hofer[3] described the cultural characters of similar streptococci isolated by him, which he designated as *Str. dysgalactiæ*.

Lancefield[4] reported that certain nonhemolytic strains of streptococci were members of the serological Group C. In a personal communication she stated that these nonhemolytic strains were from cases of bovine mastitis.

In the serological study of 15 strains of *alpha* hemolytic mastitis streptococci of Group II *(Str. dysgalactiæ)*, isolated here or obtained from 3 other laboratories, it was found that all 15 strains possessed an antigen which gives a group-specific precipitin-reaction with sera of Lancefield's serological Group C. Furthermore, the group-specific antibodies in Group C sera obtained from Dr. Lancefield could be removed by absorbing with the Group II mastitis strains. Antisera prepared from formalin-killed cultures of Group II streptococci were precipitated by the extracts of hemolytic strains of Group C.

In a personal communication of April 18, 1939, Dr. Wayne Plastridge informed the writer that mastitis strains originally classified by him as *S. pseudo-agalactiæ* belong to serological Group C and are culturally identical with cultures described by Diernhofer[3] as *Str. dysgalactiæ*.

The results show that strains of *alpha* hemolytic mastitis streptococci (Group II) either are related to or belong to Lancefield's serological Group C.

## 10640

## Effect of Neoprontosil on Bacterial Toxins.

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Domagk[1] first reported that Prontosil prevented the death of mice injected with lethal doses of hemolytic streptococci, even though it had no bactericidal effect on the microörganisms *in vitro*. Because of Domagk's findings, the drug was used for treatment in streptococcal infections in man and subsequently in other infectious diseases.

[3] Diernhofer, K., *Milchwirtsch. Forsch.*, 1932, **13**, 368.

[4] Lancefield, R. C., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 473.

[1] Domagk, Gerhard, *Deutsche Med. Wchnschr.*, 1935, **61**, 250.

The favorable results obtained in the clinical use of the compound prompted several investigators to study its mode of action. Although sulfanilamide soon became more widely used than Prontosil, the fact that the latter is much less toxic warrants further investigation of its therapeutic value.

Because Neoprontosil* has little, if any, bactericidal effect *in vitro,* our studies have been concerned with the action of the drug on bacterial toxins. This report describes the effect of the compound in mice when it is given orally, intraäbdominally, or subcutaneously after the injection of fatal doses of the following toxins: staphylococcal, streptococcal, gonococcal, and those formed by *Clostridium botulinum* and *Clostridium welchii.* Rockland mice, weighing 20 g each, were used in all the experiments. A 5% solution of Neoprontosil in distilled water was used throughout. The preparation was introduced directly into the stomach by means of a hypodermic syringe equipped with a blunted 18-gauge needle, 32 mm in length. This procedure is referred to hereafter as "oral administration."

*Staphylococcal Toxin.* The toxins from 3 hemolytic strains of *Staphylococcus aureus* were employed. Two of the strains were recovered from purulent discharges—one from otitis media, the other from a furuncle. The third strain was isolated from the nasal mucosa of a patient with small boils in and about the nose. The toxin was prepared according to Dolman's technic.[2] A 36-hour culture grown on semi-solid agar was mixed with Douglas' broth, filtered through cheese-cloth and filter-paper, and then centrifugalized. The supernate constituted the toxin. Merthiolate was added to give a final concentration of 1:8,000.

The toxin administered orally produced no ill effect, but when injected intraäbdominally was fatal in doses of from 0.1 to 0.2 cc. Within ½ hour after the injection of the toxin, the coat became rough, the mice assumed a crouching position, gradually became comatose, and died within 24 hours. When the drug was administered orally in doses of 10, 15, or 25 mg at 1, 2, 5, and 24 hours following injection of staphylococcal toxin, 386, or 87.7%, of 440 mice survived, while none of 110 controls lived (Table I). The treated mice showed the same symptoms as the controls until the drug became effective, which usually occurred after the third dose. Then, they recovered within 48 hours. It was also observed that

* Prontosil was named "Neoprontosil" when approved by the Council on Pharmacy and Chemistry of the American Medical Association. Neoprontosil was supplied by the Winthrop Chemical Company.

2 Dolman, C. E., *Canadian Public Health J.*, 1932, **23**, 125.

TABLE I.

Therapeutic Effect of Neoprontosil Administered Orally to Mice Injected Intraäbdominally with Bacterial Toxins.

| Toxin | No. of mice injected | M.L.D. of toxin, cc | Amt Neoprontosil administered in mg | Treated Mice | | | Control Mice | | | Difference* / S.E.D.M. | Odds to 1 against difference being result of chance |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | No. injected | No. survived | % survived | No. injected | No. survived | % survived | | |
| Staphylococcal | 550 | 0.1 | 40<br>60<br>100 | 440 | 386 | 87.7 | 110 | 0 | 0.0 | 18.0 | $\rightarrow \infty$ |
| Streptococcal | 110 | 1.5 | 100 | 80 | 62 | 77.5 | 30 | 2 | 6.7 | 6.7 | $> 5 \times 10^8$ |
| Gonococcal | 80 | 0.2 | 100 | 60 | 3 | 5.0 | 20 | 0 | 0.0 | 1.0 | 2.15 |
| *Clostridium welchii* | 140 | 0.08 | 125 | 100 | 84 | 84.0 | 40 | 4 | 10.0 | 8.2 | $\rightarrow \infty$ |
| *Clostridium botulinum* | 80 | 0.15 | 100 | 60 | 52 | 86.7 | 20 | 1 | 5.0 | 6.7 | $> 5 \times 10^8$ |

*Difference ÷ standard error of the difference of the means.

TABLE II.

Therapeutic Effect of Neoprontosil Administered to Mice Injected Intraäbdominally with Bacterial Toxins.*

| Toxin | No. of mice injected | M.L.D. of toxin, cc | Dose of neoprontosil in mg | Treated Mice | | | Control Mice | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | No. injected | No. survived | % survived | No. injected | No. survived | % survived |
| Subcutaneous Administration of Drug. | | | | | | | | | |
| Staphylococcal | 75 | .1 | 75 | 60 | 0 | 0.0 | 15 | 0 | 0 |
| Gonococcal | 75 | .2 | 75 | 60 | 1 | 1.6 | 15 | 0 | 0 |
| *Clostridium welchii* | 75 | .08 | 75 | 60 | 4 | 6.6 | 15 | 3 | 20 |
| Intraäbdominal Administration of Drug. | | | | | | | | | |
| Staphylococcal | 75 | .1 | 100 | 60 | 2 | 3.3 | 15 | 0 | 0 |
| Gonococcal | 84 | .2 | 100 | 64 | 5 | 7.6 | 20 | 0 | 0 |
| *Clostridium welchii* | 75 | .08 | 100 | 60 | 5 | 8.3 | 15 | 2 | 13.3 |

*The negative results obtained from the injection of neoprontosil are without statistical significance.

25 mg of Neoprontosil given orally 2 hours prior to the toxin protected 43, or 71.7%, of 60 mice, while all of 20 controls died.

*Streptococcal Toxin.*† The toxin employed in this experiment was produced from a strain of hemolytic streptococci isolated from a patient with puerperal septicemia. The intraäbdominal injection of 1.5 cc of the toxin killed 28, or 93%, of 30 mice within 72 hours. Immediately after injection, the mice showed severe twitching of the muscles. This symptom, which may have been caused in part by the phenol used as a preservative in the toxin, subsided within an hour. The untreated mice became listless, assumed the usual crouching position, and died within 72 hours. Neoprontosil in doses of 25 mg given orally at 1, 2, 5, and 24 hours after the toxin prevented death in 62, or 77.5%, of 80 mice. The treated mice showed similar symptoms until after the last dose of Neoprontosil at 24 hours, when improvement was noted. Recovery occurred in from 4 to 5 days.

*Gonococcal "Toxin"* The preparation referred to as gonococcal "toxin" was a lyophilized and regenerated whole broth culture of the gonococcus containing no viable organisms. It was so concentrated that 0.2 cc consistently killed the mice within 24 hours. We[3] have previously reported that sulfanilamide affords a high degree of protection to mice injected with this toxin. Attempts to duplicate this work by the oral administration of Neoprontosil, however, have been unsuccessful. Doses of 25 mg were given 1, 2, 5, and 24 hours after the toxin. Only 3, or 5%, of 60 mice so treated survived the intraäbdominal injection of the toxin. After injection with the toxin, both the treated and untreated mice were listless, their coats became rough, and they remained in a crouching position until death. Diarrhœa usually developed shortly after inoculation, the temperature became subnormal, and the eyelids became stuck together with a mucopurulent exudate. The drug also failed to protect mice when administered intraäbdominally or subcutaneously (Table II).

*Clostridium welchii Toxin.* The toxin of *Clostridium welchii* in doses of 0.08 cc injected intraäbdominally or intramuscularly killed 36, or 90%, of 40 mice. An area of necrosis developed at the site of injection within from 48 to 60 hours. Extensive edema then developed in the skin and muscles of the abdominal wall. The mice were active until death, which occurred suddenly 5 or 6 days after injection. Autopsy findings in untreated mice were: generalized edema, subcutaneous hemorrhage, and focal areas of necrosis in the

† Puerperal septicemia toxin Rx 017471A was supplied by Parke, Davis & Company.

[3] Carpenter, C. M., Hawley, P. L., and Barbour, G. M., *Science*, 1938, **88**, 530.

kidneys and liver. Eighty-four of 100 mice treated with Neoprontosil, as described above, survived. A small brownish area always appeared at the site of injection in these mice. Neither necrosis nor edema occurred. An autopsy performed 2 weeks later revealed no gross pathological changes.

*Clostridium botulinum Toxin.*‡ Toxins of *Clostridium botulinum,* types A and B, were used. Doses of 0.15 cc of either type uniformly killed the control mice within 48 hours. Within 24 hours, the mice showed extreme muscular weakness. Control of head movements was impaired, because the muscles of the neck were particularly affected. Within 36 hours the mice were moribund, and death occurred within 48 hours. The oral administration of Neoprontosil prevented the death of 52, or 86.7%, of 60 mice (Table I). These mice showed some weakness and loss of muscular control, but responded noticeably to the drug within 36 hours and were fully recovered in 3 days.

*Summary.* Neoprontosil was administered orally, subcutaneously, intraäbdominally, or intramuscularly to a total of 740 mice which had received lethal doses of 5 bacterial toxins by intraäbdominal injection. Oral administration of the drug prevented death in 87.7% of 440 mice given staphylococcal toxin, 77.5% of 80 mice given streptococcal toxin, 84% of 100 mice given the toxin of *Clostridium welchii,* and 86.7% of 60 mice given the toxin of *Clostridium botulinum.* On the other hand, only 5% of 60 mice injected with gonococcal "toxin" survived. In all instances, the compound was ineffective when administered parenterally.

‡ Toxins of *Clostridium botulinum,* A 172 and B 161, were supplied by the Lederle Laboratories.