Mucolytic Enzyme Systems. XIV. Effect of Certain Quinolines on Hyaluronidase and Its Serum Inhibitor.* (18046)

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The occurrence of the invasive factor, hyaluronidase, in many bacteria is well known. That it might also be employed by animal parasites in their invasive functions is a possibility that has been relatively neglected. However, Levine *et al.*(1) have recently demonstrated that cercariae of *schistosoma mansoni* contain hyaluronidase, and, with the collaboration of Dr. F. G. Wallace of our Zoology Department, an investigation is now underway to determine whether it is present in other parasites as well.

It was observed by Bieter and coworkers(2) that certain 8-amino quinolines exerted a pronounced chemotherapeutic effect in mice infected with *schistosoma mansoni,* while other chemically related compounds were relatively ineffective. The present communication is concerned with an attempt to determine whether the difference in the action of some of these compounds is connected to their effect, *in vitro,* on hyaluronidase or its serum inhibitor.

Materials and methods. The preparation of the hyaluronidase from bull testes followed the procedure used previously(3), and the original method for the preparation of the hyaluronic acid from human umbilical cords was used(3) with the modifications subsequently introduced(4,5). The enzyme activity and its inhibition were determined by the viscosimetric method(3) utilizing those changes in detail that were later employed(5).

The reaction mixture for studies of the effect of compounds on the hyaluronidase was prepared by adding 1.5 cc of the aqueous solution of the compound to be tested to 0.5 cc of the borate buffered enzyme, allowing to stand 6-10 min at 38°C, and then mixing with 4.0 cc of the veronal buffered substrate. 5.0 cc was pipetted into the viscosity tube for measurements at 38°C. The reaction mixture used for studies of the effect of compounds on the serum inhibitor of hyaluronidase was made up in a similar manner; the only difference being that normal human blood serum was first added to the solution of the compound to be tested so that 1.5 cc of the solution contained 0.04 cc of serum. When serum alone was used as the inhibitor, 0.04 cc of it was diluted with distilled water to 1.5 cc for addition to the 0.5 cc of buffered enzyme. The results were expressed as percent inhibition of the hyaluronidase activity(4).

Results and discussion. Two compounds, rosaniline and plasmochin, that had little chemotherapeutic effect in mice infected with *schistosoma mansoni,* were compared with two chemically related effective compounds, 6-methoxy-8-(β-diisobutylaminomethyl) amino-quinoline dihydrochloride (SN 2842) and 6-methoxy-8-(diisobutylaminoisopropyl) amino-quinoline dihydrochloride (SN 3501). However, of the above compounds, only rosan-

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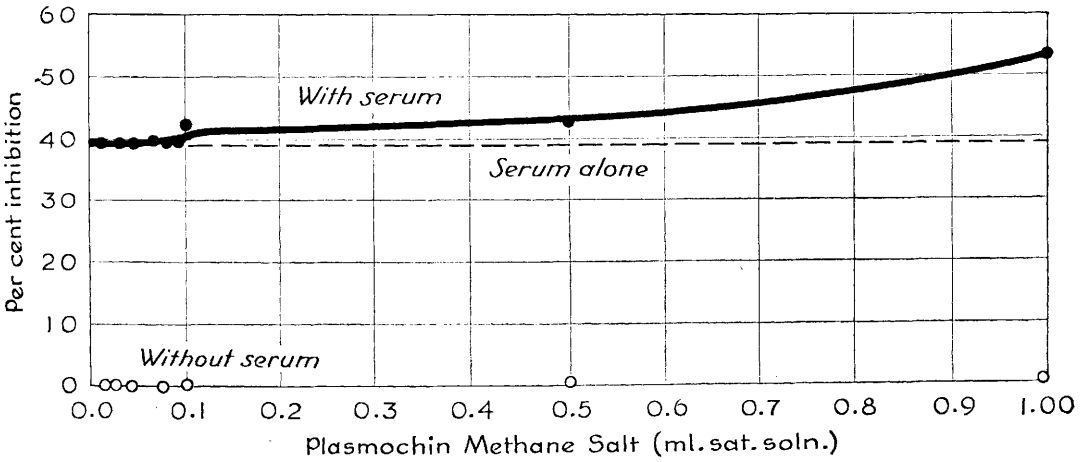

FIG. 1.
Effect of plasmochin on hyaluronidase and its serum inhibitor.

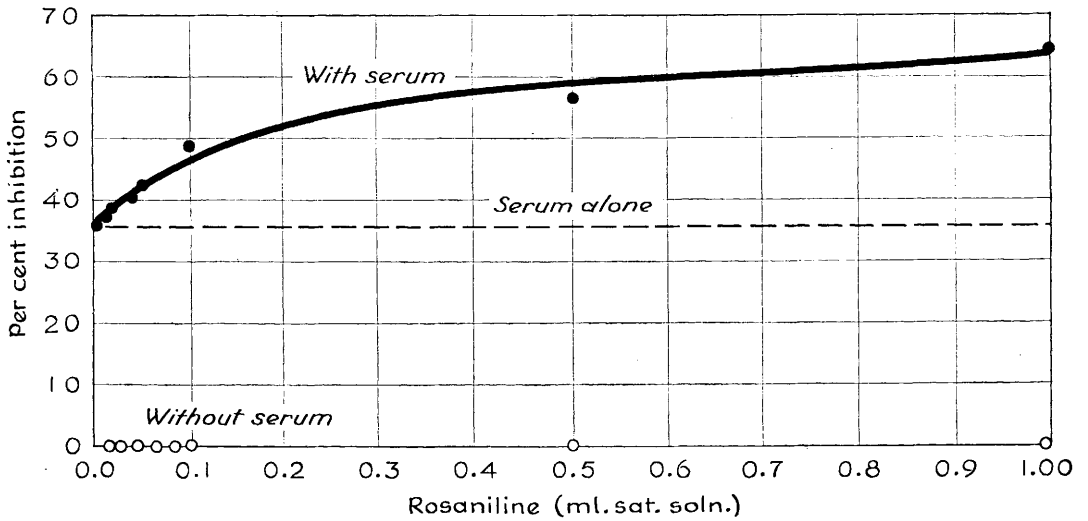

FIG. 2.
Effect of rosaniline on hyaluronidase and its serum inhibitor.

iline was found to exert a prophylactic effect in the infection. To produce this effect the compound must be administered daily for several weeks before, or within the first 2 weeks after, infection. The results are given in Fig. 1-3. The abscissae in all figures refer to quantity of drug per cc final reaction mixture.

It is apparent that, at the concentrations used, neither plasmochin nor rosaniline, Fig. 1-2, had a demonstrable direct effect on the hyaluronidase. Both exhibited some potentiation of the serum inhibitor, the influence being more pronounced with rosaniline. In both instances it will be noted that saturated (25°C) aqueous solutions of the drugs were employed; this was necessitated by the relative insolubility of the substances.

In contrast to these findings, the two compounds chemotherapeutically effective were potent inhibitors of the hyaluronidase at concentrations greater than 0.32 mg per cc reaction mixture; complete inhibition was attained at 1.28 mg per cc, Fig. 3. It may also be seen that these two substances inactivate the naturally occurring serum inhibitor at the same time they exert their direct inhibition on the hyaluronidase. The

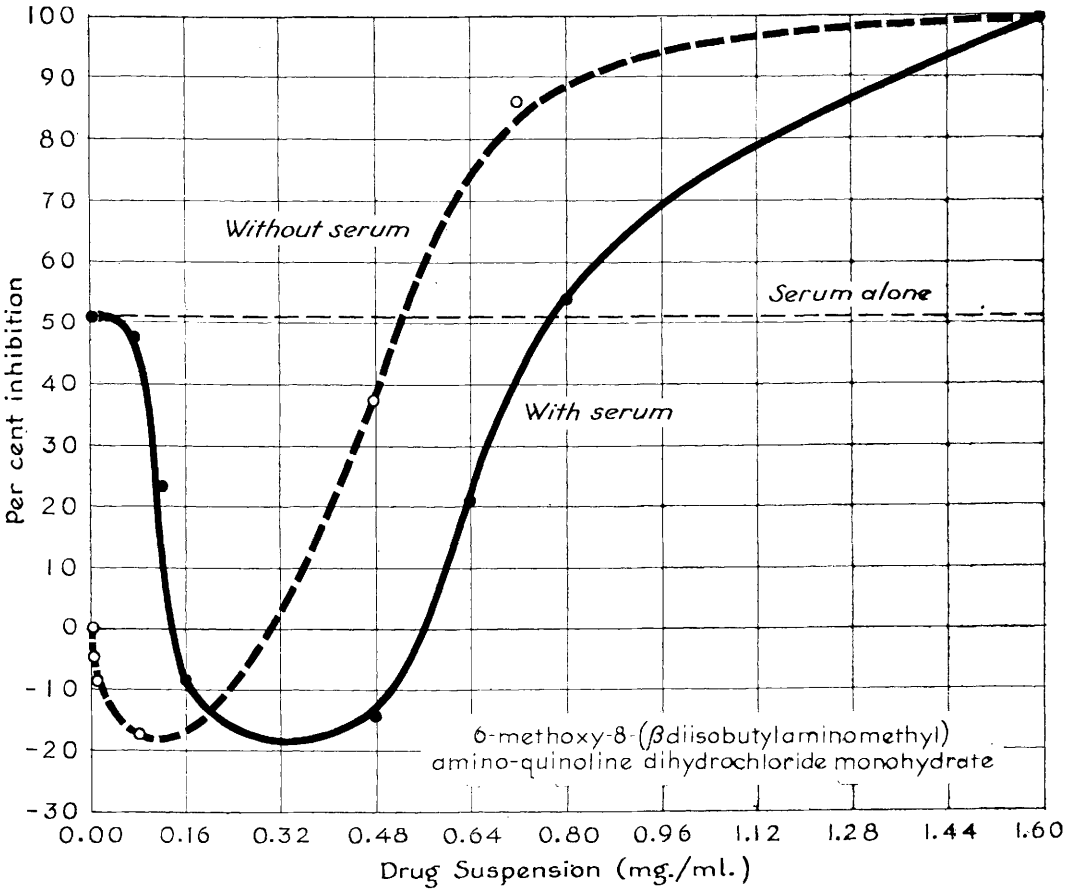

FIG. 3.

Effect of 6-methoxy-8-(β-diisobutylaminomethyl) amino-quinoline dihydrochloride monohydrate on hyaluronidase and its serum inhibitor. Curves practically identical with these were obtained for 6-methoxy-8-(diisobutylaminoisopropyl) amino-quinoline dihydrochloride.

inactivation of the serum inhibitor is manifest at the lowest concentrations of the drugs, and this effect is overtaken by the direct inhibition of the enzyme by the compounds at higher concentrations. Thus, with or without the presence of the serum inhibitor, the drugs produce a complete cessation of the enzyme activity at 1.60 mg per cc. No explanation can be offered at present for the slight degree of activation of the hyaluronidase in the absence of serum that was observed at the lowest drug concentrations.

Summary. Two compounds, 6-methoxy-8-(β - diisobutylaminomethyl) aminoquinoline dihydrochloride and 6-methoxy-8-(diisobutylaminoisopropyl) aminoquinoline dihydrochloride, which are chemotherapeutically effective in mice infected with *schistosoma mansoni,* have been shown to be potent inhibitors of hyaluronidase although they inactivate the serum inhibitor of the enzyme. Chemically related rosaniline and plasmochin, that have little chemotherapeutic effect, had no demonstrable direct effect on the hyaluronidase, although both potentiated the serum inhibitor to some degree.

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