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STUDIES ON THE TOXICITY AND THE ACTION OF DIAMINO-DIPHENYL SULPHONE (DDS)
IN AVIAN AND SIMIAN MALARIA. RAPID SELECTION OF DDS RESISTANT STRAIN OF
P. CYNOMOLGI. ABSENCE OF CROSS-RESISTANCE TO PYRIMETHAMINE.¹

by

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INTRODUCTION

Early in 1941 Coggeshall and co-workers investigated the effectiveness of disodium p.p' diaminodiphenyl sulphone N, N'-didextrose sulphonate (DDS, "Promin") against P. cathemerium in canaries and against P. knowlesi, P. inui and P. cynomolgi in Rhesus monkeys. The drug was also tried against induced vivax malaria as well as against naturally acquired vivax and falciparum malaria. The workers noted the antimalarial activity of promin but did not consider it suitable for adoption in preference to other than established antimalarials.

After a lapse of about 15 years, attention was drawn to the possible suppressive activity of DDS against malaria which was absent in lepers treated with DDS in contrast to the general population in a holoendemic area (Leiker, 1956). Actual successful treatment of P. falciparum cases with dextrose diglucoside of DDS (Tarabini, 1958) revived the interest in sulphone as an antimalarial. Human trials of DDS showed that it had substantial schizontocidal activity although slower than that of 4-aminoquinoline group of drugs. Considering its low cost, the possibility of its use in African countries for the mass chemoprophylaxis of malaria has been suggested (Archibald & Ross, 1960). Keen interest has been evinced recently in DDS as a possible antimalarial potentiating the activity of pyrimethamine.

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This report deals with the investigations carried out against P. gallinaceum¹ in chicks and fowls, against P. cynomolgi² and P. knowlesi³ in Macaca mulatta monkeys. Endeavour has been made to estimate the activity of DDS in comparison with some antimalarials.

Toxicity of DDS

Repeated oral administration of DDS⁴ to monkeys for seven days in doses of 5 to 25 mg/kg daily did not produce symptoms of poisoning. The animals maintained the body weight comparable to normal monkeys (Table 1). Daily doses of 50 and 100 mg/kg for seven days, although did not kill the animals, produced some reduction in body weight. Doses of 200, 300 and 450 mg/kg daily killed all animals even before the drug could be administered for full seven days.

The daily dosage of 25 mg/kg of DDS for seven days was found non-toxic. Repeated oral administration of DDS is comparable in its toxicity to chloroquine (Findlay, 1951) and mepacrine, which cause slight toxæmia in monkeys in equivalent doses. Pyrimethamine 20 mg/kg daily for seven days is slightly toxic (J. Singh, Ray, Misra & Basu, 1953), whereas proguanil is non-toxic in 80 mg/kg daily for seven days (Nair, Ray & J. Singh, 1951). It would seem that on repeated administration DDS is more toxic than proguanil but as safe as chloroquine, mepacrine and pyrimethamine.

¹ The strain of P. gallinaceum is the same as used by Jaswant Singh, Basu & Ray (1952).

² Strain maintained at Malaria Institute of India isolated by Sinton & Mulligan (1932).

³ Nuri strain of P. knowlesi as described by Singh, Ray & Nair (1953).

⁴ "Avlosulfon" (Imperial Chemical Industries), tablets of 0.1 g.

TABLE 1. TOXICITY OF DDS ON SEVEN-DAY TREATMENT
IN MONKEYS

Daily dosage mg/kg	Number of animals	Number survived	Changes in body weight	Remarks
450	2	Nil	-	Toxic
300	2	Nil	-	"
200	2	Nil	-	"
100	1	1	Loss	"
50	1	1	Slight loss	Slightly toxic
25	3	3	No change	Not toxic
20	2	2	"	"
10	3	3	"	"

PARASITICIDAL ACTIVITY

1. Schizontocidal properties in chick test

Activity of DDS was determined against P. gallinaceum by the technique routinely followed at the Institute (J. Singh, Basu & Ray, 1952). Five schedules of DDS dosages, namely, 1/24, 1/16, 1/4 of and 1 and 4 times the minimum effective dose of quinine¹ were investigated. DDS showed activity only at a dosage level of 4 times the quinine MED, i.e., a dosage of 6.4 mg per 50 g. Thus the quinine equivalent of DDS was found to be 0.25 (Table 2) and the schizontocidal activity of DDS inferior to that of quinine, mepacrine, proguanil (J. Singh, Basu & Ray, 1952), chloroquine, amodiaquin and pyrimethamine (J. Singh, Ray & Chandrasekhar, 1953) for which the quinine equivalents are 1, 4, 16, 16, 32 and 1066 respectively in P. gallinaceum infection.

¹ Quinine equivalent (Q.E.) = $\frac{\text{MED of quinine}}{\text{MED of X}}$ where MED (minimum effective dose) of quinine is 1.6 mg per 50 g.

TABLE 2. ACTIVITY OF DDS AGAINST P. GALLINACEUM

Dosage in terms of quinine MED	Number of chicks used	Activity
1/24	5	Inactive
1/16	10	"
1/4	10	"
1	5	"
4	5	Active

2. Causal prophylactic activity of DDS against P. gallinaceum

DDS was administered orally to five fowls at a dosage of 60 mg/kg daily for two days prior to, on the day of sporozoite inoculation, and on the following two days. Parasites appeared in the peripheral blood of all the birds in 6 to 7 days' time from the date of inoculation with two mosquito equivalent of sporozoites per fowl. In the control fowls the prepatent period was also 6 to 7 days. Thus DDS had no action on the primary tissue schizonts.

3. Sporontocidal activity of DDS in P. gallinaceum

Two fowls showing gametocytes in peripheral blood were used. Aedes aegypti mosquitos were fed on the birds on two days (referred to as D-1 and 0 day in Table 3) prior to the drug administration. Each bird received a single dose of DDS 120 mg/kg on the 0 day. Mosquitos were fed on these birds for the subsequent ten days. After the expiry of extrinsic incubation period, dissection showed high sporozoite rates in the lots of mosquitos up to the seventh day following the drug administration. Whatever decline was observed in the rates could also be noted in mosquitos fed on untreated but infected fowls. Thus, DDS had neither gametocidal nor sporontocidal activity in P. gallinaceum infection.

TABLE 3. INVESTIGATIONS OF SPORONTOCIDAL ACTIVITY OF DDS IN P. GALLINACEUM (SPOROZOITE RATE IN AÉDES AEGYPTI, AVERAGE OF TWO PARALLEL LOTS FED ON TWO FOWLS)

Time of feeding	Number of mosquitos dissected	Number showing sporozoites	Sporozoite rate (per cent.)
D - 1 day	25	20	80.0
0 "	25	19	76.0
D + 1 "	30	19	63.3
D + 2 "	33	17	51.5
D + 3 "	22	8	36.3
D + 4 "	11	3	27.3
D + 5 "	14	3	21.4
D + 6 "	10	2	20.0
D + 7 "	10	1	10.0
D + 8 "	10	Nil	Nil
D + 9 "	12	"	"
D + 10 "	15	"	"

4. Therapeutic properties in acute P. knowlesi (Nuri strain) infection

Twenty-six monkeys were infected intravenously with the Nuri strain of P. knowlesi each with the standard inoculum of five million parasitized erythrocytes per kg body weight. Treatment commenced at the stage of 0.1 to 0.2 per cent. red cell infection. DDS in doses from 0.5 to 3 mg/kg was administered orally to each of the respective groups of animals while two monkeys were kept as controls. Treatment continued for seven days. Criteria for the assessment of results were based mainly on the disappearance of parasites from the peripheral blood on the day following the last dose - Class II effect of Shannon (Wiselogle, 1946). The results (Table 4) showed that minimum effective dose (MED) of DDS was 0.25 mg/kg and the parasite clearance was obtained within 96 hours of the commencement of the treatment. Recrudescences were not

uncommon even with a dose of 1 mg/kg daily for seven days. Thus dose for dose DDS was as effective against this strain of P. knowlesi as proguanil (Nair, Ray & Jaswant Singh, 1953) which has the MED of 0.2 mg/kg. Pyrimethamine which has the MED of 0.05 mg/kg is much more active than DDS (Nair, Ray & Jaswant Singh, 1953). The MED of quinine, mepacrine, chloroquine ("Aralen") and sulphadiazine are 30, 6, 2.1 and 0.8 mg/kg respectively in P. knowlesi (Nuri strain) infection (Ray & Nair, 1955; Nair & Ray, 1955). The speed of clearance of parasites from the peripheral blood was comparable to that obtained with the above antimalarials.

TABLE 4. THERAPEUTIC EFFECT OF DDS IN P. KNOWLESI INFECTION

Dose mg/kg daily for seven days	Number of monkeys used	Effective (Class II)		Ineffective Number of animals	Recrudescences within 15 days of sub-patency
		Number of animals	Parasite clearance (in hours)		
3	5	5	72-96	Nil	Nil
2	2	2	72	Nil	Nil
1.5	2	2	72	Nil	Nil
1.0	2	2	72	Nil	One after 7 days
MED 0.25	3	3	72-96	Nil	-
0.15	6	4	72-96	2	-
0.05	4	Nil	-	4	
Control	2	(Both animals died on the fifth day after inoculation due to 81 to 93 per cent. cell infection)			

5. Therapeutic properties in acute P. cynomolgi infection

The technique similar to that employed in the preceding investigation was adopted to determine the efficacy of DDS in P. cynomolgi infection. Fourteen monkeys were used for the purpose. DDS in doses of 0.1 to 1 mg/kg daily for seven days was used. The minimum effective dose (MED) was 0.5 mg/kg daily for seven days. Parasite clearance was obtained within 96 to 144 hours from the commencement of treatment. Recrudescences occurred within 5 to 7 days following the completion of treatment in three out of six monkeys which received the MED of the drug (Table 5). Lower doses of 0.2 and 0.1 mg/kg daily for seven days were partially effective.

Thus in P. cynomolgi infection DDS appeared to be, dose for dose, more effective than quinine, mepacrine, pyrimethamine, chloroquine and proguanil, the MED of these drugs being 20, 5, 1.1, 1 and 1 mg/kg respectively for seven days (Ray et al., 1954; Jaswant Singh et al., 1953; Nair et al., 1953). But, dose for dose, DDS was less active than sulphadiazine in P. cynomolgi infection, the MED of the latter being 0.15 mg/kg (Jaswant Singh et al., 1956).

TABLE 5. THERAPEUTIC EFFECT OF DDS ON P. CYNOMOLGI INFECTION

Dose mg/kg daily for seven days	Number of monkeys used	Effective (Class II)		Ineffective	Recrudescences within 15 days of sub-patency
		Number of animals	Parasite clearance (in hours)	Number of animals	
1.0	2	2	96	Nil	Nil
MED 0.5	6	6	96-144	Nil	In three monkeys within 5-7 days
0.2	2	1	144	1	
0.1	2	1	120	1	
Control	2	Parasitaemia continued for 12-21 days and then passed on to chronic stage			

RESISTANCE TO DDS

1. Selection of resistant strain of *P. cynomolgi*

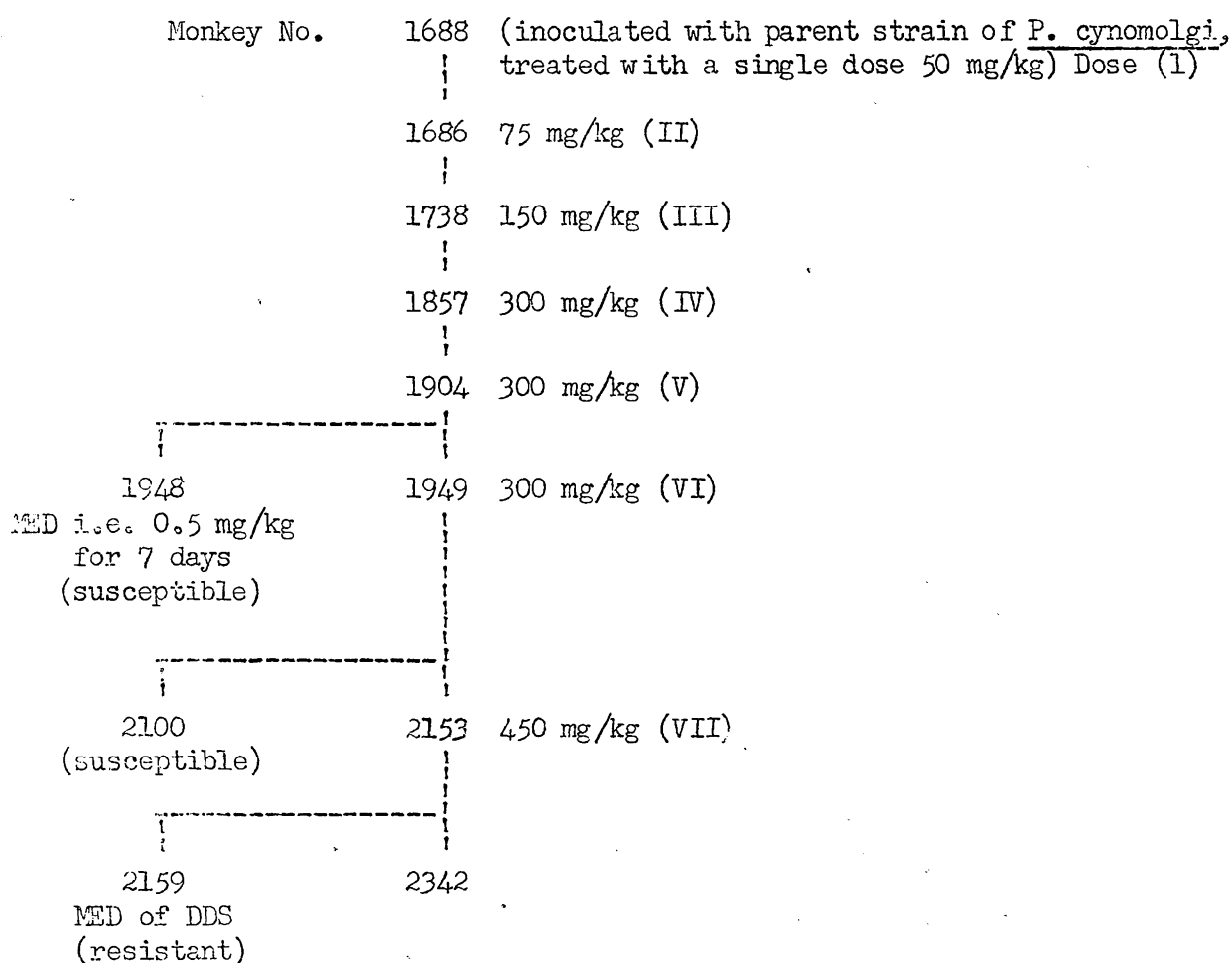
In laboratory selection of drug resistant strains of plasmodia, the general procedure has been to use sub-minimal doses of the drug and gradually to increase it in the course of subsequent passages. A departure from the above technique was resorted to in selecting the resistant strain of *P. cynomolgi* against DDS. The faster selection of sulphadiazine resistant strain of *P. berghei* (Ramakrishnan et al., 1960) where large parasite population was exposed to large drug doses provided the necessary lead.

The process involved inoculation of monkeys intravenously with a standard inoculum of five million parasitized erythrocytes. The infection was allowed to reach about 4 per cent. cell involvement when a large single dose of the drug (50 mg/kg) was administered by mouth. Sub-passages were effected invariably 48 hours after the drug dose except in the first sub-passage which was made 24 hours after the drug. Although a standard inoculum was attempted in all sub-passages, the low count of the surviving parasites did not ensure it in some. The dose was rapidly increased in the sub-passages - this being raised serially to 75, 150, 300, 300, 300 and 450 mg/kg single dose. The sensitivity of the parasites was checked at the stages following the administration of the V, VI and VII drug doses (fig. 1) by challenging the parasite with the MED of DDS, i.e. 0.5 mg/kg daily for seven days. The strain apparently remained susceptible after the second and third doses of 300 mg/kg but after the administration of 450 mg/kg in single dose, resistance to the MED of DDS was manifest. Even a dose of 450 mg/kg daily for three days after the seven-day treatment with the MED of DDS failed to secure parasite clearance. This strain was not subjected to any further exposure to DDS (fig. 2).

Measurable resistance was available only after stage VII of exposure to the drug. The very fact that some parasites were obviously surviving through drug exposures I to VII, showed that those individuals must have been resistant to the drug from the commencement. It was possible that sufficient number of such individuals were propagated only at the end of the time taken for the experiment at drug exposure VII.

Even at this stage there was a proportion of susceptible individuals in the population (fig. 2). This raises the question whether all the asexual progeny of a resistant merozoite consisted entirely of resistant individuals or a mixture of resistant and susceptible individuals. The answer to this question must await further investigation. The indications, however, would seem to suggest that the progeny of a given resistant merozoite consisted of a mixture of resistant and susceptible individuals. Thus, large

FIG. 1. SCHEMATIC REPRESENTATION OF THE PROCEDURE INVOLVED IN THE SELECTION OF DDS RESISTANT STRAIN OF *P. CYNOMOLGI*. SUBINOCULATION 48 HOURS AFTER THE SINGLE DOSE OF THE DRUG EXCEPT IN THE FIRST SUB-PASSAGE



parasite populations were exposed to seven progressively high doses of the drug. This precipitated the selection of a DDS-resistant strain of P. cynomolgi. The build-up of a large population necessarily involved a longer period after inoculation and consequent rising immunity. On an average, parasitaemia for about seven days preceded the administration of the single drug dose. In untreated infection reduction of parasitaemia by crisis occurred on the tenth day of parasitaemia.

2. Degree of resistance

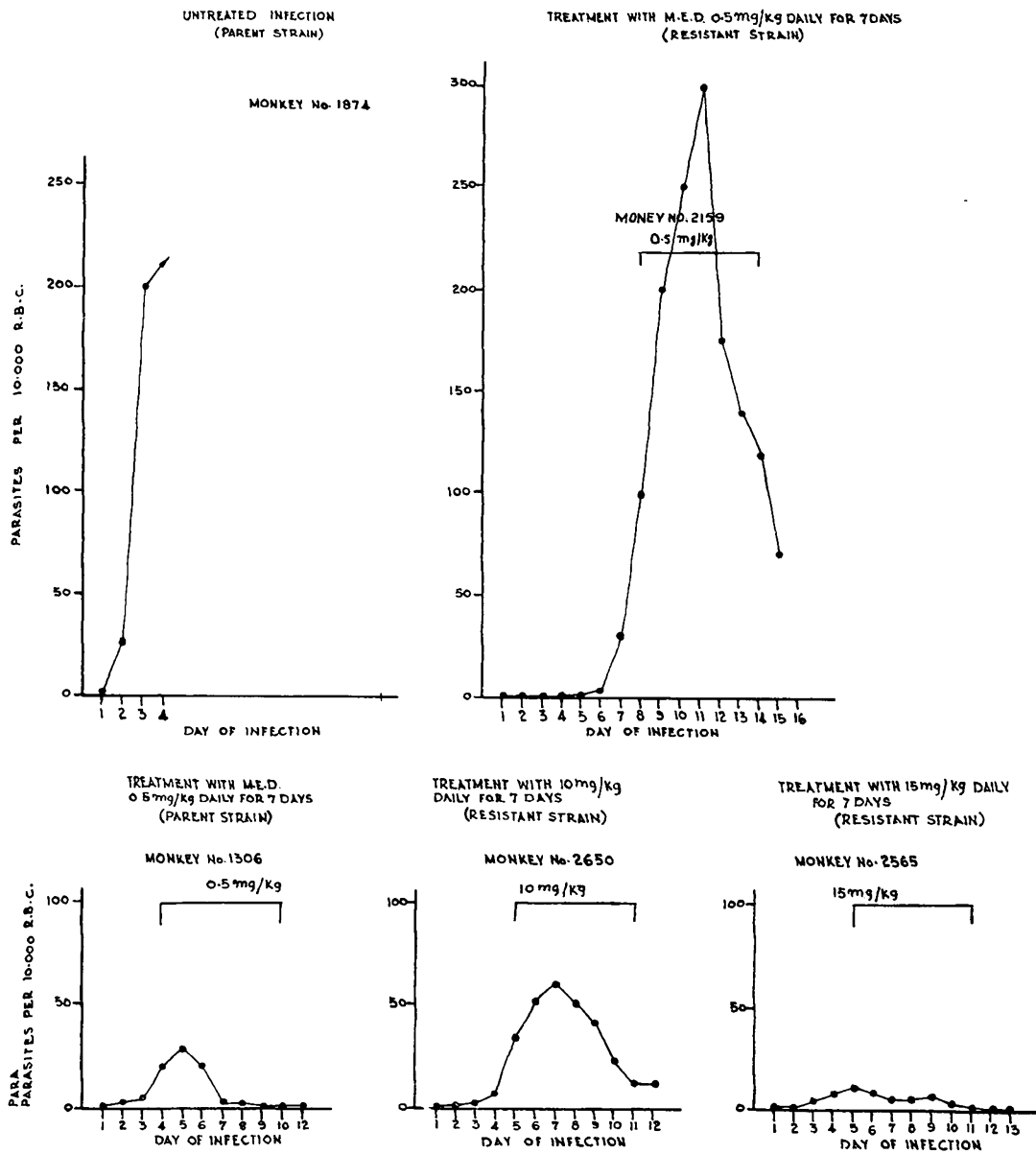
Three monkeys were inoculated intravenously with the DDS-resistant strain of P. cynomolgi, the standard inoculum being five million parasitized erythrocytes per kg. When the cell infection reached a stage of 0.1 to 0.2 per cent., DDS in doses of 10 and 15 mg/kg daily for seven days was administered orally, the first monkey receiving 10 mg and the second and third monkeys receiving 15 mg/kg dosages each. The course of infection (fig. 2) showed that parasite clearance was not obtained even with a dosage of 10 mg/kg for seven days and the infection passed on to chronic stage, whereas the parent strain showed that 0.5 mg/kg for seven days (MED) secured complete clearance of the parasites. Thus a twenty-fold resistance to DDS was noted. However, with a dosage of 15 mg/kg daily for seven days, although the parasites continued to be present up to the last day of treatment, they were cleared on the day following the completion of treatment. This delayed clearance of parasites indicated partial refractoriness to this high dose of DDS.

3. Cross resistance to sulphadiazine

Two monkeys were inoculated with the DDS-resistant strain according to the standard technique. When the parasitaemia reached 0.1 to 0.2 per cent. cell infection, the monkeys received a dose slightly higher than MED of sulphadiazine (0.2 mg/kg daily for seven days). No parasite clearance was obtained and the infection passed on to chronic stage (fig. 3). Thus the DDS-resistant strain of P. cynomolgi was observed to possess some cross resistance to sulphadiazine.

In order to determine the degree of this cross resistance, another monkey was inoculated similarly with the resistant strain and the infection was challenged with ten times the MED (0.15 mg/kg daily for seven days) of sulphadiazine, i.e. 1.5 mg/kg daily for seven days. Parasites were not cleared following the treatment and the

Fig.2 DEGREE OF RESISTANCE TO SULPHONE IN *P. Cynomolgi*

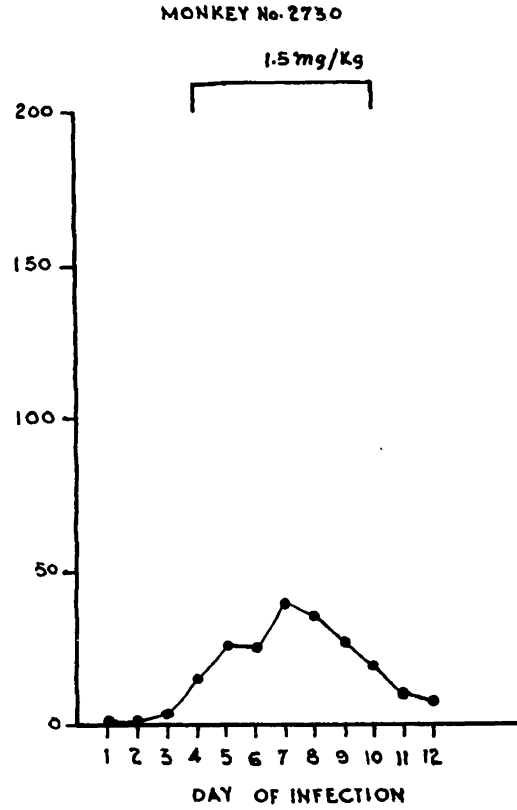
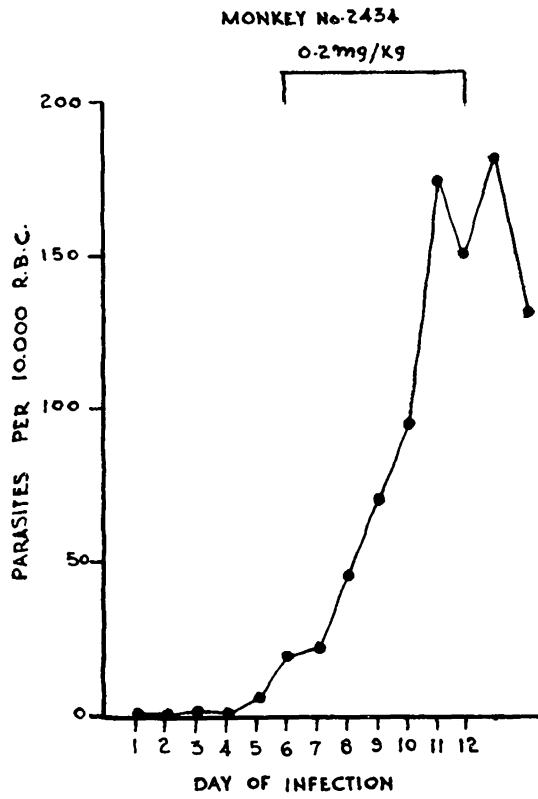


**Fig 3 CROSS RESISTANCE TO SULPHADIAZINE
BY THE SULPHONE RESISTANT STRAIN OF**

P. Cynomolgi

TREATMENT WITH 0.2mg/Kg
SULPHADIAZINE DAILY FOR 7 DAYS
(RESISTANT STRAIN)

TREATMENT WITH 1.5mg/Kg
SULPHADIAZINE DAILY FOR 7 DAYS
(RESISTANT STRAIN)



infection passed on to chronic stage (fig. 3). Subsequently, another monkey infected with the resistant strain received 3 mg/kg of sulphadiazine daily for seven days. The parasites could be detected in the peripheral blood after completion of treatment. Thus, the cross resistance to sulphadiazine amounted to at least twenty-fold.

4. Absence of cross resistance to other antimalarials

Four groups consisting of two animals each were inoculated with the DDS-resistant strain. The first group was challenged with the MED of proguanil; the infection was cleared by the treatment. The second group was similarly challenged with the MED of chloroquine; complete parasite clearance was obtained. The third group was challenged with the MED of pyrimethamine and the peripheral blood was rendered negative for parasites. The fourth group was challenged with the MED of primaquine (determined previously to be 0.5 mg/kg daily for seven days); parasites were absent from the peripheral blood at the close of the treatment. Thus, the DDS-resistant strain of P. cynomolgi did not reveal any cross resistance to proguanil, chloroquine, pyrimethamine and primaquine.

CONCLUSIONS

Diamino-diphenyl sulphone (DDS) shows an activity in rhesus monkeys against both P. knowlesi (Nuri strain) and P. cynomolgi infections, more so against the latter. In P. gallinaceum infection of fowl the drug has low antimalarial activity. Similar results were obtained with promin in simian and avian (P. cathemerium) malaria (Coggeshall et al., 1941). DDS does not possess any causal prophylactic, gametocytocidal or sporontocidal activity in P. gallinaceum. Lack of causal prophylactic activity was also noted with promin in P. cynomolgi infection (Coggeshall et al., 1941). In P. cynomolgi infection, the schizontocidal activity of DDS is of a higher order than that of all other established antimalarials, although inferior in this respect to sulphadiazine. DDS has been shown to possess some antimalarial activity in human malaria (Coggeshall et al., 1941; Archibald & Ross, 1960), though this action is much inferior to chloroquine.

The antimalarial activity and the slow excretion of DDS (found in the blood in appreciable amount for period up to several weeks after stoppage of treatment - according to Lowe, 1952) are comparable only with those of pyrimethamine. Consequently a combination of these two drugs holds a certain promise for prolonging the duration of action. Such a combination is further desirable in view of the facts that individually both the drugs are prone to selection of the resistant strain of parasites and that both probably act by competing either with p-aminobenzoic acid or its metabolic product pteroylglutamic acid for certain enzymes in the parasite. Above all, the absence of cross resistance between DDS-resistant strain and pyrimethamine shows that the drugs act at different points in the metabolic processes of the parasite. Thus the combination of the two drugs is likely to reduce the chances of parasite survival and consequently resistance. The combination might provide both schizontocidal as well as sporontocidal protection. The possibility of potentiation between sulphones (which have a relatively low toxicity in primates) and pyrimethamine is under active study.

SUMMARY

1. Diamino-diphenyl sulphone (DDS) in doses of 5 to 25 mg/kg daily for seven days by mouth was well tolerated in monkeys. Doses of 50 and 100 mg/kg daily for seven days, although did not kill the animals, produced some loss in body weight.
2. Against P. gallinaceum in chicks, the quinine equivalent of DDS was only 0.25, which is much lower compared with other established antimalarials.
3. The drug has no causal prophylactic, gametocidal or sporontocidal activity in P. gallinaceum infection.
4. The MED (minimum effective dose) of DDS in P. knowlesi (Nuri strain) was 0.25 mg/kg daily for seven days; the MED in P. cynomolgi was 0.5 mg/kg daily for seven days.
5. The DDS-resistant strain of P. cynomolgi was selected by exposing large parasite population to high doses of the drug. Such selection was obtained after seven exposures to drug doses and rapid sub-passage. The resistance obtained to DDS was twenty-fold that of the original strain.

6. The DDS-resistant strain of P. cynomolgi was cross-resistant to sulphadiazine. Such cross resistance was at least twenty-fold.
7. The DDS-resistant strain was susceptible to the MED of proguanil, chloroquine, primaquine and pyrimethamine.

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