Effect of Azosulfamide (Neoprontosil) and Sulfanilamide on Experimental Welchii Infection in Mice[1]

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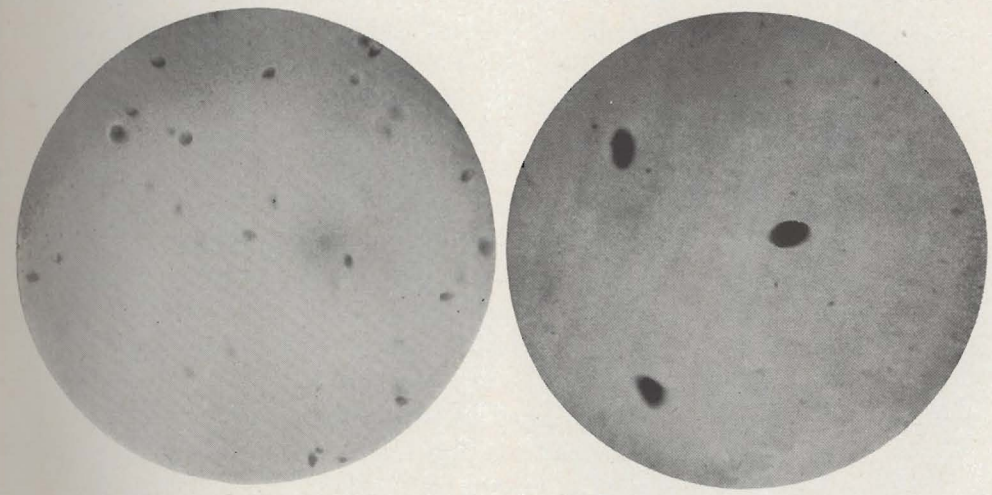
Bliss and Long[2] have studied the use of sulfanilamide in the treatment of experimental *Clostridium welchii* infection in mice, and have found that the beneficial results obtained are due to the bacteriostatic action of the drug. Spray[3] studied the direct bacteriostatic action of sulfanilamide, disulfamide, and Prontosil soluble upon a series of sporulating anaerobes, and found that the drugs exert an apparent specific selective action against certain species such as *Clostridium tetani*, *Cl. lentoputrecens*, *Cl. novyi*, *Cl. septicum*, and *Cl. histolyticum;* while *Cl. welchii*, *Cl. sporogenes*, *Cl. bifermentans*, as well as *Cl. botulinus* type A, are scarcely affected. He also finds that the bacteriostatic activity increases in the following order: Prontosil soluble, sulfanilamide, and disulfamide. Sadusk and Manahan[4] report that sulfanilamide exerts a definite inhibiting action on the growth of *Cl. welchii*, and that its bacteriostatic action is inversely proportional to the number of organisms used in the inoculum. Kendrick[5] studied the therapeutic value of Neoprontosil, sulfanilamide, and sulfapyridin in gas gangrene on infections produced experimentally in guinea pigs. He reports that these drugs in the doses used by him did not provide protection against infection. Carpenter and Barbour[6] report that the oral administration of Neoprontosil prevented death in mice given the toxin of *Cl. welchii*.

There are numerous reports in the literature on the clinical use of sulfanilamide and Neoprontosil in the cases of gas gangrene, but the results disagree as much as those cited above in the cases of experimental infection. Due to these discrepancies, we decided to study the effect of Neoprontosil and sulfanilamide on experimental *Cl. welchii* infection in mice.

Strain used. The strain of *Cl. welchii* used in the following experiments was isolated from a case of gas gangrene, and is similar in its biological characteristics to the classical *Cl. welchii* described in text books. A culture was passed through Swiss mice before culturing in glucose broth, in order to retain its virulence. Its minimal lethal dose for mice under these conditions was 0.1 cc. of a 24-hour glucose broth culture.

1. Received for publication December 3, 1940.
2. A. L. Bliss and P. H. Long, *J.A.M.A.*, 109:1524. 1937.
3. R. S. Spray, *J.Lab.Clin.Med.*, 23:609. 1938.
4. J. F. Sadusk and C. P. Manahan, *J.A.M.A.*, 113:14. 1939.
5. D. B. Kendrick, *J.Clin.Investigation*, 18:593. 1938.
6. C. M. Carpenter and G. M. Barbour, *Proc.Soc.Exp.Biol.&Med.*, 41:255. 1939.



I II

FIGURE I: *Clostridium welchii* colonies plated on agar from a three-hour broth culture. Culture diluted 1 x 100.

FIGURE II: *Clostridium welchii* colonies plated on agar from a three-hour broth culture containing 1% of sulfanilamide. Culture diluted 1 x 100.

PLACA I: Colonias de *Clostridium welchii* en placa de agar inoculado con un cultivo en caldo de tres horas. Cultivo diluído al 1 x 100.

PLACA II: Colonias de *Clostridium welchii* en placa de agar inoculado con un cultivo en caldo de tres horas, conteniendo sulfanilamida al 1 por ciento. Cultivo diluído al 1 x 100.

Experiment one. Ninety mice, separated into three groups of 30 each, were inoculated intramuscularly with ascending doses of a 24-hour glucose broth culture of *Cl. welchii*. The first group was injected with 0.05 cc., the second with .075 cc., and the third with 0.1 cc. Seventy-nine of these mice (87.7%) died before the lapse of 72 hours, 20 (66%) in the first group, 29 (96.6%) in the second, and all (100%) in the third.

Ninety additional mice were similarly grouped and inoculated, but besides the corresponding dose of the organism each was given 1 cc. of Neoprontosil (5% solution) intramuscularly at the time of inoculation. Eighty-five (94.4%) mice died within 72 hours, 25

(83.3%) in the first group, and all (100%) in the second and third groups (see Table I, Experiment 1).

Ten mice were similarly inoculated with 1 cc. of Neoprontosil only, and all survived.

In the above experiment we found that Neoprontosil did not protect mice from *Cl. welchii* infection.

Experiment two. A 24-hour glucose broth culture was centrifuged at high speed. The supernatant was set aside and the sediment was repeatedly washed with saline. After repeated centrifugations and washings, enough saline was added to the cells to restore the original volume of the culture, and 0.1 cc. of this suspension was used for the inoculations.

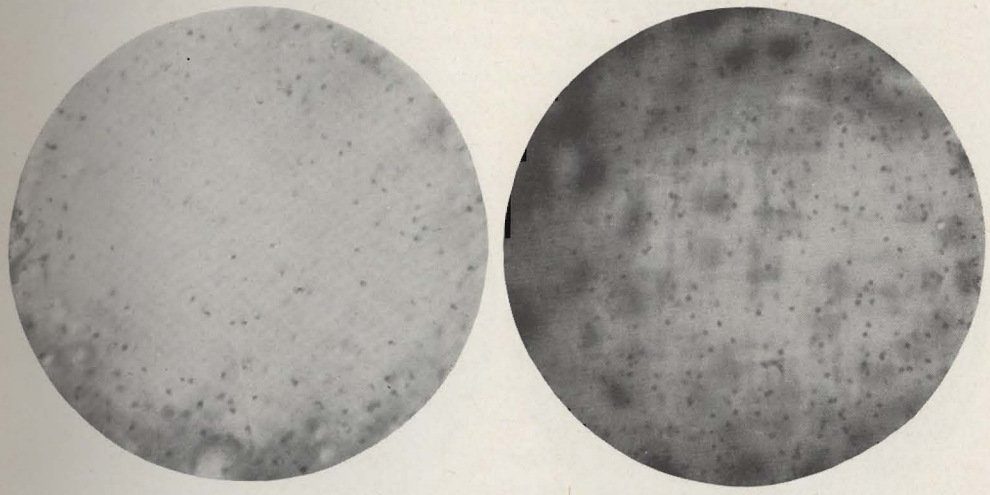
One hundred and fifty mice were separated into five groups of 30 mice each. Group One was inoculated intramuscularly with 0.1 cc. of washed cells of *Cl. welchii*. Group Two was inoculated intramuscularly with 0.1 cc. of washed cells and 0.1 cc. filtrate from the same culture. Group Three was inoculated with 0.1 cc. of washed cells and 0.1 cc. filtrate, plus 1 cc. of Neoprontosil. Group Four was inoculated with 0.1 cc. of washed cells plus 1 cc. of Neoprontosil, and Group Five, with 0.1 cc. filtrate plus 1 cc. of Neoprontosil.

Four mice (13.3%) died in Group One; 7 (23.3%) died in Group Two; 27 (90%), in Group Three; 27 (90%), in Group Four; and none in Group Five. (See Table II, Experiment 2.) In this case the injection of Neoprontosil with washed cells of *Cl. welchii* led to the development of the corresponding infection, while the washed cells alone showed little tendency to develop in the tissues after inoculation.

In repeating the above experiment, the dose of Neoprontosil was lowered, 0.75 cc., 0.50 cc., and 0.25 cc. being used, and in every case when the dose of Neoprontosil was given with washed cells of *Cl. welchii*, the animal succumbed to infection. On examining the lesions produced, one could observe normal phagocytic activity and numerous organisms within the tissue of the lesions produced.

We decided to investigate the effect of the drug administered orally.

Experiment three. One hundred and fifty mice were divided into five groups of 30 mice each. Group One was given 1 cc. of Neoprontosil orally, and none of the animals died. Group Two was given one lethal dose of the culture of *Cl. welchii* by mouth, and all of the mice survived. Group Three was given ½ cc. of Neoprontosil and one minimal lethal dose of a culture of *Cl. welchii* by mouth, and all survived. Group Four was given one minimal lethal dose of the culture of *Cl. welchii* intramuscularly; 29 animals (96.6%) died and one survived. Group Five was given ½ cc. of Neoprontosil orally and one minimal lethal dose of *Cl. welchii* culture intramuscularly; 23 (76.6%) died and 7 survived. In this experiment the culture of *Cl. welchii* was inactive when given by mouth. Neoprontosil, when given orally, gave a slight protection against *Cl. welchii* infection in mice. (See Table III, Experiment 3.)



III IV

FIGURE III: *Clostridium welchii* colonies plated on agar from a three-hour broth culture containing 2½% of Neoprontosil. Culture diluted 1 x 100.

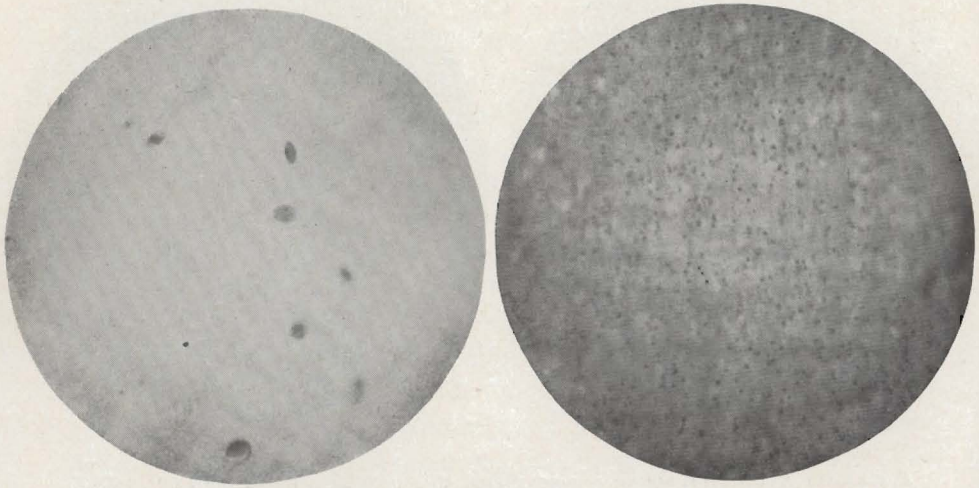
FIGURE IV: *Clostridium welchii* colonies plated on agar from a six-hour broth culture. Culture diluted 1 x 100.

PLACA III: Colonias de *Clostridium welchii* en placa de agar inoculado con un cultivo en caldo de tres horas, conteniendo 2½ por ciento de neoprontosil. Cultivo diluído al 1 x 100.

PLACA IV: Colonias de *Clostridium welchii* en placa de agar inoculado con un cultivo en caldo de seis horas. Cultivo diluído al 1 x 100.

Experiment four. In this experiment we compared the action of Neoprontosil with that of sulfanilamide, using repeated doses. A 1 per cent solution of sulfanilamide was used. One hundred mice were divided into five groups of 20 mice each. Group One was given one minimal lethal dose of the culture of *Cl. welchii* intramuscularly. Nineteen mice (95%) died. Group Two was given one minimal lethal dose of the culture of *Cl. welchii* plus 1 cc. of Neoprontosil intramuscularly every 12 hours—two doses in all. All the 20 animals

died. Group Three was given one minimal lethal dose of the culture of *Cl. welchii* plus ½ cc. of Neoprontosil orally every 12 hours—three doses in all; 13 animals died (65%) and 7 survived. Group Four was given one minimal lethal dose of the culture of *Cl. welchii* plus 1 cc. of a one per cent solution of sulfanilamide intramuscularly every 12 hours—3 doses in all; 2 animals (10%) died and 18 survived. Group Five was given one minimal lethal dose of a culture of *Cl. welchii* plus ½ cc. of a 1 per cent solution of sulfanilamide orally every 12 hours—three doses in all; 6 animals died (30%), and the rest survived the experimental period. (See Table IV, Experiment 4).



V VI

FIGURE V: *Clostridium welchii* colonies plated on agar from a six-hour broth culture containing 1% of sulfanilamide. Culture diluted 1 x 100.

FIGURE VI: *Clostridium welchii* colonies plated on agar from a six-hour broth culture containing 2½% of Neoprontosil. Culture diluted 1 x 100.

PLACA V: Colonias de *Clostridium welchii* en placa de agar inoculado con un cultivo en caldo de seis horas, conteniendo 1 por ciento de sulfanilamida. Cultivo diluído al 1 x 100.

PLACA VI: Colonias de *Clostridium welchii* en placa de agar inoculado con un cultivo en caldo de seis horas, conteniendo 2½ por ciento de neoprontosil. Cultivo diluído al 1 x 100.

Under the conditions used in these experiments, sulfanilamide has been effective in the treatment of experimental *welchii* infection of mice.

We decided to take advantage of the results established by these experiments, and therefore planned to study the effect of Neoprontosil and sulfanilamide *in vivo*.

Experiment five. Three groups of 4 mice each were inoculated. In Group One, 4 mice were inoculated intraperitoneally with one minimal lethal dose of *Cl. welchii* culture plus 1 cc. of saline. In Group Two, 4 mice were inoculated intraperitoneally with one minimal lethal dose of *Cl. welchii* culture plus 1 cc. of a 1 per cent solution of sulfanilamide. In Group Three, 4 mice were inoculated intraperitoneally with one minimal lethal dose of *Cl. welchii* culture with 1 cc. of a 5 per cent solution of Neoprontosil. One mouse of every group was punctured every hour for 18 hours and the peritoneal exudate was stained with Wright's stain and examined for the number of bacteria in the smear, number and character of the cells in the exudate, and number and character of bacteria within the cell.

In all the specimens the phagocytosis was marked and the number of bacteria ingested by the cells was large. In the control, where saline was used instead of the drugs, some lymphocytes were observed in the field.

The preparations made from the mice injected with sulfanilamide had apparently less bacteria in the field than those injected with Neoprontosil or saline.

It is difficult to draw definite conclusions from this experiment. From our observations, however, phagocytosis does not seem to be altered by any of the drugs. Sulfanilamide apparently had a bacteriostatic effect *in vivo*, since the number of organisms per field was less in the case of sulfanilamide than in the case of the others.

Experiment six. This experiment was designed to find out the bacteriostatic action of Neoprontosil and sulfanilamide. A 24-hour glucose broth culture of *Cl. welchii* was used. Test tubes were prepared in the following manner: 2 cc. of plain broth plus 2 cc. of a 2 per cent solution of sulfanilamide; 2 cc. of plain broth plus 2 cc. of a 5 per cent solution of Neoprontosil; 2 cc. of plain broth plus 2 cc. of normal saline. The tubes were inoculated with a straight platinum needle immersed in the 24-hour broth culture of *Cl. welchii* and then immersed in the inoculated test tube, the contents of which were well shaken. The test tubes so inoculated were put under partial anaerobiasis in an anaerobic jar and incubated at 37 C. Then we prepared pour plates of plain agar in dilutions of 1:100 and 1:10,000 in sterile water from the contents of each tube after 3, 6, and 8 hours' incubation. These plates were incubated for 48 hours at 37 C. and then counted.

A good idea of the results may be obtained from study of the photographs of each plate (Figures I–VI).

It can readily be observed that under the conditions used in these experiments, Neoprontosil does not show any bacteriostatic action on the cultures of *Cl. welchii*, while sulfanilamide shows a definite bacteriostatic action.

DISCUSSION

Feinstone, Bliss, Ott, and Long[7] present evidence indicating that the activity of Neoprontosil depends on its reduction to sulfanilamide *in vivo*. In contrast, Rosenthal[8] and Barlow[9] have published observations indicating the therapeutic superiority of the oral as compared to the parenteral route of giving Prontosil under experimental conditions, while the chemo-therapeutic efficacy of sulfanilamide by the subcutaneous route is superior to that noted after oral administration (Rosenthal[10] and Proom[11]). Sulfanilamide is excreted rather slowly; Prontosil, much more rapidly.

We were unable to protect mice from minimal lethal dose of *Cl. welchii* by intramuscular injections of Neoprontosil. In the instances in which Neoprontosil was given orally, slight protection was observed. Sulfanilamide protected mice from minimal lethal dose of *Cl. welchii* when used either orally or parenterally.

When Neoprontosil was used with washed cells under the conditions described by us, the animals succumbed to infection. On examining the lesions produced, one could observe normal phagocytic activity and numerous organisms within the tissue of the lesion produced. Sulfanilamide produced a definite bacteriostatic effect *in vivo* as well as *in vitro*. Neoprontosil, under *in vitro* conditions used by us in these experiments, produced no demonstrable bacteriostatic action.

CONCLUSIONS

We were unable to protect mice from minimal lethal doses of *Cl. welchii* by intramuscular injections of Neoprontosil.

Under the conditions employed in these experiments we found sulfanilamide to be effective in protecting mice from a minimal lethal dose of *Cl. welchii*.

Thus, we conclude sulfanilamide to be superior to Neoprontosil in the treatment of experimental *welchii* infection in mice.

7. W. H. Feinstone, E. A. Bliss, E. Ott, and P. H. Long, *Bull.Johns Hopkins Hosp.*, 62:565. 1938.
8. S. M. Rosenthal, *Pub.Health.Rep.*, 52:48. 1937.
9. O. W. Barlow, *Proc.Soc.Exp.Biol.& Med.*, 37:315. 1937.
10. S. M. Rosenthal (*loc. cit.*).
11. H. Proom, *Lancet*, 1:16. 1937.

TABLE I

Experiment 1

Groups studied	*No. of mice inoculated*	*No. of mice that died*	*Percentage of mice that died*
Control			
1	30	20	66
2	30	29	96.6
3	30	30	100
Totals	90	79	87.7
Experimental			
1	30	25	83.3
2	30	30	100
3	30	30	100
Totals	90	85	94.4

TABLE II

Experiment 2

Groups studied	*No. of mice inoculated*	*No. of mice that died*	*Percentage of mice that died*
1	30	4	13.3
2	30	7	23.3
3	30	27	90
4	30	27	90
5	30	0	0

TABLE III

Experiment 3

Groups studied	*No. of mice inoculated*	*No. of mice that died*	*Percentage of mice that died*
1	30	0	0
2	30	0	0
3	30	0	0
4	30	29	96.6
5	30	23	76.6

TABLE IV

Experiment 4

Groups studied	*No. of mice inoculated*	*No. of mice that died*	*Percentage of mice that died*
1	20	19	95
2	20	20	100
3	20	13	65
4	20	2	10
5	20	6	30