isolation procedures involving protein mixtures and complexes.

Summary. 1. A continuously recording counter adapted to the scanning of radio chromatograms has been described. 2. This method has been applied to a chromatographic method of demonstrating and measuring the reaction between radioactive iodine labelled bovine serum albumin and its antiserum. 3. Preliminary experiments have shown a reasonable agreement between the zone of maximum antigen fixation obtained by a chromatographic method and the zone of maximum precipitation obtained by the precipitin method. 4. The method appears to have useful possibilities in characterizing soluble antigen-antibody complexes as well as other protein-protein interactions. 5. It has been demonstrated that normal sera have the property of permitting a homogeneous movement of the labelled bovine serum albumin whereas the buffered solutions thus far tried have resulted in streaking of the albumin.

Received April 20, 1951. P.S.E.B.M., 1951, v77.

Effect of 2,4-diamino-5-(p-chlorophenoxy)-6-methylpyrimidine and 2,4-diamino-6,7-diphenylpteridine on Chlorguanide-resistant Strain of *Plasmodium gallinaceum.* (18716)

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In previous reports(1,2), resemblances were shown to exist in the biological activities of certain biguanide-type compounds including chlorguanide (Paludrine), certain 2,4-diaminopyrimidines and 2,4-diaminopteridines. The antimalarial activity of these compounds against *Plasmodium gallinaceum* was potentiated by sulfadiazine and partially inhibited by folic acid. It was considered possible that the mode of action of these compounds might be identical, and one would therefore expect that a strain of parasites resistant to one of them would also be resistant to the others. Recently Falco *et al.*(3) have shown that some 2,4-diaminopyrimidines are over 700 times as active as quinine and 60 times as active as chlorguanide aginst *P. gallinaceum.* Such compounds are clearly of potential significance in the treatment of human malaria. Their application in this field might be limited, however, if strains of malaria now resistant to chlorguanide were also resistant to the pyrimidines.

This paper deals with the effect of one representative, 2,4-diamino-5-aryloxypyrimidine and one 2,4-diaminopteridine on a chlorguanide-resistant strain of *P. gallinaceum.*

Methods. Two strains of *P. gallinaceum* were used: a chlorguanide-resistant strain and its "normal" 8A parent strain. According to the criteria used in this laboratory(4) the chlorguanide-resistant strain was, at the time of these experiments, about 16 times more resistant to the activity of the drug than was the parent strain. The hosts were week-old New Hampshire Red chicks weighing 45 to 55 g. The chicks were inoculated at random; each received 16 x 10^6 parasitized erythrocytes intravenously in 0.1 cc of inoculum. Drugs were administered twice daily for 4 days beginning 4 to 5 hours before inoculation. Blood smears were made and stained with Giemsa the morning following the last dose of drug, and parasite counts were made on the basis of parasitized cells per 10^4 erythrocytes. The

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TABLE I. Effect of Chlorguanide, 2,4-diamino-5-(p-chlorophenoxy)-6-methylpyrimidine, and 2,4-diamino-6,7-diphenylpteridine on the Parent and Chlorguanide-resistant Strain of *P. gallinaceum*.

Drug	Minimum effective dose, mg/g b.i.d. for 4 days	
	Parent strain*	Chlorguanide-resistant strain*
Chlorguanide	<.002, .002, .002	>.016, .032, .032
Pteridine	<.016, .016	>.064, >.064
Pyrimidine	.002, .004, .002	.002, <.004, .002

* Each result represents one series.

minimal effective dose of a test drug is that amount which will cause a 75% reduction in the mean parasitemia in 5 treated birds as compared with 10 untreated controls. The diaminopyrimidine and the diaminopteridine were tested for antimalarial activity against both strains of the parasite either simultaneously or during the same week.

Experimental. As seen in Table I the minimum effective dose of chlorguanide against the parent strain was about 0.002 mg/g; against the resistant strain approximately 0.032 mg/g. The chlorguanide-resistant strain of the parasite was at least four times as resistant to the activity of 2,4-diamino-6,7-diphenylpteridine as the parent strain. Where 0.016 mg/g was more than the minimum effective dose of the pteridine against the parent strain, 0.064 mg/g was less than the minimum effective dose of the compound against the chlorguanide-resistant strain. The pteridine could not be tested in doses greater than 0.064, since this was the chick's limit of tolerance. On the other hand, 2,4-diamino-5-(p-chlorophenoxy)-6-methyl-pyrimidine was equally effective against both the chlorguanide-resistant and the parent strains of the parasite.

It was unlikely that the mode of action of the pyrimidine and chlorguanide was identical against *P. gallinaceum*. However, it was possible that the two compounds were acting on the same metabolic system of the parasite but at different loci. If this were the case, then one might expect that the two compounds would be synergistic in their antimalarial activity. However, when the two compounds were administered concurrently at less than the minimum effective dose no evidence of synergism was obtained.

Discussion. Since the discovery of chlorguanide (Paludrine) as an active anti-malarial there has been considerable interest in the mode of action of this acyclic biguanide as well as in certain condensed biguanides. The results of this paper and previous findings pertinent to them are summarized in Table II.

The 2,4-diaminopteridines are so similar in their biological characteristics to chlorguanide that the mode of action of these compounds is probably identical. The 2-arylamino-4-alkylaminoalkylaminopyrimidines (the structural precursors of chlorguanide)(11) bear little resemblance to chlorguanide aside from the fact that both compounds have antimalarial activity. The 2,4-diamino-5-aryloxypyrimidines are similar to chlorguanide in certain biological characteristics, but since they exhibit no cross-resistance with chlorguanide, their mode of action is probably different. We have found (unpublished data) that it was impossible to produce resistance to these 5-aryloxy-diaminopyrimidines by methods which easily produced chlorguanide-resistant parasites. The fact that the 2,4-diamino-5-aryloxypyrimidine is effective against chlorguanide-resistant parasites, and that resistance to these compounds is not easily produced may be of considerable importance in the use of these drugs in treatment of human malaria.

It is obvious that the diaminopyrimidines form a heterogeneous group of compounds. Falco *et al.*(3) have pointed out that the antimalarial activity of 2,4-diaminopyrimidines substituted with an alkyl, benzyl or aryloxy

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TABLE II. A Comparison of Biological Activity of Chlorguanide, Certain Pteridines and Diaminopyrimidines.

Activity against		Chlor-guanide	Pteridine, 2,4-diamino-6,7-diphenyl	Pyrimidines: 2,4-diamino-5-aryloxy	Pyrimidines: 2-arylamino-4-alkylamino-alkylamino
P. gallinaceum in vivo	Potentiated by sulfonamides	Yes(6)	Yes(1)	Yes(2)	No(2)
	Inhibited by folic acid	" (7)	" (1)	" (2)	—
	Cross-resistant to chlorguanide	—	" (5)	No(5)	No(10)
Lactobacillus *in vitro*	Potentiated by sulfonamides	—	" (9)	—	—
	Inhibited by folic acid	Yes(8)	" (9)	Yes(8)	—

group in the 5-position is decreased by substitution on the 2- and/or 4-position amines. The antimalarial activity of the diaminopyrimidines with no or an alkyl substitution on the 5-position is dependent on substitutions on the 2- and 4-position amines. These findings accentuate the differences noted in Table II.

Summary. A chlorguanide-resistant strain of *Plasmodium gallinaceum* was cross-resistant to 2,4-diamino-6,7-diphenylpteridine. The same chlorguanide-resistant strain was not cross-resistant to 2,4-diamino-5-(p-chlorophenoxy)-6-methylpyrimidine. Chlorguanide and 2,4-diamino-5-(p-chlorophenoxy)-6-methylpyrimidine were not synergistic in their antimalarial activity.

Received April 30, 1951. P.S.E.B.M., 1951, v77.

Chemotherapeutic Effects of a Naphthoquinonimine on Infection of Mice with Columbia-SK Group of Viruses.* (18717)

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Schnitzer, Buck, and Steiger(1) described the chemotherapeutic effects obtained with a certain naphthoquinone derivative (compound Ro 2-3532, *i.e.* 3-(1,4-dihydro-3-hydroxy-4-oxonaphthylideneamino) N - [β-(β-hydroxyethylamino)-ethyl] benzamide) in mice infected with Col-SK or MM virus. This substance is not soluble in water but may be rendered highly water-soluble through coupling with a reducing agent such as sodium bisulfite. This process is reversible by oxidation. The solubilized compound is designated as Ro 2-3532/1. Through cooperation with the above workers we were able to examine the chemotherapeutic properties of this substance in this Laboratory. The results are presented here. In chemotherapeutic studies of a neurotropic viral disease it is obviously of advantage to limit the experimental approach to efforts designed to restrain the virus from entering the central nervous system rather than to attempt to influence is intraneural spread. Hence, the Columbia-SK type of virus was chosen for this work. As is well-known, Col-SK virus—in contrast to Yale-SK, Lansing, or MEF virus—possesses a high and constant degree of infectivity in rodents by various peripheral channels of inoculation.

* Aided by a grant from the Sister Elizabeth Kenny Foundation.

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