(A) Selection of Host for Chemotherapeutic Studies With
P. yoelii nigeriensis

Rodent malaria parasite P. yoelii is discovered by Landau and Killick Kendrick (1966) which was followed by the discovery of subspecies P. yoelii nigeriensis by Killick and Kendrick (1973). The natural resistance of this parasite to chloroquine was reported by Peter (1970). Very little information on the chemotherapeutic response of P. yoelii nigeriensis is available. Preliminary work done by Pande (1984), reported that Swiss mice was most suitable model for infection of P. yoelii nigeriensis. It's infection is 100 % fatal which is accompanied by high level of parasitaemia.

The present study shows that the random bred Swiss mice are best host since they develop very high level of parasitaemia before death, are susceptible to infection with low inocula of parasites and show cent percent mortality after infection. The level of parasitaemia ranges from 62.5 to 80 % and mortality pattern and mean survival time depends upon the inoculum of the parasite.

(B) Chemotherapeutic Studies with P. yoelii nigeriensis

Swiss mice are ideal host for carrying out drug screening studies on P. yoelii nigeriensis. According to Diggen and Gregory (1969) P. yoelii nigeriensis, has certain degree of natural resistance to chloroquine. Pande and Dutta
(1982) used *P. yoelii nigeriensis* for their studies and have reported natural (innate) resistance of this parasite to several antimalarial drugs such as chloroquine, amodiaquine, mefloquine and quinine, although this parasite was never exposed to the action of these drugs. Thus this model is ideal to screen and develop new drugs for the control of multiple drug resistant malaria infection.

(i) **Long-Acting Antimalarial Efficacy of Antimalarial and Sulfa Drugs:**

The use of long-acting sulfa drugs like sulphadoxine or sulfadene with pyrimethamine, chloroquine and other antimalarials as a single dose therapy has gained popularity in the treatment of malaria in the recent past. Number of papers reporting the use of sulphanilamides and sulphones in treatment human malaria were published for instance by Diez DeLeon (1938, 1940), Niven (1938), Hall (1938), Chopra (1939), Chin *et al.* (1966), Powell *et al.* (1967); Yoshinga *et al.* (1970), Ramos and Cabrera (1972), Clyde *et al.* (1971), Williams *et al.* (1978), Coggeshall *et al.* (1941), Archibald and Ross (1960), Laing (1955 b), Degowin *et al.* (1966 a), Laing (1970), Rieckmann (1967). It was stated that long-acting drugs have longer half-lives. In human mean half life of sulfadiazine was observed by several workers i.e.
Kruger-Thiemer et al. (1965) 16 hrs; Ohnaus and Spring (1975) 10 hrs; Reeves et al. (1973) 13 hrs; Shastri et al. (1979) 18 hrs.

Half life of sulphadoxine was reported 135 hrs (Bohni et al. 1969), 123 hr (Bunger, 1967), 195 hrs (Peak et al., 1975). Half life of dapsone was reported 21, 30, 29 hrs (Glazko et al., 1968; Peters et al., 1972; Ahmad et al., 1980). Half life of acedapsone was reported as 43 days and 46 days (Glazko et al., 1968; Peters et al., 1977).

Pyrimethamine has same half life as sulphadoxine i.e. about one week (Nityanand, 1983). Laboratory data on the studies testing of of long-acting nature of different antimalarials against P. yoelii nigeriensis have shown that acedapsone is most long-acting drug. 4 x M.E.D of acedapsone when administered on day -9, suppressed parasitaemia for 21 days. This shows that excretion of acedapsone is very slow. Acedapsone at 4 x M.E.D. dose given 9 day in advance of infection, had curative effect.

8 x M.E.D. of pyrimethamine, metakelfine, dapsone and sulphanilamide suppresses parasitaemia till day 4. Persistence of these drugs in Swiss mice was found to be about 4 days.

Sulphadiazine is inactive at 8 x M.E.D. therefore excretion of sulphadiazine is very quick from the body of
the host. These findings are very close to the above reports on half lives of antimalarial drugs. Poor efficacy of sulphadiazine in field had been reported by several workers.

Findlay (1946) used sulphadiazine in treatment of falciparum malaria in Europe, it was found to be inferior to other standard antimalarials. Chin et al. (1966) and Powell et al. (1967) achieved poor efficacy of sulphadizine. Peters (1970 a) using Swiss mice as host, tested several sulfa drugs and pyrimethamine against P. berghei in Ranea test. At highest dose of sulphanilamide 1280 mg/kg, mean survival time was 13.3 days. At 160 to 640 mg/kg dose of pyrimethamine, deaths due to toxicity were observed in mice. M.S.T. of dapsone treated mice at 80 mg/kg was ≈60 day and 640 mg/kg dose of dapsone was found to be toxic. It was observed that DAODS at 640 mg/kg dose protected all mice.

Our findings are in agreement with those of Peters (1970 a). Among the sulfa drugs sulphanilamide and sulphadazine showed very little activity against P. yoelii nigeriensis. They were able to cure the infection only at the highest dose i.e. 640 mg/kg. M.S.T. of sulphanilamide treated mice at 640 mg/kg dose was found to be ≈14.4 days and M.S.T. of dapsone treated mice at 80 mg/kg dose was found to be ≈20.7 days. The mice were observed till +21 days for survi-
It was observed that dapsone was toxic at 640 mg/kg dose. Highest activity was observed in acedapsone treated mice. No mortality was observed in 20-320 mg/kg treated mice. Pyrimethamine was found to be toxic at same doses i.e. 160-640 mg/kg as reported above by Peters.

Successful single dose treatment with long acting sulphadoxine was reported in fields against human malaria by several workers such as Laing (1968, 1970) and Shute and Dawling (1966).

In the present study sulphadoxine was also found to be active. It was seen that at 80 mg/kg single dose of sulphadoxine, all mice were cured.

In literature mefloquine was reported to be very active, specially against chloroquine resistant falciparum malaria (Rieckmann et al., 1974; Trenholme et al., 1975; Pearlman et al., 1980; Eliot et al., 1980; Jiang et al., 1982; Harinasute et al., 1983, 1985). Half life of mefloquine was reported from 12 to 27.5 days and 6.4 - 22 days (Derjardins et al., 1979b; Schwartz et al., 1980). We have found contradictory results in our study. It was observed that sensitivity of P. yoelii nigeriensis was very low towards mefloquine, when given in single dose treatment. Excretion of mefloquine was found to be very quick from
the body of Swiss mice. Similar findings were reported by Peters et al. (1977 a). The activity of mefloquine in mice was lost after 48 hrs as determined by the appearance of parasite in treated animals infected 48 hrs after subcutaneous administration of single dose of 60 mg/kg.

Efficacy of fansidar in fields had been reported by several workers (Eliot et al., 1980; Spencer et al., 1984; Overbosch et al., 1984). Combination of fansidar with mefloquine was reported to delay the emergence of resistance to mefloquine as suggested by clinical studies using tablets containing 250 mg of mefloquine, 500 mg of sulphadoxine and 25 mg of pyrimethamine (T.D.R. 1984).

The present study has shown that at 40 mg/kg sulphadoxine + 2 mg/kg pyrimethamine dose (fansidar) cured the infection and triple combination of sulphadoxine/pyrimethamine/mefloquine was found to be active at very low doses i.e. sulphadoxine 2 mg/kg + pyrimethamine 0.25 mg/kg + mefloquine 1 mg/kg dose cured the infection of P. yoelii nigeriensis.

Activity of metakelfine was found to be very low. In the present study, mice were found to survive beyond day +21 post-infection after single dose treatment with several drugs when challenged with P. yoelii nigeriensis infection (normal). IInd challenge was given to mice which
Thompson et al. (1963) have recorded protection to a challenge infection of *P. berghei*, lasting for 1 to 9 weeks after subcutaneous 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).

Laboratory data has shown that no protection was observed with sulfadiazine and sulphanilamide treated mice. After challenge mice were protected at only 640 mg/kg dose. Sulphadoxine and dapsone treated mice were partially protected after challenge and rechallenge with *P. yoelii nigeriensis*. Thompson et al. (1965 c) have reported prolonged plasmocidal action with some of the sulphones e.g. DADDS after single dose 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.

The present study has shown that maximum protection after two challenges was observed with DADDS treated mice at 160-640 mg/kg dose.

(ii) Efficacy of New Antimalarial Compounds in Single Dose Treatment

Using single dose treatment several compounds no. 1, 2, 4, 5 (received from Chemistry Department of Lucknow
University) 42/183, 80/695, 82/142, 83/495, 42/183, 83/494, 80/693, 82/628, 83/498, 83/496, 83/38, 82/143 were tested. It was found out that above compounds could not eradicate parasitaemia by day 4 but in one compound i.e. 83/695 mean survival time of the treated mice extended to nearly twice at 640 mg/kg dose. Rest of the compounds were found to be inactive or of very low activity.

(iii) Blood Schizontocidal Activity of New Antimalarial Drugs Received from Chemistry Department of Lucknow University

Standard 4-days test for the blood schizontocidal activity of antimalarial new compounds no. S8, S4 and Sl2 was carried out with P. yoelii nigeriensis in Swiss mice. M.E.D. of compound no. S8, S4 and Sl2 was 2 mg/kg, ≥1 mg/kg and 1 mg/kg respectively. Extension of mean survival time at above doses was found to be twice as compared to control.

W.H.O. (1973) has laid the criterion that extension of survival time to double that of control should be considered as significant for demonstrating the antimalarial activity. According to above criterion we would conclude that compound nos. S8, S4 and Sl2 had antimalarial activity.
Chemotherapeutic studies on *P. knowlesi* (W₁) have been carried out with some standard antimalarial i.e. chloroquine, amodiaquine, quinine and sulphadiazine.

There is some evidence in literature showing the sensitivity of chloroquine and amodiaquine against *P. knowlesi*.

Using the Nuri strain of *P. knowlesi*, Nair and Ray (1955) found that the M.E.D. of three brands of chloroquine varied from 1.8 to 2.5 mg/kg, given daily for 7 days, the M.E.D. of amodiaquine was found to be 2.5 mg/kg.

Singh et al. (1950 a) reported that chloroquine or amodiaquine given in doses of 20 mg dose for 7 days subsequent to inoculation suppressed the *P. knowlesi* infection and 10 mg chloroquine or 20 mg of amodiaquine given daily for 7 days prior and 7 days subsequent to infection was also completely suppressive. Chloroquine was the best of the suppressive tried and amodiaquine came next.

The present study on *P. knowlesi* has shown that 5 mg/kg dose of chloroquine for 3 successive days completely suppressed the parasitaemia whereas at 5 mg/kg dose of amodiaquine for 3 days showed recrudescence of parasit-
taemia. Here also response of chloroquine is better than amodiaquine as shown above by Singh et al. (1950 a).

Several investigators have published data on the effect of quinine on P. knowlesi. Raw, Dalal and Cullerpiri (1933) state that a dose of 2.5 grains of quinine checked the infection but a recrudescence occurred within a few days whereas in present study 100 mg/kg dose of quinine for 3 days was given when the parasitaemia was allowed to reach 50%. After this treatment very early recrudescence was observed.

Schizontocidal activity of sulphadiazine against P. knowlesi infection had been described by several workers (Dasgupta, 1938; Coggeshall et al., 1941; Coggeshall and Marier, 1941). Curative dose of sulphadiazine was recorded as 3 gm for 3 days (Dixhit and Ganapathi, 1940). The present study has shown that 250 mg/kg dose of sulphadiazine cured one monkey out of 2 monkeys and 500 mg/kg dose for 3 days cured all the monkeys.

(D) Drug Resistance Studies in Malaria

The drug resistant laboratory strains occupy a very important place in malaria research in the present day because these can be used as useful laboratory models for screening and development of new antimalarials for the control of drug resistant strains.
The first observation of chloroquine resistance were made in 1959 in Latin America and in Thailand. A report by Maberti (1960) from Venezuela was quickly followed by that of Moore and Lainer (1961) from Colombia. Chloroquine resistance was suspected in Thailand as early as 1957 but the first proven cases were those reported by Harinasuta et al. (1962) since that time numerous reports have appeared indicating that chloroquine resistance is restricted to P. falciparum but that resistant strains of this parasite are widely distributed i.e. in Colombia (Moore and Lanier, 1961; Powell et al. 1963 a; Young and Moore, 1961; Comer et al., 1968), Cambodia (Contacos et al., 1963, Eyles et al., 1963), Malaya (Contacos et al., 1963; DeGowin et al., 1965), Brazil (Box and Young, 1963), Thailand (Jeffery, Collins and Skinner, 1963), Ghana (Schwendler, 1965), Singapore (Ng et al., 1967), Malaysia (Mcklvey et al., 1968), Phillipine (Smrkovski et al., 1985), Burma (Clyde et al., 1973), Mozambique (Pillay et al., 1975), Inodnesia (Verdrager, 1976), Vietnam (Bertelloni et al., 1967), Malawi (Alver et al., 1985), Kenya (Turner, 1984), Benda (Bac et al., 1985), Zambia (Kofiekve et al., 1983). Burundi (LeBras et al., 1984), Tanzania (Onori et al., 1982), Angola (Lindberg et al., 1985), Pakistan (Fox et al., 1985) and some reports are from Africa (Niewveld, 1982; Jepsen et al., 1983; Kroger et al., 1983).
In India foci of chloroquine resistance have been identified under *P. falciparum* containment programme of N.M.E.P. in areas of Assam, Uttar Pradesh, Haryana, Gujrat, Maharashtra, Nagaland, Meghalaya, Orissa (Pattanayak et al., 1979; Dwivedi et al., 1979; De et al., 1979; Das et al., 1979; Chakravarty et al., 1979).

The problem was further aggravated when the multiple drug resistant strains of *P. falciparum* started appearing in 1962, particularly in South East Africa and South America, which were highly resistant to almost all of the available drugs such as chloroquine, amodiaquine, proguanil, pyrimethamine, mepacrine and even to the synergestic combinations of sulfa and antifoles like fansidar and metakelfine etc. (Contacas et al., 1963; Montgomery et al., 1963; Young et al., 1963, 1972; Jeffery et al., 1963; Powell et al., 1964; DeGowin et al., 1965; Clyde et al., 1973 b; Miller et al., 1974; Glew et al. 1974; Willerson et al., 1974; Hurwitz et al., 1981; Bygbjerg et al., 1981; Darlow et al., 1982; Ferranoni et al., 1983).

The above field reports focus the urgency of developing experimental resistant lines of rodent malaria.

(1) **Resistance to Sulphadoxine/Pyrimethamine/Mefloquine Combination in Rodent Model**

*P. yoelli nigeriensis* used in present study has
natural low level of resistance to chloroquine, mefloquine and quinine and it exhibits sensitivity to acedapsone, dapsone, pyrimethamine, sulphadiazine, sulphanilamide, fansidar and triple combination of sulphadoxine/pyrimethamine/mefloquine.

Resistance to combination of sulphadoxine + pyrimethamine (fansidar) is gradually emerging and efficacy of this combination to control resistant P. falciparum is gradually decreasing as indicated by reports of fansidar resistance from Thailand, Burma, Vietnam, Columbia and Brazil (Eugene et al., 1979; Larry et al., 1979; Darlow et al., 1980; Hurtwitz et al., 1981; Tin et al., 1981; Bygbjerg et al., 1981; Black et al., 1981; Chongsuphajasisiddhi et al., 1981; Dixon et al., 1982; Jimmermanns et al., 1982; Eichenloub et al., 1983). There is suspicion that mefloquine initially developed as blood schizontocide for control of drug resistant falciparum strains might not maintain its efficacy in the field and resistance to mefloquine may eventually develop if it is used alone for the control of drug resistant strains of falciparum W.H. O. (1984) have advised the use of mefloquine in combination with fansidar which is expected to prevent the emergence of mefloquine resistance or this combination might delay significantly the development of resistance to the triple combination.
In the present study an experimental evidence has been presented to suggest the possible emergence of triple drug combination (sulphadoxine + pyrimethamine + mefloquine) in a strain of *P. yoelii nigeriensis* exposed to subcurative doses of this combination for 64 passages over a period of 464 days in Swiss mice. The present study provides strong evidence to show that use of fansidar + mefloquine combination does not prevent the emergence of resistance of *P. yoelii nigeriensis* to the triple combination. 16-fold increase of resistance to the mefloquine-fansidar combination has been first time recorded.

In field study reports on resistance (RII and RIII type) to above combination is recently published from Indonesia (Haffman et al., 1985).

**Cross chemotherapeutic studies** - Studies on chemotherapeutic response of normal strain of *P. yoelii nigeriensis* had been reported earlier by Pande and Dutta (1982). The M.E.D. of this strain for antimalarial drugs in 4 day test was as follows: chloroquine 32 mg/kg, amodiaquine 16 mg/kg, quinine 450 mg/kg, mefloquine 8 mg/kg, mepacrine 8 mg/kg dapsone 2 mg/kg, pyrimethamine 2.0 mg/kg.

M.E.D. of sulphadiazine and sulphanilamide was reported by Pande (1985) on 0.04 mg/kg and 100 mg/kg respectively.
Strain of *P. yoelii nigeriensis* resistant to fansidar/mefloquine combination shows 16-fold cross resistance to mefloquine and amodiaquine. There is also increase in M.E.D. of mepacrine, sulphadoxine, sulphadiazine, dapsone to 8 fold, 6-fold, 3,12-fold, 2-fold respectively M.E.D. of fansidar raised to 17-fold.

Above strain was found to be highly resistant to chloroquine and quinine at maximum tolerance dose.

This strain maintains its sensitivity to pyrimethamine, acedapsone and sulphanilamide. Combination of acedapsone + pyrimethamine and dapsone + pyrimethamine show synergistic action. It was seen that doses of dapsone and acedapsone with pyrimethamine could be reduced to half when melfoquine was added to these combinations. Metakelfine has also been found to be very active.

Although acedapsone alone is very effective against *P. yoelii nigeriensis* multiple resistant strain, but in triple combination 1/8th of M.E.D. of acedapsone with very low doses of pyrimethamine and mefloquine is found to be active against highly multiple resistant *P. yoelii nigeriensis*.

Our study strongly suggests that fansidar can not protect the emergence of mefloquine resistance if both the drugs are used in subcurative stages and there is a need for caution.
to use mefloquine + fansidar combination in fields against multi-resistant strains of *P. falciparum*.

Areas where resistance to above mentioned triple combination (fansidar + mefloquine) might develop, the use of triple combination of acedapsone + pyrimethamine + mefloquine for the ultimate control of malaria may be fruitful and should be given trial.

(II) Resistance to Chloroquine and Amodiaquine in *P. knowlesi*

There is no report on resistance to chloroquine or amodiaquine against *P. knowlesi*. In present study exposure of *W.1* 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 at high parasitaemia. Recrudescence after various dosage of both chloroquine and amodiaquine was occasionally observed but when these parasites were subinoculated in healthy monkeys, the resistance of *P. knowlesi* to high doses of chloroquine or amodiaquine was not demonstrable.

The present study shows that though recrudescence at 15 mg/kg dose of chloroquine and 20 mg/kg of amodiaquine was observed but there is no stability of resistance as indicated by sensitivity tests in healthy monkeys. The strains selected by subcurative therapy were sensitive to 5-10 mg/kg of these drugs.
(E) Immune Status After Curative/Subcurative Treatment

Radical cure of *P. knowlesi* infection in rhesus monkey or *Selius* rhesus is generally believed to impart little or no protection against reinfection or challenge (Shortt *et al.*, 1938). On the other hand, partial or complete protection after radical cure has also been recorded by various workers (Coggeshall *et al.*, 1938; Voller *et al.*, 1969). Dutta and Singh (1980) reported that radical cure infection conferred 41% protection upon challenge. In subcurative therapy 67% protection was observed after challenge.

The present study has shown that poor protection was observed after curative treatment with chloroquine against *P. knowlesi* in rhesus monkeys, whereas after subcurative therapy 33% monkeys were protected after challenge.

Antimalarial drugs in very low doses given over a long period could stimulate indirectly the host's defence mechanism in such a way that *P. knowlesi* infection became chronic and resulted in a premunition type of immune response (Mulligan *et al.*, 1933; Brown *et al.*, 1970).