

A NEW BACTERIAL DISEASE OF FRESH-WATER FISHES.

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Contribution from the U. S. Fisheries Biological Station, Fairport, Iowa.

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INTRODUCTION.

While working on the protozoan parasites of fishes at the U. S. Fisheries biological station, Fairport, Iowa, during the summer of 1917 a number of fish which had been recently placed in aquaria were found to be dying from the effects of a bacterial infection. Later in the season the disease was quite prevalent among fishes confined in aquaria, but owing to the pressure of other work no particular attention was paid to it at that time.

Early the following summer (1918) the disease again made its appearance, this time in one of the ponds. In the course of a series of feeding experiments being carried on at the Fairport station a large number of fingerling buffalofish were held in a pond in which had been placed a considerable quantity of horse manure. These fish grew rapidly for some time and appeared to be in a healthy, vigorous condition. However, early in July they began to die in large numbers, and a careful examination showed that the dying fish were infected with the same species of bacteria found the previous summer on fish in aquaria. A little later in the season the disease appeared among fishes that had recently been transferred to troughs for feeding experiments and caused considerable mortality.

On account of its evident importance it was decided to undertake an extended investigation of the disease to determine, if possible, a practicable method of control. This investigation was begun during the latter part of the summer of 1918 and continued during the summer of 1919. During the summer of 1919 the writer was assisted by Miss Miriam Mackenzie.

DESCRIPTION OF THE DISEASE.

The disease is easily recognizable, being distinguished by well-defined characteristics. Ordinarily the first indication of the disease is the appearance of one or more characteristic dirty-white or yellowish areas on some part of the body. The infected areas are usually quite conspicuous and increase rapidly in size. In some cases a large proportion of the body may eventually become infected. With the increase in the size of the lesions the fish become greatly weakened and usually die from 24 to 72 hours after the lesions first become visible.

The lesions may occur on any part of the body, but in the majority of cases first appear on the fins, especially the caudals, and from there spread to adjoining portions of the body (figs. 231-237). In late stages of the disease the lesions may cover from one-half to two-thirds of the body, while the fins become badly frayed, the caudal sometimes becoming worn to a mere stub (figs. 233 and 234). There is considerable variation in the site of the initial infection. As will be shown later the disease is usually the result of injuries and first makes its appearance in the injured region. There is also considerable variation dependent on the species of fish infected. In the crappies, for instance, the disease is usually confined to the fins and gills (figs. 231, 232, and 243) and only rarely does the infection spread to the body. Possibly this may be due to the fact that these fishes are especially susceptible to the disease and die before there is time for the bacteria to become widely spread over the surface of the body.

Lesions on the gills first appear as small, white patches (figs. 243 and 244). These spread rapidly, and the fish usually dies within a few hours (fig. 245).

On bullheads (*Ameiurus melas* and *A. nebulosus*) the lesions have a somewhat different appearance from those on scaled fishes. They usually first appear as numerous small, circular areas with sharp, distinct outlines (fig. 238).

The centers of the lesions are dark blue overlaid by a whitish veil or cloudiness. Surrounding this is a well-defined zone about 5 mm. wide characterized by a distinctly reddish tinge due to hyperemia. This region, like the central portion of the lesion, is overlaid by a slight cloudiness. Later the lesions often become confluent and cover the greater portion of the body (figs. 239-241). For some reason, in fingerling bullheads the disease more commonly starts on the caudal fin, as in other fishes, and the infected area gradually advances toward the anterior end, the entire posterior end of the body becoming a dirty white (fig. 242).

In many respects the lesions resemble those produced by Saprolegnia and, in fact, have usually been confused with them. A careful examination will, however, readily enable one to distinguish between the two, since the bacterial lesions do not present the fuzzy appearance so characteristic of those infected with fungus. Of course they can easily be distinguished by a microscopical examination, but this is usually not necessary.

In some instances a bacterial infection may be followed by an infection with Saprolegnia, but in such cases the fungus is of secondary importance. During the summer very little fungus has been observed at Fairport and infection with Saprolegnia appears to be dependent on the temperature of the water. When the temperature is high (above 75° F.) the bacteria develop rapidly and the fish die so quickly that the fungus does not have time to develop to any appreciable extent. However, the bacteria are much more susceptible to any decrease in temperature than is the Saprolegnia, the result being that

at temperatures below 75° F. the bacteria develop much more slowly and the fungus growth may become evident before the fish succumbs.

This is notably the case early in the fall. With the advent of cooler weather a considerable number of the diseased fish may show characteristic fungus growths in connection with the bacterial lesions. But even in these cases there can be little doubt that the lesions were primarily due to bacteria and that the *Saprolegnia* was a secondary invader.

Occasionally fish may die from the disease without showing any lesions on the body or fins. In such cases the gills are always badly infected, a large part of them being entirely destroyed. In late stages of gill infection the fish often show characteristic signs of suffocation, such as swimming at the surface and gasping for breath. In the majority of cases, fish with infected gills will also show lesions on the surface of the body or fins, but here again there is considerable variation in different species.

OCCURRENCE OF THE DISEASE.

It seems probable that most species of fishes are liable to attack by this disease, although some species are much more susceptible than others. While it has been necessary to confine our observations to a comparatively small number of species the disease has been observed on the following fishes: The buffalofishes (*Ictiobus bubalus* and *I. cyprinella*), the sunfishes (*Lepomis incisor* and *L. humilis*), the carp (*Cyprinus carpio*), the black basses (*Micropterus salmoides* and *M. dolomieu*), the crappies (*Pomoxis sparoides* and *P. annularis*), the warmouth (*Chaenobryttus gulosus*), the yellow perch (*Perca flavescens*), the white bass (*Roccus chrysops*), the brook trout (*Salvelinus fontinalis*), the minnow (*Pimephales notatus*), the channel catfish (*Ictalurus punctatus*), and the bullheads (*Ameiurus nebulosus* and *A. melas*). From the above list it will be readily seen that the disease is widespread, and there is little reason to doubt that under favorable conditions it may occur on nearly all species of fresh-water fishes.

In one instance several tadpoles which were confined in a tank with a number of infected buffalofish developed well-defined lesions on the tail. The tadpoles were just beginning to metamorphose, which may have made them more susceptible to infection. Attempts to inoculate adult frogs with the disease have proved unsuccessful.

So far it has not been possible to obtain any data regarding the geographical distribution of the disease. However, while on a visit to the St. Lawrence River at Ogdensburg, N. Y., during the first week in July, 1919, the writer found the bacteria in lesions on the smallmouth black bass and the common perch. In this case the fish did not appear to be seriously injured by the bacteria which were evidently growing very slowly.

CAUSE OF THE DISEASE.

The disease is produced by an apparently undescribed species of bacteria for which I propose the name *Bacillus columnaris*. It has not yet been possible to grow the organism in pure culture, but its appearance is so characteristic that it is always easily recognizable if present in any numbers. It is a long, slender, flexible, rod-shaped organism, 5 to 12 μ long and 0.5 μ wide (figs. 246 and 247). The bacteria are very transparent and unless present in considerable numbers are difficult to distinguish on this account. They usually appear perfectly homogeneous, but rarely may exhibit a slightly granular structure. The bacteria are motile and probably possess flagella, although owing to

lack of facilities this has not been determined with certainty. They can often be seen to move in a straight line for a considerable distance, the movement being accompanied by a sinuous bending of the rod which may temporarily assume an S-shape. One of the characteristic movements is to turn one end slowly in a circle, the other end remaining stationary and forming a pivot on which the entire rod revolves. This movement is very noticeable along the edge of scales or bits of infected tissue when placed on a slide. Large numbers of bacteria can usually be seen in such places with one end attached while the free end moves back and forth in the manner described above.

Another characteristic is the formation of small chains of bacteria from one object to another while on the slide. The chains are formed by the bacteria arranging themselves in rows joined end to end, the ends slightly overlapping. Usually these chains consist of two or three rows of bacteria arranged parallel to each other, but occasionally there may be but a single row. Bacteria can be seen continually moving back and forth along these living chains in a very characteristic manner.

However, the most characteristic movement and the one which usually serves to make the species easily recognizable is well shown when a little material scraped from a lesion is placed on a slide in a drop of water. Possibly owing to the pressure of the cover glass the bacteria soon collect in immense numbers on the edge of bits of infected tissue, scales, etc. Here they form short columnlike masses, each column being separated a short distance from its neighbor (figs. 248 and 249). The columns usually taper slightly toward the free end, which is ordinarily rounded but in rare cases may be pointed. In some cases the ends of the columns may be distinctly enlarged. In favorable cases a whole series of these columns may form along the edge of a scale. These columnar masses of bacteria are very characteristic, and it is apparently in this way that they free themselves from the gelatinous matrix in which they are embedded while growing on the fish. Along the sides and rounded ends of the columns can be seen bacteria with one end attached while the free end waves back and forth in the manner already described. Bacteria continually break loose from the columns while others swarm out to take their place. Occasionally short chains may be formed extending out from the ends of the columns, the bacteria at the ends continually becoming free but often managing to work their way back along the chain after a time. When free, the bacteria at first exhibit a peculiar vibratory movement somewhat different from the Brownian movement and which, when once seen, is easily recognized. After being free a short time they usually collect on the underside of the cover glass and become perfectly motionless.

These characteristic swarming movements of the bacteria are no doubt continually taking place on the fish, at least in late stages of the disease, for it has been found that badly diseased fish are continually shedding bacteria in enormous numbers.

Owing to the fact that a variety of stains was not available the staining reactions of the bacteria were not studied in detail. They stain readily with fuchsin and Giemsa's stain, the stained preparations appearing perfectly homogeneous.

No evidence of sporulation has been observed, and that they do not form spores is also indicated by the fact that the bacteria are easily killed by chemicals and drying.

All attempts to grow the bacteria on artificial media have so far proved unsuccessful. During the summer of 1918 the writer made several attempts to isolate the bacteria, and these experiments were continued by Miss Mackenzie during the summer

of 1919. On account of the limited facilities at our disposal it was only possible to try a few of the simpler media. Standard beef-broth agar and fish agar were tried, and although bacteria appeared in the plates in large numbers no trace of *columnaris* could be found. Several lots of media with a less acid reaction than the standard were tried, including one with the same reaction as the river water, but the results were all negative. Attempts to grow the bacteria on fish serum were equally unsuccessful. Possibly with facilities for employing a wider range of media it may be possible to isolate the bacteria. However, the failure to isolate the bacteria did not prove so serious a handicap in the study of the disease as would ordinarily be the case. The bacteria can easily be procured in large numbers from the lesions of infected fish, and their appearance is so characteristic that they can be readily recognized.

While, of course, the failure to isolate the bacteria in pure culture has rendered it impossible to demonstrate the cause of the disease beyond question, the evidence is so conclusive as to leave little room for doubt. The bacteria can always be found in abundance in the lesions, and the disease can easily be produced in healthy fish by scraping off a few scales and applying a few bacteria from a diseased fish. In advanced stages of the disease there are usually large numbers of bacteria present in addition to *columnaris*, but it has been found that in small lesions *columnaris* is invariably enormously more abundant than any other organism, and in some cases when the lesions first become visible there is a nearly pure culture of this species present.

Strong evidence for the causal relationship of *columnaris* to the disease can be seen in infected bullheads. The reader will recall that the lesions on these fish are characterized by an outer reddish zone about 5 mm. wide, while the center of the lesion has a quite different appearance. When very small the entire lesion presents the same appearance as the peripheral zone in later stages. As the lesions increase in size the characteristic blue center appears and enlarges with the growth of the lesion. It is obvious that the red zone is found only where the skin has been recently infected and that the darker color is due to destructive changes produced by the bacteria. If a little material is scraped from the darker central portion of the lesion it will be found that considerable numbers of bacteria are present in addition to *columnaris*. On the other hand, if material from the red zone is examined it will be found that nearly all the bacteria present are *columnaris*. Other species are so few as to be scarcely noticeable. Of course the same thing is true of lesions on other species of fishes, but owing to the absence of scales it is more striking in the case of the bullheads than elsewhere. The great abundance of *columnaris* around the advancing edge of the lesions is so noticeable that it soon became a matter of routine in our work to always procure the bacteria from this region. The smears from which the photomicrographs (figs. 246 and 247) were taken were made from such material.

PATHOGENESIS.

As previously stated the bacteria grow only on the surface of the body or on the gills. No case has been observed where they had penetrated any distance into the tissues. When growing on the body they destroy the integument, the muscles sometimes being exposed in late stages of the disease. For obvious reasons the development of the lesions can be more easily studied in the case of scaleless fish, such as the common

bullhead. Figure 250 is a cross section through a lesion which is just beginning to develop. The outer layer of the epidermis has begun to disintegrate, but the inner layer is not yet noticeably affected. Figure 251 shows a little later stage. The epidermis is now entirely destroyed in one place at the right in the figure. On either side the epidermis is rapidly disintegrating. A somewhat later stage is shown in figure 252. The outer layer of the corium which has been exposed by the destruction of the epidermis is now beginning to show signs of disintegration. In late stages of the disease the corium may be entirely destroyed in the center of the lesion, as shown in figure 253.

As previously described, the lesion grows rapidly outward in all directions from the center of infection. Figures 254 to 257 are from photomicrographs of sections through the margins of lesions and show the disintegration of the epidermis caused by the outward growth of the bacteria. Usually there is a noticeable hyperemia just underneath the epidermis in this region. This is well shown in figures 255 to 257. The capillaries in the outer portion of the corium become gorged with blood; eventually their walls disintegrate and the blood fills the interstices of the corium and crowds between the corium and the epidermis. Occasionally the blood corpuscles may even penetrate a short distance into the epidermis (fig. 255). This is the cause of the reddish zone which is often quite distinct around the margin. Later as the epidermis is destroyed the corpuscles also disintegrate. It is scarcely necessary to add that the hyperemic zone advances outward as the lesion enlarges. Usually the hyperemia is noticeable only underneath that portion of the epidermis which is undergoing disintegration, but occasionally may occur before the overlying epidermis shows any change. No case has been observed where the muscles showed any appreciable pathologic changes. Possibly this may be due to the fact that the fish usually die by the time the lesions have developed to the stage shown in figure 253.

While the above account is based on a study of the lesions in bullheads the same conditions are found in other fishes with some slight modifications due to the presence of scales. In these fishes the scales become loosened and slough off as the integument disintegrates.

In all cases there is a thick, matted layer of bacteria covering the lesion. This layer is not shown in the figures since it invariably drops off during the treatment to which the tissues are subjected in preparation for sectioning. Although the bacteria occur in enormous numbers in this superficial layer only a few scattered individuals can be found among the disintegrating cells of the epidermis and corium.

When growing on the gills the bacteria produce much the same effects as on the integument. The epithelium and blood vessels are entirely destroyed until only the skeletal parts are left. As would be expected gill infections are much more quickly fatal than infections on the surface of the body.

METHODS OF INFECTION.

Since the bacteria grow only on the surface of the body and gills it is evident that infection can easily take place by their being carried in the water from one fish to another. As previously pointed out the bacteria are continually being set free from infected fish, especially in late stages of the disease. Where fish are crowded closely together, as in aquaria, the bacteria can thus readily pass from one individual to another. They are

also easily rubbed off onto the nets or hands when diseased fish are handled and then can readily be transferred to healthy fish. We have several times infected healthy fish by simply holding them in our hands after having previously handled diseased fish. There is also a possibility that the disease may be transmitted by predacious insects, but no experiments have as yet been carried out along this line.

Undoubtedly the most important factors in the spread of the disease under ordinary conditions are those which render the fish more susceptible to infection. All the evidence at hand indicates that the bacteria rarely or never attack healthy, uninjured fish under ordinary circumstances. If, however, a fish is injured or its vitality lowered in any way it is liable to contract the disease. Most outbreaks of the disease which have come under the writer's notice have occurred among fish which had been recently handled. As is well known it is almost impossible to handle fish without some slight injury to the fins or body, and even a very slight injury is all that is necessary to enable the bacteria to gain a foothold, and once started they are able to spread rapidly over the body. During warm weather, when the temperature of the water at Fairport averages about 75 to 80° F., an outbreak of the disease is almost certain to occur within 48 to 72 hours after the fish are handled. Well-defined lesions may sometimes appear in 24 hours, but the fish usually do not begin to die until after 48 hours. The greatest mortality usually occurs on the third and fourth days, after which there is a gradual decrease until the disease finally disappears. If the fish are again handled, however, there is almost certain to be another outbreak.

In a large percentage of cases the disease first makes its appearance on the fins, especially the caudal. This is, of course, readily explained since no other part of the body is so liable to be injured by the struggles of the fish when taken in a net. Of course, any slight abrasion on the surface of the body, such as the removal of a few scales or even a slight injury to the epithelium, is equally liable to infection. It has been found very easy to infect fish artificially by simply scraping away a few scales with a scalpel and applying a small amount of material scraped from a diseased fish. Usually a characteristic lesion will develop around the site of infection in 24 to 48 hours.

There is also evidence that in some cases lowered vitality unaccompanied by mechanical injuries may result in the fish becoming infected. This is probably the explanation of the epidemic which appeared in one of the ponds at Fairport in which young buffalofish were being held for a feeding experiment. These fish had not been handled for some weeks previous to the outbreak of the epidemic. Just before the disease appeared the water had become noticeably foul and this, no doubt, so lowered the vitality of the fish as to make them susceptible to infection. The writer has also been informed by fishermen that they had seen fish with the same disease in isolated ponds and sloughs along the Mississippi River late in summer. At this time the water in these ponds becomes very warm and stagnant, conditions which would favor the development of the bacteria while tending to lower the vitality of the fish.

Another important factor influencing the spread of the disease is the temperature of the water. As already pointed out *columnaris* is very susceptible to temperature changes and grows rapidly only at a comparatively high temperature. The disease is distinctly a warm-weather disease, and so far as our observations go is not ordinarily of great importance when the temperature of the water is low. This was very noticeable at Fair-

port during September, 1919. With the advent of cooler weather there was a considerable decrease in the temperature of the water in the aquaria in which the experimental fish were kept. After the first week in September the average temperature of the water decreased from about 75 to 80° to about 70° F. and there was a notable decrease in the severity of the disease. The percentage of infected fish decreased, the disease developed more slowly, and a considerable percentage of the infected fish recovered.

The writer does not, however, wish to give the impression that there is no danger of an outbreak of the disease when the water temperature falls below 70° F. There is some evidence that an epidemic may occur at a comparatively low temperature, but it is probable that under such conditions the disease is much less severe and more easily controlled. That the bacteria may develop at lower temperatures is shown by an experiment with brook trout. A number of fingerlings were shipped to Fairport from the hatchery at Manchester, Iowa, and placed in a trough supplied with running water from a well. The temperature of the water averaged about 62° F. On September 13 two badly diseased fish, one buffalofish and one bluegill, were placed in the trough among the trout. At a temperature of about 75° F. both fish would in all probability have died in less than 24 hours. However, after being placed in the trough with the trout the bluegill lived 48 hours and the buffalofish about 72 hours. One of the trout was found dying with the disease on September 17. Unfortunately, owing to the writer's departure from Fairport, it was impossible to continue the experiment longer.

During the first week in July, 1919, the writer found a slight infection on a yellow perch and one on a smallmouth black bass from the St. Lawrence River at Ogdensburg, N. Y. In each case the infection was in wounds on the side of the body. These fish were in a tank with a large number of other fishes, and during the three days they were under observation none of the other fish contracted the disease. Furthermore, the lesions on the infected fish did not increase noticeably in size during this time and the fish showed no ill effects from the disease. Since the temperature of the water averaged about 65° F. it is probable that the failure of the disease to develop rapidly and spread to the other fishes in the tank was largely due to this fact. Many of the other fishes confined in the tank had been more or less injured when captured, so there would seem to have been every opportunity for the disease to spread. However, in this case it was impossible to determine definitely whether the failure of the disease to develop was due to the low temperature or to the fact that the bacteria may have belonged to a much less virulent strain than that at Fairport. It has not yet been possible to carry out any detailed experiments to determine the effect of temperature on the virulence and growth of the bacteria, but it is hoped to do so in the near future.

There is also evidence that, in general, fish are more likely to contract the disease from fish of the same species than from one of another species. This means, of course, that physiological strains of bacteria may be developed on different species of fish. As is well known such physiological strains are common among many species of bacteria. The strongest evidence of the occurrence of physiologically distinct strains in *Bacillus columnaris* was obtained in the course of some experiments with the black bullhead (*Ameiurus melas*). This species is ordinarily not very susceptible to the disease, and during the summer of 1919 a number of these fish were kept in the same tank with infected buffalofish and bluegill without any of them contracting the disease. After several weeks one of the bullhead was artificially inoculated with bacteria obtained

from a diseased buffalofish. Inoculation was made as usual by scraping away the epidermis from a small area on one side of the body and rubbing the bacteria into the wound. The bullhead developed a characteristic lesion, and within 3 or 4 days practically every bullhead in the tank became infected. The epidemic among the bullhead was not confined to this tank but soon spread to bullhead in adjoining tanks. This can be easily explained. On account of their apparent immunity to the disease no precautions were taken to prevent the bacteria getting from one tank to another, and they could easily have been transferred on the hands or nets. The epidemic developed with remarkable rapidity, and within a few days nearly all the bullhead which had been held in the tanks from one to several weeks without showing any signs of the disease were dead or dying. Only by the use of control measures to be described later was the epidemic checked and a few fish saved. It was noticeable that although the infected bullhead were in several instances kept in the same tanks with buffalofish and bluegill the disease did not spread among them nearly so rapidly as among bullhead, although ordinarily both buffalofish and bluegill are more susceptible to the disease.

It has also been observed in making artificial inoculations that while it is usually not difficult to infect a fish with bacteria from another species a larger percentage of positive results is often obtained when the bacteria are taken from a fish of the same species. Further investigations along this line are greatly to be desired.

As intimated above, while most species of fresh-water fishes are liable to be attacked by the disease some species are much more susceptible than others. It is, of course, by no means easy to determine accurately the relative susceptibility of different species, but there can be no question that there is a great deal of variation in this respect. It is possible to divide the fishes which have been most studied into two classes. The first includes those species which are very susceptible and are almost certain to contract the disease in large numbers if handled during warm weather. In this class we would include the buffalofishes (*Ictiobus bubalus* and *cyprinella*), the crappies (*Pomoxis annularis* and *sparoides*), and possibly the bluegill (*Lepomis incisor*). The second class includes those fishes which are ordinarily only moderately susceptible and do not usually contract the disease in such numbers, even in warm weather. Furthermore, a considerable percentage of the diseased fish may recover if kept under favorable conditions. In this class we would include the largemouth black bass (*Micropterus salmoides*), the channel catfish (*Ictalurus punctatus*), the bullheads (*Ameiurus nebulosus* and *melas*), the sunfish (*Lepomis humilis*), the carp (*Cyprinus carpio*), the warmouth (*Chænobryttus gulosus*), and the white bass (*Roccus chrysops*). Of course it is not to be understood that all of the species in each class are equally susceptible, for that is certainly not the case, but in the present state of our knowledge it does not seem advisable to attempt a more detailed analysis of their relative susceptibility to the disease.

It is also noticeable that the young of any species are, in general, more susceptible than are the adults. This is especially true in the case of the carp, the young of which are quite susceptible, while the adults are nearly immune.

At first it was assumed that the bacteria must be widely distributed in the water, since this would most readily explain the appearance of the disease when the fish are handled, but we have no proof that this is the case, and there is some evidence that the bacteria do not live for any length of time off the fish. If this is true, we are forced to assume that they are able to live in small numbers on perfectly healthy fish without

injuring them, but are capable of increasing enormously whenever proper conditions arise. Such a state of affairs is not unknown as in the case of streptococci and pneumococci in man.

TREATMENT AND CONTROL OF THE DISEASE.

On account of the great economic importance of the disease special efforts have been made to develop effective methods of control. Early in the investigation it became evident that reliance must be placed chiefly on methods for preventing the spread of the disease rather than to attempt to cure fish already infected. This is due to the fact that the bacteria, although living exclusively on the exterior of the fish, form a thick, matlike growth which protects the bacteria underneath from the effects of chemicals. Furthermore, the bacteria soon make their way underneath the scales which form an additional protection. To kill bacteria in such protected situations requires a comparatively strong solution.

A number of chemicals have been tried in the effort to find an effective control for the disease, but only potassium permanganate and copper sulphate have been found to be of any value. In all our experiments with chemicals we have first determined the maximum concentration of the chemical which the fish can stand without serious injury. Different species differ greatly in this respect, and unfortunately the fishes which are least resistant to chemicals are often very susceptible to the disease. It was soon found that the buffalofishes are among the most susceptible species, both to chemicals and to the disease, and it is believed that any treatment which will succeed with these fish will be equally successful with most of our common fishes. Having ascertained the strength of the solution which the more susceptible fishes could stand without injury, we then attempted to determine the minimum concentration which will kill the bacteria when fully exposed to the chemical. Since the bacteria can not be grown on culture media it is obviously impossible to determine with certainty when they are killed. It was found, however, that fairly accurate results could be obtained by placing the bacteria on a slide and then treating them with solutions of various strengths. The cessation of all movement by the bacteria was taken as evidence that they were seriously injured if not killed. While, of course, there are serious objections to this method it was found in practice to work out remarkably well.

Of course the time element is important in the use of any chemical. Treatment with a very weak solution may be as effective as a much stronger solution if the time is sufficiently increased. Temperature may also be an important factor, but we have only meager data as to its effects. With few exceptions our experiments have been carried on with river water at a temperature of 75 to 80° F.

During the summer of 1918 an extensive series of experiments with potassium permanganate was undertaken. It was found to greatly weaken or kill the bacteria in solutions so dilute that most species of fish could be kept in them for some time without serious injury. In order to kill the bacteria it is necessary to treat the fish with a 1 to 50,000 solution for 10 to 15 minutes. Weaker solutions have little effect. Buffalofishes can undergo this treatment without serious injury, while the black bass and sunfishes can stand a much stronger solution. A 1 to 20,000 solution was used on black bass for the same length of time with good results. However, very few fishes can stand a 1 to 20,000 solution, and weaker solutions do not seem to penetrate far into the bacterial

mat covering the lesions. Furthermore, potassium permanganate is expensive and dissolves slowly in water. For these reasons the potassium-permanganate treatment was later abandoned in favor of copper sulphate.

A number of experiments with copper sulphate were made during the summer of 1918, and it was found that a 1 to 30,000 solution is more effective than a 1 to 50,000 solution of potassium permanganate and not so injurious to the fish. A solution of this strength will not, however, kill bacteria which are well protected, such as those beneath the scales. Our experiments showed that normal fingerling buffalofish are not appreciably injured when placed in a 1 to 30,000 solution for 30 minutes, but diseased or weakened fish are not so resistant. Buffalofish over 1 year old are not injured by treatment with a 1 to 25,000 solution for 30 minutes, and black bass and bullheads can stand a 1 to 20,000 solution for the same length of time without injury. As a result of these experiments the following treatment was recommended and used with much success: The fish previous to the appearance of any lesions are placed in a 1 to 30,000 solution of copper sulphate for 20 minutes and are then removed at once to running water. If properly handled they rarely suffer any permanent injury. Wooden vessels are preferable, and if it is necessary to use galvanized vessels they should be painted to prevent chemical action between the copper sulphate and the metal sides of the vessel. A thin coating of melted paraffin over the inside of a galvanized tank serves the purpose admirably. During the treatment some of the less resistant species of fishes may swim around at the surface and show more or less signs of distress, but this does not necessarily indicate any serious injury.

It should be distinctly borne in mind that the treatment to be effective must be given within a few hours after the fish have been handled and before any signs of infection appear. If the fish are to be confined in aquaria, two or three successive treatments at 12 to 24 hour intervals are advisable. When this treatment is properly used it is believed it will effectually prevent any serious outbreak of the disease. It must be repeated, however, every time the fish are handled or subjected to injury in any way.

In an experimental test of this treatment 30 fingerling buffalofish were seined from one pond. They were handled quite roughly, and 14 controls were placed in a small aquarium without being treated. The remaining 16 fish were treated with a 1 to 30,000 solution of copper sulphate for 20 minutes and then placed in a small aquarium like that in which the controls were held. Throughout the experiment both aquaria were supplied with running water, and the conditions in each were as nearly identical as it was possible to make them. Two of the treated fish died within 12 hours, probably as a result of mechanical injuries. At the end of 24 hours the fish were again treated with a 1 to 30,000 solution for 20 minutes. Twenty-four hours later two of the treated fish were found dead, but a careful examination failed to disclose any signs of *Bacillus columnaris*. Since the remaining fish showed no signs of infection no treatment was given at this time, but 6 hours later one fish showed a well-defined lesion at the base of the tail. This fish was at once removed and the rest given the same treatment as before. Since no further signs of infection appeared in this lot in three days the experiment was discontinued.

Among the controls 3 fish showed well-defined lesions after 24 hours, and at the end of 48 hours 11 out of the 14 were dead, while 2 of the remaining 3 had well-developed

lesions on the tail and died a few hours later. The remaining control died the next day with a large lesion on the side of the head. In short, there was a loss of $31\frac{1}{4}$ per cent among the treated fish and 100 per cent among the controls. Four of the five fish which died among the treated lot were in all probability killed by the direct effects of the copper sulphate. A large number of experiments have shown that fish when weakened in any way, as by handling or being kept in water deficient in oxygen, are much more susceptible to the injurious effects of the treatment than perfectly normal fish and are often killed by solutions which will not appreciably injure fish in good condition. It is this fact which has made it impossible to devise a treatment which will be successful under all conditions. Of course it is evident that the fish which are so weakened as to be injured by the treatment are the very ones which are most liable to contract the disease if not treated.

It has not been practicable to conduct many experiments so completely under control as this one, but we have in a number of instances successfully checked by this treatment an outbreak of the disease among fish which were being kept in aquaria for other purposes. Another test of the treatment under somewhat different conditions is of considerable interest: On July 20, 1919, in connection with a series of feeding experiments which were being carried on at the Fairport station, a number of 3-year-old buffalofish were seined from the pond in which they had been confined for some time and distributed among four different ponds. This necessitated handling the fish rather roughly and transferring them some distance in a galvanized tank. To make matters worse it was a very hot day and under ordinary circumstances there would undoubtedly have been considerable loss, especially since many of the fish were badly rubbed during the transfer. Before being liberated in the ponds all the fish were treated for 20 minutes with a 1 to 30,000 solution of copper sulphate. Of the 551 fish treated only 4 died as a result of the transfer, and none of these showed any evidence of the disease. It is unfortunate that in this case it was not practicable to keep any controls for comparison with the treated fish.

While the treatment described above has been very successful it has the great disadvantage of requiring considerable time, and this is a serious objection where many fish are to be treated. During the summer of 1919 extensive experiments were carried on with a view to shortening the treatment. As a result a rapid treatment has been devised which is believed to be even more effective than the longer treatment previously in use. Briefly, the new method consists in treating the fish with a 1 to 1,000 solution of copper sulphate for one to two minutes. This treatment is so great an improvement on the earlier treatment with a 1 to 30,000 solution that in our later experiments we entirely discarded the latter.

When the fish are in good condition they can be placed in the 1 to 1,000 solution for two minutes without injury, but if they have been previously weakened in any way they should not be exposed to the solution for more than one minute. Such hardy fishes as the black bass, sunfishes, and bullheads can be safely left in the solution for three minutes if in good condition. In some cases it is advisable to repeat the treatment after 12 to 24 hours.

A number of experiments with the rapid treatment were carried out during the latter part of the summer of 1919, but it will suffice to describe a few representative experiments.

Experiment No. 1.—September 6 a number of buffalofish and crappie were seined from a wire inclosure in the river (see fig. 258) where they had been held since September 2. The fish were in good condition and were divided into two lots before being placed in aquaria well supplied with running water. Except for the copper-sulphate treatment both lots were kept under as nearly identical conditions as possible. Sixteen crappie and eight buffalofish were treated with a 1 to 1,000 solution of copper sulphate for two minutes (water temperature 71° F.) before being placed in the aquarium. The controls, 10 crappie and 8 buffalofish, were placed directly in an aquarium without treatment. September 8 among the controls there were two crappie dying with the disease, while most of the remaining fish showed considerable infection on the fins and sides of the body. Among the treated fish, one buffalofish and two crappie were slightly infected. At this time there was a very striking difference in appearance in the two lots of fish. The untreated fish were nearly all in bad shape, with well-developed lesions and considerable mucous on various parts of the body. On the other hand, the treated fish appeared bright and clean, and those infected showed only slightly developed lesions. September 9 two more crappie and one buffalofish were found dead among the controls. There were no new infections among the treated fish and one infected crappie was improving. September 10 there were one dead buffalofish and one dead crappie among the controls. The treated lot were in good condition, and those infected, with the exception of one crappie which died, were recovering. Since there were no new infections and the slightly infected fish in both lots were recovering, the experiment was discontinued on September 11. The treated lot at this time was noticeably in better condition than the controls.

SUMMARY OF EXPERIMENT NO. 1.

Species.	Treated fish.			Controls.		
	Number.	Loss from disease.		Number.	Loss from disease.	
		Number.	Per cent.		Number.	Per cent.
Crappie.....	16	1	10	5
Buffalofish.....	8	0	8	2
Total.....	24	1	4.16	18	7	38.88

Experiment No. 2.—September 13 a number of buffalofish and crappie were removed from the same inclosure and treated as in the preceding experiment. Since the details of the experiment do not differ in principle from the preceding, it will be sufficient to summarize the results:

SUMMARY OF EXPERIMENT NO. 2.

Species.	Treated fish.			Controls.		
	Number.	Loss from disease.		Number.	Loss from disease.	
		Number.	Per cent.		Number.	Per cent.
Crappie.....	3	0	3	2
Buffalofish.....	12	0	10	5
Total.....	15	0	0	13	7	53.84

These two experiments indicate what the copper-sulphate treatment can accomplish when used under favorable conditions. If the fish have been greatly weakened before being given the treatment, however, the results are far from encouraging.

As pointed out, fish which have been seriously weakened by previous treatment are often killed by the copper sulphate, and it is doubtful if any treatment can be devised which can be safely used while the fish are in this condition. This is especially true if the fish just previous to being treated have been confined for some time in a small vessel where the oxygen supply is deficient. Several experiments were carried out with fish seined from small ponds which had been isolated from the river when the waters receded after the spring floods. These fish were transported in galvanized washtubs, often for a distance of 2 or 3 miles, and held in the tubs an hour or two before reaching the station. A large number of fish were carried in each tub, and although attempts were made to keep the water aerated they were partially asphyxiated when reaching the station. All our experiments in treating such fish were unsuccessful, the great majority being killed by the copper sulphate. Possibly the fish could be successfully treated if first held in running water for several hours. The two following experiments are typical of results with these exhausted fish.

Experiment No. 3.—The fish were brought to the station at 5 p. m., September 10, in a greatly weakened condition. Part were treated with 1 to 1,000 copper sulphate for two minutes and then placed in an aquarium well supplied with running water. The remainder were placed in a similar aquarium without being treated. Most of the fish in the treated lot died within 24 hours. In the following summary all fish which died during the first 48 hours are tabulated as having died from the effects of the copper-sulphate treatment or injuries sustained on their way to the laboratory. These fish showed no well-developed bacterial lesions, and our previous experiments had shown that fish rarely die from the effects of bacteria until more than 48 hours have elapsed.

SUMMARY OF EXPERIMENT NO. 3.

Species.	Treated fish.					Controls.				
	Num-ber.	Loss from treat-ment and injuries.		Loss from disease.		Num-ber.	Loss from injuries.		Loss from disease.	
		Number.	Per cent.	Number.	Per cent.		Number.	Per cent.	Number.	Per cent.
Crappie.....	16	11	0	21	1	1
Buffalofish.....	20	9	0	15	0	5
Bluegill.....	3	1	0	7	1	1
Total.....	39	21	53.84	0	0	43	2	4.65	7	16.27

This case is remarkable for the small number of infected fish among the controls. Possibly this can be explained by the fact that although the fish had been much weakened from lack of oxygen they had evidently been handled carefully and showed only slight mechanical injuries.

Experiment No. 4.—September 11 a number of buffalofish and bluegill were brought to the laboratory from the same pond as in the preceding experiment and were treated in the same way.

SUMMARY OF EXPERIMENT NO. 4.

Species.	Treated fish.					Controls.				
	Num-ber.	Loss from treat-ment and injuries.		Loss from disease.		Num-ber.	Loss from injuries.		Loss from disease.	
		<i>Number.</i>	<i>Per cent.</i>	<i>Number.</i>	<i>Per cent.</i>		<i>Number.</i>	<i>Per cent.</i>	<i>Number.</i>	<i>Per cent.</i>
Buffalofish.....	15	10	0	0	16	0	11
Bluegill.....	9	2	0	0	10	1	3
Total.....	24	12	50	0	0	26	1	3.85	14	53.84

As in the case of the more dilute solutions, the 1 to 1,000 solution of copper sulphate to be effective must be used before the appearance of any lesions. When the bacteria have become abundant enough to produce visible lesions many of them are too well protected to be reached by the solution during the short time the fish are exposed to it. However, while this treatment is not recommended as a cure, it has been shown experimentally that fish may be cured by its use, provided the gills are not infected.

Experiment No. 5.—At 5 p. m., September 9, seven badly diseased fish, two crappie and five buffalofish, were treated with a 1 to 1,000 solution for two minutes. These fishes were so badly diseased that they would undoubtedly have died within a few hours if not treated. They were so weak from the disease that three buffalofish turned on their backs during the treatment, but two recovered later. The next morning, September 10, another buffalofish was found dying and the other fish showed no improvement. The remaining fishes, two crappie and three buffalofish, were again treated with a 1 to 1,000 solution for two minutes at 11 a. m. One buffalofish died shortly after treatment. At 11.30 a. m., September 11, the fish were again given a 1 to 1,000 treatment for two minutes. No further treatments were given, and one buffalofish and one crappie died on the 13th. The remaining fishes, one buffalofish and one crappie, showed great improvement, and on the 16th, when the experiment was discontinued, had entirely recovered. No doubt the relatively low temperature of the water, which averaged about 70° F. during this experiment, was an important factor in aiding their recovery.

Of course a longer treatment with the copper sulphate would be more destructive to the bacteria but would not be advisable on account of the bad effects on the fish. All our experiments indicate that too strong a solution or too long a treatment with a weaker solution is worse than no treatment at all. While the fish may not be noticeably injured at the time, they may be so weakened as to easily succumb to a later infection. This is well illustrated by one of our experiments in which five bluegill were treated with a 1 to 1,000 solution of copper sulphate for six minutes. These fish showed no ill effects from the treatment at the time, but two days later all had become infected and died within 24 hours. In another series of experiments to determine the effects of various chemicals six normal buffalofish were treated with a 1 to 1,000 solution of formalin for eight minutes. The fish appeared in good condition after the treatment, but during the next three days all developed a fatal case of the disease.

In most cases it will probably not be worth while to attempt to cure fish with well-developed lesions, but in case it should be thought advisable the most effective treatment consists in local applications of a 1 per cent solution of copper sulphate. We have cured a number of badly diseased fish in this way, and the treatment is successful in a large

percentage of cases, provided the gills are not affected. The solution can best be applied by gently swabbing the lesion with a small piece of cotton which has been previously dipped in the solution. Two or three applications at intervals of 6 to 12 hours should be sufficient. After each local application of the copper sulphate the fish should be placed in a 1 to 1,000 solution for one minute.

Since, in most cases, the disease is primarily due to injuries or weakened vitality the old adage, "An ounce of prevention is worth a pound of cure," is peculiarly applicable in this case. Above everything else, anyone handling fish should exercise the greatest care to prevent injuring the fish in any way. Even the slightest injury, such as the rubbing off of a few scales or even a small portion of the mucous covering, may lead to infection. When taken in a net or seine the delicate caudal fin is very easily injured by the struggles of the captured fish and every effort should be made to reduce the injury to a minimum. A large percentage of infections among fish taken in this way first appear on the caudal fin, and undoubtedly injury to the fins is one of the most common causes of infection.

Great care should be taken to prevent the spread of the disease through the use of infected nets or vessels. This can be easily prevented, since the bacteria are entirely destroyed by thorough drying for several hours in direct sunlight.

Finally, in the case of fish confined in aquaria it is essential that all infected individuals be removed at once. As in the case of any contagious disease all contact of healthy with diseased individuals should be guarded against. It has been shown that bacteria are continually leaving diseased fish and thus may readily get on any healthy fish in the same aquarium.

It should be said in passing that this treatment is also very effective for ectoparasitic Protozoa. Experiments on fish infected with *Costia*, *Chilodon*, and *Cyclochæta* have shown that these parasites are entirely destroyed by a single treatment with copper sulphate. In the case of fish infected with *Ichthyophthirius* the treatment is not so successful, since the encysted stages are not affected. The exposed parasites are, however, entirely destroyed.

A number of other chemicals have been tried, but none of them has given encouraging results. Lysol and creolin were found to stop all movements of the bacteria in four to five minutes when diluted 1 to 5,000. The use of such strong solutions is, however, not practicable, since buffalofish and bullheads placed in a 1 to 5,000 solution for 1 minute died within 24 hours. Formalin was also found to be of no value for the same reason.

Since sodium chloride is used a great deal by fish-culturists for fungus a number of infected fish were treated with solutions of various strengths. It was soon found that the bacteria are not appreciably affected by solutions which seriously injure the fish, and it is not believed that this treatment is of any value against the disease. In fact our experiments indicate that it may actually aggravate the disease by weakening the fish.

ECONOMIC IMPORTANCE OF THE DISEASE.

At the present time it is impossible to make any general statements regarding the importance of this disease. So far, with the exception of two slightly infected fish at Ogdensburg, N. Y., the disease has been recognized only at Fairport. Here it has caused

a very considerable mortality each summer for several years. This mortality for the most part has occurred in fishes which were being transferred from one pond to another, or to aquaria for experimental purposes. It was not until the past summer (1919) that the disease was shown to occur on fish in the Mississippi River as well as in the ponds. However, fish-culturists are well aware that it is impossible to handle many species of fish during warm weather with any degree of success. Undoubtedly part of this mortality among fish which have been handled is due to the direct effects of the treatment to which they have been subjected, but there is no question in the writer's mind that a much larger part is directly due to this disease and only indirectly to the handling which has simply rendered the fish more susceptible to infection. We are convinced that a great part of the mortality usually ascribed to fungus is in reality caused by *Bacillus columnaris*. In fact the writer is inclined to doubt if fungus is ever an important cause of fish mortality. In all probability *Bacillus columnaris* is widely distributed over the country and during warm weather, at least, is the most important agent in the destruction of fish which have been injured in any way.

Anglers are often advised to remove small fish from the hook and return them to the water. In the light of our experience with the disease at Fairport it is doubtful if many fish which have been handled in this way actually survive. Since the disease would not make its appearance until two or three days later, it is obvious that only through carefully conducted experiments can their chance of survival be ascertained. An incident which occurred at Fairport during the summer of 1918 is very suggestive in this connection. A number of largemouth black bass were needed for some experimental work in mussel propagation. They were taken from one of the ponds on a hook and line and placed in a large tank supplied with running water. Within two or three days nearly every fish had become infected with *Bacillus columnaris*. As previously mentioned, this species of fish is not very susceptible to the disease, and, furthermore, the fish in this case were several years old. As the reader will recall the older fish are much less susceptible than the young.

During the last few years the Bureau of Fisheries has been rescuing large numbers of young fishes from the small pools and ponds in which they are imprisoned when the spring floods recede. These pools and ponds are widely scattered over the flood plain of the Mississippi, often only a few hundred yards from the river. In some instances they may be 2 or 3 miles from the main channel. As the waters recede these ponds continually grow smaller and may eventually become entirely dry. They are often crowded with fish and the shallow waters exposed to the hot sun of July and August become heated to a relatively high temperature. These conditions combined with a limited supply of oxygen often result in the death of large numbers of fish. The conditions are such as to render the fish susceptible to infection by *Bacillus columnaris*, and fishermen have told the writer of having seen large numbers of diseased fish in such ponds.

In transferring the fish to the river they must first be seined from the ponds, a process which necessitates considerable rough handling, and then carried in galvanized washtubs (often under a broiling sun) to the river. It is obvious that such treatment is likely to result in infection by *Bacillus columnaris*. That the fish when liberated in

the river quickly swim away and are seen no more proves nothing. The disease would not be evident for two or three days in any case.

In order to determine the prevalence of the disease among rescued fishes a long series of experiments will be required, but owing to the limited time at the writer's disposal only a few preliminary experiments have as yet been carried out. In these experiments a special effort was made to duplicate so far as possible the average conditions found in rescue work. The fish were seined from small, isolated ponds, one of which is shown in figure 259, and then carried in washtubs to the river, where they were placed on a launch to be carried to the station. These fish were in the tubs from one-half to one hour, which the writer was assured by the fishermen is somewhat less than the average time they are held in tubs in ordinary rescue work. On reaching the station the fish were at once placed in an inclosure surrounded by fine-meshed poultry wire. This inclosure (fig. 258) was constructed in shallow water along the river shore and was sufficiently large to obviate any danger of the fish being crowded. A small stream carrying the overflow from the fishponds flowed into the inclosure, so there was a good supply of running water at all times. The cross partition shown in the figure was added later for some special experiments which are not considered in this paper. It is believed that the fish held in this inclosure were in fully as favorable an environment as they would have been if liberated in the river.

Experiment No. 6.—September 2, 1919, 42 buffalofish, 41 crappie, and 8 bluegill were seined from the pond shown in figure 259. This pond was believed to be typical of those from which fish are taken in rescue work. It was about 400 feet long by 100 feet wide and quite shallow, being not over 3 or 4 feet in depth at the deepest part. The bottom, as in most ponds of this kind, was composed of fine, soft mud. The temperature of the water at the time the fish were removed was 83° F. All the fish appeared in good condition when placed in the inclosure. The next day (Sept. 3) six crappie were found dead. Since no bacteria could be found on these fish they are believed to have died from injuries due to handling. September 4 one badly diseased buffalofish was removed, and the next day there were two dead buffalofish, both with well-developed lesions. The inclosure was seined on September 6 and all the fish removed. Only four dead fish were found, three buffalofish and one crappie. However, many of the living fish were badly diseased and in all probability would have died in a short time, but it was not thought best to leave them in the inclosure longer, since there was great danger of the disease spreading from the infected to the healthy fish. It is not believed that any of the fish which are classed as infected at this time had contracted the disease in this way, since all were in advanced stages of the disease. The diseased fishes included 19 buffalofish and 10 crappie. They were removed to aquaria well supplied with running water, but all died within 24 hours. It is believed justifiable to assume that all the fish classed as diseased became infected as a result of the treatment to which they had been subjected and would have eventually died if set free in the river.

SUMMARY OF EXPERIMENT No. 6.

Species.	Number of fish.	Fish lost from injuries.		Fish lost from disease.		Total loss of fish.	
		Number.	Per cent.	Number.	Per cent.	Number.	Per cent.
Buffalofish.....	42	0	0	25	59.52	25	59.52
Crappie.....	41	6	14.63	11	26.83	17	41.46
Bluegill.....	8	0	0	0	0	0	0

Experiment No. 7.—September 6, 1919, 36 buffalofish and 3 crappie were seined from the same pond as in the preceding experiment and placed in the inclosure. The next day eight buffalofish were found dead, evidently from the effects of injuries due to handling. All the fish were removed from the inclosure on September 9. There were 17 dead buffalofish, 8 living badly diseased buffalofish, and 3 badly diseased crappie. The remaining fish were in good condition.

SUMMARY OF EXPERIMENT No. 7.

Species.	Number of fish.	Fish lost from injuries.		Fish lost from disease.		Total loss of fish.	
		Number.	Per cent.	Number.	Per cent.	Number.	Per cent.
Buffalofish.....	36	8	22.22	25	69.44	33	91.67
Crappie.....	3	0	0	3	100	3	100

Experiment No. 8.—Since there was a possibility in the above experiments that the fish might have injured themselves on the poultry wire in trying to escape from the inclosure a fine-meshed seine was attached to stakes so as to form another inclosure by the side of the one constructed of poultry netting. September 10, 21 buffalofish and 12 crappie were placed in this inclosure. These fish were taken from a different pond from that used in the preceding experiments, but conditions were much the same, except that the pond was somewhat farther from the station.

SUMMARY OF EXPERIMENT No. 8.

Species.	Number of fish.	Fish lost from injuries.		Fish lost from disease.		Total loss of fish.	
		Number.	Per cent.	Number.	Per cent.	Number.	Per cent.
Buffalofish.....	21	0	0	12	57.14	12	57.14
Crappie.....	12	8	66.67	2	16.67	10	83.33

While the above experiments are only preliminary and too much weight should not be placed on the results, it is evident that the conditions are especially favorable for the development of this disease among rescued fish and that in all probability it causes a very considerable mortality during warm weather. Much of the rescue work is carried on in the fall and early winter, and with the low temperatures then prevailing there is no doubt much less danger of infection by bacteria, but on this point we have no data at present.

It is obvious that great care should be exercised to injure the fish as little as possible and that, whenever practicable, rescue operations should not be undertaken until

cool weather. If the fish have not been greatly weakened by unfavorable conditions, especially lack of oxygen, treatment with 1 to 1,000 copper sulphate just before they are liberated in the river would doubtless cause a marked decrease in the loss from disease. Some experiments were attempted along this line in September, 1919, but owing to an accident the results were of no value.

EXPLANATION OF FIGURES.

All figures, with the exception of figure 249, are from photographs by the author. Abbreviations used are as follows: bl, blood; cor, corium; ep, epidermis; and mus, muscles.

FIGS. 231 and 232.—Crappie, *Pomoxis sparoides*, with dorsal, caudal, and anal fins infected. Note lesions just starting in three places on anal fin of fish in figure 232.

FIG. 233.—Bluegill, *Lepomis incisor*, with dorsal, caudal, and anal fins infected.

FIGS. 234 and 235.—Fingerling buffalofish, *Ictiobus bubalus*, with caudal fins badly infected.

FIG. 236.—Largemouth black bass, *Micropterus salmoides*, showing infection on dorsal, anal, and caudal fins.

FIG. 237.—White bass, *Roccus chrysoptis*, with infection developing on dorsal, anal, and caudal fins.

FIG. 238.—Bullhead, *Ameiurus melas*, with a number of small lesions on dorsal surface of body.

FIGS. 239 and 241.—Bullheads, *Ameiurus melas*, in late stages of the disease.

FIG. 240.—Bullhead, *Ameiurus melas*, with nearly entire side of body covered with lesions.

FIG. 242.—Small bullhead, *Ameiurus melas*, with posterior end of the body infected and another lesion just back of pectoral fin.

FIG. 243.—Head of crappie, *Pomoxis sparoides*, with operculum removed to show small lesion on gill.

FIG. 244.—Head of buffalofish, *Ictiobus bubalus*, with operculum removed to show a somewhat larger lesion on gill than in figure 243.

FIG. 245.—Head of buffalofish, *Ictiobus cyprinella*, with operculum removed to show gills in late stages of the disease.

FIGS. 246 and 247.—*Bacillus columnaris*. From a dried smear stained with carbolfuchsin. $\times 800$.

FIG. 248.—Small portion of scale of bluegill to show formation of columns by bacteria along the edge. Photomicrograph from preparation mounted in glycerin jelly and stained with eosin. Columns are more slender and pointed than normal, due to shrinkage by the preserving fluid.

FIG. 249.—Showing formation of columns by bacteria along edge of a bit of infected tissue after being removed to slide. Somewhat diagrammatic. $\times 340$.

FIGS. 250 to 257 are from cross sections through lesions in integument of bullhead, *Ameiurus melas*.

FIG. 250.—Lesion just beginning to develop. $\times 70$.

FIG. 251.—A little later stage in development of lesion. The epidermis is entirely destroyed at one place to the right in the figure. $\times 70$.

FIG. 252.—A somewhat later stage than figure 251. The corium is beginning to disintegrate where the overlying epidermis has been destroyed. $\times 70$.

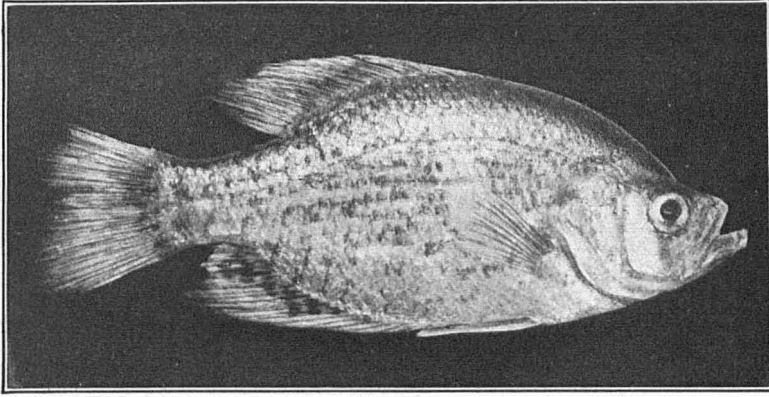
FIG. 253.—Cross-section through lesion in late stage of disease. The corium is entirely destroyed at center of lesion. Less highly magnified than preceding figures.

FIG. 254.—Showing disintegration of epidermis in early stage of development of lesion. $\times 108$.

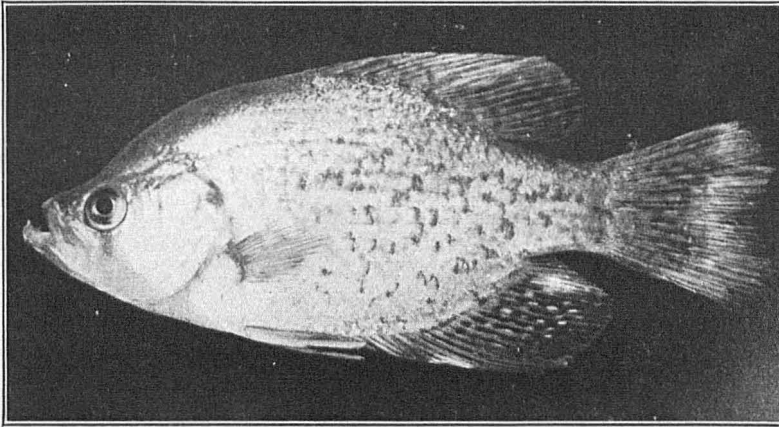
FIGS. 255 to 257.—Sections through margin of well-developed lesions, the epidermis at the right has been entirely destroyed. Note the blood corpuscles (*bl*) in the outer layer of the corium and between the corium and the epidermis. In figure 255 the blood has broken through into the epidermis for a short distance. $\times 108$.

FIG. 258.—Inclosure constructed of poultry netting in which fish were held in experiments 6, 7, and 8.

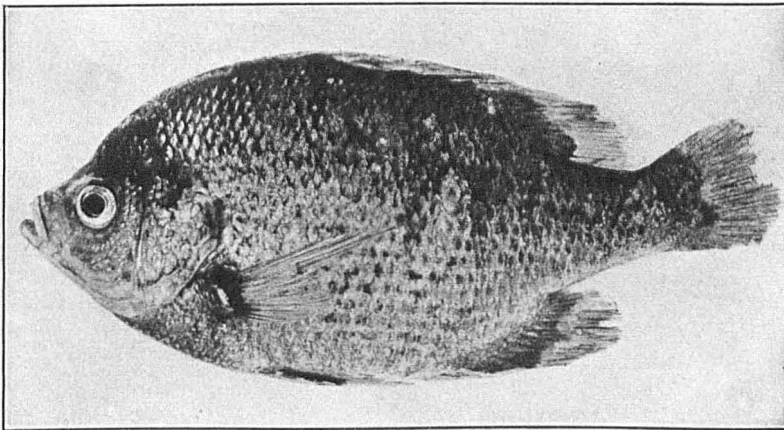
FIG. 259.—Small pond from which fish were taken in experiments 6 and 7.



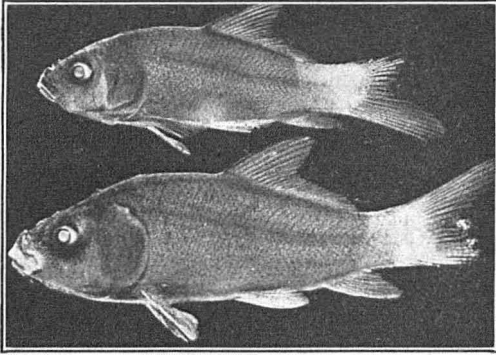
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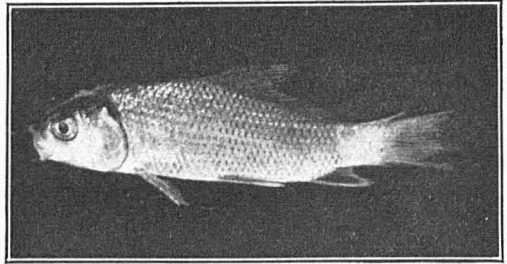
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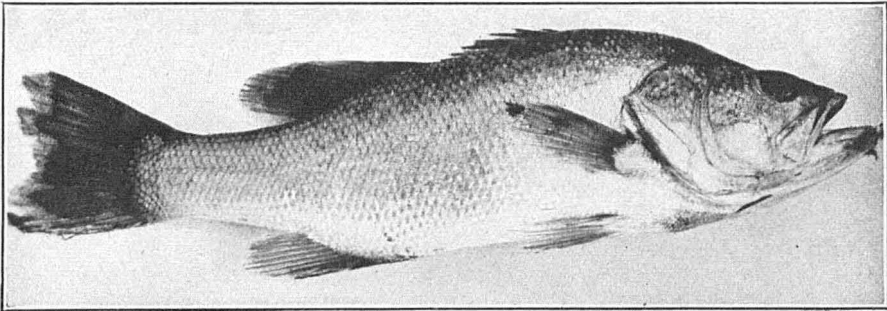
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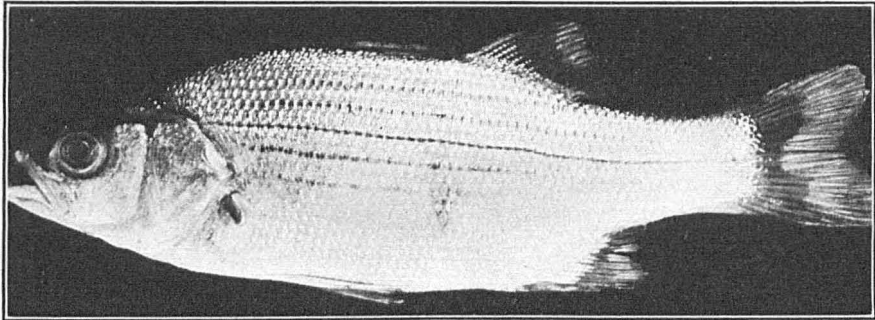
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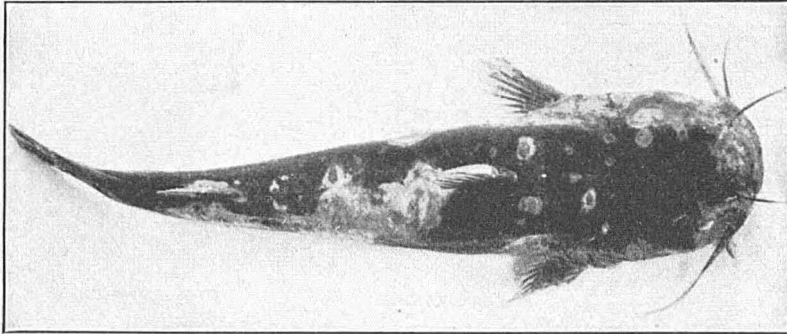
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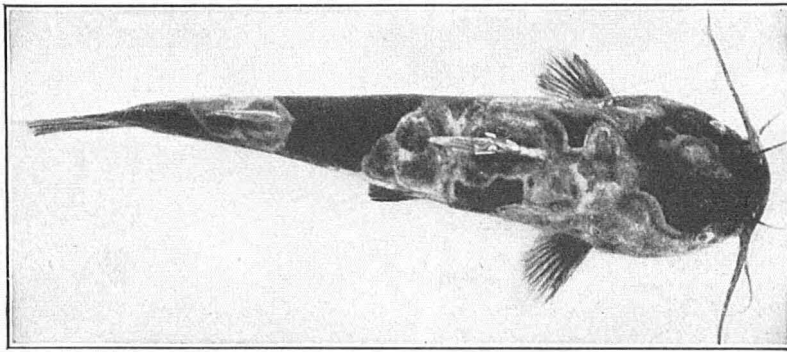
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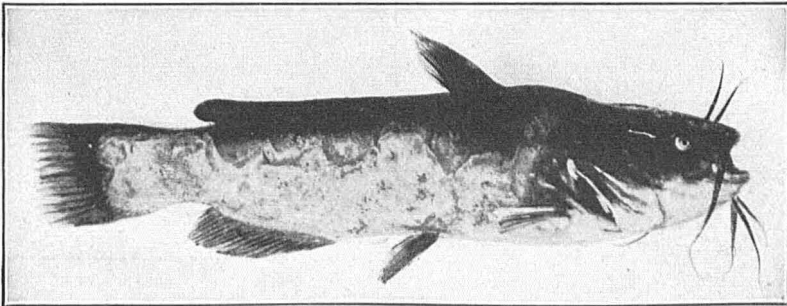
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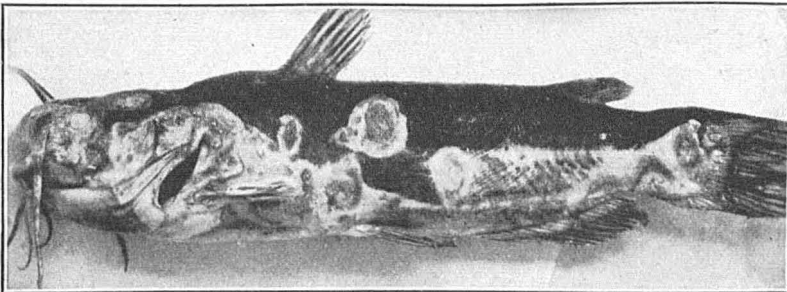
238



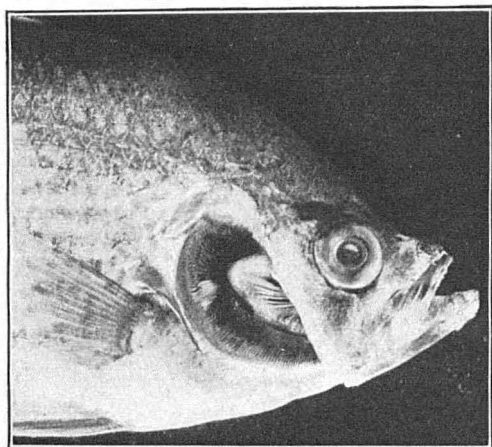
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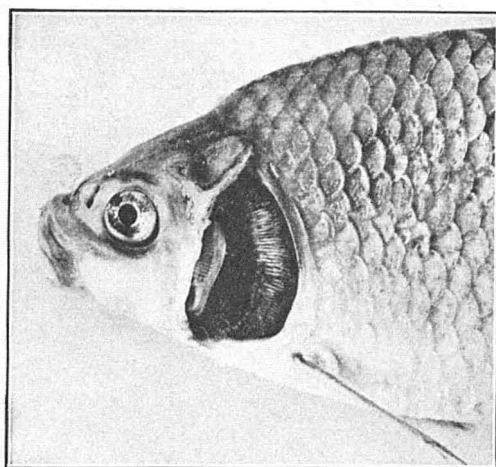
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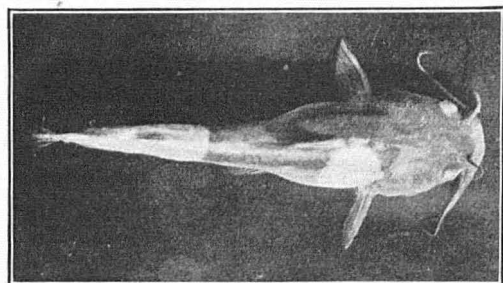
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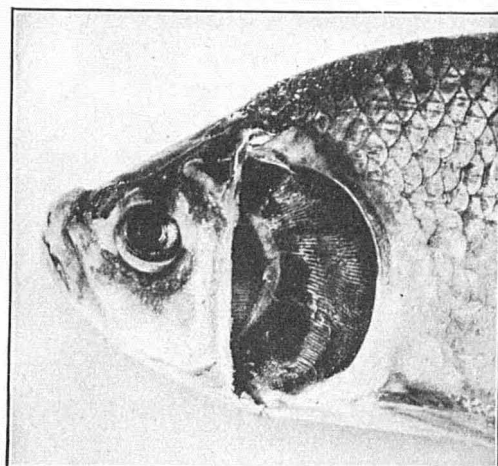
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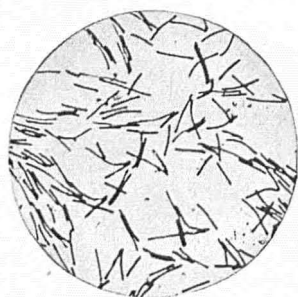
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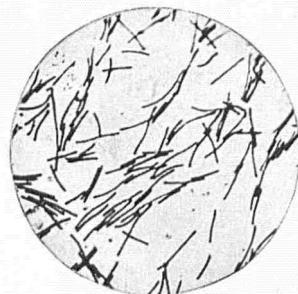
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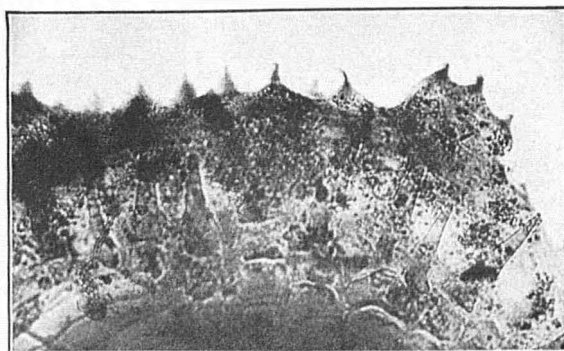
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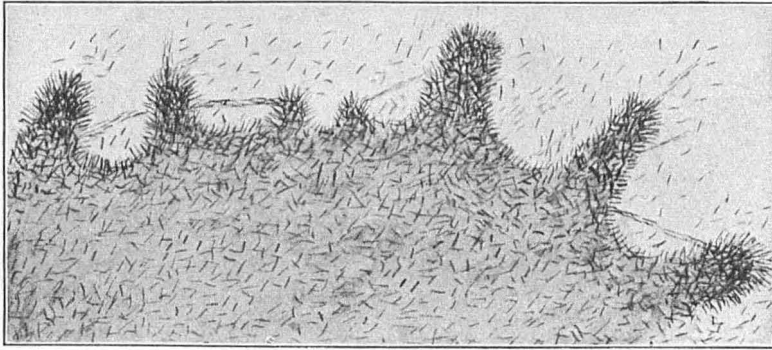
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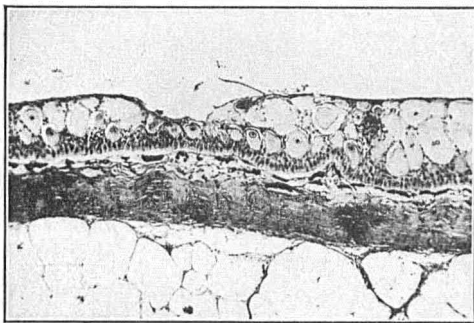
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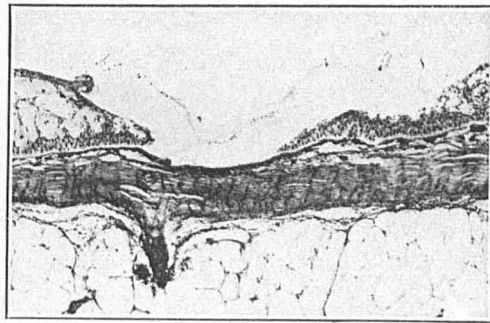
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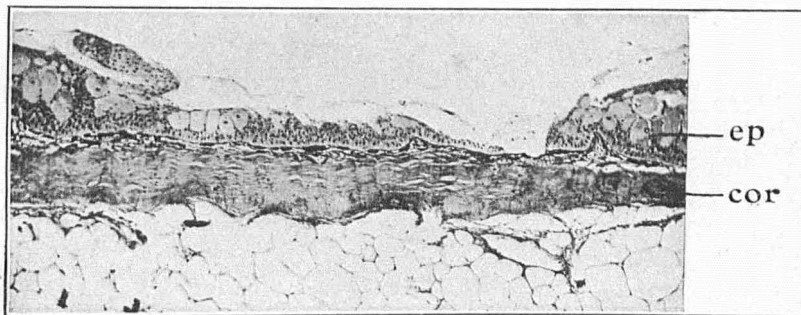
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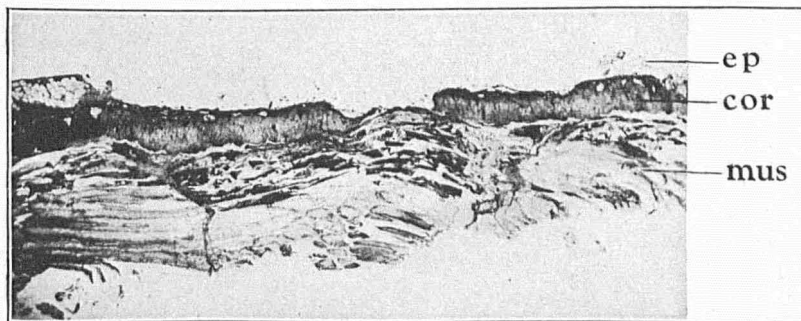
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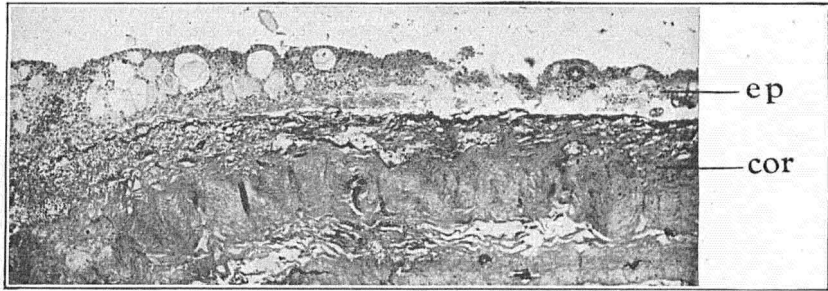
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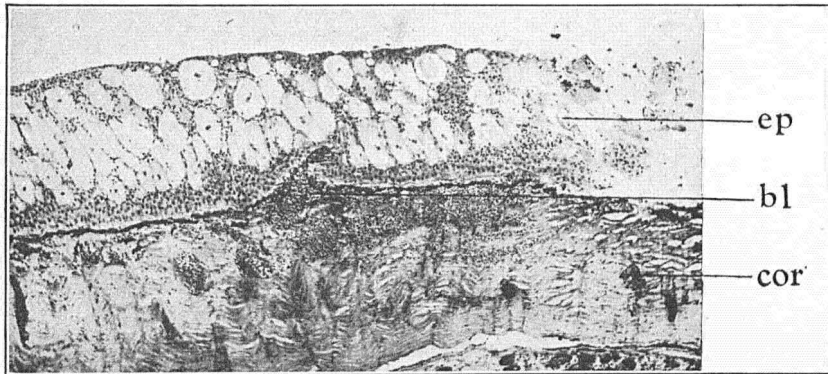
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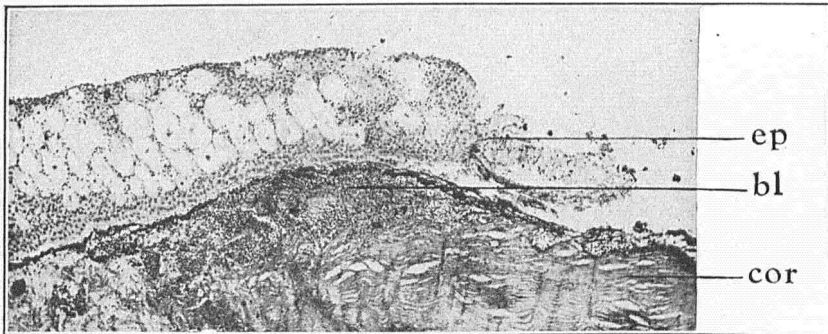
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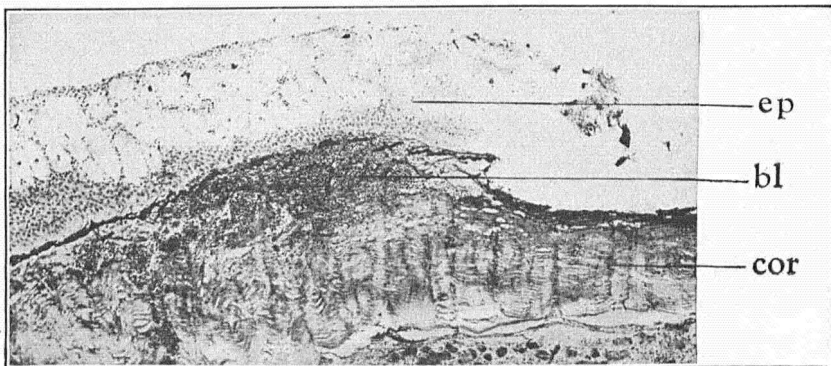
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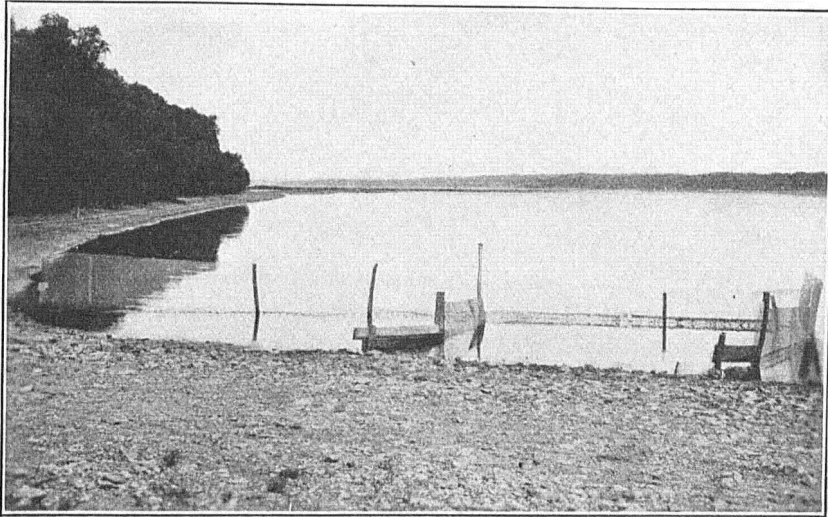
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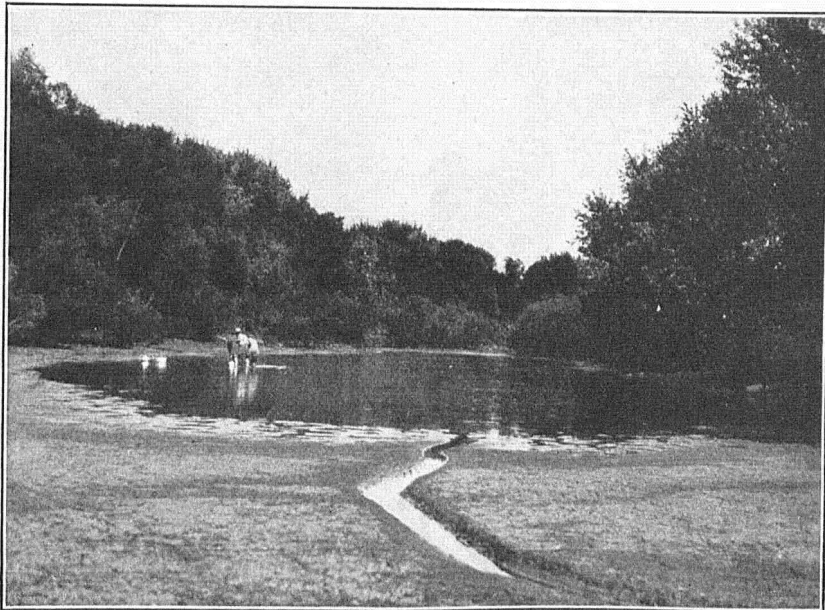
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