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Influence of Prontosil-soluble on Beta Hemolytic Streptococci Growing in Tissue Culture Media.*

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On the basis of *in vitro* experiments with the hydrochloride of 4′ sulfonamide-2, 4 diaminoazobenzol (Prontosil) and other sulfonamide compounds, Levaditi and Vaisman[1] concluded that certain of these drugs neutralized streptococcal hemolysin and leukocidin.

Meyer[2] reported that Prontosil shows some neutralizing power for streptococcal hemolysin. He also states that "Prontosil" added to serum-broth " . . . prevents the production of toxins." We are not certain whether Meyer's conclusions should be interpreted as applying to Prontosil or Prontosil-soluble, *i. e.,* the disodium salt of 4′ sulfamidophenyl-2 azo-7 acetylamino-1 hydroxynaphthalene-3, 6 disulfonic acid, (or both). He lists both in his general statement on chemotherapy but does not refer to Prontosil-soluble again, although some of his expressions in the tables might be taken to imply that he was using Prontosil-soluble.

Recently Gross, Cooper, and Lewis[3] investigated the hemolysin-neutralizing power of a number of drugs including Prontosil-soluble. Of the related compounds, *i. e.,* sulfanilamide, Prontosil and Prontosil-soluble, the latter was the only one showing any significant neutralizing power.

With a view to determining the influence of Prontosil-soluble on streptococcal hemolysin under experimental conditions which are as physiological as can be attained *in vitro,* we have studied its effect on the hemolysing power of colonies of β streptococci growing in tissue-culture medium containing the drug (concentration 1: 1000).

The culture-clots were made of 1 part autogenous heparinized rabbit-plasma and 2 parts autogenous serum-extract of 6-day chick

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1 Levaditi, C., and Vaisman, A., *Compt. rend. Soc. de biol.,* 1935, **120**, 1077.

2 Meyer, F., *Quart. Bull. Sea View Hosp.,* 1937, **2**, 148.

3 Gross, P., Cooper, F. B., and Lewis, M., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 275.

embryos. The extract was inoculated with suitable dilutions (to make 10^{-5} to 10^{-8} in the final medium) of a strain of β streptococcus recovered from a fatal septicemia in man. Sufficient rabbit's erythrocytes were added to the extract to make a 5% suspension in the final medium. Clotting takes place promptly on mixing the plasma and extract. Cultures are incubated at 37.5°C in Maximow moist chambers. Details of the methods have been described previously.[4, 5, 6]

Colonies become visible in the cultures before lysis starts. Measurements of the colonial diameter and the zone of lysis can be made with accuracy at 60× magnification. The measurements are made with an eyepiece-micrometer previously standardized with a stage-micrometer (114 units = 1 mm). Photomicrographs can be made on Process film to show the diameter of both colony and zone of hemolysis.

The term "hemolytic index" is used to describe the hemolyzing power of a colony in relation to its diameter, *i. e.*, the diameter of the zone of hemolysis over the diameter of the colony.

Colonies of β streptococci growing in this medium containing 1:1000 Prontosil-soluble are qualitatively identical with colonies in the same medium without the drug.

Prontosil-soluble exhibits no bacteriostatic effect under these experimental conditions.

It is found, however, that colonies in the medium containing the drug show smaller zones of lysis in relation to their size, *i. e.*, lower hemolytic index.

In Table I an experiment is summarized showing the average size of the colonies and hemolytic zones in control and experimental sets. The hemolytic index is shown for both sets and the inhibition of the hemolytic zone (on the basis of diameter) for the experimental set.

It may be noted that hemolysin-production is continuous, once

TABLE I.

	Controls			Prontosil-soluble			
Time	Diam. colony	Diam. H zone	Hem. index	Diam. colony	Diam. H zone	Hem. index	% Hem. inhib.
18 hr	39.76	64.99	1.63	40.23	52.71	1.31	18.9
24 "	78.49	132.86	1.69	76.3	106.2	1.39	21.0
48 "	214.7	354.0	1.64	219.2	273.9	1.24	22.6
3 days	252.4	399.5	1.58	262.6	310.3	1.18	22.4

[4] King, J. T., *Arch. f. Exp. Zellforsch.*, 1930, **9**, 341.

[5] King, J. T., *Arch. f. Exp. Zellforsch.*, 1931, **10**, 467.

[6] King, J. T., *Arch. f. Exp. Zellforsch.*, 1937, **20**, 208.

started, under these conditions. In other experiments it has been observed to continue into the 5th day.

In some experiments we have observed somewhat greater inhibition of lysis than that seen in the series summarized in Table I. We have never seen anything approaching complete inhibition of lysis, however.

The technic used measures the inhibition of hemolytic damage in relation to control preparations. It does not distinguish between reduction in the amount of hemolysin formed and subsequent neutralization.

The results are in harmony with those obtained by others using neutralization-tests on active culture fluids. If Meyer's conclusions were intended to apply to Prontosil-soluble, then our results are not in accord with his finding that hemolysin-formation is prevented when β streptococci are grown in presence of the drug.

It seems to us that tissue-culture clots holding erythrocytes in fixed position in relation to the developing colonies provide a means of registering quantitatively the activity of hemolysin as formed and under conditions as nearly comparable to those existing in the body as can be arranged *in vitro* at present.

Conclusion. *Beta* hemolytic streptococci growing in tissue-culture media containing Prontosil-soluble, 1:1000, produce hemolysin. The amount available for erythrocytic destruction is moderately reduced as compared with controls. This effect is not secondary to bacteriostasis.

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Response of Leukocytes to Colonies of Streptococci Growing in Tissue-culture Media.*

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In discussing the mechanism of action of sulfanilamide, Mellon and Bambas[1] state, " . . . the growth inhibition brought about by the indirect action of sulfanilamide predicates a suspension in the pro-

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1 Mellon, R. R., and Bambas, L. L., *Med. Rec.*, 1937, **146**, 247.