tion of the deposition of the labeled antibodies in freeze-dried sections of various tissues suspected of being sources of orosomucoid.

Summary. A homogeneous glycoprotein, orosomucoid, isolated from normal human serum was found to be weakly antigenic in chickens. The antibody-antigen system appeared to consist of a single antigen and its specific antibody. A procedure for determining immunochemically the orosomucoid content of serum has been described. The results show a reasonably good correlation of orosomucoid with the levels of the seromucoid fraction (mucoprotein) of normal and pathological serum.

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Received October 20, 1955. P.S.E.B.M., 1955, v90.

Inhibition Studies with *Streptococcus faecalis*. Quinolines. (22122)

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Farber and his associates(1) observed that the anti-folics may be used to treat leukemia. This observation gave impetus to the synthesis and examination of metabolite blocking compounds. One outgrowth of this was the observation by Hitchings and his co-workers (2,3) that some anti-folics may have antimalarial properties. In view of these findings we have examined compounds initially prepared for testing against malaria(4-9) for their ability to interfere with the utilization of folic acid.

In this communication the results of tests on 93 quinolines for anti-folic properties are given. It may be indicated at the outset that some of the most useful anti-malarials are without effect against folic acid. While some specificity of activity was suggested by the study, the compounds do not compare favorably with some of the known anti-folics. Accordingly, the results appear to be only of theoretical interest at this time.

Experimental. The method of test was based on the use of *Streptococcus faecalis* (ATCC No. 8043) with a slightly modified version of the medium of Teply and Elvehjem (10). The modifications consisted of omitting the xanthine from the medium and of substituting "vitamin-free" casein hydrolysate (Nutritional Biochemicals) for the charcoal treated preparation suggested by the above authors. Under our conditions of test, with increasing amounts of folic acid in the medium up to 1 mμg per ml of medium, the

TABLE I. Quinolines Which Interfere with the Utilization of Folic Acid.

Compound*	Derivatives	Activity vs. folic acid, μg/ml
4-$NH(CH_2)_2N(C_6H_{13})_2$	3-CH_3 7-Cl(4)	1.6/ 8
4-$NH(CH_2)_4N(C_2H_5)_2$	3-NO_2 7-Cl	40 /200
4-$CH(C_6H_5)COO(CH_2)_2N(C_2H_5)_2$(8)		"
4-$COO(CH_2)_2S(CH_2)_2N(C_2H_5)_2$	2-OC_4H_9	"
4-$COO(CH_2)_2S(CH_2)_3NC_5H_{10}$	"	"
4-$SCH_2CONH(CH_2)_2N(C_2H_5)_2$	3-CH_3 7-Cl(9), 3-Br 7-Cl(9), 3-CH_3 6-OCH_3(9), 3-CH_3 6-Br(9)	"
8-$NH(CH_2)_3N(C_2H_9)_2$	6-OCH_3	8 / 40

* Six publications, considered representative, are cited for the chemistry of compounds given in the Tables. A more complete listing of compounds tested against malaria may be seen in Wiselogle(12) or in the F. S. A. monograph(13).

growth, as judged by acid production, increased from blanks of about 0.5 ml of N/10 alkali per tube up to 9.5 to 10 ml of alkali. For the test, about half maximum growth was obtained with 0.32 mμg of folic acid per ml of medium. Accordingly, the test compounds were run against this amount of folic acid in the medium. They were added to duplicate tubes in amounts of 1.6, 8.0, 40 or 200 μg per ml of medium. The tubes were plugged, sterilized for about 12 minutes at 120°C, cooled, inoculated(11) with a drop of a suspension of the organism, and incubated for 3 days at 37°C. At the end of this time, the growth was estimated titrimetrically.

Results. The compounds tested and their ability to interfere with the utilization of folic acid are summarized in Tables I and II. An arbitrary ratio term is given in the Table to evaluate the compounds which interfere with the growth of the organism. The lesser amount is that at which little or no interference with the growth of *S. faecalis* occurred, while the larger amount is that at which growth was essentially completely prevented.

From the results summarized in Table I it may be seen that 10 of the quinolines were toxic to the organism. To determine the nature of the toxicity, the inhibitory activity was further differentiated. With 2.0 mμg of citrovorum factor(14) per ml of medium as the limiting nutrient, in place of folic acid, the mercapto quinoline N-(2 diethylaminoethyl) - (7-chloro-3-methylquinolyl-4) - mercaptoacetamide was no longer inhibitory. With 2.5 μg of thymine(15,16) per ml of medium as the limiting nutrient in place of folic acid 7 - chloro-4-(4-diethylaminobutylamino)-3-nitroquinoline was no longer inhibitory. Finally, with 2.5 μg of thymidine(17) per ml of medium as the limiting nutrient in place of folic acid, the mercaptoquinoline N-(2 - diethylaminoethyl) - (6 - methoxy-3-methylquinolyl - 4) - mercaptoacetamide was no longer inhibitory.

The inhibitory properties of the foregoing 3 quinolines, while interesting, are considerably below those of some of the better known anti-folics. For example, in the presence of 0.32 mμg of folic acid per ml of medium under the above conditions of test the inhibition ratios were 0.008/0.04 μg for aminopterin, 0.00032/0.0016 μg for amethopterin, and 0.0016/0.008 μg for pyrimethamine (Daraprim) per ml of medium. Thus the latter were 5000-fold (or more) as active as the above 3 quinolines. Accordingly, the results appear to be of no more than theoretical interest at this time.

Seven of the quinolines of Table I which blocked the utilization of folic acid were still inhibitory with thymidine in the medium as the limiting nutrient. Whether the inhibitory activity of these compounds was due to a block in the nucleic acid metabolic sequence or whether it was due to interference in some other metabolic sequence is not known.

The anti-malarials given in Table II, in amounts up to 200 μg per ml of medium, did not inhibit the organism in the presence of limiting amounts of folic acid. This included chloroquine (Aralen), hydroxychloroquine†

† Not to be confused with oxychloroquine.

(Plaquenil), sontoquine, primaquine, and isopentaquine. The biguanide anti-malarial Paludrine in our hands was likewise inert, although it is clearly an anti-folic with the *L. casei* test system(18). Thus, the foregoing anti-malarials differ from pyrimethamine, which is a strong inhibitor of folic acid with *S. faecalis*.

Accordingly, it may be concluded that an examination of 93 quinolines, prepared in

TABLE II. Quinolines Which Do Not Interfere with the Utilization of Folic Acid.

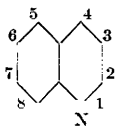
Compound 	Derivatives
a. 4-amino quinolines	
bis[(4-NH_2,2-CH_3,6-quinolyl)-NHCO]$=C(CH_3)(C_3H_7)$	
4-$NH(CH_2)_2NHCH_2CH_2OH$	7-Cl, 3-CH_3, 7-Cl
" $N(C_2H_5)_2$	3-CH_3 7-Cl
" $N(C_4H_9)_2$	"
4-$NH(CH_2)_3N(C_2H_5)_2$	7-Cl(5), 3-CH_3 7-Cl (brachysan)
4-$NHCH_2CHOH\ CH_2N(C_2H_5)_2$	7-Cl (oxychloroquin)(6), 3-CH_3 7-Cl(6), 3-Br 7-Cl, 3-I 7-Cl, 5-Cl
4-$NHCH(CH_3)(CH_2)_2NC_4H_8O$	7-Cl
4-$NH(CH_2)_4N(C_2H_5)_2$	7-Cl(5), 7-Br(5), 6-OCH_3, 3-CH_3 7-Cl (norsontoquine)(6)
4-$NH(CH_2)_4N(C_4H_9)_2$	3-CH_3 7-Cl
" $_2CHOHCH_2N(C_2H_5)_2$	7-Cl
4-$NHCH(CH_3)(CH_2)_3N(C_2H_5)_2$	" (chloroquine)(5), 7-Br(5), 3-CH_3 7-Cl (sontoquine)(6), 3-CH_3 7-Br(6), 2-CH_3 7-Cl, 5-Cl
" $N(C_2H_5)(C_2H_4OH)$	7-Cl (hydroxychloroquine)
" NC_4H_8	7-Cl
4-$NHCH_2CH(C_6H_4OCH_3)(CH_2)_2N(C_2H_5)_2$	3-CH_3 7-Cl
" $(C_6H_5)(CH_2)_2N(C_2H_5)_2$	"
4-$NH(CH_2)_2S(CH_2)_2N(C_2H_5)_2$	7-Cl(7)
" $SO_2(CH_2)_2N(C_2H_5)_2$	"
b. Carbon linked at position 4	
4-$CH(C_6H_5)CONH(CH_2)_2N(C_2H_5)_2$	7-Cl(8)
" $(CH_2)_2N(CH_3)_2$	5-Cl(8)
" $(CH_2)_2N(C_2H_5)_2$	7-Cl(8)
4-$C(C_6H_5)(CN)(CH_2)_2N(CH_3)_2$(8)	" , 6-OCH_3(8)
" $(CONH_2)(CH_2)_2N(CH_3)_2$	5-Cl(8)
" $(CONH_2)(CH_2)_2N(C_2H_5)$	7-Cl(8)
4-$CH(C_6H_5)COO(CH_2)_2N(C_2H_5)_2$	"
c. 4-mercaptoquinolines	
4-$SCH_2CONH(CH_2)_2N(C_2H_5)_2$(9)	7-Cl(9), 2-CH_3 7-Cl(9), 5-Cl(9), 3-CH_3(9), 3,8-$(CH_3)_2$(9), 3-CH_3 8-OC_2H_5(9), 6-OCH_3(9)
4-$S(CH_2)_3N(C_2H_5)_2$	7-Cl(4), 3-CH_3 7-Cl(4)
" NC_5H_{10}	7-Cl(4)
4-$SCH_2COO(CH_2)_2N(CH_3)_2$	7-Cl(9), 5-Cl(9)
" $N(C_2H_5)_2$(9)	" " , 6-OCH_3(9)
" NC_5H_{10}	" "
" NC_4H_8O	5-Cl(9)
4-$SCH(CH_3)COO(CH_2)_2N(C_2H_5)_2$	7-Cl(9)
4-$S(CH_2)_5NHC_3H_7$	" (4)
4-$S(CH_2)COO(CH_2)_2N(C_2H_5)_2$	" (9)
4-$S(CH_2)_2COO(CH_2)_3N(C_2H_5)_2$	" (9)
d. Oxygen linked at position 4	
4-OH	3-CH_3(6), 7-Cl 2-COOH(5)
4-$OCH(CH_3)(CH_2)_3N(C_2H_5)_2$	7-Cl(4)
4-$O(CH_2)_2S(CH_2)_2N(C_2H_5)_2$	7-Cl

TABLE II (continued).

Compound	Derivatives
e. 8-amino quinolines	
8-NH_2	6-OCH_3(amichin)
8-$NHCH(CH_3)CH_2NHC_3H_7$	6-OCH_3
8-$NH(CH_2)_3N(C_4H_9)_2$	"
" $(CH_2)_4NH_2$	"
8-$NHCH(CH_3)(CH_2)_3NH_2$	" (primaquine), 4-CH_3 6-OCH_3
" NHC_3H_7	" (isopentaquine)
" $N(C_2H_5)_2$	6-OH
8-$NH(CH_2)_5NH_2$	6-OCH_3
" $(CH_2)_2S(CH_2)_2N(C_2H_5)_2$	" (7)
" $(CH_2)_3S(CH_2)_2N(C_2H_5)_2$	" (7)
" $S(CH_2)_3N(C_2H_5)_2$	"
f. 2-mercapto quinolines	
2-$S(CH_2)_2N(C_2H_5)_2$(4)	
" $(CH_2)_3N(C_2H_5)_2$	4-CH_3(4)
g. Carbon linked at position 1	
1-$CH_2C_6H_4SO_2NH_2$	1-Br

connection with an anti-malarial program, has not disclosed compounds with immediate application.

Summary. 1. Ninety-three quinolines were tested against *Streptococcus faecalis* in the presence of limiting amounts of folic acid. Ten of the quinolines were toxic to the organism when they were in the medium at concentrations of 8.0 to 200 μg/ml. The toxicity of 3 of the 10 inhibitors could be abolished. The inhibition of N-(2-diethylaminoethyl)-(7-chloro-3-methylquinolyl-4) - mercaptoacetamide was reversed by citrovorum factor, that of 7-chloro-4-(4-diethylaminobutylamino)-3-nitroquinoline was abolished by thymine, and that of N-2-diethylaminoethyl-6-methoxy-3-methylquinolyl - 4) - mercaptoacetamide was overcome by thymidine. The remaining 7 were not studied further since their degree of inhibition did not compare sufficiently favorably with that of some of the better known anti-folics. 2. None of 5 anti-malarials examined (chloroquine, hydroxychloroquine, sontoquine, primaquine, and isopentaquine) interfered with the utilization of folic acid. Thus, these differ metabolically from pyrimethamine which has been shown to be both an anti-folic and an anti-malarial.

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Received October 24, 1955. P.S.E.B.M., 1955, v90.