

SIMULTANEOUS INJECTIONS OF STREPTOCOCCI AND DAHLIA IN THE GUINEA-PIG *

W. H. HOFFMAN, W. B. McCLURE, AND L. W. SAUER

From the Otho S. A. Sprague Memorial Institute Laboratory of the Children's Memorial Hospital, Chicago

The effect of anilin dyes on micro-organisms in vitro has been investigated by a number of workers, among whom may be mentioned Rozsahegyi,¹ Stilling,² Drigalski and Conradi,³ Simon and Wood,⁴ and Churchman.⁵ A review of the literature up to 1914 occurs in the first article by Simon and Wood. Since that time, reports by Shiga,⁶ Hall and Taber,⁷ and a second communication by Simon and Wood have appeared. The latter found that certain dyes in dilutions of 1:100,000, regardless of their color, may be capable of inhibiting the growth of certain bacteria. One of the dyes investigated, dahlia (a mixture of methyl violet and fuchsin), was found effective in inhibiting the growth of certain strains of streptococci when it was present in culture media in a concentration of 1:100,000.

Later, Ruhräh⁸ used dahlia in cases of tonsillitis and in various skin infections. Several cases of extensive furunculosis of the scalp in this hospital were treated locally with dahlia solution with apparently good results in one case. The possibility then occurred to us of intravenous injections of the dye in systemic streptococcus infections.

First, we determined the minimal lethal dose of a 1:1000 solution of dahlia in normal salt solution to be 5 c.c. for a guinea-pig of 200 gm. when injected intravenously. When the dahlia was given in doses of from one-eighth to one-half of the minimal lethal dose, the animal showed practically no ill effects, with the possible exception of an occasional slight loss in weight. In determining the effect of the intravenous injections of dahlia on streptococcus infections, we never have used more than the sublethal quantities of dahlia mentioned. Further control animals which were injected intraperitoneally with sterile

* Received for publication October 19, 1915.

¹ Centralbl. f. Bakteriöl., I. O., 1887, 11, p. 418.

² Anilin Farbstoffe als Antiseptika und ihre Anwendung in der Praxis, 1890.

³ Ztschr. f. Hyg. u. Infektionskrankh., 1912, 39, p. 283.

⁴ Am. Jour. Med. Sc., 1914, 142, p. 247; p. 524.

⁵ Jour. Exper. Med., 1912, 16, pp. 221, 822; 1913, 17, p. 373.

⁶ Ztschr. f. Immunitätsf., 1914, 18, p. 65.

⁷ Jour. Infect. Dis., 1914, 15, p. 566.

⁸ Am. Jour. Med. Sc., 1915, 149, p. 661.

broth, instead of a 24-hour broth culture of streptococci, showed no effects of broth injections.

We used various strains of hemolytic streptococci, isolated from scarlet-fever sore throats. The strains used, with the exception of D, grew in dahlia broth, 1:100,000 concentration. Strain D did not grow in this dilution, but showed scant growth in dahlia broth, 1:200,000 concentration. Churchman, working with gentian violet, and Simon and Wood, working with dahlia, etc., have shown that the dyes do not affect all strains of streptococci alike. Measured quantities (0.3 to 1.5 c.c.) of 24-hour broth cultures of streptococci were injected intraperitoneally before or soon after an intravenous injection of the 1:1000 dahlia solution. Duplicate sets of animals were frequently injected.

Early in our work we obtained an unexpected result: control animals receiving streptococci survived without presenting any appreciable signs of illness, while the other animals, receiving the same amount of broth culture intraperitoneally and an intravenous injection of dahlia much below the fatal dose, died within 24 hours. That this did not remain an isolated occurrence is seen from Table 1.

Unfortunately, it was not always possible to work with animals of the same weight, and this difference may have influenced the result. For instance, in Experiment 7, the one control animal which died weighed one-fifth less than the heavier animal which was killed by the combined injections. Nevertheless, the fact stands out clearly that in the combined injections the dahlia acts detrimentally. Experiment 8 is of special interest because the animal weighing 200 gm. received in the 2.5 c.c. of dahlia solution an amount of dahlia corresponding to 1:80,000 of the body weight. This same streptococcus failed to grow in dahlia broth, 1:100,000. The concentration of the dye in the animal (its total weight being taken into consideration) was greater than that necessary to inhibit the growth of the streptococcus in vitro. In one instance only did it happen that, in a set of 4 animals, 2 receiving streptococci died, while only 1 of 2 receiving dahlia and streptococci succumbed. In another set of experiments 1 animal of each group died and 1 survived. None of the animals receiving Strain C died; on the other hand, some streptococci were so virulent that all the animals succumbed.

In an attempt to investigate the failure of dahlia to act beneficially in streptococcus infections, even when the strain was killed by the dye in vitro, some experiments were made testing the effect of serum as the culture medium. Blood was withdrawn from the hearts of guinea-pigs,

allowed to coagulate, centrifuged, and the serum collected under aseptic conditions. To measured quantities of the serum thus obtained concentrated solutions of dahlia were added in various proportions.

TABLE 1

RESULTS OF SIMULTANEOUS INJECTIONS OF STREPTOCOCCI AND DAHLIA INTO GUINEA-PIGS

Experiment	Weight of Guinea-pig in Grams	Strain of Streptococcus	Dosage	Result
1	200 200	A A	1 c.c. 24 hour broth culture The same with 1 c.c. dahlia	Survived Death in 16 hours
2	250 230 190	A A A	1 c.c. 24 hour broth culture The same with 1 c.c. dahlia The same with 1 c.c. dahlia	Survived Death in 70 hours Death in 70 hours
3	405 350 315	B B B	2 c.c. 24 hour broth culture 1.5 c.c. of 24 hour broth culture with 1.5 c.c. dahlia 1.5 c.c. of 24 hour broth culture with 1.5 c.c. dahlia	Survived Survived Continuous loss of weight until death on 8th day Streptococcus recovered from heart
4	410 380	B B	2 c.c. 24 hour broth culture The same with 1 c.c. dahlia	Survived Death in 36 hours. Streptococcus recovered from heart
5	310 400 380 370 320	B B B B B	2 c.c. 24 hour broth culture The same The same with 1.5 c.c. dahlia The same with 1 c.c. dahlia The same with 1 c.c. dahlia	Survived Survived Continuous loss of weight until death on 7th day Survived Death in 60 hours. Streptococcus recovered from heart
6	540 580	B B	2 c.c. 24 hour broth culture The same with 1 c.c. dahlia	Survived Continuous loss of weight until death on 14th day
7	215 300 225 270	D D D D	0.5 c.c. 24 hour broth culture The same The same with 2 c.c. dahlia The same with 2 c.c. dahlia	Death in 33 hours. Streptococcus recovered from heart Survived Death in 18 hours. Streptococcus recovered from heart Death in 48 hours. Streptococcus recovered from heart
8	195 230 200 235	D D D D	0.5 c.c. 24 hour broth culture The same The same with 2.5 c.c. dahlia The same with 2.5 c.c. dahlia	Survived Survived Death in 18 hours. Streptococcus recovered from heart Survived
9	230 270 240 250	D D D D	1 c.c. 24 hour broth culture The same The same with 2.5 c.c. dahlia The same with 2.5 c.c. dahlia	Survived Death in 23 hours. Streptococcus recovered from heart Death in 18 hours. Streptococcus recovered from heart Death in 19 hours. Streptococcus recovered from heart

The tubes were then inoculated with Strain D (controlled anew). The cocci failed to grow in dahlia broth, 1:100,000, but there was an occasional scant growth in dahlia broth, 1:200,000. In the presence

of serum a concentration of the dye of 1:10,000 was necessary to inhibit growth, while a concentration of 1:20,000 was not effective. The dye therefore fails to fulfil the postulate made by Wright:⁹ "that a bactericidal drug in order to have chemo-therapeutic effect must not only be capable of killing the micro-organisms in vitro, but also must not combine with any of the chemical constituents of the normal body, otherwise that portion will be rendered inert towards the bacteria." These results explain why dahlia, under the conditions of our experiments, fails to act beneficially, but they do not explain the injurious action of the combined injections.

DISCUSSION

Our experiments show that a substance capable, in high dilutions of broth cultures, of destroying streptococci, when injected intravenously may not only fail to have a beneficial effect on experimental streptococcal infections, but, on the contrary, may act injuriously. It is not unlikely that the virulence of the different strains enters into consideration. Thus, it may happen that neither the controls nor the animals receiving the combined injections are affected in any way. The other extreme is that all animals succumb with or without dahlia. Between these two extremes we have strains of such degree of virulence that the animals receiving the streptococci alone, survive, while those receiving the combined injections die. We incline to the assumption that the dye renders the organism less resistant to the attack of the streptococci—in the sense suggested by Ehrlich, combining with the elements of the living body and thus interfering with their physiologic function.

The question naturally arose whether the dahlia would injure some of the defensive mechanisms of the organism. Therefore, one of us (Sauer) tested the influence of dahlia on the opsonic power of the guinea-pig serum as well as the possibility of an influence on the complement of the blood. In the opsonic experiments guinea-pig leukocytes and guinea-pig serum were used together with streptococci or staphylococci. The dahlia was added in a total concentration of 1:4000. In the control experiments the dahlia salt solution was replaced by physiologic salt solution. In 10 series of experiments no influence of the dahlia on the opsonic index could be observed.

On the addition of dahlia, in total concentrations varying between 1:400,000 and 1:2000, to a hemolytic system there was no influence

⁹ *Lancet*, 1912, 183, pp. 1633, 1701.

whatever on the reaction. Neither did the serum of a guinea-pig (300 gm.) that had received 3 c.c. of a dahlia solution, 1:1000, intravenously 15 minutes previously, behave differently from the serum obtained before the dahlia injection.

In conclusion, it seems evident from these experiments that intravenous injection of dahlia in general streptococcal infections is not warranted as a therapeutic measure.