17173. Inhibition of the Antimalarial Activity of Chlorguanide by Pteroylglutamic Acid.

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Chlorguanide (1-(p-chlorophenyl)-5-isopropylbiguanide) is a widely used antimalarial compound, the activity of which was first described by Curd, Davey and Rose.[1] It has been shown to have several interesting relationships with sulfadiazine. When the two drugs are given together there is definite potentiation of effect against *Plasmodium gallinaceum* in the chick.[2] Some chlorguanide-resistant strains are hypersensitive to sulfadiazine,[3] while others are resistant to sulfadiazine.[4] P-aminobenzoic acid will antagonize the antimalarial activity of sulfadiazine[5,6] but not that of chlorguanide.[4] The mode of action of these two drugs is thus not identical but is probably very closely related. It is known that pteroylglutamic acid (PGA) can inhibit the activity of sulfonamides.[7] Experiments were, therefore, undertaken to see if PGA would interfere with the activity of chlorguanide.

Materials and Methods. The tests were conducted according to a procedure previously described.[8] New Hampshire Red Chicks, maintained on a commercial starting mash (Purina Startena), were used throughout. The birds were one week old at the start of an experiment and weighed 42 to 45 g. Treatment was administered twice daily for 4 days beginning 4 to 5 hours before intravenous inoculation with 16 x 10^6 chick erythrocytes parasitized by *P. gallinaceum*. Parasite counts (parasitized erythrocytes per 10^4 erythrocytes) were made on the morning of the 4th day after inoculation of parasites (day of inoculation is day O). A dosage level of drug which causes a 75% reduction in parasitemia in the treated chicks as compared with untreated controls is considered the minimum effective dose. Chlorguanide hydrochloride was administered orally in aqueous solution by catheter and pteroylglutamic acid was administered at the same time and by the same route, in gelatin capsules.

Experimental. The minimum effective dose of chlorguanide has been found to be about 0.002 mg/g twice daily for 4 days. Pteroylglutamic acid alone has no significant effect on parasitemia. When the 2 compounds were administered together (Table I), pteroylglutamic acid at 0.002 mg/g did not inhibit the antimalarial activity of chlorguanide. However, PGA at 0.02 mg/g significantly but not completely inhibited the effect of chlorguanide; no further inhibition was produced by 0.2 mg/g. Larger doses of chlorguanide were partially inhibited by PGA.

Pteroylglutamic acid at 0.2 mg/g body weight completely inhibited the minimal effective dose of sulfadiazine, but it had no effect on the antimalarial activity of quinine, chloroquine or pamaquine (Table II).

Discussion. The fact that pteroylglutamic acid can inhibit the antimalarial activity of chlorguanide is not proof that chlorguanide is a direct antagonist of PGA. It is possible that the parasite may be able to use PGA to synthesize another metabolite with which

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TABLE I.
Inhibition by Pteroylglutamic Acid of the Antimalarial Activity of Chlorguanide Against *Plasmodium gallinaceum* in the Chick.

No. chicks	Dose of Chlorguanide HCl (mg/g b.i.d. for 4 days)	Dose of Pteroylglutamic acid (mg/g b.i.d. 4 days)	4th day parasitemia (parasitized erythrocytes per 10^4 erythrocytes)
Exp. 1			
10	—	—	8070 ± 175
5	.002	—	1674 ± 364
5	.002	.002	990 ± 404
5	.002	.02	3540 ± 558
5	.002	.2	3425 ± 748
Exp. 2			
10	—	—	6840 ± 319
5	.002	—	44 ± 25.1
5	.004	—	44 ± 36.0
5	.008	—	6 ± 1.9
5	.002	.2	5140 ± 276
5	.004	.2	1858 ± 937
5	.008	.2	278 ± 219
Exp. 3			
10	—	—	6790 ± 213
5	.002	—	628 ± 197
5	.002	.02	4520 ± 1010
5	.002	.2	3790 ± 263

TABLE II.
Effect of Pteroylglutamic Acid on the Antimalarial Activity of Quinine, Pamaquine, Chloroquine and Sulfadiazine. (Five chicks were used in each group).

	Dosage mg/g twice daily for 4 days	Dosage of pteroylglutamic acid mg/g twice daily for 4 days	4th day parasitemia (parasitized erythrocytes/ 10^4 erythrocytes)
Untreated	—	—	7244
Quinine hydrochloride	.01	—	6060
	.02	—	942
	.02	.2	796
Chloroquine diphosphate	.001	—	4196
	.002	—	30
	.002	.2	8
Pamaquine citrate	.0005	—	4762
	.001	—	208
	.001	.2	108
Sulfadiazine	.015	—	4120
	.03	—	2170
	.03	.2	7280

chlorguanide directly competes. The fact that it requires about 5 molecules of PGA to inhibit significantly the effect of one molecule of chlorguanide, and that not even 50 molecules of PGA can completely inhibit the effect of one molecule of chlorguanide might indicate that chlorguanide is doing more than competing with PGA. However, the hypothesis that chlorguanide is a PGA antagonist is an appealing one in view of the relationship between chlorguanide and sulfadiazine, and between the latter and pteroylglutamic acid. In this connection we have found (unpublished data) that 2, 4-diamino-6, 7-diphenylpterin which is believed to stop bacterial growth by preventing the use of

PGA,[9] behaves similarly to chlorguanide in *P. gallinaceum* infections. It potentiates sulfadiazine to about the same degree as chlorguanide and its activity is partially inhibited by the same amounts of PGA as are required to inhibit chlorguanide. If chlorguanide should prove to be a PGA antagonist, this fact would be highly significant in the development of chemotherapeutic agents for malaria and other diseases as well as for the study of the metabolism of the malaria parasite.

Summary. The antimalarial activity of chlorguanide against *Plasmodium gallinaceum* in the chick could be significantly but not completely inhibited by pteroylglutamic acid *in vivo*. Pteroylglutamic acid had no significant effect on the antimalarial activity of quinine, chloroquine or pamaquine, but completely inhibited the effect of sulfadiazine.

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17174. Pancreatic Islet Hyperplasia in Rats Force Fed High Carbohydrate Diets.

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Although both the cat and the dog may develop islet degeneration and irreversible diabetes after prolonged periods of hyperglycemia,[1-6] permanent diabetes has not been produced in the rat by methods short of partial pancreatectomy or alloxan treatment. After noting the ease with which Ingle produced chronic glycosuria in the rat by force feeding a high carbohydrate diet,[7] it occurred to us that it might be of interest to perform a similar experiment and examine the islets of Langerhans.

Plan of experiments. Two groups of 6 male albino rats of Sprague-Dawley strain were subjected to force feeding of gradually increasing quantities of high carbohydrate rations until glycosuria was sustained. Weights and urinary glucose excretion were determined at frequent intervals. Rats which died during the experiment as well those which survived for the entire experimental period of 56 days were examined grossly and histologically, especial attention being given the appearance of the pancreatic islets.

Rations, method of feeding and urine glucose determinations. A liquid ration containing about 50% carbohydrate was force fed to each group. The ration fed Group I (Table I) was slightly modified from that described by Ingle.[7] The ration fed Group II was similar except that the carbohydrate was all glucose. The latter diet was employed with the thought that intestinal absorption might be faster and more complete, thus making it possible to obtain higher levels of blood glucose. The rations were force fed 2 times per day at about 8 a.m. and 5 p.m. by means of the technic described by Shay and Gruenstein.[8] Starting with 6 ml per rat the daily portions administered were increased by 4 ml per day until 26 ml were being given. This quantity was maintained for a week to accustom the rats to the method of feeding and to avoid "food-shock".[7] Following this the quantities of ration were gradually in-

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