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## The Biological Action of Rotenone on Freshwater Animals

Howard Laverne Hamilton  
*State University of Iowa*

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## THE BIOLOGICAL ACTION OF ROTENONE ON FRESH-WATER ANIMALS

HOWARD LAVERNE HAMILTON

The physiological properties of rotenone have been extensively studied by Van Hasselt (1); Haag (2), and many others.<sup>1</sup> The history, distribution, and chemical properties of the alkaloid, which has been isolated as a white crystalline material (Greshoff, 5), are adequately reviewed by Leonard (4) and also by Van Hasselt and Haag. Rotenone first attracted scientific attention when it was found that preparations of powdered derris root had excellent insecticidal properties. Subsequent experiments were devised to test its action on various vertebrate animals, in order to make certain that the lethal concentration for insects would not be deleterious to humans, even after prolonged exposure. Recently, plant derivatives containing rotenone have been used for removing rough fish from ponds and lakes which were to be stocked with game fish. Little consideration has been given, however, to the possibility that the poison might kill other fresh-water animals (thus breaking the food chain) and seriously affect the survival of any fish with which the waters might be restocked.

The present studies describe the mode of action of rotenone on typical fresh-water animals, principally invertebrate, and present data on the relative susceptibilities of these organisms to the drug.<sup>2</sup>

### MATERIALS AND METHODS

The source of rotenone in these experiments was powdered derris root, kindly supplied by Dr. D. H. Thompson of the Illinois Natural History Survey, and assayed to contain 5.0 percent rotenone. This preparation was used for making solutions rather than the pure drug, and special solvents for rotenone such as alcohol or ethylene glycol were omitted, in order that the experimental conditions might approximate the normal conditions under which the drug is used in field work.

<sup>1</sup> For a bibliography of Derris, consult Roark (3), and the more recent paper by Leonard (4).

<sup>2</sup> This problem was suggested by Dr. A. B. Taylor, and I am very grateful to him and to Dr. T. L. Jahn for much helpful advice during the course of the work. I am especially indebted to Prof. J. H. Bodine for my appointment at the Iowa Lakeside Laboratory which made this investigation possible.

All solutions were made up in fresh lake water. A concentrated 1/1,000 suspension of derris root (50 mg. of rotenone per liter) was first prepared, and the required amount of this suspension was diluted to the desired volume and concentration. A minimum number of ten animals was placed in each concentration of rotenone, except in rare cases when the required number of organisms was not available, and an equal number was placed in control vessels containing equivalent volumes of lake water. In preliminary experiments compressed air was bubbled through the solutions; in later experiments volumes of solution were used which far exceeded the requirements of the number of animals involved. Small animals were placed in glass vessels of 8 liters capacity or less; cement aquaria with volumes of 2,000 liters were used for fish, salamanders, and crayfish. The depth of the solution was adjusted to the optimum level whenever necessary (crayfish, salamanders).

The rotenone preparation contained approximately 0.7 percent iron filings (apparently from the grinding mill). The presence of this contamination, however, probably did not affect the accuracy of the experimental results, since the percentage of rotenone in the mixture of powdered root and metal had been standardized.

#### EXPERIMENTAL RESULTS

*Fish.*—Buffalo (*Ictiobus* sp.), carp (*Cyprinus carpio*), and bullheads (*Ameiurus melas*) were exposed to concentrations of rotenone ranging from 8.3 to 25 gamma per liter of water. The responses of each species of fish to its lethal concentration (that which killed all animals in approximately twenty-four hours are graphically shown in Fig. 1. Within an hour after exposure to the poison the fish responded by swimming uneasily and rising frequently to the surface to gulp in air. Buffalo and carp attempted to jump out of the tanks at this stage. During the next four or five hours the fish swallowed enough air to float them on their sides or upside down, and they writhed on the surface in this position until death resulted from suffocation. In later stages some of the animals remained passively on or near the bottom of the tank, ventral side uppermost, and violently moved the gill operculum in their efforts to get more oxygen.

Autopsies of fish which were near suffocation showed that the stomach was full of swallowed air; other internal organs were normal. The heart was often beating strongly in animals which showed no external signs of life. A gross examination of the gills of normal and derris-treated fish showed a marked difference in

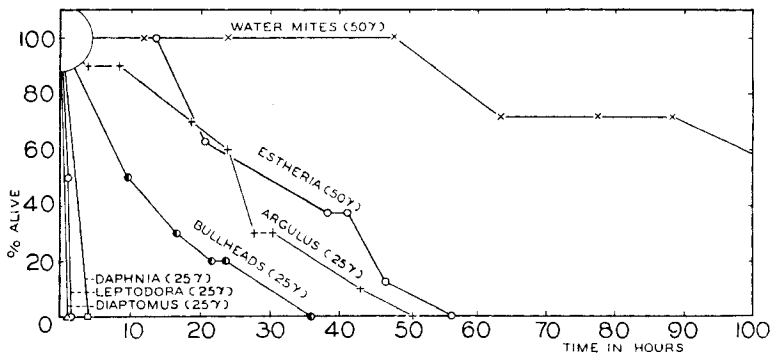
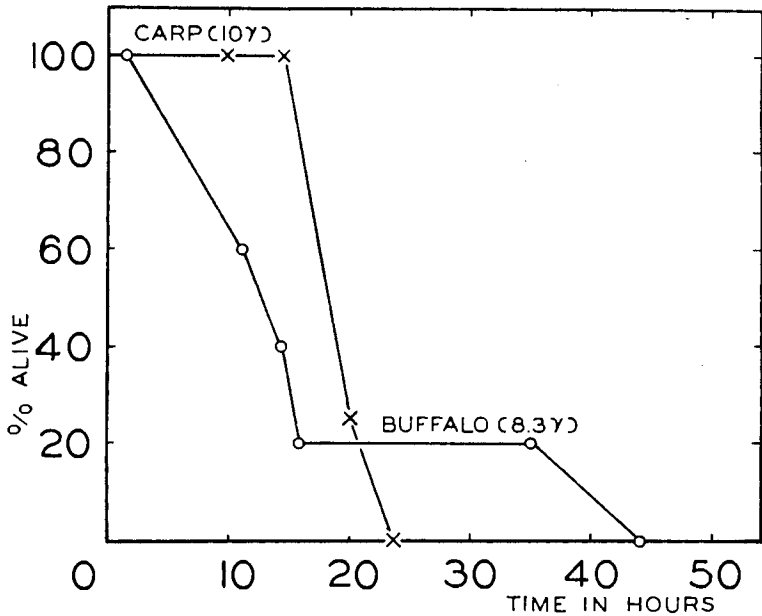


Fig. 1. Susceptibilities of various animals to dilute concentrations (expressed as  $\sqrt{1.}$ ) of rotenone.

blood supply. The pale pink gills of poisoned animals, in contrast to the normal bright red color, indicated that suffocation was due to decreased circulation of blood through the gill filaments.

Comparative histological studies were made of the gills of normal and rotenone-treated bullheads, in order to determine the exact point of action of the drug on the circulatory system. The fish were placed in their lethal concentration of rotenone ( $25 \sqrt{1.}$ ), and the gills were removed and fixed when the animals were near death, but still able to move slightly. Little difference was found in the condition of the gill epithelium except for a greater amount of mucus in treated animals. The erythrocytes were entirely normal. In control animals the afferent branchial arteries were filled with blood cells, whereas the efferent vessels were nearly empty. Both were approximately half-filled with blood cells in poisoned animals. The condition of the capillaries was most significant; in normal gills they were of sufficient diameter to allow passage of a single row of red blood corpuscles (Fig. 3), but in rotenone-treated fish the capillaries had narrowed so that corpuscles could not enter. Accordingly, the capillaries were nearly empty, even though their feeding arterioles were packed with blood cells (Fig. 4), and the few corpuscles which had been trapped in them were compressed and distorted.

*Amphibia.*—Tadpoles of *Rana pipiens*, which were obtained in stages of metamorphosis, were exposed to concentrations of rotenone ranging from 10 to 500 gamma per liter, and observations were made on changes in susceptibility to rotenone as pulmonary respiration replaced gill respiration. Tadpoles which had not begun to metamorphose responded to the drug with typical symptoms of suffocation by swimming to the surface and gasping for air. Metamorphosing tadpoles which were resorbing the tail remained more or less permanently at the surface of the rotenone solution where they could get a continuous supply of air. Muscular movements gradually became sluggish after several hours. The lethal concentration for all stages was 100 gamma per liter (Fig. 2). However, the time of death varied with the stage of metamorphosis. Tadpoles which were either partially or entirely dependent upon gill respiration were killed within eight hours, but metamorphosed animals could tolerate the lethal concentration for twenty-four hours. Metamorphosing salamanders (*Ambystoma tigrinum*) were similar to frogs in their responses to rotenone, for they showed the same initial struggle for oxygen followed by gradual slowing of muscular activity. The lethal concentration was

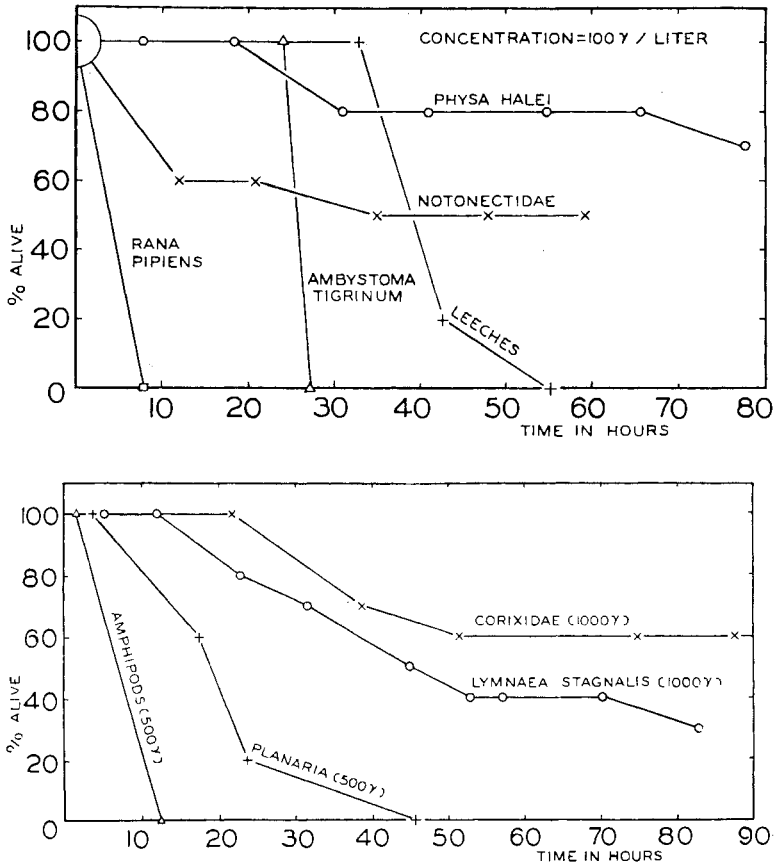


Fig. 2. Responses of animals to high concentrations of rotenone. Note that a certain percentage of more resistant individuals can live beyond the effective period of the poison (2-3 days).

100  $\sqrt{}$  /1. for salamanders which had completed metamorphosis (Fig. 2), but before resorption of the gills, concentrations as low as 16.6  $\sqrt{}$  /1. were toxic though not necessarily fatal. The results of these experiments on fish and amphibia indicated that rotenone was acting principally upon the circulatory and respiratory systems.

*Mollusca*.—The aquatic snail, *Lymnaea stagnalis*, was treated with concentrations of rotenone ranging from 10 to 1,000 gamma per liter. An exposure of 3.5 days to a concentration of 1 mg. /1. resulted in the death of seventy percent of the animals (Fig. 2). Thirty percent were killed by a concentration of 500  $\sqrt{}$  /1. in the same length of time. Other solutions were ineffective. *Physa haley* was treated with concentrations ranging from 10 to 100 gamma per liter, but little effect was noted (Fig. 2).

*Arachnida*.—Water mites (Hydrachnidae) were placed in concentrations of rotenone of 16.6 and 50 gamma per liter. The continual swimming movements which are characteristic of these animals were gradually slowed until most of the animals lay quietly on the bottom of the dish. Swimming movements were resumed if the animals were disturbed. The stronger concentration (50  $\sqrt{}$  /1.) caused a gradual narcosis or paralysis which resulted in the death of approximately forty-three percent of the animals during a period of four days (Fig. 1). Surviving individuals resumed normal swimming movements after remaining in the test solution four or five days.

*Insecta*.—Notonectids were not affected by concentrations of 10 or 25 gamma per liter, but fifty percent were killed by a solution of 100  $\sqrt{}$  /1. within twenty-four hours (Fig. 2). Concentrations as strong as 500 and 1,000 gamma per liter were not fatal to Corixids (Fig. 2). These insects swam to the surface of the solution and rested on the surface film unless disturbed. Untreated animals were nearly always actively swimming under water.

The intact heart of the grasshopper (*Melanoplus bivittatus* and *M. differentialis*) was treated with a concentrated suspension of rotenone (50 mg. /1.) in physiological salt solution (Bělař). The typical response was an initial decrease in rate of beat followed by a momentary slight stimulation, after which the rate and amplitude of beat decreased steadily until the heart was completely paralyzed (Fig. 5, A-C). An extremely long, slow beat could be restored by rinsing the heart with Bělař solution (Fig. 5, D).

*Crustacea*.—The crayfish (*Cambarus immunis*) was unaffected by solutions of rotenone as concentrated as 500 gamma per liter.

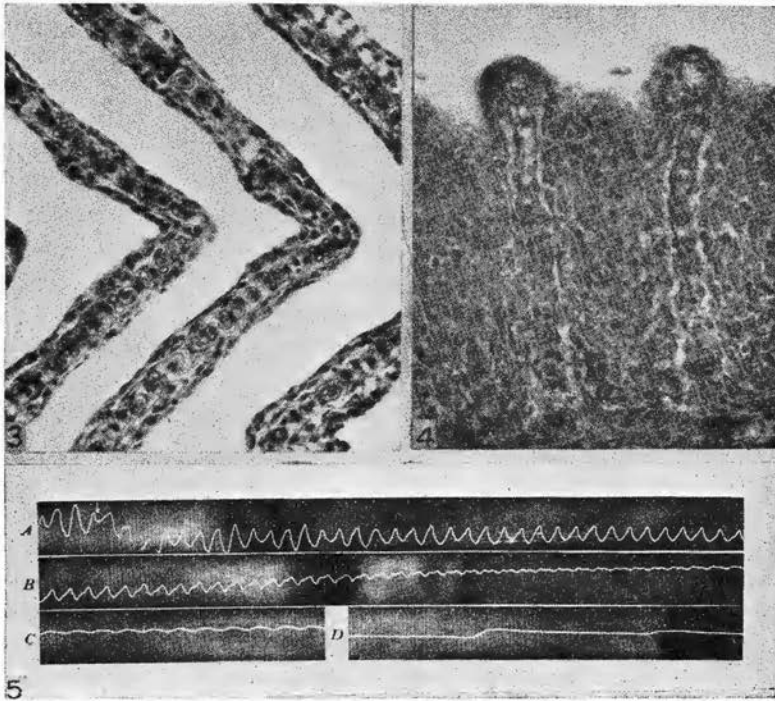


Fig. 3. Transverse section of gills from an untreated bullhead. Note the large number of erythrocytes within the capillaries.

Fig. 4. Transverse section of gills from a bullhead which was suffocating from rotenone. Note that erythrocytes are present in the terminal arterioles, but that the capillaries are so constricted that they prevent the passage of blood cells.

All sections prepared by the hot celloidin technique. Bouin's fixative. Mallory's triple stain. X 248.

Fig. 5. Mechanocardiogram of the grasshopper heart after addition of rotenone. In all records the mechanograph was on cardiac segment 2. Systole is represented by a downward deflection. The distance between successive dots of the time record represents 0.57 second. Temperature, 29.2° C. X 140.

A-B: Rotenone (50 mg./l.) added at the point marked by an arrow.

C: Response of the heart 6 minutes after the initial treatment in record A. Complete paralysis occurred soon after this record was taken.

D: Recovery, after rinsing with fresh Bělař solution.



This same concentration was lethal to amphipods within twelve hours (Fig. 2).

During an autopsy of a poisoned bullhead, a fish louse was found actively swimming in the fluid surrounding the animal. Since the reactions of such ectoparasites to poisons may be practically important, a large number of *Argulus* sp. was collected and exposed to a series of concentrations ranging from 10 to 500 gamma per liter. The lethal concentration, which was  $25 \sqrt{1}$ . (Fig. 1), caused a gradual paralysis of movements.

The planktonic crustacea, *Diatomus siciloides*, *Leptodora kindtii*, and *Daphnia pulex*, were strikingly similar in their high sensitivities to rotenone, for a concentration of  $25 \sqrt{1}$ . was sufficient to kill all animals within three hours (Fig. 1). Other unidentified cladocera, ostracods, and copepods of the plankton were equally sensitive to the drug. The reactions of all these micro-crustacea to rotenone was a gradual slowing of movements, followed by complete narcosis and death.

The conchostracan, *Estheria mexicanus*, was killed by a rotenone concentration of  $50 \sqrt{1}$ . (Fig. 1). The first restless swimming movements toward the surface of the solution were similar to the suffocation responses of vertebrates. In later stages the animals lay on the bottom with the shell valves closed, and the only signs of life were respiratory movements of the thoracic limbs, which could be seen indistinctly through the shell. When the poison stopped respiratory movements, death resulted, and the shell valves opened slightly.

*Annelida*.—Leeches were treated with rotenone concentrations ranging from 25 to 1,000 gamma per liter. The lethal concentration was  $100 \sqrt{1}$ . (Fig. 2). In this and stronger concentrations the animals lay widely separated with their bodies extended and relaxed. In the strongest concentration they lay practically motionless with their suckers unattached but would contract slightly or curl when pressed gently with a glass rod; the action of rotenone was evidently similar to that of an anaesthetic. Control animals tended to clump together in a mass and showed many muscular movements.

Experiments on the fresh water annelid, *Stylaria*, were complicated by the rapidity with which the animal multiplies by transverse fission. However, a rotenone solution of 50 gamma per liter caused a twenty percent reduction in a given number of animals, whereas the controls had multiplied more than sixfold in the same length of time.

*Platyhelminthes*.—The action of rotenone in lethal concentrations (500  $\sqrt{1}$ ., Fig. 2) on *Planaria* was similar to its action on leeches. The drug gradually slowed the swimming movements of the flatworms until they were completely anaesthetized in a relaxed condition.

*Decomposition of Rotenone*.—In many of the experiments it was noted that animals placed in sublethal concentrations of rotenone were profoundly affected by the drug for many hours, but that they usually recovered after one or two days. Either the animals had developed a tolerance for the drug, or the solution of rotenone had decomposed so that it was no longer effective. The first possibility could be eliminated by showing that animals would die in slightly sublethal concentrations if they were placed in fresh solutions every twelve or twenty-four hours. Ginsburg (6) found that rotenone is readily decomposed in sunlight, losing its toxic properties in a few days. Since the solutions used in the present experiments were never exposed to direct sunlight, it seemed desirable to know something about the decomposition of rotenone in lake water. A fresh solution of rotenone (25  $\sqrt{1}$ .) was stocked with five bullheads. As soon as the animals were dead, another group of untreated fish was added, and this process was continued until the solution no longer showed toxic properties. The same experiment was repeated with a solution of 100  $\sqrt{1}$ ., using tadpoles of *Rana pipiens*. In each case the solution had lost nearly all its potency after twenty-four hours. Finally, bullheads were placed in a rotenone solution of 500  $\sqrt{1}$ .; even this very concentrated solution had lost all toxic properties within forty-eight hours.

#### DISCUSSION

The responses of fish and frogs to rotenone have been described in detail by Van Hasselt (1) and Haag (2), and death has been attributed to respiratory failure (Haag) or stoppage of the heart (Van Hasselt). Van Hasselt further inquired into the manner of action of rotenone on mammals, and found that it did not affect the respiratory exchange of gasses in the blood or its psychochemical properties, but rather worked directly on the respiratory center in the central nervous system. He described a number of other reactions produced by rotenone on cats, such as stimulation of the pilomotor system causing erection of hair, and secretion of sweat from foot pads, which are characteristic of sympathetic ner-

vous activity, but are produced by rotenone only in those regions of the body where the central nervous system is intact. The present experiments confirm the fact that respiratory failure is responsible for the death of the fish, and show further that this "failure" is due to a greatly decreased circulation of blood in the gills, apparently caused by capillary constriction. Since the heart beat was still strong even after nearly complete circulatory stasis in the gills, it seems probable that the alkaloid was exerting a specific vaso-constrictor action (or an action on a vaso-constrictor center). This is in line with the other sympathetic-like actions of rotenone, but it should be emphasized that the drug does not reproduce all actions of the sympathetic system (e.g., rotenone does not alter contractions of the bladder; Van Hasselt, 1). It is clear from these data that death is due to the vaso-constrictor action of rotenone and not to a histolysis of the gill epithelium. The latter effect, which was obtained by Daneel (7) and Scheuring and Heuschmann (8), using a rotenone concentration much higher than one which is just lethal, could scarcely cause the death of the animal unless the destruction were so complete as to cause extensive hemorrhage. It is difficult to understand how erosion of an epithelium could have a negative effect on the ease of diffusion of oxygen to the capillaries.

Further evidence that histolysis of the gill epithelium is not the primary cause of death is afforded by the responses of fish to sub-lethal concentrations of rotenone. The animals may exhibit, for hours, the most acute respiratory distress, such as swallowing air and the loss of equilibrium, but eventually will recover as soon as the rotenone has decomposed sufficiently. If loss of epithelium were the cause of such responses, the rapid recovery of fish (which may occur within a few hours) would imply a remarkable capacity for gill regeneration.

Rotenone acts uniformly on invertebrates, causing a gradual narcosis or paralysis. In some cases (e.g., *Estheria*) a respiratory inhibition is apparently involved. It is uncertain whether the drug has a direct narcotic action, for if it functions, in general, as a respiratory poison, a carbon dioxide narcosis would naturally occur.

The relative sensitivities of those animals on which rotenone has an effect may be summarized:

The order of sensitivity with respect to time is graphically shown in Fig. 6. A striking correlation is seen between the species order of sensitivity of fish to rotenone and their oxygen require-

Table I

Animal	Lethal Concentration ( $\sqrt{1.}$ )
Buffalo	8.3
Carp	10
Leptodora	25
Diaptomus	25
Daphnia	25
Bullheads	25
Argulus	25
Estheria	50
Rana	100
Ambystoma	100
Leeches	100
Amphipods	500
Planaria	500

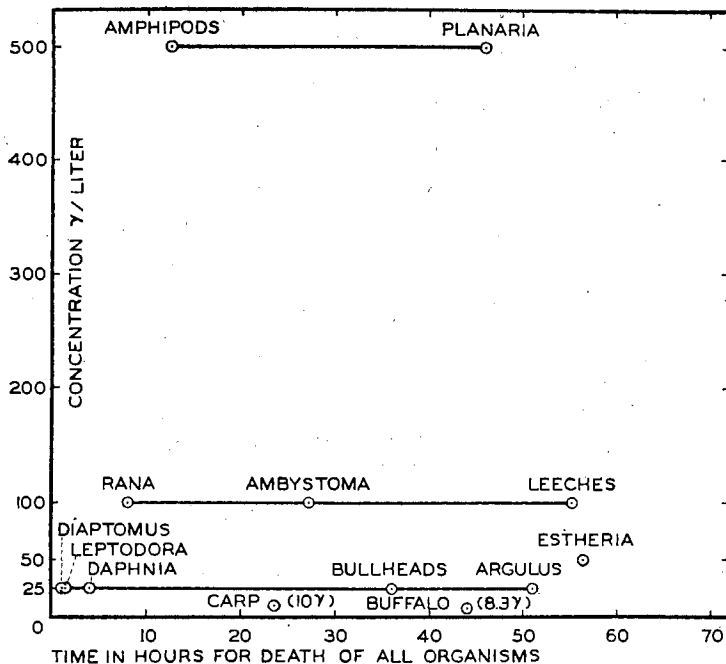


Fig. 6. Order of sensitivity of fresh water animals to rotenone with respect to lethal concentrations of the drug and time of death. The graph should be read from right to left and from top to bottom. Thus, Planaria are the least sensitive and Buffalo the most sensitive. At any single concentration level, the nearer the point lies to the ordinate, the more sensitive is the organism (i.e., less time is required for death of all individuals).

ments. Irving (9) has demonstrated a similar species order in the case with which the blood of fish becomes saturated with oxygen, trout requiring the most oxygen for half-saturation of the haemoglobin, carp less, and catfish least. These species differences in the properties of the blood corpuscles are apparently the physiological basis for the species order of sensitivity to rotenone, for a drug which impairs gill circulation will be more toxic, the more exacting the respiratory requirements of the fish.

Air-breathing animals are relatively insensitive to rotenone in solution, in contrast to those which are gill-breathing or completely aquatic. The lethal concentration of rotenone requires a much longer time to take effect on completely metamorphosed frogs and salamanders than it does to act on larvae with gills. The difference may be in the ease of access of the drug to the animal; apparently it can enter only through the skin of adult amphibia but penetrates readily to the blood stream through the thin gill epithelium of tadpoles. Aquatic insects easily avoid the action of rotenone by resting on the surface film or by flying to a more favorable environment. The ectoparasite, *Argulus*, shows a relatively high sensitivity to rotenone in comparison with more completely parasitic forms, for Haag (2) reports that rotenone has no apparent action on either round worms from hogs or dog tapeworms. However, the sensitivity of *Argulus* is far less than that of the most resistant fish, so that rotenone has no practical value for ridding the host of its parasite.

The concentrations of rotenone which killed fish were too dilute to affect other animals, except for the micro-crustacea, which were killed within one to four hours by solutions that were lethal to bullheads. Even if the initial destruction of plankton forms were rather complete, it is possible that no permanent damage would result, since the eggs of micro-crustacea are often very resistant. It is concluded, therefore, that derris root, if carefully used, may be useful for removing undesirable fish from a lake, provided that the animals with which the waters are to be restocked are not immediately dependent upon the micro-crustacea of the plankton for their food.

#### SUMMARY

1. The action of rotenone as a respiratory poison for fish is shown to be due to a vaso-constrictor property of the alkaloid. The drug greatly reduces circulation of blood corpuscles through

the gill filaments by occluding the capillaries, but apparently has no marked action on the heart or larger blood vessels.

2. Studies on metamorphosing amphibia show that there is a marked decrease in sensitivity to rotenone as gills are functionally replaced by lungs.

3. Rotenone acts on a variety of invertebrates by causing a gradual narcosis or paralysis. In general, invertebrates are much less sensitive to the drug than fish, except for the micro-crustacea, which are very sensitive to those rotenone concentrations which kill fish. The importance of this action on plankton animals is briefly discussed.

4. Decomposition of solutions of rotenone is so rapid that even high concentrations (500  $\sqrt{1}$ .) have lost their toxicity within three days.

DEPARTMENT OF ZOOLOGY, AND  
IOWA LAKESIDE LABORATORY,  
STATE UNIVERSITY OF IOWA,  
IOWA CITY, IOWA.

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