reported in his studies with rhesus monkeys infected with sporozoites of B strain of *P. cynomolgi* that mefloquine is devoid of prophylactic or radical curative properties. During studies with human volunteers Clyde et al. (1976) found that persons bitten by 10-15 mosquitoes infected with chloroquine and pyrimethamine resistant *P. falciparum* when treated with
mefloquine at 200 mg per week or 500 mg per 2 weeks or 1000 mg per 4 weeks did not show any patent infection during the period of drug delivery or during the follow up period of 60 days. On the other hand, studies with P. vivax showed that (Clyde et al. 1976) treatment with 250 mg/week suppressed the sporozoite induced P. vivax infection but malaria developed after the completion of the course.

d). Chemoprophylaxis with antibiotics: Out of the eight antibiotics tested for causal prophylaxis against P. gallinaceum, minocycline and doxycycline have shown highest prophylactic activity at relatively low dose (15 mg/kg). Other antibiotics viz: demeclocycline, chlortetracycline, tetracycline and oxytetracycline gave prophylactic activity at higher dose level (135 mg/kg) and erythromycin was active only at highest level (405 mg/kg). Causal prophylactic activity of minocycline, demeclocycline and erythromycin has been determined for the first time in the present study. Results with the doxycycline in this study are in agreement with those of Hill (1975) who recorded activity of this antibiotic at 10 mg/kg against P. berghei tissue stages. The observations with chlortetracycline are in agreement with those of Coatney and Greenberg (1952) who found total protection against P. gallinaceum at 300 mg/kg dose. The same authors recorded complete protection with chloramphenicol against P. gallinaceum at 500 mg/kg but in the present study it protected only 2 out of 6 chicks at 405 mg/kg. Darrow et al. (1952) observed extension in the prepatent period after 4 day treatment with oxytetracycline at 1000-2500 mg/kg; chlortetracycline at 500-600 mg/kg and chloramphenicol at 3500 mg/kg but cent per cent survival was not achieved with any of these. Extension of prepatent period by 4 days with oxytetracycline in case of mosquito induced P. cynomolgi ceylonensis infection was reported by Garnham et al. (1971) using a dose of 33 mg/kg for 2 days.
(day 3 and 4 post infection). In the present study chlortetracycline, tetracycline and oxytetracycline have shown similar extension of prepatent period for 1-4 days at dose of 45 mg/kg, but complete protection was achieved only at 135 mg/kg.

Detailed studies presented above on the causal prophylactic activity and blood schizontocidal activity with 8 antibiotics clearly show that antibiotics do not produce radical cure when used as blood schizontocides whereas they possess demonstrable causal prophylactic activity against avian malarial parasite. Furthermore amongst all the antibiotics studied during the present work, doxycycline and minocycline have shown antimalarial activity against both tissue and blood stages at relatively very low doses. The above data would provide a baseline information for evaluation of potential antimalarial compounds for causal prophylaxis activity against malaria.

**Drug Resistance**

There have been a number of reports on the selection of strains of *Plasmodium* resistant to most of the anti-malarial drugs (Goodwin and Rollo 1955; Bishop 1959; Hill 1963; Peters 1967, 1970). The drug resistant strains occupy a very important place in malaria research in the present day because of their significance in the development of new antimalarials for the control of drug resistant strains, Peters (1975) has also strongly emphasized this point and he has suggested that the drug resistant strains of rodent malaria can be of great value in secondary screening and development of new antimalarial compounds for the treatment of drug resistant malaria in man. Since the drug resistant strains are not presently available in this country, efforts have been made in the present study to select strains highly resistant to
commonly used antimalarial drugs. Since parasites have developed resistance to all the synthetic antimalarials, it was considered worthwhile to attempt selection of a strain resistant to mefloquine which is latest addition to the battery of antimalarials.

Studies with the rodent malaria show that most of the resistant strains, have been selected in mice. Rapid passage of the parasites under treatment at increasing drug pressure has been the method of choice with most of the workers. In the present study, rats have been employed for the selection of drug resistant strains to a few antimalarial drugs. The method of interrupted sub-curative therapy used in the present study has been found to be very useful since it ensures that the parasite population is exposed to drug pressure at each step when the parasites tend to increase in number. Use of weanling rats as the experimental host avoids the interference of host immunity which might inhibit the multiplication of small number of parasites which survive drug action during the course of selection.

I. Pyrimethamine resistant strain: The strain of *P.berghei* reported in this study was resistant to pyrimethamine treatment at daily dose of 133 mg/kg, which is very high level as compared to the earlier reports of Kollo (1952a), Thurston (1953b) and Jacobs (1965) where the strains were made resistant to 13, 5 and 50 mg/kg/day dose level respectively. All these strains were selected in the mouse model and the present strain when tested in other hosts including mice was found to be resistant to treatment at daily dose of 132 mg/kg. The present strain agrees with the observations of Jacobs (1965) that the pyrimethamine resistance is stable in the absence of drug pressure and also of Singh *et al.* (1954) who found the resistant strain of *P.knowlesi* to be stable after drug free passages in rhesus monkey for 2½ months. Cross
sensitivity studies in the different host parasite-systems show that pyrimethamine resistant strain of *P. gallinaceums* was cross resistant to proguanil and cycloguanil and sensitive to dapsone and sulphadiazine (Bishop 1962); the resistant strain of *P. berghei* in mice was sensitive to quinine and chloroquine (Jacobs 1965) but cross resistant to proguanil and its active metabolites (Thurston 1953b). Singh et al. (1951) and Schmidt et al. (1953) reported the cross resistance of pyrimethamine resistant strain of *P. cynomolgi* to proguanil and similar cross resistance to proguanil has also been observed in *P. knowlesi* (Singh et al. 1954). In the present study, the pyrimethamine resistant strain has been found to be sensitive to all the anti-malarials tested at the dose levels effective against the sensitive strain except for sulphadiazine to which a two fold resistance was observed. Kaddu et al. (1974) reported a slight decrease in the antimalarial activity of minocycline against pyrimethamine resistant strain of *P. berghei*, and the index of resistance was found to be nearly 3 and 2 times respectively at ED$_{50}$ and ED$_{90}$ levels. In present study index of resistance of minocycline to pyrimethamine resistant strain was found to be nearly 1.5. Besides the antimalarial activity of some other antibiotics against the pyrimethamine resistant strain has been recorded for the first time in present study and except for some decrease in sensitivity to demeclocycline (index of resistance 1.5 at ED$_{50}$ and 2.0 at ED$_{90}$ levels) there was no significant variation in the activity of other antibiotics.

II. Chloroquine resistant strain: Various methods have been used by the earlier workers for the selection of chloroquine resistant strains of *P. berghei*, Hawking and Gammage (1962) and Hawking (1966) using the drug diet method considered the blocking of reticuloendothelial system of the host by ethyle-palmitate necessary for the induction of resistance. The method of increasing drug pressure used in this study was similar to that of most of
the earlier workers (Ramakrishnan et al. 1957; Peters 1965a; Jacobs 1965) though the level of resistance attained in this study was maximum. The level of resistance attained by *P. berghei* to chloroquine treatment varies with the route of drug administration. The strain developed by Ramakrishnan et al. (1957) was resistant to 60 mg/kg/day by subcutaneous route; Hawking's strain (1966) was resistant to 50 mg/kg/day by intraperitoneal route; strain reported by Peters (1965a) was resistant to 100 mg/kg/day by subcutaneous route and the strain selected by Jacobs (1965) was resistant to 200 mg/kg/day by the oral route. The strain reported in this study was tested for the tolerance to drug after treatment through different routes and was found to be resistant to maximum tolerated dose of 248 mg/kg/day by oral route, 93 mg/kg/day by the subcutaneous route and to 62 mg/kg/day by intraperitoneal route.

The stability of resistance to chloroquine after drug free passages has been studied by a few workers. Jacobs (1965) found that the resistance to chloroquine was lost after 8-15 passages. Peters (1965a) observed that the resistance was stable up to 15 weeks in the absence of drug after which it was found to have lost. Rosario (1976) reported the resistance to chloroquine to be stable after 25 passages in his strain of *P. chabaudi*, but, the level of resistance to drug was very low. The present strain agrees with the observations of Rosario (1976) although this strain was highly resistant to chloroquine. Stability of resistance after cryopreservation reported in this study is in agreement with the observations of Hawking (1966) and Schneider et al. (1968).

The cross sensitivity patterns of the present strain were also found to be similar to that of earlier workers. Peters (1965a) and Jacobs (1965) had described their chloroquine resistant strains to be cross resistant to amodiaquine, mepacrine, quinine and primaquine. The strain
developed by Hawking (1966) was cross resistant to amodiaquine and mepacrine while that of Macomber et al. (1966) was cross resistant to mepacrine and quinine. Schneider (1968) has also reported that chloroquine resistant strain of *P. berghei* in mice was susceptible to treatment with Cycloguanil, pyrimethamine, DDS and primaquine but the parasites were cross resistant to treatment with quinine, amodiaquine and mepacrine (quinacrine). The resistant strain developed in this study has also shown cross resistance at maximum tolerated dose level to amodiaquine and mepacrine in rats. When tested in mice, though the strain was resistant to chloroquine at 248 mg/kg, the cross resistant to amodiaquine and mepacrine was less pronounced than in rats and it was only 12 fold and 8 fold respectively when compared with the corresponding values of the sensitive strain at MED level. Besides a 2 fold resistance to primaquine was also noticed in mice. The present strain differs from the reported observations of Peters (1977a) that the chloroquine resistant strain is less sensitive than the sensitive strain to treatment with mefloquine when tested at similar doses. In the present study, the MED of mefloquine against chloroquine resistant strain was equal in rats and \( \frac{1}{2} \) in mice to that of the corresponding value with the sensitive strain. Likewise the \( \text{ED}_{90} \) values obtained for mefloquine against the chloroquine resistant strain was nearly \( \frac{1}{2} \) to that of the sensitive strain, thus showing more activity against the resistant strain. Kaiku et al. (1974) have evaluated the comparative efficacy of minocycline against sensitive and chloroquine resistant strains of *P. berghei*. The \( \text{ED}_{50} \) and \( \text{ED}_{90} \) values of minocycline against the resistant strain were found to be 6 and 4 times the corresponding value in sensitive strain. Erythromycin was reported by Warhurst et al. (1976) to exert activity against both the sensitive and chloroquine resistant strain at similar dose level. In the present study also the \( \text{ED}_{50} \) and \( \text{ED}_{90} \) values of minocycline were found to be nearly 1.5 time to that in the normal strain. Besides some decrease in
sensitivity to demeclocycline has also been observed, though there was not any significant change in the activity of other antibiotics.

III. Primaquine resistance: A survey of the primaquine resistant strains selected in various host-parasite models by earlier workers (Table 4), show that except the work of Ramakrishnan et al. (1961) with *P. knowlesi*, all other workers obtained resistant strains after a large number of serial passages in presence of drug pressure. Bishop (1967) and Beaudoin et al. (1970) treated *P. gallinaceum* for 55 passages each for the selection of primaquine resistant strain. Satya Prakash et al. (1961) and Peters (1965c) made 41 and 61 passages respectively in mice for selection of resistant strain in *P. berghei*. In another study Merkli and Peters (1976) compared the speed of selection of resistant strains by using the two different techniques namely (i) the modification of 2% relapse technique and (ii) selection under continuous drug pressure. Comparing the level of resistance at ED$_{50}$ level, they found that while there was no significant change from the sensitive strain in the ED$_{50}$ values for primaquine in the line developed after 30 passages by first method, the value was two fold after 15 passages for the line selected by the second method. The authors concluded that a single heavy dose does not create the same selection pressure as produced when parasites are exposed to drug at 24 hours intervals and the latter is needed to select tolerance in an infection of *P. berghei*. However, in the present study, the resistant strain could be selected in only 9 passages after the interrupted therapy with the sensitive strain. The level of resistance attained in this study was not as high as that of Satya Prakash et al. (1961) who reported 15 fold increase. The difference is attributable to the variation in the maximum tolerated dose of the drug in two studies, while in their work Satya Prakash et al. (1961) administered drug upto a daily
dose of 225 mg/kg to mice, in the present study doses above 70 mg/kg/day were toxic to both rats and mice. Peters (1966) in his observations also has reported that the $ED_{90}$ value of primaquine against the resistant strain (130 mg/kg/day) was above the maximum tolerated dose and was recorded only from graphical evaluation.

The results on the stability of primaquine resistance of *P.berghei* after drug free maintenance are in agreement with the earlier reports (Satya Prakash *et al.*1961; Peters 1965; Beaudoin *et al.* 1970). In agreement with the work of Peters (1966) the primaquine resistant strain has been found to be sensitive at normal dose level to chloroquine, amodiaquine and mepacrine, though there was no cross resistance to pyrimethamine or dapsone. Corresponding studies with avian plasmocidia show that the strain developed by Bishop (1967) was cross resistant to pamaquine but maintained its normal sensitivity to proguanil and pyrimethamine. Beaudoin *et al.* (1970) also found their primaquine resistant strain of *P.fallax* to be sensitive to pyrimethamine at normal dose levels. The cross sensitivity pattern observed in this study, therefore, agrees with the observations recorded with the avian malaria.

IV. Mefloquine resistance: The mefloquine resistant strain developed during this study was selected in a much shorter time (10 passages) and was resistant to the maximum tolerated dose level of the drug. Peters *et al.* (1977b) reported the selection of mefloquine resistant strain over a period of 3-4 months which was resistant to a total dose of 240 mg/kg administered subcutaneously in four days. The observations on the loss of pigment formation and also the loss of resistance after drug free passage for a few weeks are in agreement with Peters *et al.*
(1977b). The cross sensitivity of the mefloquine resistant strain to other antimalarials has been determined for the first time in this study. It was found that mefloquine resistant strain remained sensitive to other antimalarials viz. chloroquine, amodiaquine, mepacrine, pyrimethamine and sulphadiazine at normal doses excepting primaquine to which it showed 2-fold resistance.