
TREATMENT OF THE EPIDEMIC DISEASE RED LEG¹

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ABSTRACT

Reg leg was cured in the frog, *Rana pipens*, with chloromycetin, using a dosage of 5 mg initially and 3 mg twice daily, or sulphadiazine with 15 mg initially and 10 mg twice daily. The drugs are best administered in suspension by gastric intubation, using a calibrated rubber bulb pipette and suspensions of a 250 mg capsule of chloromycetin in 22 cc water yielding 3 mg per $\frac{1}{4}$ cc of suspension. Other species of frogs or toads may be treated with the above methods with dosages proportional to body weights.

"Red leg" is an epidemic disease attacking most species of frogs and toads. It apparently is not too common in wild frogs or toads, but produces death at an astounding rate in the animals when in captivity. The result is the loss of much time and money when performing any laboratory experiments which must use live frogs. Therefore, it is considered worthwhile to report on the successful treatments and control of red leg in the frog, *Rana pipiens*.

Ernst (1890) first isolated a bacterium from infected frogs, called *Bacillus ranicida*. Sanarelli (1891) next studied an organism isolated from frogs infected with red leg and named it *Bacillus hydrophilus fuscus*. Russell (1898) reported a detailed study of the pathology of red leg and mentioned the production by *Bacillus hydrophilus fuscus* of toxins producing effects on cardiac and skeletal muscles of frogs as well as effects on the central nervous system.

In the fifth edition of the *Manual of Determinative Bacteriology* (Bergey, 1939) the nomenclature was revised to *Proteus hydrophilus* and then to *Pseudomonas hydrophila* in the sixth edition (Breed, 1948). Making an extensive study of red leg, Kulp and Borden (1942) described two strains of *Pseudomonas hydrophila* from different sources. The existence of carrier frogs harboring the pathogen in their gall bladders was also indicated. Smith (1950) reported the use of chloromycetin in successful treatment of red leg, which will be described later.

Reg leg is supposedly caused by the pathogen *Pseudomonas hydrophila*. The disease is septicemic and involves all organs. It is characterized by the reddening of the lower abdominal region and legs due to the dilation of the blood vessels. The disease usually terminates in death.

METHODS

After several trials, it was found that the best growth of possibly pathogenic bacteria could be obtained by spreading blood from an infected frog on a blood agar slant and incubating the slant at 37 C. The resulting growth was washed off one tube with distilled water and diluted with distilled water to about 2,000:1. A loop of this dilution was streaked on another blood agar slant and incubated.

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In three days many small circular colonies, 1 to 2 mm in diameter, appeared. Several individual colonies were observed microscopically and compared with bacteria found in blood smears of infected frogs. A third slant was inoculated from a colony chosen as likely to be infective. After three days of incubation, a suspension was made of the growth on this third blood agar slant. Four frogs, each in its own container, then received injections of $\frac{1}{4}$ cc of this suspension; two were left uninoculated, in a separate container. All of the injected frogs died in 5 to 7 days with outstanding cases of red leg infection.

The bacterium isolated in the above manner was presumably *Pseudomonas hydrophila*. It possessed two distinguishing features: flagella, evidenced by motion of the suspensions, and a large protective capsule.

With a successful method for infecting frogs at hand, various treatments could be tested. Sixteen frogs were injected with $\frac{1}{4}$ cc *Pseudomonas hydrophila* suspension. Three days later the frogs were divided into 4 groups of 4 each. Group 1 received chloromycetin (chloramphenicol) with a dosage of 5 mg initially and 3 mg twice daily as suggested by Smith (1950). Group 2 received sulphadiazine with a dosage of 15 mg initially and 10 mg two times daily, in view of reports that sulphadiazine combats some types of gram negative pathogens such as *Pseudomonas hydrophila*.

Group 3 was chilled at 10 C on the basis of evidence, noted during previous experiments, that cold temperatures slowed the course of red leg. Group 4 was left as a control. Treatment lasted five days.

RESULTS

The results were conclusive. The symptoms of Group 1 disappeared and the frogs again became healthy. Group 2 showed the same results. However, 50 per cent of the frogs in Group 3 died in 13 days. All of Group 4 was dead within 6 days, with smears of their blood showing an abundance of the *Pseudomonas hydrophila* pathogen.

DISCUSSION

It would not be unreasonable to assume that these cures would work as successfully on any species of frogs or toads infected with *Pseudomonas hydrophila*. However, when administering these drugs to different species, size and weight must be considered in determining the dosage. *Rana pipiens* weighs from 30 to 50 g, but *Rana sylvatica* weighs only about 15 to 20 g, or even less, while *Rana catesbeiana* weighs as much as 100 g. It would probably be safe to assume that if one gives *Rana pipiens* (weighing 50 g) 20 mg of sulphadiazine per day, one should give *Rana catesbeiana* twice as much. It is also probable that *Rana sylvatica* could be given half the dosage and still be cured.

Since the drugs were difficult to dissolve, except in ethyl alcohol, which produced harmful side effects, it was found to be easier and apparently quite satisfactory to administer the drugs in suspension. Rather than measure out 10 mg or 3 mg of the drug each time a frog was treated, it was simpler to put a capsule of chloromycetin (250 mg) into 22 cc of water, resulting in 3 mg to a $\frac{1}{4}$ cc of water, and a tablet of sulphadiazine (600 mg) into 15 cc of water, resulting in 10 mg to each $\frac{1}{4}$ cc of water. The drugs were then given by gastric intubation with a rubber bulb pipette marked off at $\frac{1}{4}$ cc. The initial doses were measured individually.

Laboratory observations indicate that both chloromycetin and sulphadiazine prevent red leg when simply put into the water in which frogs are kept. However, it is considered better to treat infected frogs by intubating the drugs. Also, frogs stored at 10 C have greater resistance to red leg than those kept at room temperature. Apparently, the cold slows the course of the disease enough to allow some natural recovery. However, chilling laboratory frogs is not a satisfactory cure or even a preventive measure in most cases.

SUMMARY

1. Chloromycetin and suphadiazine in the above dosages may be used to cure frogs infected with *Pseudomonas hydrophila*.
2. Chloromycetin and sulphadiazine may be used in the habitat water with some success in preventing infection.
3. Frogs may be stored at 10 C to some advantage in preventing the spread of red leg.

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