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STUDY ON THE JOINT ACTION OF DIAMINO-DIPHENYL SULFONE (DDS)  
AND PYRIMETHAMINE AGAINST PLASMODIUM GALLINACEUM AND  
PLASMODIUM CYNOMOLGI BASTIANELLII;<sup>1</sup>  
POTENTIATION BETWEEN THE TWO DRUGS<sup>1</sup>

by

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The development of chemotherapy of infectious diseases has been accompanied by an increased number of reports on cases of drug resistance. To meet this problem it has been suggested that along with the synthesis of new drugs, existing chemicals should be evaluated for their potentiating properties.

Rollo (1955) and Hurly (1959) reported potentiation of pyrimethamine by sulfadiazine against avian and human malaria respectively.

DDS (diamino-diphenyl sulfone) had been shown to possess low schizontocidal activity against P. gallinaceum; it was also less active than sulfadiazine in P. cynomolgi. DDS, however, was found to be superior to sulfadiazine in P. knowlesi infection as well as in human malaria (P. falciparum) (Archibald & Ross, 1960; Ramakrishnan et al., 1961; Basu et al., 1962). A DDS-resistant strain of P. cynomolgi did not show any cross-resistance to pyrimethamine, although it was cross-resistant to sulfadiazine (Ramakrishnan et al., 1961) indicating a similar mode of action as that of sulfadiazine. Further DDS, like sulfadiazine, was antagonized by PAB and folic acid (unpublished data, Malaria Institute of India, 1962).

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Investigations were undertaken to study the joint action of DDS and pyrimethamine against P. gallinaceum in chicks<sup>1</sup> and P. cynomolgi bastianellii<sup>2</sup> in Macaca mulatta monkeys. The results of these studies are presented in this paper.

## 1. MATERIAL AND METHODS

### 1.1 P. gallinaceum

Seven- to nine-day-old chicks (Rhode Island Red) were inoculated intravenously with  $0.5 \times 10^6$  parasitized cells per g body-weight. The chicks were hatched in the laboratory and were fed on standard diet and kept at a temperature between  $30^{\circ}$  and  $32.2^{\circ}\text{C}$ . The inoculated birds were then chosen at random for treatment with different dosage of drugs as stated below. The antimalarial drugs were given twice a day, orally. The first dose was given about four hours after inoculation and a total of seven doses was given over three-and-a-half days.

Infection was assessed on the fourth day after inoculation. Two blood smears were prepared from each of the experimental and control birds. The stained smears were examined by two persons independently.

### 1.2 P. cynomolgi bastianellii

Monkeys each weighing 2-4.5 kg were inoculated intravenously with  $5 \times 10^6$  parasitized cells per kg body-weight. Treatment commenced when the erythrocyte infection was 0.1 to 0.2%. The antimalarials were administered orally, once a day, and the treatment continued for seven days. Infection was assessed by daily examination of blood smears from the experimental as well as the control animals during the treatment and on the day following the completion of the treatment.

### 1.3 Design of the experiment and biometric analysis of data

P. gallinaceum. Different dosages of DDS and pyrimethamine<sup>3</sup> were prepared in water as suspension. The range of dosages for each drug was selected (based on available data) to get a reduction of about 10 and 90% cell infection. Within this range three to four concentrations of the drugs were selected at equal logarithmic intervals.

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

<sup>2</sup> Strain of P. cynomolgi bastianellii obtained through the courtesy of Professor P. C. C. Garnham, London, and maintained at this Institute in M. mulatta monkeys by blood inoculation since 1961.

<sup>3</sup> Avlosulfon (Dapsone), tablets at 0.1 g. Daraprim brand pyrimethamine, tablets 25 mg.

Groups of birds were treated with the two drugs separately and in a number of combinations. Attempts were made to keep two to five birds in each treatment regimen. The experimental birds were serially numbered and allotted to different regimens at random. A relatively large number of birds (nine) was observed as control. There was some mortality in the birds during the period of experiment and it disturbed the equality in allotment. The readings, however, in each dose regimen represented the average values of a minimum of two birds and a maximum of five birds.

The factorial design with scalar dosage was used to study precisely the different types, if any, of joint action of different dosage combinations. For enumeration of parasitized cells  $10^4$  red blood cells were examined in each slide.

P. cynomolgi bastianellii. The number of monkeys in the different dosage regimens varied from two to five. The criterion of the successful treatment was judged by the complete disappearance of parasite in all the animals on any dose regimen on the day following the completion of treatment. The minimum effective dose of DDS and pyrimethamine thus determined provided the starting point for the combination of the two drugs. Combinations, therefore, always contained doses of each, lower than the minimum effective dose of either drug.

In the tests carried out against P. gallinaceum, the results of the parasite count in the two smears by two different persons were pooled and expressed as the number of parasites per  $2 \times 10^4$  red blood cells. Geometric means were used to estimate the mean parasite density (MPD) in each dose regimen.

The response index (RI) was worked out as the ratio of MPD in the treated to the corresponding value observed in the control birds. The response index thus obtained was converted to probit for purposes of drawing the probit/log dose regression lines. These regression lines in the majority of cases were found to be straight lines. From the regression lines  $ED_{85}$  and  $ED_{50}$  values (effective dose that gives 85% and 50% reductions in parasite density when compared with the density in control) were estimated

by graphic interpolation method for each of the two drugs separately and in various combinations. The  $ED_{85}$  and  $ED_{50}$  thus estimated were used to prepare bolograms to determine the nature of the joint action of the two drugs (Gaddum, 1952).

Bolograms were also prepared for the study of the joint action of the drugs against P. cynomolgi. For this purpose a series of doses lower than the minimum effective doses of either drug which gave complete clearance of parasite was used.

## 2. RESULTS

P. gallinaceum. The results of the blood examination of treated and untreated birds are given in Table 1.

The mean parasite density was as high as 13 032 in the control series, whereas in the treated groups the values were low in many of the combinations used. The mean parasite density, however, was comparable to the control values where the combinations involved very small doses of each drug. The drug response index suggested a correlation between the dosages and the response. Therefore, the data of the response index, after appropriate conversions, were plotted on a graph (Fig. 1).

From the probit/log dose curve (Fig. 1) the  $ED_{50}$  and  $ED_{85}$  values in respect of DDS and pyrimethamine singly and in combination were estimated (Table 2).  $ED_{85}$  and  $ED_{50}$  of DDS singly were 5.9 mg/50 g and 3.5 mg/50 g respectively. Similar values for pyrimethamine were 0.00126 mg/50 g and 0.00076 mg/50 g respectively. To obtain an 85% response, the dose combinations of the two drugs were lower in five dose regimens than the dose required for the same response by any one of the drugs used singly. When the dose of DDS was 0.8 mg/50 g, the dose of pyrimethamine required to produce 85% response was the same as when pyrimethamine was used alone. The dose combinations were also lower at the  $ED_{50}$  level for all six combinations. In no case was the  $ED_{50}$  or  $ED_{85}$  found to be higher than the corresponding equipotent dosage of the two parent compounds tried alone. This suggested that the drugs did not show antagonistic action when used as mixture.

TABLE 1. PARASITE DENSITIES AND RESPONSE INDICES IN P. GALLINACEUM-  
INFECTED CHICKS TREATED WITH VARIOUS DOSAGES OF DDS AND PYRIMETHAMINE  
SINGLY AND IN COMBINATION ALONG WITH THE UNTREATED CONTROLS  
(DOSAGES IN MG PER 50 G BODY-WEIGHT)

DDS	Pyrimethamine	0.0015	0.00075	0.000375	0.0001875	0.00009375	Pyrimethamine - nil
12.8	MPD	1.0	1.0	36.3	1.7	-	60.5
	RI	0.008	0.008	0.278	0.013	-	0.464
6.4	MPD	1.7	2.0		33.0		1 499.7
	RI	0.013	0.015		0.253		11.507
3.2	MPD	3.3		35.8	382.8	5 382.7	7 568.3
	RI	0.025		0.274	2.937	41.303	58.074
1.6	MPD	3.7	70.3	792.50	6 441.7	7 961.6	11 967.0
	RI	0.028	0.539	6.081	49.429	61.092	91.827
0.8	MPD			8 590.1	10 940.0	12 134.0	14 555.0
	RI			65.915	83.947	93.109	111.686
(DDS - nil)	MPD	1 039.9	9 141.1	9 763.0	94 890.0	13 397.0	13 032.0
	RI	7.979	70.143	74.915		102.800	(control without drugs)

MPD = mean parasite density per bird per  $2 \times 10^4$  erythrocytes

RI = response index =  $\frac{\text{MPDT}}{\text{MPDC}} \times 100$  (the more effective the treatment, the lower the "response index")

MPDT = mean parasite density per bird per  $2 \times 10^4$  red blood cells in treated group

MPDC = mean parasite density per bird per  $2 \times 10^4$  red blood cells in control

TABLE 2. ED<sub>85</sub> AND ED<sub>50</sub> VALUES IN RESPECT OF DDS AND PYRIMETHAMINE  
SINGLY AND IN COMBINATION IN P. GALLINACEUM INFECTION  
(DOSAGES IN MG PER 50 G BODY-WEIGHT)

Dose	ED <sub>85</sub>	ED <sub>50</sub>
	<u>Dosages of DDS</u>	
DDS + 0 pyrimethamine	5.9	3.5
DDS + 0.00009 pyrimethamine	4.4	2.2
DDS + 0.0001375 pyrimethamine	2.3	1.4
DDS + 0.000375 pyrimethamine	1.4	0.9
	<u>Dosages of pyrimethamine</u>	
Pyrimethamine + 3.2 DDS	0.00014	0.00009
Pyrimethamine + 1.6 DDS	0.00032	0.00020
Pyrimethamine + 0.8 DDS	0.00126	0.00050
Pyrimethamine + 0 DDS	0.00126	0.00076

Two bolograms (Fig. 2 and Fig. 3) also supported the above conclusion. Further, these bolograms indicated potentiation of the two drugs when used in the mixture. In the most effective dose combinations of the two drugs, evaluated at ED<sub>85</sub>, the effective combinations which required the smallest dose of either drug, were found to be DDS 1.4 mg/50 g + pyrimethamine 0.00032 mg/50 g. The isobols for these dosages were found to be close to the origin of the bologram. However, the magnitude of potentiations was relatively lower at ED<sub>50</sub> than ED<sub>85</sub> (Fig. 3). The shape of the bologram indicated that when the proportion of any one drug was reduced the type of joint action gradually changed from potentiation to addition.

The above observation of the joint action of DDS and pyrimethamine was found to be tenable also in the tests carried out against P. cynomolgi bastianellii in monkeys. The minimum effective doses of DDS and pyrimethamine were found to be 0.15 mg/kg and 0.05 mg/kg respectively (Table 3). The dosages lower than the minimum effective dose of the two drugs combined showed high potentiation (Table 3 and Fig. 4).

TABLE 3. THE RESPONSE OF P. CYNOMOLGI BASTIANELLII INFECTION TO DDS AND PYRIMETHAMINE SINGLY AND IN COMBINATION

Drug dosages in mg/kg for 7 days		Number of animals used	Number of animals showing clearance of parasite	Number of animals showing persistence of parasite	Parasite clearance in hours		
DDS	Pyrimethamine						
	0.5	1	1	Nil	120		
	0.25	2	2	Nil	96	120	
	0.05	5	5	Nil	120 (4)	144 (1)	
	0.025	5	4	1	120 (1)	144 (3)	
0.5		4	4	Nil	96 (1)	144 (2)	168 (1)
0.25		5	5	Nil	120 (2)	144 (2)	168 (1)
0.15		5	5	Nil	120 (2)	144 (1)	168 (2)
0.05		2	2	2	-		
0.05	+0.02	2	2	Nil	168	168	
0.005	+0.02	2	2	Nil	144	144	
0.075	+0.025	2	2	Nil	168	168	
0.005	+0.002	2	2	Nil	144	144	
0.05	+0.002	2	2	Nil	120	96	
0.0025	+0.001	1	1	Nil	144		
0.00125	+0.0005	3	1	2	168		

Note: Numbers within parenthesis indicate the number of animals.

The effective combination attained by the smallest dose of either drug was found to be DDS 0.0025 + pyrimethamine 0.001 mg per kg. The isobols for this dosage was found to be very close to the origin of the bologram. The clearance of parasites was, however, quicker with DDS 0.05 + pyrimethamine 0.002 mg per kg than with the other dose schedules.

### 3. DISCUSSION

The bologram method of interpretation of results was suitable for the present study which aimed to explore the joint action of DDS and pyrimethamine. The bolograms illustrate a potentiation of the two drugs at certain dose levels. In certain dosages only additive action was noted. This was observed in two instances in P. gallinaceum infection when the proportion was (i) DDS 3.2 and pyrimethamine 0.00009 mg per 50 g and (ii) 0.8 and 0.00126 mg per 50 g of the respective drugs (Fig. 2).

The strong potentiation between pyrimethamine and sulfadiazine is probably due to two drugs acting on different points on the same metabolic pathway (Rollo, 1955). It has been further suggested that an additive effect occurs when the drugs act at the same point or upon different pathways. The action of both sulfadiazine and pyrimethamine was competitively antagonized by either PAB or folic acid and there was no cross-resistance between pyrimethamine and sulfadiazine in P. gallinaceum (Rollo, 1955). Similarly, the action of DDS was antagonized by PAB and folic acid (unpublished reports of the Malaria Institute of India) and there was no cross-resistance between DDS and pyrimethamine (Ramakrishnan et al., 1961). Therefore, DDS and pyrimethamine probably acted on the same metabolic pathway but the points of attack might be different.

Similar potentiation between sulfadiazine and pyrimethamine has been shown in human malaria (Hurly, 1959). According to Basu et al. (1961) small doses of DDS combined with a small dose of pyrimethamine (100 and 50 mg of the former and 12.5 mg of the latter) proved adequate for treatment of both P. falciparum and P. vivax infections and indicated probable potentiation.

In chicks, the dosages of DDS (5.9 mg/50 g, i.e., 118 mg/kg) required for the control of P. gallinaceum were found to be much higher than the dosages of DDS required in monkeys for the control of P. cynomolgi bastianellii infection (0.5 mg/kg). This might explain why the dosages of the two combined drugs to obtain a potentiating effect were lower in P. cynomolgi bastianellii than in P. gallinaceum.

#### SUMMARY

1. DDS and pyrimethamine were assayed separately and in various combinations against P. gallinaceum in chicks and against P. cynomolgi bastianellii in monkeys for plasmodicidal activity.
2. In certain dosages the combination of two drugs showed a potentiating effect. Combinations containing lower dosages of either drug showed simple addition.
3. There was a difference in the response of P. gallinaceum in chicks and of P. cynomolgi bastianellii in monkeys to the combined use of the two drugs.

#### ACKNOWLEDGEMENT

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FIG. 1  
 SHOWING THE RELATION BETWEEN DOSE AND RESPONSE INDEX IN METAMETRIC UNITS  
 ( IN *P. gallinaceum* IN CHICKS )

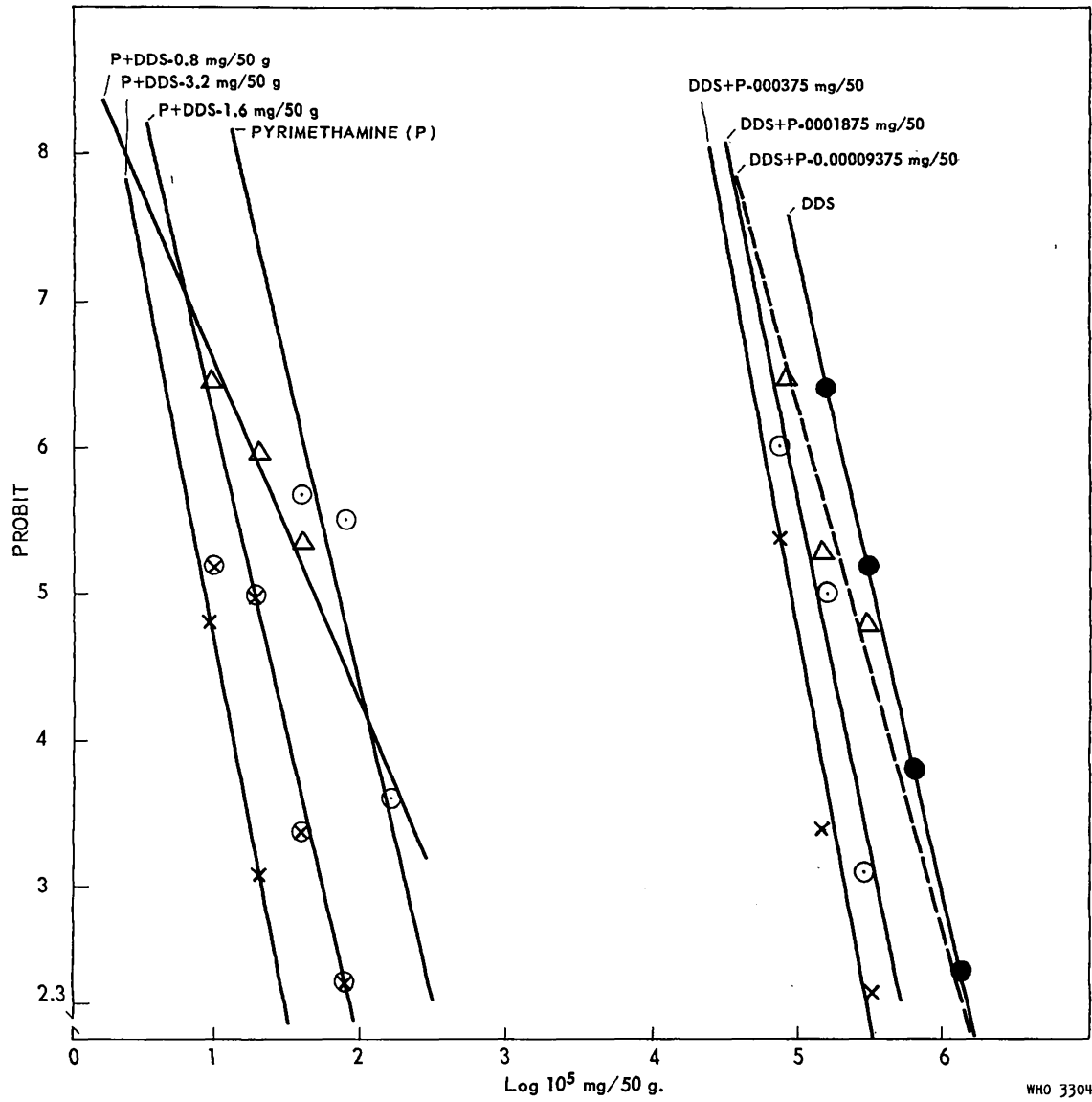
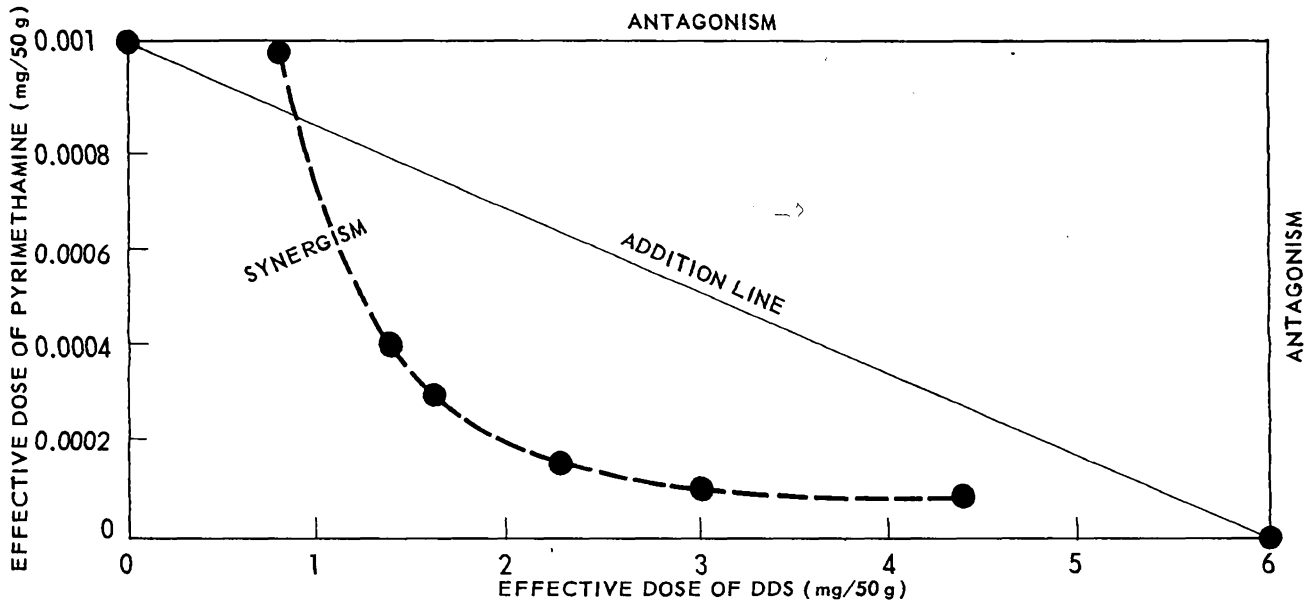


FIG. 2

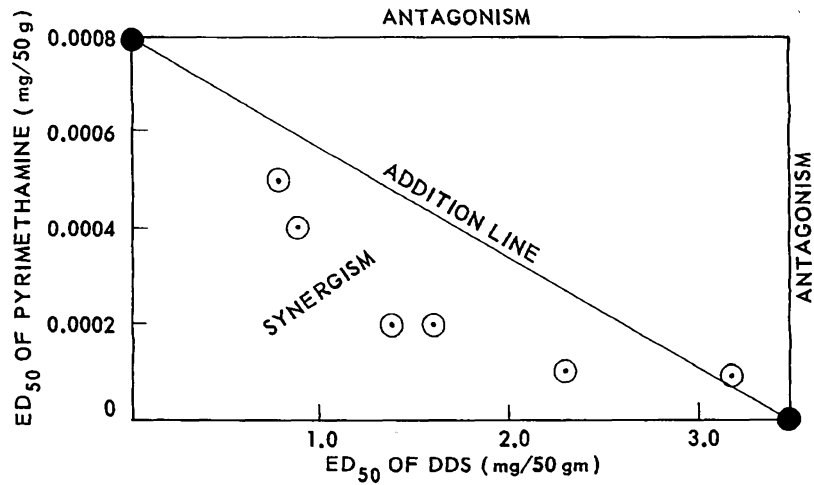
SHOWING THE  $ED_{85}$  OF PYRIMETHAMINE AND DDS ADMINISTERED BOTH  
SINGLY AND TOGETHER IN VARIOUS PROPORTION IN CHICKS INFECTED  
WITH *P. gallinaceum*



WHO 3305

FIG. 3

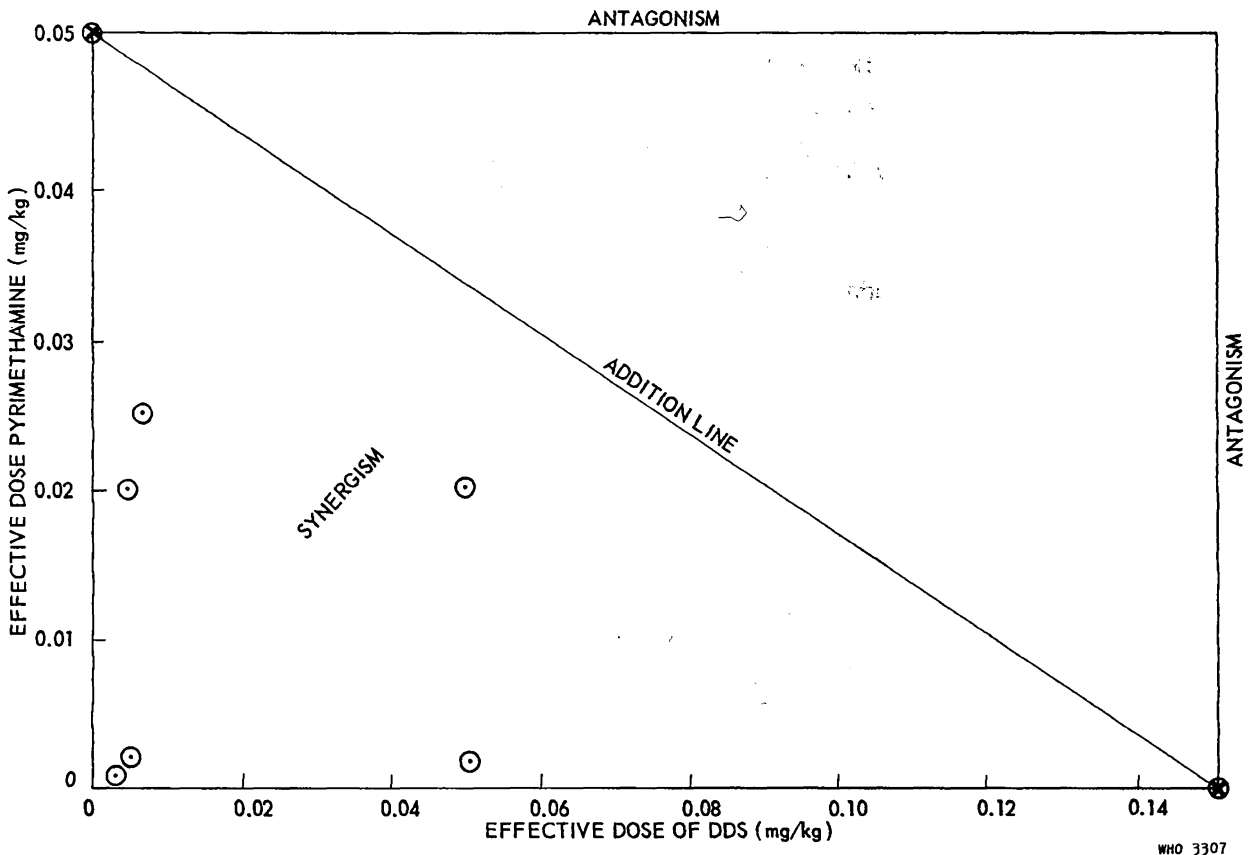
SHOWING THE  $ED_{50}$  OF PYRIMETHAMINE AND DDS BOTH  
SINGLY AND TOGETHER IN VARIOUS PROPORTION IN  
CHICKS INFECTED WITH *P. gallinaceum*



WHO 3306

FIG. 4

JOINT ACTION OF DDS AND PYRIMETHAMINE AGAINST *P. cynomolgi bastianelli* IN MONKEYS



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