Hyperactivity of Metachloridine against *Plasmodium gallinaceum* in Chicks Maintained on a Purified Diet.* (22096)

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In a previous report(1), it was shown that sporozoite-induced *Plasmodium gallinaceum* infections behave similarly in chicks fed a purified diet or a commercial stock diet. It was thought, however, that the effect of therapeutic agents might differ in chicks fed different dietary regimens, since several antimalarials are known to be, or are suspected of being, antimetabolites. Commercial stock diets are known to vary in their composition from time to time and conceivably could vary in their content of critical natural components which might inhibit the activity of certain antimalarials. The variability in the content of diet of such natural metabolites might account for the minor variations occasionally encountered in the activity of antimalarials. Alternatively, the presence of antibiotics currently being added to stock feed might have some influence on the results of tests with antimalarials.

We undertook to determine the effect of a purified diet (C-2(1,2)) on the parasitemia produced by *P. gallinaceum* in untreated chicks after the intravenous inoculation of erythrocytic parasites and the effect of such a diet on the antimalarial activity of certain compounds. As will be shown, the use of the purified diet had little or no effect on the antimalarial activity of most of the compounds tested. However, metachloridine (2-metanilamido-5-chloropyrimidine) and sulfadiazine were strikingly more active in chicks fed the purified diet than in those fed the stock diet. The hyperactivity of sulfadiazine might have been the result of the absence of *p*-aminobenzoic acid from the purified diet; however, neither *p*-aminobenzoic nor *m*-aminobenzoic acid had any effect on the antimalarial activity of metachloridine(3), nor, to date, has any natural substance been isolated which can reverse the activity of metachloridine. This drug was placed by Josephson *et al.*(4) in a class of antimalarials characterized as plasmodistatic, acting only during the dividing stages of the parasite. It was predicted that the parasite could be made resistant to members of this class of antimalarials, and Bishop and McConnachie(5) found that resistance to metachloridine can be readily induced. Josephson *et al.*(4) predicted that members of this class would be antimetabolites. The fact that metachloridine was more active in chicks fed the purified diet than in those fed stock diet suggested that the latter diet contained an inhibitor, possibly a substance essential for the parasite. A search for such a substance was undertaken, and the results are reported in this paper.

Methods. New Hampshire chicks from a single source were used throughout the experiments. They were received in the laboratory when one day old, separated into 2 groups, and each group immediately placed on one of the test diets, on which they continued until the end of the experimental period. Weights of the chicks were recorded after they had been on the diets for 7 days and again 4 days later. The drug tests were conducted according to a procedure previously described(6). Week-old chicks were inoculated intravenously with 16×10^6 erythrocytes parasitized with *P. gallinaceum*. Parasite counts (parasitized erythrocytes per 10^4 erythrocytes) were made on the morning of day 4 (day of inoculation is day 0). Drugs were administered orally, twice daily for 4 days, beginning 4 to 5 hours before parasite

* The authors are grateful to the following: the Archer-Daniels-Midland Co. for furnishing the soy grits and hulls; the Soya Products Division of the Glidden Co. for the soy granules; Drs. M. R. Spivey Fox and G. M. Briggs for helpful suggestions; Anne Jowett, G. W. Rusten, and J. Phillips for technical assistance.

TABLE I. Comparison of Antimalarial Activity of 9 Compounds Tested in Chicks Infected with *P. gallinaceum* and Fed Either the C-2 Diet or the Stock Diet.

Compound	Minimum effective dosage* (mg/g body wt) C-2 diet	Stock diet
Quinine	.005	.01
Chloroquine	.001	.002
Atabrine	.005	.01
Chlorguanide	.0005	.002
	.002	.002
Terramycin	.04	.08
Fumagillin	>.04	>.04
Pamaquine	.0003125	<.000625
Sulfadiazine	<.025	.1
	≦.003125	.05
Metachloridine	.000125	.002
	.0000625	.002
	<.0000625	.0005

* Each result derived from one experiment.

inoculation. The stock diet used was Purina Startena; the purified diet was prepared in this laboratory according to published procedures(1,2). Henceforth, the purified diet will be termed the C-2 diet. Variations in the C-2 diet were made by replacing a portion of the Cerelose with the test quantity of the nutrient to be studied. In the experiments designed to test the effect of nutrient variables of the C-2 diet on the antimalarial activity of metachloridine, the drug was given at 5 levels to those chicks given the varied C-2 diet and at 3 levels to those fed the stock diet. In late experiments, the C-2 diet served as the control diet. The corn-soy diet (a complete all-vegetable diet)(7) contained the following constituents per kilo of diet: 575 g of ground, whole, yellow corn; 350 g of soybean meal; 10 g of Cerelose; 5 g of corn oil; 60 g of Briggs chick salts-A(2); 0.02 mg of vit. D_3, 8 mg of riboflavin; and 0.02 mg of vit. B_{12}.

Results. The mean 4-day weight gain of 60 parasitized chicks in 12 series fed stock diet was 20.1 ± 0.6 g; the corresponding 60 chicks fed the C-2 diet gained 18.5 ± 0.9 g. The difference between the means was not significant ($p<0.2$).

The mean 4-day parasitemia of the above 60 chicks fed the stock diet was 7289 ± 251/10^4 erythrocytes; the parasitemia of the corresponding 60 chicks fed the C-2 diet was 6890 ± 225. There was no significant difference between the means ($p<0.3$).

The antimalarial activity of 9 compounds was compared in chicks fed C-2 diet and stock diet (Table I). With 7 of these compounds, the difference in antimalarial activity between the 2 diets was small and probably of doubtful significance. On the other hand, sulfadiazine was at least 16 times as active in chicks fed the C-2 diet as it was in chicks fed stock diet. In preliminary trials metachloridine was about 16 times as active when used to treat chicks fed C-2 diet as it was in chicks fed stock diet.

In repeated trials, it was established that metachloridine was 16 times as active in C-2 diet-fed chicks (Table II). At the suggestion of Dr. George M. Briggs, metachloridine was tested in chicks fed an all-vegetable diet (see Methods), in which the 2 most abundant constituents were ground corn and soybean meal. Metachloridine was no more active with this diet than it was with the stock diet. Ground corn and soybean meal were tested separately as part of the C-2 diet in approximately the concentrations in which they occurred in the corn-soy diet (Table II). When 41% soybean meal (either expeller type or hexane-extracted) was incorporated in the C-2 diet, the hyperactivity of metachloridine associated with the purified diet was almost completely obliterated. Ground corn at 60% of the diet appeared to reverse partially the hyperactivity of metachloridine, but soybean meal appeared to be a richer source of inhibitor and to account for most of the inhibition of metachloridine activity observed in the all-vegetable diet.

The protein of soy at a 33% level was put into the C-2 diet at the expense of Cerelose. In chicks fed this diet, metachloridine was only slightly less active than in chicks fed the unmodified C-2 diet (Table II).

Other fractions of soybean meal were tested in single or duplicate tests (Table III). Metachloridine was as active in chicks fed 4% soybean oil in place of the same amount of corn oil of the C-2 diet, or the residue after alkaline extraction of the meal ("soybean

TABLE II. Effect of Diet on Antimalarial Activity of Metachloridine against *P. gallinaceum* Infected Chicks.

Dosage of metachloridine, mg/g b.i.d. for 4 days	Mean fourth day parasitemia (parasitized rbc/10^4 rbc)				
	Stock	C-2	C-2* 41% soybean oil meal§	C-2† 60% ground corn	C-2‡ 33% soy protein‖
.002	970 ± 106¶				
.001	2164 ± 190¶		1504 ± 297	298 ± 134	369 ± 130
.0005			2582 ± 365	1012 ± 361	402 ± 89
.00025			4008 ± 432	1786 ± 416	949 ± 183
.000125		760 ± 158**	5629 ± 501	3335 ± 490	2034 ± 462
.0000625		2150 ± 280††	6084 ± 277	4449 ±1040	3917 ± 540
.0000313		3836 ± 350‡‡			
None	7622§§	7268 ± 596	7196 ± 236		

* 5 series, 25 chicks each dose.
† 2 " , 10 " " " .
‡ 3 " , 15 " " " .
§ Expeller type and hexane extracted, data pooled.
‖ Both alpha protein and sodium proteinate, data pooled.
¶ 22 series, 109 chicks.
** 8 " , 40 " .
†† 8 " , 37 " .
‡‡ 10 " , 50 " .
§§ 23 " , 197 " .

granules"), as it was in those fed the regular C-2 diet. The residue remaining after the acid extraction of the meal ("acid-washed grits") appeared to have as much of the inhibitor as the soybean meal. Soybean hulls did not appear to be a rich source of the inhibitor.

Incorporation into the C-2 diet of *p*-aminobenzoic acid (100 mg/kg of diet). 5% brewer's yeast, 5% dried whole beef liver, or twice the concentration of the water-soluble vitamins of the C-2 diet, failed to inhibit the antimalarial activity of metachloridine.

Discussion. Of the 9 antimalarials tested against *P. gallinaceum* in chicks fed the C-2 diet, only sulfadiazine and metachloridine were significantly more active than in chicks fed stock diet. The hyperactivity of sulfadiazine is not surprising, since the C-2 diet has no added *p*-aminobenzoic acid and only a normal amount of folic acid.

TABLE III. Effect of Various Soy Bean Fractions, Incorporated into C-2 Diet, on Antimalarial Activity of Metachloridine against *P. gallinaceum* Infected Chicks.

Fractions of soy beans	Minimum effective dose of metachloridine, mg/g body wt
4% soy bean oil	.000125
" " " "	.000125
41% " " hulls	.0005
" " " granules	.00025
" " " grits (acid washed)	.001

The hyperactivity of metachloridine in chicks fed the purified diet could indicate the absence from this diet of an inhibitor present in the stock diet. Such an inhibitor appears to be present in vegetable products such as soybeans and corn, though the former appears to be a richer source. The inhibitor is not *p*-aminobenzoic acid, nor does it appear to be concentrated in such sources of vitamins as dried yeast or dried liver. It is probably not one of the known water-soluble vitamins for no significant inhibition of metachloridine resulted when the vitamin content of the C-2 diet was doubled.

Neither the fatty fraction nor the protein fraction of soybeans appears to contain significant amounts of the inhibitor. The residue left after the alkaline extraction of soybean meal ("granules") had little or no inhibitor, but the residue after acid extraction ("grits") seemed to have significant amounts of the inhibitor.

The conclusions that can be drawn at this stage of the investigation must be tentative. The data strongly suggest that there is a substance in soybean meal which inhibits the antimalarial activity of metachloridine. This substance is neither fat nor water-soluble protein, nor is it significantly soluble in hexane. It would appear to be alkali soluble, or labile, and acid insoluble. Such a substance could conceivably be a growth factor, a natural product essential for the parasite but not for

the host, and not produced in any abundance by the host. The isolation of such a substance might give some further clues as to what kind of natural products become growth factors for parasites and what kind of chemicals serve as inhibitors of such substances.

Summary. 1. The erythrocytic form of the malarial parasite *Plasmodium gallinaceum* was found to grow at a comparable rate in chicks fed either a purified diet (C-2) or a commercial stock diet. The antimalarial activity of 7 compounds was approximately the same in parasitized chicks fed either of the above diets, while sulfadiazine and metachloridine exhibited a marked increase in effectiveness in those chicks fed the C-2 diet. 2. The hyperactivity of metachloridine in chicks fed the C-2 diet was reversed almost completely when this diet contained 41% soybean meal. Neither the fatty nor protein fractions of soybeans, nor several other dietary nutrients appeared to contain significant amounts of the inhibitor.

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Received May 26, 1955. P.S.E.B.M., 1955, v90.

ERRATUM

Article on Serum Lactic Dehydrogenase . . . by Kuang-Mei Hsieh *et al.*, Vol. 89, p. 627, Tables I, II, and III, second column heading should read "mM substrate oxidized per L serum per hr."