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STUDIES ON THE CONTROL OF WHIRLING DISEASE (*Myxosoma cerebralis*).

I. The Effects of Chemicals on Spores *in vitro*, and of Calcium Oxide as a Disinfectant in Simulated Ponds

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Abstract: Based on presumptive evidence of death (extrusion of polar filaments and disintegration of sporoplasm) 1.0%, 0.5%, and 0.25% calcium oxide or potassium hydroxide killed the spores of *Myxosoma cerebrallis in vitro*. Chlorine at 400 ppm destroyed 36% to 90% of the spores but 13% to 37% of those in the controls perished. Calcium hydroxide, ammonium chloride, sodium borate, potassium permanganate, Roccal (alkyl dimethylbenzylammonium chloride), and copper sulfate allowed survival of 38-96% of the spores, usually not much less than the rate of survival of the controls.

In simulated pond testing, quicklime at 380 grams or more per square meter (3360 lbs/acre) of pond bottom prevented whirling disease in rainbow trout *(Salmo gairdneri)*.

INTRODUCTION

Whirling disease, caused by *Myxosoma cerebralis* (Protozoa: Myxosporida), has caused great concern among trout culturists ever since it first became evident in Germany about 1900.[2,7] Control and eradication of the disease involves cleaning and disinfecting the ponds. Schäperclaus,[8] Tack,[11] and Ghittino[1] used calcium cyanamide ($CaCN_2$), and Schäperclaus[8] also recommended quicklime (CaO) for disinfection. In addition, a noncontaminated water supply must be provided and, where possible, concrete raceways should be built. Whirling disease does not exist in properly designed raceways supplied with spore-free water.

Although widely used in Europe, neither calcium cyanamide nor calcium oxide has been tested under laboratory conditions. Calcium cyanamide is a rich source of nitrogen and its effect on natural waters should be considered. Calcium oxide does not cause significant pollution when used in drained trout ponds. Quicklime and fresh hydrated lime also have been used to control ectoparasites in ponds.[5,10] Fresh calcium oxide is also bactericidal; a 1% aqueous solution of calcium oxide in 20% chicken feces killed *Staphylococcus aureus* in 90 min.[6]

The spore wall of *M. cerebralis* appears to be very resistant and it has long been assumed that only rather drastic disinfection procedures would kill the spores.

Hoffman and Putz[4] found in preliminary experiments that the following chemicals and concentrations would kill the spores by releasing the polar filaments, destroying the sporoplasm, or causing disintegration of the spore valves: calcium hydroxide, 0.5% and 2%; available chlorine (as sodium hypochlorite) 1600 ppm; Roccal (alkyl dimethylbenzylammonium chloride), 200 and 800 ppm active ingredient.

The work reported consists of (1) a search for better or additional disinfectants by observing the treated spores microscopically, and (2) a test of calcium oxide against *M. cerebralis* under simulated pond conditions.

MATERIALS AND METHODS

1. In vitro effects of disinfectants.

To prepare the spore concentrate, the heads of 8-10 month old infected fish were cut off and as much soft tissue as possible removed under a dissection microscope. The remaining parts were then cut into small pieces and macerated with a small mortar and pestle. The resulting mass was diluted and strained and the cleaned material allowed to sediment in the refrigerator. Although satisfactory suspensions in which the spores could be counted were prepared, they were never free of fish tissue. A fresh batch was prepared for the work represented by each table. Spore preparations were tested in small vials at room temperature *(ca* 22 C). About 0.75 ml of disinfectant dissolved in water was added to an equal amount of spore concentrate. Untreated spores in similar vials served as control. After the spores and test materials were placed in loosely stoppered vials they were observed at intervals of 2 to 4 days for 2 weeks or more. From 10 to 183 spores (average 69) were counted for each observation. Numbers were dependent on availability. Death of a spore was assumed when the polar filaments were extruded, the sporoplasm disintegrated, or the spore wall opened.

The chemicals tested were ammonium chloride (NH_4Cl) 0.1%; calcium hypochlorite ($CaClO)_2$) 200 and 400 ppm chlorine; sodium borate ($Na_2B_4O_7.10\ H_2O$) 0.1%; ammonium carbonate 0.1%; potassium hydroxide (KOH) 0.01%, 0.1%, and 1.0% calcium hydroxide ($Ca(OH)_2$) 0.125%, 0.25%, and 0.5%: potassium permanganate ($KMnO_4$) 0.01%, 0.1%, and 1.0%; calcium oxide 0.25%, 0.5%, and 1.0%; Roccal (alkyl dimethylbenzylammonium chloride) 50, 100, and 200 ppm of active compound; and copper sulfate ($CuSO_4.5H_2O$) 0.1%, and 0.5%. These chemicals were selected because they have been used as disinfectants for bacteria, coccidia, nematodes, or fish parasites. Inadvertently; calcium cyanamide, which has been used for whirling disease control, was omitted.

2. Testing quicklime in simulated ponds.

To each of four 340-liter fiberglass tanks were added 20 liters of mud (about 3 cm deep) from a source contaminated with *M. cerebralis.* The entire lot of mud was thoroughly mixed prior to placement in the tanks. While the mud was still wet, three of the tanks were treated with quicklime.[1] We added 240 grams of calcium oxide to one tank bottom (6180 sq cm) equivalent to approximately 380 grams per square meter (3360 lbs per acre). In addition, 300 (475 grams per square meter) and 3400 (5380 grams per square meter) grams of quicklime were used in other tanks. The fourth tank served as control. After 2 weeks, each tank was supplied with about 1800 ml per minute of 12 C disease-free spring water and 100 two-week-old rainbow trout *(Salmo gairdneri)* were added to each tank. Control fish were kept in standard facilities with the same water supply and they did not become infected. The fish were fed commercial crumbles and pellets *ad libitum.* Six months later, samples were netted randomly, and the fish measured and examined for *M. cerebralis* spores as in Hoffman, Snieszko, and Wolf.[3]

RESULTS

1. Microscopic effect of chemicals on spores.

Of the chemicals tested, only calcium oxide and potassium hydroxide, each at 1.0%, 0.5%, and 0.25% concentration, produced 100% mortality in spores under test-tube conditions. Other chemicals produced less severe alterations in spores and presumably a lower kill. Tables 1-4 present the data obtained in the experiments.

[1] Labelled "Washington High Calcium Chemical Hydrated Lime" containing 73.7% calcium oxide and supplied by Standard Lime and Refractories Company, Martin Marietta, Baltimore, Maryland.

TABLE 1. Effect of various chemicals on the spores of **Myxosoma cerebralis (in vitro)**, trial 1.

Disinfectant	Percentage "killed" 2 days	4 days	8 days	14 days
1. Control	00.0	14.3	13.0	16.3
2. NH_4Cl (0.1%)	00.0	12.0	16.3	38.3
3. *$Ca(ClO)_2$ (400 ppm)	100.0	100.0	100.0	90.0
4. *$Ca(ClO)_2$ (200 ppm)	28.6	61.9	42.2	40.8
5. $Na_2B_4O_7$ $10H_2O$ (0.1%)	32.4	19.6	35.8	34.3
6. $(NH_4)_2CO_3$ (0.1%)	6.45	4.00	21.6	27.3
7. KOH (.01%)	18.2	26.7	20.6	39.2
8. KOH (0.1%)	25.0	11.8	17.5	30.3
9. KOH (1.0%)	100.0	100.0	100.0	100.0
10. $Ca(OH)_2$ (0.125%)	29.4	45.6	37.0	22.8
11. $Ca(OH)_2$ (0.25%)	26.7	33.3	22.5	36.9
12. $Ca(OH)_2$ (0.5%)	29.3	24.6	35.4	33.9

*Parts active chlorine. Subsequent experimentation produced insignificant destruction of spores.

TABLE 2. Effect of various chemicals on the spores of **Myxosoma cerebralis (in vitro)**, trial 2.

Disinfectant	Percentage "killed" 2 days	4 days	6 days	8 days	10 days	14 days
1. Control I	18.2	12.5	20.0	17.4	15.7	37.2
2. KOH (1.0%)	100.0	100.0	100.0	100.0	100.0	100.0
3. $Ca(ClO)_2$ (400 ppm)	40.0	43.8	42.8	33.3	20.0	33.3
4. $Ca(ClO)_2$ (200 ppm)	5.0	45.5	14.3	13.0	36.9	33.3
5. $Ca(OH)_2$ (0.5%)	35.7	55.0	41.7	38.5	18.7	61.5
6. $Ca(OH)_2$ (0.25%)	17.7	53.0	33.8	27.3	40.0	6.66
7. NH_4Cl (0.1%)	18.7	35.0	16.1	27.8	38.1	30.0
8. $Na_2B_4O_7.10H_2O$ (0.1%)	8.33	53.8	16.7	13.6	23.6	17.9
9. Control II	8.34	14.8	17.9	26.1	17.9	22.2

TABLE 3. Effect of various chemicals on the spores of **Myxosoma cerebralis (in vitro)**, trial 3.

Disinfectant	Percentage "killed" 2 days	6 days	14 days
1. Control I	12.5	18.5	13.7
2. $KMnO_4$ (1.0%)	20.5	4.17	25.3
3. $KMnO_4$ (0.1%)	10.2	16.0	24.5
4. $KMnO_4$ (0.01%)	14.5	14.3	15.6
5. $KMnO_4$ (1.0%)	21.4	25.9	26.7
6. $KMnO_4$ (0.1%)	20.8	25.0	26.7
7. $KMnO_4$ (0.01%)	12.3	15.9	17.7
8. CaO (1.0%)	100.0	100.0	100.0
9. CaO (0.5%)	100.0	100.0	100.0
10. CaO (0.25%)	80.3	100.0	100.0
11. Cl ($Ca(ClO)_2$ (400 ppm)	21.4	25.7	36.4
12. Cl (200 ppm)	19.3	15.0	28.6
13. Control II	6.94	19.6	19.3

TABLE 4. Effect of various chemicals on the spores of **Myxosoma cerebralis (in vitro)**, trial 4.

Disinfectant	Percentage "killed" 2 days	4 days	8 days	14 days
1. Control	5.00	8.33	7.55	9.6
2. Roccal (200 ppm)	7.14	8.05	11.6	11.9
3. Roccal (100 ppm)	7.27	6.05	0.00	6.66
4. Roccal (50 ppm)	12.5	17.3	18.8	10.9
5. KOH (0.5%)	100.0	100.0	100.0	100.0
6. $CuSO_4$ (0.5%)	17.6	4.00	10.0	13.3
7. $CuSO_4$ (0.1%)	0.00	8.33	0.00	5.72

2. Effect of quicklime in simulated ponds.

Our results show that fish remained uninfected in those tanks treated with quicklime and that fish were significantly larger than the control fish (Table 5). It must be noted that the mud in the aquaria was not stirred after the fish were added; therefore, it is not known if the disinfection was complete to the bottom.

(see addendum)

TABLE 5. Effect of quicklime on whirling disease in simulated ponds.

Treatment	No. of fish measured	Size of fish, cm	Number infected	Degree of infection
Control	26	7.6 (4.5-11.4)	6 of 10	mild
3400 grams of CaO	26	10.5 (8.0-12.3)	0 of 26	none
300 grams of CaO	25	10.8 (7.8-12.6)	0 of 25	none
240 grams of CaO[1]	26	10.7 (7.8-13.1)	0 of 26	none

[1] Approximately 380 grams/sq meter (3360 lbs/acre).

DISCUSSION

These results verify that, although difficult, the chemical disinfection of *M. cerebralis* is possible. Ghittino[2] stated that calcium cyanamide is more effective than calcium oxide in Italy. Some German workers[3] prefer calcium oxide because it does not have the undesirable nutrient fertilizing effect of calcium cyanamide. Calcium oxide reacts with carbon dioxide in air and is converted to calcite; therefore, one should make certain the chemical is fresh when used. Because of the importance of whirling disease in the United States, further testing of disinfectants would be highly desirable. Of greatest importance is the need to thoroughly test the potential disinfecting ability of chlorine - bearing compounds such as calcium and sodium hypochlorite.

In the present experiments some of the lower kills at 8, 10, and 14 days were probably due to complete chemical destruction of killed spores.

Because the tissue residue was not completely removed, the data given cannot be considered exact but rather as an indication of the probable range of effectiveness for the chemicals. These chemi cals should be tested with fish, but time permitted only the examination of calcium oxide.

[2] Ghittino, P. 1970. Personal communication. Institute Zooprofilattico Sperimentale, Via Bologna 148, 10154 Torino, Italy.

[3] Wiesner, E. R. 1968. Personal communication. Oberfishereirat bei der Regierung von Schwaben in Augsburg, West Germany.

ADDENDUM

Six months after the completion of Results 2 (above), the calcium oxide treated mud and the control mud were stirred thoroughly. Newly hatched rainbow trout were added as before. Five months later when the fish were examined, the controls displayed signs of whirling disease, and spores were present; the fish in the treated tanks were apparently normal and no spores were found in them.

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