SOME GENERAL ASPECTS CONCERNING THE
TOXIC AND PHYSIOLOGICAL EFFECTS
OF COPPER SULPHATE ON FISH

DISSertation
Presented in Partial Fulfillment of the Requirements
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By

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** ** ** ** **

The Ohio State University
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# TABLE OF CONTENTS

Introduction .............................................. 1
Phase I ....................................................... 7
  Methods Utilized in Obtaining Fish Specimens .... 8
  Method of Holding Test Specimens ................. 9
  Methods Used in Pre-testing Procedures .......... 10
  Source of Water ....................................... 12
  Description of Test Area ............................ 13
  Testing Procedure ................................... 13
  Results .................................................. 16
Phase II .................................................... 23
  Methods Utilized in Continuous-Flow Tests .... 25
  Procedure of Tests .................................. 26
  Results of Continuous-Flow Tests ............... 27
Phase III .................................................. 29
  Description of Copper-64 .......................... 29
  Source of Radioactive Copper-64 ................. 29
  Method of Identification of Copper-64 .......... 30
  Method of Preparing Tissue for Counting ....... 34
  Method of Irradiating Copper-63 ................. 37
  Test Procedure ...................................... 37
    1. Aquarium Tank Test ............................ 37
    2. Continuous-Flow Split Chamber Test .......... 38
  Results of Radiation Tracer Tests ............... 41
Discussion of Results of All Phases ............... 51
Conclusions ............................................. 61
Miscellaneous Observations ..................................................... 64
Bibliography ............................................................................... 66
Autobiography ................................................................................. 70
LIST OF TABLES

1. Summary of the Positive Results of Phase I . . . 17
2. Summary of the Results of Phase I for
   Micropterus d. dolomieui. ......................... 21
3. Summary of Results of the Relationship
   between Concentration of Copper Sulphate
   and Oxygen Consumption ......................... 28
4. Results of Goldfish Radiation Experiment
   Run #1 ...................................................... 42
5. Results of Goldfish Radiation Experiment
   Run #2a .................................................... 43
6. Results of Goldfish Radiation Experiment
   Run #2b .................................................... 44
7. Results of Goldfish Radiation Experiment
   Run #3 ...................................................... 45
8. Results of Goldfish Radiation Experiment
   Run #4; Continuous-Flow Split Chamber
   System .................................................... 46
9. Summary of the Results of Goldfish
   Radiation Experiments ............................... 55
LIST OF ILLUSTRATIONS

1. The Calculated Regression Curve Based on Positive Experimental Results of Phase I ........ 20
2. Drawing of Continuous-Flow System in Phase II ......................................................... 24
3. Goldfish Radiation Experiment Run #1 Energy Calibration Curve ........................ 32
4. Goldfish Radiation Experiment Run #1 Linearity Curve ............................................. 33
5. Goldfish Radiation Experiment Run #1 Decay Curve of Cu$^{64}$ .............................. 35
6. Aluminum Covered Plastic Planchet Chamber Utilized in the Detection of the .51 Energy of Cu$^{64}$ ............................................................... 36
7. Drawing of the Continuous-Flow Split Chamber System Utilized in the Goldfish Radiation Experiment Run #4 ................................................. 40
8. Goldfish Radiation Experiment Run #4 Energy Calibration Curve ........................... 47
9. Goldfish Radiation Experiment Run #4 Linearity Curve ............................................ 48
10. Goldfish Radiation Experiment Run #4 Decay Curve of Cu$^{64}$ Utilizing a Scintillation Spectrometer Apparatus ............................... 49
11. Goldfish Radiation Experiment Run #4 Decay Curve of Cu$^{64}$ Utilizing a Geiger Counter Apparatus ...................... 50
INTRODUCTION

Copper, a heavy metal, is an element which is present in many situations. It can usually be found as a trace constituent of ore, as the by-product of industrial processes, and the main metal ingredient of many commercial products. Because of its toxicity to living organisms it has, for more than a half century, found wide use as a biological control reagent. In this manner it has been used as a molluscicide, parasiticide, and an algicide. Such uses have involved the treatment of natural waters which has exposed the aquatic inhabitants to the toxic properties of the metal. The attempt to determine the physiological effect that copper and the other heavy metals have on aquatic life has resulted in an extensive literature on the subject.

As a biological control, the algicidal use of copper, in the form of copper paints, copper silicates, and copper sulphate (Ingram, and Tarzwell, 1954), has been one of the more constant sources of its introduction into natural waters. It is used both by public and private agencies in the treatment of obnoxious algae in ponds, lakes, bathing ponds, and reservoirs. Since this metal is extremely toxic to both animals and plants it became very important to determine the specific concentrations that were lethal,
not only to algae, but to the fish in the treated waters. Fish in these waters are an important part of our national resources. Thus the factors that govern this lethal level and the relationship that this metal, at these levels, has on the metabolic activities of the animal must be established in order to prevent the unnecessary destruction of useful fish populations.

In 1863 Penny and Adams reported that a concentration of 10 ppm of copper sulphate was lethal to goldfish, *Carassius auratus*. In 1905 Moore and Kellerman reported on the lethal concentration for several species of fishes. These were catfish, Ictaluridae; goldfish and carp, Cyprinidae; sunfish and black bass, Centrachidae. The limits cited for each species were within the range of 0.1 ppm to 2 ppm. In 1927 Carpenter reported that the minnow, *Leuciscus phoxinus*, died in 62 minutes at a level of 399 ppm in distilled water. Ellis, 1937, reported 2 ppm as being fatal to goldfish, *Carassius auratus*, in 24 to 96 hours.

It was apparent that a variety of limnological factors affected that concentration of copper sulphate which would be lethal to fish. Thus studies concerning this metal turned to the relationship of the metal to such factors as dissolved oxygen concentration, oxygen consumption, temperature, pH, alkalinity, and soluble salts such as sodium nitrate and calcium chloride.
White, 1912, studied the absorption of metal salts by fish. Carpenter, 1927, studied the effect of several metallic salts, including lead. Powers, 1929 reported the relationship between fish and such factors as respiration, pH, and alkalinity. In an earlier paper, Powers, 1920, also related these factors with the toxicity of metallic salts. Jones, 1938, studied the synergistic action of calcium salts to lead and zinc. Jones, 1947, related oxygen consumption to toxic solutions. Cook, 1926, studied the effect of copper on respiration. These are only a few of the extensive publications that exist today concerning this metal, and its relatives such as lead and zinc. Copper has also been explored in terms of other uses. Thompkins and Bridges, 1958, reported it as an aid to commercial fisheries; and Titcomb, 1914, Atkins, 1933, and Cott, 1934, expressed its possible use as a fish poison in the management areas. However, these beneficial uses have not superseded the remaining toxic problem that still exists today.

In recent years, the ever increasing recognition of the problems relative to industrial wastes, civic water supplies, and civic sewage treatment, has greatly increased the study of toxic agents in waters, and with this has increased the knowledge of the effect of copper and other heavy metals. Thus, it has become
generally accepted that at a lethal concentration the primary cause of death to fish by copper sulphate is asphyxiation. The reaction being the precipitation of the muco-protein secreted over the gills. It is also generally accepted that at lower concentrations the metal may be internally toxic by disrupting metabolic systems (Ellis, 1937). However, the available data is still contradictory. It does not present a means for the formation of a standard index of lethal concentration which can be safely applied to other aquatic environments. For example, as in the earlier reports, concentrations lethal to fish in a body of water of a given alkalinity do not hold up if applied to other bodies of water. It is generally accepted that a higher concentration of copper sulphate is necessary to be lethal in hard water than in soft water (Ellis, 1937 and Doudoroff and Katz, 1953). However, it is found that under similar test conditions these levels are non-fatal. Ellis, 1957 reports goldfish, Carassius auratus, as surviving 11 to 72 hours or more in hard water at a concentration of 10 ppm. The author, in preliminary studies using the same type of test system found that only 8 ppm was required to cause death, in soft water. Thus it is apparent that a combination of factors that will affect the tolerance of each specific species must be present in a given body of water.
This problem was instigated by the need for more
decisive information concerning the specific tolerance
levels of commercial and game species inhabiting treated
waters. In a sense, this paper is directed more toward
the need of the algologist than the water pollution
engineer. The problem was organized in a manner which
would produce the above information yet cover a broad
scope of the relationship of the metal to fish. It was
hoped that this general approach would establish an
index pattern, or trend, between the lethal limits of
fish and such factors as alkalinity, temperature, pH,
etc. Such an index, though certain to be variable,
would have value. Such an approach, it was felt, would
also open new directions of study which might shed light
on the existing situation.

Thus, the problem was set up in three phases. The
first was an attempt to secure information on the lethal
limit of as many species as could be acquired within the
Put-In-Bay area of Lake Erie, and to relate these limits
to other environmental factors.

The second phase was to be a brief study of the
probable relationship between lethal limits, oxygen
consumption, alkalinity, and temperature. This was also
designed as a laboratory problem and was carried out with
the facilities of the Zoology and Entomology Department
at Ohio State University.
The third phase was an attempt to trace the course of copper throughout the organ system of the goldfish, Carassius auratus. It also attempted to determine the specific organs affected, the relative amounts of copper deposited in these organs, and the relationship between these factors and the concentration of copper sulphate used.

Any miscellaneous reactions that were observed during the project were also recorded. The net result, it was hoped, would be the acquisition of new information or, at least, the verification of pre-existing information.
PHASE I

This phase was carried out at the Franz Theodore Stone Laboratory situated at Put-In-Bay, Ohio, on Lake Erie. The project was conducted over a period of two consecutive summers, 1957 and 1958. The species of fish that were chosen to be tested were those species that readily inhabit the Bass Island region of Lake Erie. The plan was to test as many of the larger fishes as it was possible to obtain. It was felt that any information obtained as to the minimum lethal dosage tolerated by several species would be of more value in terms of present application rather than the information obtained from the study of one species.

Many problems concerning the trapping of suitable test specimens developed under this plan. The larger game species tend to be elusive and difficult to trap. Also, the nature of the problem required healthy test specimens to assure that any recorded lethal concentration represented the tolerance level of a "normal" specimen rather than that of an injured or diseased specimen. Thus such factors as injury during trapping, disease, and suitable pre-test holding tanks had to be considered in the attempt to produce a continuous supply of these specimens. Also, it was necessary to avoid crowding in the holding tanks as this usually results in undesirable specimens because of injury, secondary
disease, attempted predation by larger fish, etc. It became apparent that only a relatively small number of fish could be kept available at any one time. This necessitated constant procurement of specimens throughout the project.

**Methods Utilized in Obtaining Fish Specimens**

Three methods were used throughout this phase: seining, hoop netting, and spin-casting.

Seining proved suitable only for small specimens. Although those acquired were in good condition, the operation was necessarily confined to shallow water and was not used to any great extent.

The second method, the hoop netting, proved fairly successful at the start of each season. However, the variety of species and number of fish obtained decreased markedly after the second week in July. From this point on only a small number of fish of the pan fish variety was obtained in this manner. The condition of the specimens obtained were in many cases poor. Injury sustained within the net with resulting fungus disease was quite prevalent; the resulting number of usable specimens being about 10 per cent of the catch. However, since the smaller members of the Centrachidae family were easily obtained by this method, it was used throughout the project. Some species caught by this method were the yellow perch, *Perca flavescens*:
the common blue gill, *Lepomis macrochirus*; the white crappie, *Pomoxis annularis*, and the black crappie, *Pomoxis nigra*.

The third method, spin-casting with a small 1/4 ounce lure, proved to be the most successful of the three in numbers, condition, and variety. Some of the specimens obtained in this manner were yellow perch, *Perca flavescens*; white bass, *Roccus chrysops*; small mouth black bass, *Micropterus d. dolomieu*; large mouth black bass, *Micropterus salmoides*; white crappie, *Pomoxis annularis*; black crappie, *Pomoxis nigra*; common blue gill, *Lepomis macrochirus*. The specimen was allowed to remain in the water until exhausted. It was then gently removed from the water and placed in a large covered tub, unhooked, and allowed to recover. Approximately 90 per cent of the specimens tested were acquired in this manner. They were usually in excellent condition and did not develop a fungus infection from the hook injury. After recovery the specimens were transported to the test area and placed in the holding tanks.

**Method of Holding Test Specimens**

The specimens were held in a large hatchery tank with dimensions of approximately 10 ft. x 3 ft. x 4 ft. The tank was equipped with a constant flow and aeration system, and was located in the State Hatchery of the Ohio Division of Wildlife on South Bass Island, Lake Erie.
This building stands directly next to the Stone Laboratory Research Building.

The specimens remained in the holding tank until just prior to the test period. In this manner any specimen showing detrimental effects was observed and removed before transferring to the test tanks. As needed, specimens were removed from this tank and placed in a 55 gallon glass aquarium utilized as a combination holding tank and control tank. The number of fish kept in this tank varied with the species and size, but never exceeded eight in number. Any specimens showing unhealthy signs were removed from this tank and not tested. In its use as a control tank, it was felt that any adverse effects brought about by other substances in the lake water would affect the specimens. At no time was this tank subjected to copper sulphate treatment.

Methods Used in Pre-testing Procedures

1. Weighing of Specimens: All specimens from tests which resulted in death to the animal (positive tests) were weighed by means of a Toledo Springless Scale apparatus calibrated to the one-half ounce. A 2½ gallon bucket was weighed with an amount of water. The specimen was then removed from the test tank, placed in the bucket and the whole weighed. The fish was then removed and the bucket and water reweighed. This was done to compensate for the error involved by the water introduced with the
specimen. However, it was determined that the amount of water added approximated the amount of water removed with the specimen with the resulting error falling within the limits of the apparatus.

If a specimen was approximately 4 inches or less it was weighed directly in gram weight by means of a Harvard Trip Balance.

2. Measurement of Standard Length of Specimen: The standard length of specimens from all positive tests was taken according to the procedure outlined by Hubbs and Lagler, 1949.

3. Temperature: All temperature readings were taken by means of a standard centigrade mercury thermometer at a level approximately half way between the top of the water surface and the bottom of the tank.

4. pH Measurements: All pH readings were made with a Beckman pH apparatus.

5. Water Chemistry Measurements: All chemical analyses followed the procedures in Welsh's Limnological Methods.

a) Free Carbon dioxide: The procedure used involved titration of the sample with N/44 sodium hydroxide using phenolphthalein as an indicator. Since all test tanks were aerated, and over a period of time all results consistently fell below .5 ppm the tests were discontinued. Intermittent checks were run to ensure that no change had occurred in the situation.
b) Dissolved oxygen concentration: The unmodified Winkler method was employed. Samples of water were taken from each test tank. Since each of these tanks were constantly aerated with standard pumps and air-stones, the oxygen values were significant only as a check to ensure that an adequate oxygen content was always present during the test period.

c) Alkalinity measurements: Both the phenolphthalein and methyl orange tests were conducted. Samples from all tanks were checked before each test.

d) Copper sulphate test chemical: A commercial grade of powdered, anhydrous copper sulphate was used in the preparation of all toxic solutions in all phases of the project.

e) Copper sulphate stock solution: A basic stock solution of 1000 ppm was prepared by dissolving 1 gm of copper sulphate in 1000 cc of double distilled water. One cc of this stock solution is equivalent to 1 ppm when diluted by 999 cc of water. This dilution factor was used to introduce the desired concentration of copper sulphate into the test tanks.

Source of Water

All water utilized in the tests of this phase was lake water. This water was first pumped into a small reservoir located in the State Fish Hatchery, and thence fed to the Research Building. As far as it could be
determined none of the pipes through which the water flowed from the State Hatchery to the test area was of copper or its alloys.

Description of Test Area

The test area was located in the Research Building of the Franz Theodore Stone Laboratory on South Bass Island, directly adjacent to the State Fish Hatchery. The test tanks consisted of five 10 gallon aquaria of the standard type having a slate bottom, chrome frame, and glass sides. The tanks were situated on a tin-covered bench that slanted toward and ended at a well type sink. Thirty liters of water was used in each test with all copper sulphate concentrations being based on this figure. Each aquarium was aerated from a standard single or double piston type air pump attached to small air stones.

All remaining apparatus, including the 55 gallon Holding-Control aquarium was located on small tables; the whole forming a closed rectangular working area.

Testing Procedure

Each of the specimens to be tested was placed in a test aquarium. They were then left for a period of not less than 3 hours in these tanks to allow for adjustment. After this adjustment period, each specimen was momentarily transferred to a tub and the test tank filled to the 30 liter mark. The fish was then replaced
in the tank. This procedure was repeated for each fish tested.

Before each test run the following water characteristics were determined for all tanks including the Holding-Control tank: dissolved oxygen, temperature in degrees centigrade, pH, and methyl orange alkalinity. After these data were recorded, an amount of water equivalent to the amount of copper sulphate stock solution to be introduced was removed from each. The test period ran for 48 hours after the last tank had received the copper sulphate solution. During the test period the specimens were checked periodically and notes on behavior were recorded. If the test concentration proved positive; that is, the specimen died, the time to death was recorded as being that point when ventilation by the animal could no longer be perceived.

At the end of the 48 hour test period, all test tanks including those in which the fish survived (negative test) were rehabilitated. A tank was partially drained by means of a siphon. The fish, if present, was removed momentarily and placed in a tub. The tank was then completely drained and thoroughly scrubbed with a sponge and rubber scraper. The tank was refilled and the procedure repeated for four complete cycles. At no time was any cleaning reagent used in the rehabilitation procedure.
Those specimens surviving the previous test were utilized in the next test series. A minimum of 12 hours was allowed between successive runs which seemed to be sufficient time for the specimens to recover.

The concentration of copper sulphate used in the initial test on each was 1 ppm. This was increased by 2 ppm for each successive test until a positive result was obtained for an individual of the particular fish group being tested. Any supplementary tests conducted with the group were with copper sulphate concentrations which were bracketed about this initial lethal concentration.

Several of the tests conducted on a particular group, or species of fish, were varied in respect to the alkalinity of the test water. It was felt that a possible proportionate relationship might exist between the minimum lethal concentration for a species and the alkalinity of the test water. Such a proportionality, if present, could possibly, it was felt, form the basis for the formation of a practical Lethal Concentration Index.

To test this concept the following steps were taken. First, the average alkalinity of the raw lake water was calculated from data existing at that time. Secondly, the average minimum lethal dosage for a species, corresponding to this average lake water alkalinity,
was computed from existing data. Thirdly, this average ratio of lethal dosage to alkalinity was used as the base ratio in the calculation of that concentration of copper sulphate which would be lethal at a lower alkalinity.

In these tests the alkalinity was lowered by adding distilled water to raw lake water, the total volume of the mixture being 30 liters. The "predicted" lethal concentration of copper sulphate necessary at this alkalinity was calculated and used in the test.

Results

All the pertinent data from the positive test for each species were tabulated (Table 1). An attempt was made to determine whether or not a relationship existed between the minimum lethal dosage for a species and the other conditions tested. The number of tests conducted per species was entirely dependent on time and availability of the species in the region. Of the 13 species tested, only those species with two or more positive results were entered in the table and considered for analysis. Of these 13, only 7 species were found to fall in this category. Of the 7, only one species, the small mouth black bass, Micropterus d. dolomieu, had been tested, it was felt, sufficiently to portray any reliable relationships. The remaining material merely provides a basis for supplementary work in the future.
TABLE 1

Summary of the positive results of Phase I for several species of fish inhabiting the Put-In-Bay region of Lake Erie.

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>LETHAL CONC.</th>
<th>TIME TO DEATH</th>
<th>TIME TO EFFECT</th>
<th>L.D.50%</th>
<th>L.D.90%</th>
<th>NO.</th>
<th>WT. OF FISH</th>
<th>S.L./MM</th>
</tr>
</thead>
<tbody>
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<td>SPECIES</td>
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* Abbreviation: Similarity

** Abbreviation: Standard Length
No relationship existed between the lethal dosage of the positive test and such factors as temperature, pH, weight, or standard length. However, it was apparent that a possible relationship existed between the lethal dosage and the alkalinity of the water. (See *Micropterus d. dolomeiui*, Table 1.) From the experimental data it was observed that the lethal dosage decreased as the alkalinity decreased. This was to be expected as such a fact is generally accepted (Doudoroff and Katz, 1953; Ellis, 1937). However, since this relationship seemed to proportional, an attempt was made to determine whether or not such a relationship existed, and, if so, to determine whether or not this relationship was exact enough to overcome the individual variability in tolerance exhibited by the species.

The average alkalinity for the two summer test periods was determined. The per cent error of this average was determined and the average minimum lethal dosage (M.L.D.) was calculated from the established range of the average alkalinity. That is, the lethal dosages of the positive tests falling within this range were averaged. Thus for the species *Micropterus d. dolomeiui*, the average M.L.D. at an average alkalinity of 93 ppm was 18 ppm copper sulphate. It should be noted that this represents the conditions existing in the test region.
Since other factors in the tests such as excess of chemical for a given alkalinity, or lowered individual tolerance by possible inherent pathological conditions influenced the lethal concentration, it was felt that through the use of the ratio 18/93, the experimental data could be "corrected" in terms of the ratio. These theoretical values could then be compared with the experimental values. The results are shown in Table 2. Note especially the comparisons between the theoretical and experimental values at the lower experimental alkalinity.

A regression curve was calculated utilizing the experimental values (Figure 1). From this curve the regression value of the M.L.D. was calculated for each experimental value of alkalinity (Table 2). A F-population confidence level was also calculated and was found to be well within the 1 per cent confidence level. The deviations from the experimental M.L.D. for both the theoretical and regression alkalinity values was also calculated (Table 2). It should be noted that in the regression deviations the largest deviation was at a value well above the average M.L.D./alkalinity ratio; thus, this large difference is only apparent as a M.L.D. of 18 could possibly have been effective. The deviation found at the 18 ppm average M.L.D. value was only .49 ppm. To establish the variability within
FIGURE 1. The calculated regression curve based on the positive experimental results of *Micropterus d. dolomieu* acquired in Phase I.
**TABLE 2**

Summary of the results of Phase I for *Micropterus a. dolomieu* showing the relationship between experimental, theoretical and regression values.

<table>
<thead>
<tr>
<th>EXPERIMENTAL IN PPM</th>
<th>THEORETICAL IN PPM</th>
<th>REGRESSION VALUES IN PPM</th>
<th>DEVIATION OF EXPERIMENTAL CONC. OF CuSO₄ IN PPM FROM THEORETICAL REGRESSION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CuSO₄</td>
<td>NOA⁺</td>
<td>CuSO₄</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>1.94</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>5.81</td>
<td>16</td>
</tr>
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<td>4</td>
<td>4</td>
<td>4.48</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
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<td>5.87</td>
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<td>6</td>
<td>5.32</td>
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<td>7</td>
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<td>5.10</td>
<td>47</td>
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<td>25</td>
<td>25</td>
<td>88</td>
<td>17.03</td>
</tr>
</tbody>
</table>

**AVERAGE K.O. ALKALINITY/PPM FOR SUMMERS OF 1957; 1958:** 93 ± .93

**MINIMUM AVERAGE LETHAL DOSAGE OF CuSO₄/PPM:** 18.00

* Methyl Orange Alkalinity
individuals of a species it would be well to test a number of specimens at the average M.L.D./alkalinity value. However, it is felt from the data that a proportionality does exist. This will be expanded further under the heading Discussion.
PHASE II

This phase of the research was carried out in the laboratory of the Zoology and Entomology Department on the campus of the Ohio State University at Columbus, Ohio. The purpose of this phase was to determine, on a preliminary scale, the effect of copper sulphate on the oxygen consumption of the goldfish, Carassius auratus. A continuous-flow system was utilized in comparison to the aquarium tank method.

Description of Continuous-flow Apparatus

A large square tank was built of 3/4 inch plywood with overall dimensions of 2 ft. x 2 1/2 ft. x 2 ft. The tank was then partitioned into two chambers with the smaller being 2 ft. x 1/2 ft. x 2 ft. It was used as the fresh (tap) water source. The larger, being 2 ft. x 2 ft. x 2 ft., was used as the copper sulphate source.

The interior of each chamber was covered with fiberglas material and then coated with a commercial type polyester resin. A thickness of approximately 1/8 inch plastic resin was poured over the entire exterior of the chambers which rendered the unit completely waterproof. Each chamber was also equipped with a permanent air intake fitting for attachment of the aerating apparatus (Figure 2). The small fresh water chamber was equipped with a by-pass plastic valve-float system which was used to maintain a constant water level.
FIGURE 2. Drawing of continuous-flow system utilized in Phase II.
in this chamber (Figure 2). This valve was constructed of the commercial acrylic plastic, Plexiglass. A rubber tube was used as the water outlet tube and pressure shut-off unit in the valve (Figure 2, item 5). Except for small sections of rubber tubing used at flexible junctions all other tubing used in the apparatus was of the polyethylene or of the glass type.

The flow of liquid from either chamber was controlled by means of Hoffman type clamps. Water to the test chambers could be directed in this manner, as desired, from either chamber. The flow rate was controlled by means of standard glass stopcocks.

The test chambers were constructed of 1/8 inch acrylic plexiglass and were of two sizes, 4 in. x 2 in. x 2 in. and 8 in. x 3 in. x 3 in. Inside Diameter respectively. Each was equipped with intake tubes from either source chamber, and an outlet tube which led to a 250 cc wide-mouth bottle, which was used as a trap. Each was also equipped with a standard centigrade thermometer (Figure 2, item 22).

**Methods Utilized in Continuous-flow Tests**

1. **Chemical methods:** All chemical tests were conducted under the same procedures as in Phase I.

2. **Weight of specimen:** All specimens were weighed directly in grams on a Shadograph gram weight apparatus.

3. **Standard length:** The procedure in measuring
the length of the specimens was the same as that used in Phase I.

4. Copper sulphate stock solution: A 1000 ppm stock solution was used as the basic source for all test concentrations of copper sulphate. The procedure used in preparing the stock solution was that of Phase I.

Procedure of Test

A goldfish was placed in the experimental chamber (Figure 2, item 22). Water from the fresh water source was allowed to flow through both this chamber and the control chamber. The flow rates of the chambers were adjusted by means of the stopcock so that the flow of water was equal through each chamber. The specimen was allowed to remain a minimum of 24 hours in the experimental chamber before dissolved oxygen samples were taken. At the end of the adjustment period the oxygen consumption of the specimen was measured by determining the difference in dissolved oxygen content of the water in both the control and experimental chambers. This measurement was repeated at various intervals over a 48 hour test period. The results were recorded as the normal rate of consumption by the specimen in untreated water. At the end of this period, the copper sulphate solution was directed through the chambers. The oxygen consumption was again determined at various intervals throughout a 48 hour period.
The time interval between measurements was recorded as
the total time elapsing after the introduction of the
copper sulphate solution. (See Table 3, Interval In
Hours.) The rate of consumption over the test period
was then compared with the rate observed under fresh
water conditions.

Results of Continuous-flow Tests

The minimum lethal dosage for the specimens tested
was 8 ppm copper sulphate at an average M.O. alkalinity
of 27 ppm at a temperature of 19°C and 5-6 ppm at a
temperature range of 23-24°C and at the same average
alkalinity. The rate of oxygen consumption, however,
did not drop drastically from that of the normal fresh
water condition. This drop in oxygen consumption when
present, did not continue as expected but remained
fairly constant throughout the test period. In those
cases showing positive results, the oxygen consumption
remained fairly constant up until death (Table 3).
It should be noted that in general whenever a drop
occurred in oxygen consumption in respect to the normal,
or fresh water, consumption values, this drop was
usually accompanied by a decrease in water temperature.
These results will be discussed further under the section
Discussion of Results.
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<th>TEMP. °C</th>
<th>O₂ CONSUMPTION</th>
<th>TIME INTERVAL</th>
<th>TEMP. °C</th>
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*Flow Rate*
PHASE III

The objectives of this phase were to determine in the goldfish, *Carassius auratus*, the extent of transfer of the copper ion through the gill filaments, to determine the internal course of the copper ion, to determine the relative concentration of the copper in the various organs, to attempt to determine the rate of excretion of the copper through the kidneys, and to determine the relationship between uptake of copper and the test concentration of copper sulphate.

It was decided that the most efficient means of detecting any copper present in the organs of the fish was by radiation techniques. The radioisotope used was copper-64 which has a half-life of 12.84 hours.

**Description of Copper-64**

Copper-64 is the radioactive isotope of stable copper-63. It is produced by bombarding copper-63, in the form of copper sulphate, with neutrons in a nuclear reactor. The various radiations emitted by radioactive copper-64 are as follows: 39 per cent-beta minus, 19 per cent-beta positive (positrons) a gamma ray and 42 per cent by electron capture which initiates the emission of x-rays.

**Source of Radioactive Copper-64**

The radioactive copper samples used in this research were acquired through the nuclear reactor facilities of
the Battelle Memorial Institute, Columbus, Ohio.
Permission to use the isotope was obtained from
Mr. Francis Bradly, Director of Radiation Safety, The
Ohio State University.

**Method of Identification of Copper-64**

It was necessary to ensure that the activated
material utilized in the tests was definitely copper-64.
Two standard methods were employed for this determination,
the scintillation spectrometer method which was used in
the identification of characteristic nuclear emissions
from copper-64, and the decay curve method. In the
latter method measurements of the radio-copper are
taken at regular intervals until the initial radioactivity
decreases by one-half. When these measurements are
plotted on the proper graph paper, a decay, or half-life
curve, is obtained which is characteristic of the radio
copper-64.

The former, the scintillation spectrometer, is an
electronic apparatus that is capable of detecting the
various gamma and x-ray energies emitted by a
radioisotope. In this case, the instrument was utilized
to detect the "annihilation" radiation emitted as a
result of the 19 per cent beta-positive radiation of
copper-64. Annihilation radiation is derived when a
positive beta particle (positron) emitted from a
copper-64 nucleus collides with an electron. The result
of this collision is the "annihilation* of the original particles with the formation of two .51 Mev. gamma rays. It was this radiation that was used in the identification of the isotope.

The spectrometer was first calibrated by means of standard radioisotopes of known gamma energies. These standards were barium-133 with three energy peaks of .082 Mev., .300 Mev., and .357 Mev.; cesium-137 with a .662 Mev. energy peak, and manganese-54 with a .852 Mev. energy peak. The various peaks were attained by plotting the counts/minute against a series of bias voltage settings of the apparatus. The bias voltage setting of the .51 Mev. energy peak of copper-64 was then calibrated as a direct proportionate value of the standards. By plotting counts/minute against this voltage, utilizing a sample of the irradiated copper sulphate as the source of radiation, the acquired peak could be observed to fall at the bias voltage setting required to detect copper-64 annihilation radiation (Figure 3). Since this procedure is dependent on the linearity of the apparatus the standard energy peaks were plotted against the respective calibrated bias voltages. The resultant curve was observed to be linear (Figure 4). A half-life curve was then plotted utilizing the copper-64 standard sample as the source and the .51 Mev. bias voltage setting. The result
FIGURE 3. Goldfish radiation experiment, run #1. Energy calibration curve utilizing isotopic standards of Ba133, Cs137, Mn54, and showing the energy peak of Cu64.
FIGURE 4. Goldfish radiation experiment, run #4. Linearity curve indicating the proportionality of the scintillating spectrometer.
would show if the irradiated material disintegrated at the same known rate as copper-64 (Figure 5).

In order to increase the efficiency in detecting the annihilation radiation emitted by the copper sulphate sample standard, an aluminum covered plastic planchet holder was constructed (Figure 6). The thickness of the aluminum layers was calculated to be that thickness which would prevent the passage of all free positron emissions but allow all gamma rays resulting from the apparatus. Thus in all recordings only gamma rays originating from within the chamber and the sample were counted.

A Geiger-counter was employed to determine the relative amounts of copper-64 in the various organs. The Geiger tube was enclosed in an inch and a half thick cast-iron chamber, or pig, to lower normal background radiation. The Geiger-counter detects all nuclear emissions from copper-64 from within the tissues.

Method of Preparing Tissue for Counting

All tissue samples were placed in stainless steel planchets and digested with concentrated nitric acid before counting. The residue was then dried over a small electric plate. In this manner the amount of self absorption of emitted rays in the tissue itself was reduced considerably and the efficiency in detecting all nuclear disintegrations occurring was increased.
FIGURE 5. Goldfish radiation experiment, run #1. Decay curve of Cu64 utilizing a scintillating spectrometer.
FIGURE 6. Aluminum covered plastic planchet chamber utilized in the detection of the .51 Mev. energy radiation of Cu64: 1-cover-aluminum layer; 2-cover-plastic layer; 3-planchet; 4-planchet table; 5-plastic base; 6-plastic base aluminum layer.
Method of Irradiating Copper-65

For each test a one-half gram sample of anhydrous copper sulphate was sealed in a quartz tube 3 inches x 1/2 inch. The duration of the radiation was 30 minutes for sample #1; 45 minutes for samples #2, #3, and #4, respectively. The specific activity produced was approximately 2 to 2.5 millicuries per half a gram of copper sulphate or approximately $7.4 \times 10^7$ disintegrations per second. This activity depended on the neutron flux of the reactor at time of irradiation.

Since the half-life of copper-64 is only 12.84 hours, it was necessary to have one sample irradiated for each experimental run. The calibration and identification procedure was carried out for each sample irradiated. Figures 3, 4 and 5 are shown as an example of this procedure.

Test Procedure

1. Aquarium Tank Test: The activated sample was carried, in a small iron-lead cask, from the reactor site to the test area. The quartz tube was then removed behind brick shielding, and then placed in a wide-mouth honey jar. It was then scored with a file and broken open by tapping lightly with a small hammer and steel rod.

A very minute amount of the irradiated copper sulphate powder was transferred by means of an applicator stick to a glass planchet disk, covered with tape, and placed
in the aluminum planchet chamber (Figure 6). This was utilized as the sample standard in all calibration and identification procedures.

The remaining powder was then dissolved in 500 cc of water producing a 1000 ppm stock solution. The test specimen, Carassius auratus, was then placed in a small aquarium containing 3 liters of tap water. To this was added the appropriate amount of stock solution to produce the desired ppm of copper sulphate.

A 0.5 ml sample of both the stock solution and the test solution was placed on a glass planchet, evaporated to dryness, and counted by means of the scintillation spectrometer. This gave an indication of the amount of copper-64 present in each solution.

The fish remained in the test solution for a period of 12 hours. At the end of this time it was removed and rinsed in five separate baths of double distilled water. The fish was then dissected and the various internal organs were removed and weighed immediately on an analytical balance. The respective tissues were placed in stainless steel planchets of 1 inch diameter, digested with concentrated nitric acid and dried. The relative amounts of copper-64 present in each organ were determined by the Geiger counter.

2. Continuous-Flow-Split Chamber Test: This test was modified only during the actual treatment of the
specimen. All other procedures were the same as for the aquarium tank tests.

A 7 inch goldfish was anesthetized with 1 per cent Urethane. The right operculum was dissected away exposing the right gills. The specimen was then placed into the chamber in the manner shown in Figure 7 - top view. The specimen was secured by means of thread to prevent excess movement. The copper sulphate delivery tube (Figure 7, item 1) was placed in the mouth with the opening directed towards the right gill. The fresh water supply was activated by the adjustment of clamps and the rubber diaphragm checked for leakage. Double distilled water was added to the posterior chamber until the specimen was covered.

The fresh water flow was regulated to maintain a constant level of about 1/4 inch from the top of the anterior chamber. The copper sulphate flow was then activated and regulated to maintain a flow approximately 25 per cent of that of the fresh water flow. This created a steady drainage flow of copper sulphate over the right gills and out of the chamber.

A 0.5 ml sample was removed from the posterior chamber every half hour throughout the test period, evaporated on a glass planchet and counted, to determine if any copper-64 was being excreted through the kidneys. If an excessive amount of radioactivity
FIGURE 7. Drawing of the continuous-flow split chamber apparatus utilized in the goldfish radiation experiment, run #4.
comparable to that of the test solution had been detected in these samples it would have been indicative of leakage occurring through the rubber diaphragm. A difference in the water levels of both chambers was also used as a check of diaphragm malfunction. However, such malfunction did not occur throughout the test.

Results of Radioactive Tracer Tests

1. Aquarium Tank Test: Tables 4-8 give the results for each of three runs at the concentrations of 3, 5 and 8 ppm of copper sulphate. The tables also give the relative amounts of copper-64 found in the organs and are reported as counts/minute/milligram of organ. It would be well to note that one recorded count represents the disintegration of one atom of copper in the tissue at the time of counting.

2. Continuous-Flow Split Chamber Test: Table 8 gives the results of this test at a concentration of 5 ppm of copper sulphate. Figures 8-10 show the energy calibration, linearity, and half-life curves, respectively, of this run determined by the spectrometer procedure.

Figure 11 shows the half life curve of this run as determined by means of the Geiger-counter apparatus.
TABLE 4

Goldfish radiation experiment, run #1, utilizing Cu\textsuperscript{64} at a concentration of 5 ppm irradiated CuSO\textsubscript{4}.

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<th>ERROR +/-</th>
<th>BACKGROUND C/M</th>
<th>WT. OF ORGAN IN MG</th>
<th>COUNT/MIN PER MG</th>
<th>RELATIVE C/M/MG</th>
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TOTAL ACTIVITY CuSO\textsubscript{4} SAMPLE, 0.5GM, IN COUNTS/MINUTE 49,943.52

TOTAL ACTIVITY STOCK SOLUTION, 1000PPM, 0.5ML, IN COUNTS/MINUTE 27,888.56

TOTAL ACTIVITY TEST SOLUTION, 5PPM, 0.5ML, IN COUNTS/MINUTE 144.72

TEST PERIOD IN HOURS 12
Goldfish radiation experiment, run #2a, utilizing Cu$^{64}$ at a concentration of 8 ppm irradiated CuSO$_4$.

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1- GALL BLADDER INCLUDED WITH LIVER
2- BELOW BACKGROUND COUNT
TABLE 6

Goldfish radiation experiment, run #2b, utilizing Cu\textsuperscript{64} at a concentration of 8 ppm irradiated CuSO\textsubscript{4}.

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<th>ERROR</th>
<th>BACKGROUND C/M</th>
<th>WT. OF ORGAN IN MG</th>
<th>COUNT/MIN PER MG</th>
<th>RELATIVE C/M/MG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BLOOD</td>
<td>.0100</td>
<td>5.7</td>
<td>.31</td>
<td>27.1 ± 0.65</td>
<td>10.0</td>
<td>.570</td>
<td>.570</td>
</tr>
<tr>
<td>2</td>
<td>GILL</td>
<td>.3056</td>
<td>1877.2</td>
<td>13.19</td>
<td>630.6</td>
<td>6.143</td>
<td>6.393</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>LIVER</td>
<td>.2353</td>
<td>93.2</td>
<td>2.70</td>
<td>235.3</td>
<td>.396</td>
<td>404</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>GALL BLADDER</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>HEART</td>
<td>.0248</td>
<td>3.0</td>
<td>0.17</td>
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<td>24.8</td>
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<td>.128</td>
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<tr>
<td>6</td>
<td>SPLEEN</td>
<td>.0230</td>
<td>BELOW\textsuperscript{2}</td>
<td>—</td>
<td>23.0</td>
<td>BELOW\textsuperscript{2}</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>INTESTINE</td>
<td>.2676</td>
<td>534.7</td>
<td>6.95</td>
<td>267.6</td>
<td>1.998</td>
<td>2.230</td>
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</tr>
<tr>
<td>8</td>
<td>KIDNEY</td>
<td>.0487</td>
<td>BELOW</td>
<td>—</td>
<td>48.7</td>
<td>BELOW</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>BRAIN</td>
<td>.0947</td>
<td>3.7</td>
<td>0.21</td>
<td>94.7</td>
<td>.032</td>
<td>.035</td>
<td></td>
</tr>
</tbody>
</table>

TOTAL ACTIVITY CuSO\textsubscript{4} SAMPLE, 0.5GM, IN COUNTS/MINUTE: 8738.32
TOTAL ACTIVITY STOCK SOLUTION, 10000PPM, 0.5ML, IN COUNTS/MINUTE: 1092.38
TOTAL ACTIVITY TEST SOLUTION, 8PPM, 0.5ML, IN COUNTS/MINUTE: 125.44

TEST PERIOD IN HOURS: 12

\textsuperscript{1}—GALL BLADDER INCLUDED WITH LIVER
\textsuperscript{2}—BELOW BACKGROUND COUNT
Goldfish radiation experiment, run #5, utilizing Cu\(^{64}\) at a concentration of 3 ppm irradiated CuSO\(_4\).

<table>
<thead>
<tr>
<th>NO</th>
<th>TISSUE</th>
<th>WT. OF ORGAN IN GM</th>
<th>COUNT/_MIN</th>
<th>ERROR</th>
<th>BACKGROUND C/M</th>
<th>WT. OF ORGAN IN MG</th>
<th>COUNT/ MIN PER MG</th>
<th>RELATIVE C/M/MG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BLOOD</td>
<td>.0853</td>
<td>85.6</td>
<td>2.57</td>
<td>28.6 ± 0.70</td>
<td>85.3</td>
<td>1.003</td>
<td>1.003</td>
</tr>
<tr>
<td>2</td>
<td>GILL</td>
<td>.6462</td>
<td>54449.0</td>
<td>217.90</td>
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<td>646.2</td>
<td>8.426</td>
<td>85.980</td>
</tr>
<tr>
<td>3</td>
<td>LIVER</td>
<td>.4286</td>
<td>2285.8</td>
<td>20.57</td>
<td></td>
<td>428.6</td>
<td>5.332</td>
<td>5.386</td>
</tr>
<tr>
<td>4</td>
<td>GALL BLADDER</td>
<td>3002</td>
<td>175.2</td>
<td>5.43</td>
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<td>300.2</td>
<td>1.713</td>
<td>1.780</td>
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<td>5</td>
<td>HEART</td>
<td>.0788</td>
<td>77.9</td>
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<td>6</td>
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<td>20.17</td>
<td>116.0</td>
<td>3.02</td>
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<td>201.7</td>
<td>.575</td>
<td>.610</td>
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<tr>
<td>7</td>
<td>INTESTINE</td>
<td>1.1261</td>
<td>1097.9</td>
<td>14.27</td>
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<td>1126.1</td>
<td>.975</td>
<td>1.025</td>
</tr>
<tr>
<td>8</td>
<td>KIDNEY</td>
<td>2484</td>
<td>172.5</td>
<td>3.80</td>
<td></td>
<td>248.4</td>
<td>.694</td>
<td>.722</td>
</tr>
<tr>
<td>9</td>
<td>BRAIN</td>
<td>2515</td>
<td>47.4</td>
<td>1.71</td>
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<td>251.5</td>
<td>.188</td>
<td>.192</td>
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</tbody>
</table>

TOTAL ACTIVITY CuSO\(_4\) SAMPLE, 0.5GM, IN COUNTS/MINUTE 34,473.11

TOTAL ACTIVITY STOCK SOLUTION, 1000PPM, 0.5ML, IN COUNTS/MINUTE 24,304.72

TOTAL ACTIVITY TEST SOLUTION, 3PPM, 0.5ML, IN COUNTS/MINUTE 110.38

TEST PERIOD IN HOURS 12
Goldfish radiation experiment, run #4; continuous-flow split chamber system; utilizing Cu64 at a concentration of 5 ppm irradiated CuSO₄.

<table>
<thead>
<tr>
<th>NO</th>
<th>TISSUE</th>
<th>WT. OF ORGAN IN GM</th>
<th>COUNT/MIN</th>
<th>ERROR</th>
<th>BACKGROUND G/M</th>
<th>WT. OF ORGAN IN MG</th>
<th>COUNT/MIN PER MG</th>
<th>RELATIVE C/M/MG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BLOOD</td>
<td>.2133</td>
<td>217.3</td>
<td>6.08</td>
<td>29.5±0.71</td>
<td>213.3</td>
<td>1.019</td>
<td>1.019</td>
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<tr>
<td>2</td>
<td>RIGHT GILL</td>
<td>1.5142</td>
<td>54.424.0</td>
<td>217.70</td>
<td>1514.2</td>
<td>35.942</td>
<td>37.054</td>
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<tr>
<td>3</td>
<td>LEFT GILL</td>
<td>1.4249</td>
<td>33.059.0</td>
<td>165.30</td>
<td>1424.9</td>
<td>23.201</td>
<td>24.143</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>LIVER</td>
<td>1.8907</td>
<td>453.7</td>
<td>9.07</td>
<td>1890.7</td>
<td>.240</td>
<td>.250</td>
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<tr>
<td>4</td>
<td>GALL BLADDER</td>
<td>.1620</td>
<td>14.9</td>
<td>.99</td>
<td>162.0</td>
<td>.092</td>
<td>.094</td>
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</tr>
<tr>
<td>5</td>
<td>HEART</td>
<td>.1770</td>
<td>514.5</td>
<td>9.78</td>
<td>177.0</td>
<td>2.907</td>
<td>2.936</td>
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<tr>
<td>6</td>
<td>SPLEEN</td>
<td>.2572</td>
<td>90.0</td>
<td>3.96</td>
<td>257.2</td>
<td>.350</td>
<td>.350</td>
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<tr>
<td>7</td>
<td>INTESTINE</td>
<td>1.6358</td>
<td>146.3</td>
<td>4.97</td>
<td>1635.8</td>
<td>.089</td>
<td>.093</td>
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<tr>
<td>8</td>
<td>KIDNEY</td>
<td>.4622</td>
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<td>4.78</td>
<td>462.2</td>
<td>.304</td>
<td>.320</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>BRAIN</td>
<td>.4887</td>
<td>1420.3</td>
<td>17.04</td>
<td>488.7</td>
<td>2.906</td>
<td>2.996</td>
<td></td>
</tr>
</tbody>
</table>

| TOTAL ACTIVITY CuSO₄ SAMPLE, 0.5GM, IN COUNTS/MINUTE | 10,115.53 |
| TOTAL ACTIVITY STOCK SOLUTION, 1000PPM, 0.5ML, IN COUNTS/MINUTE | 9,660.30 |
| TOTAL ACTIVITY TEST SOLUTION, 5PPM, 0.5ML, IN COUNTS/MINUTE | 53.72 |
| TEST PERIOD IN HOURS | 8HR. 40MIN. |
FIGURE 9. Coldfish radiation experiment, run #4; continuous-flow split chamber system. Energy calibration curve utilizing isotopic standards of Ba133, Ca137, Mn64, and showing the energy peak of Cu64.
FIGURE 9. Goldfish radiation experiment, run #4; continuous-flow split chamber system. Linearity curve indicating the proportionality of the scintillating spectrometer.
FIGURE 10. Coldfish radiation experiment, run #4; continuous-flow solit chamber system. Decay curve of Cu64 utilizing a scintillating spectrometer.
FIGURE 11. Goldfish radiation experiment, run #4; continuous-flow split chamber system. Decay curve of Cu64 utilizing a Geiger-counter apparatus.
DISCUSSION OF RESULTS OF ALL PHASES

The tendency of the minimum lethal dosage of copper sulphate, in respect to a species, to be proportional to the methyl orange alkalinity (Welsh, 1948) of the water provides a potential basis for the formation of a treatment index which is applicable to various bodies of water. This index, in itself, is the Regression Curve derived as a result of plotting the experimental lethal dosages against the corresponding experimental values for alkalinity. The Regression Curve, or Index Curve in this case, is that curve which represents the average in respect to the experimental data. (See Figure 1.)

However, in attempting to utilize this curve as an Index, other influencing factors must be evaluated as to their possible effect on such an index curve. Some influencing factors to be considered are:

1. Temperature: The experimental data did not show any definite relationship of temperature to lethal dosage. This, however, would not be expected to hold true in cases of drastic temperature changes as such changes usually affect the metabolic rate of fishes. The metabolic rate generally increases with a rise in temperature and decreases with a lowering of temperature. Thus, at low temperatures the metabolic requirements of the animal would decrease; while at the same time
the chemical action between the muco-protein secretion on the gills and the copper ions in the water would also decrease. The net result would be an increase in tolerance to copper sulphate by the animal due to lower metabolic requirements and to the decrease in gill blockage by precipitated mucous.

Thus, the experimental data from which the Index Curve, for a particular fish, is computed should be obtained at high temperature conditions. Then, if the Index is referred to for treatments at lower temperatures the dosages used should not prove lethal to the fish.

2. **Effect of Carbon Dioxide:** Moore and Kellerman (1905) found that excessive carbon dioxide in the presence of copper compounds increases the amount of the copper ion in solution which, in turn, increases the rate of muco-protein precipitation on the gills. This would seriously affect the index values by pivoting the right end of the curve upward. Therefore, at a given alkalinity, the lethal value would be lower. The writer feels that experimentation concerning the exact effect of carbon dioxide on the index values at various concentrations of copper sulphate would result in the derivation of a relative correction factor.

3. **Synergistic Compounds:** The presence of such compounds in waters (Ellis, 1937) which by their presence increase the lethal effect of the copper, would seemingly
affect the index curve in the manner similar to that of carbon dioxide. A correction factor, for those compounds known, could likewise be determined experimentally.

4. **Antagonistic Compounds:** The presence of such compounds in waters (Ellis, 1937) which by their presence decrease the lethal effect of the copper, would lower the index curve. Thus their presence should not adversely affect the safety factor of the index values as a water treatment aid.

It should be noted that the data is extremely limited. It is felt that a probable basis for such an index has been shown; but the practical feasibility of such an index will depend on future experimentation.

Since asphyxiation by blocking of the gill filaments is generally held as the cause of death in the presence of large concentrations of copper sulphate, the oxygen consumption was expected to gradually decrease with time. As pointed out in Phase II, this gradual decrease did not occur.

The facts governing this unexpected phenomena are not clear. Since a chemical reaction is governed by the relative amounts of each reacting chemical, it would seem that over a period of time the gill filaments would become more blocked and less oxygen could be absorbed through the gills. This action would also seem logical
even though sloughing off of the precipitated mucous occurred.

In 1926 Cook noticed a latent period existing in the toxic effect of copper on respiration in algae. Atkins (1933) stated that copper sulphate when used as a fish poison in streams, was not toxic to the fish. It is felt that the factors responsible for the oxygen phenomena observed by Cook could possibly have been effective in the observed expression of oxygen consumption in the current problem. In respect to the observations by Atkins, a continuous flow system would simulate, in respect to current, a running water situation. Thus, the prospect of any copper ion remaining in close proximity to the gills would decrease; therefore, the gill filaments would remain relatively free over a period of time. The passage of oxygen over the gills would continue to take place; hence the steady oxygen consumption observed. This view is merely inferred at this time.

Next, as the tracer tests showed, the lower the concentration of copper sulphate the greater the absorption of copper by most organs. Under constant flow conditions deposition increased considerably in some of the organs. A comparison may be made by consulting Table 9. In considering the relative amounts present, it should be noted that the figures
Summary of the results of the goldfish radiation experiments showing relative amounts of Cu64 detected in the various organs in respect to concentration and experimental systems employed.

<table>
<thead>
<tr>
<th>NO</th>
<th>TISSUE</th>
<th>AQUARIUM TEST TANK</th>
<th>CONTINUOUS FLOW SPLIT—CHAMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TEST CONCENTRATION OF COPPER SULPHATE IN PPM</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>----</td>
<td>----------</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>1</td>
<td>BLOOD</td>
<td>1.003</td>
<td>2.382</td>
</tr>
<tr>
<td>2</td>
<td>GILL</td>
<td>85.980</td>
<td>18.170</td>
</tr>
<tr>
<td>3</td>
<td>GILL</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>LIVER</td>
<td>5.386</td>
<td>2.293</td>
</tr>
<tr>
<td>5</td>
<td>GALL BLADDER</td>
<td>1.780</td>
<td>.388</td>
</tr>
<tr>
<td>6</td>
<td>HEART</td>
<td>1.040</td>
<td>.337</td>
</tr>
<tr>
<td>7</td>
<td>SPLEEN</td>
<td>.610</td>
<td>.054</td>
</tr>
<tr>
<td>8</td>
<td>INTESTINE</td>
<td>1.025</td>
<td>.567</td>
</tr>
<tr>
<td>9</td>
<td>KIDNEY</td>
<td>.722</td>
<td>.977</td>
</tr>
<tr>
<td>10</td>
<td>BRAIN</td>
<td>.192</td>
<td>.078</td>
</tr>
</tbody>
</table>

1—GALL BLADDER INCLUDED  
2—BELOW BACKGROUND COUNT
represent activated copper at the time of counting and does not indicate the total amount of copper, both active and stable, that is present in the organ. Viewed thusly, the amount present increases considerably, since a radioactive element possesses the same chemical properties as the stable counterpart. Thus, under continuous-flow conditions it is inferred that the effect of copper is possibly internally lethal rather than physically lethal, as in asphyxiation. This could account for the latent phenomenon of oxygen consumption.

Also, in the continuous-flow split chamber test, no excretion of the metal was recorded in the posterior chamber although the kidney showed .320 counts/minutes/milligram of tissue over the test period. As expected, the treated right gill showed a higher copper reading than the left, or untreated gill, although this gill showed a fairly high copper content. This fairly high content of the left (untreated) gill was thought to be the result of two possible causes. The more probable cause seemed to be inadequate flow valves with consequent turbulence within the oral cavity causing contamination of the left gill. Secondly, that the left gill was a site of excretion of the copper ions which had been absorbed through the right (treated) gill. Smith (1930) showed that excretion occurred in the gills of marine teleosts. More delicate flow
valves, and slight modifications of the apparatus, are required before a more definite conclusion can be obtained. However, the basic principle of the system is experimentally sound.

The amounts of copper detected in the tissues in relation to the various copper concentrations used may be seen in Table 9. It is interesting to note the relative concentration in liver, gall bladder, and intestine as well as the amount of copper detected in the brain at low concentrations and in the continuous-flow split chamber test.

The processes that lead to the deposition of copper ions in the various tissues are not clear, although the entrance of the ions over the gills is evident. At high concentrations of copper sulphate death seems to be caused by asphyxiation. But the variability between fish in respect to death time at all concentrations hints at the presence of a more complex reaction which precedes asphyxiation. The fact that the copper ion is absorbed over the gills so readily must be considered in any final conclusion as to the physiological processes involved. The variability in copper uptake at various concentrations and between various test systems must also be considered, as well as the apparent effect on oxygen consumption and the possibility of internal pathological disturbances. The following inference has
been formulated in an attempt to consolidate these variables. However, it should be taken only as an inference with final conclusions being reserved until further data can be obtained.

The copper ion in the water diffuses through the gills into the blood stream. It is carried directly by the blood to the liver which acts as a detoxifying organ. At this point the number of copper ions that remain in the area of the mucous secretion of the gill is decreased due to their constant diffusion towards the blood stream which, at this point, is low in copper. Thus a concentration gradient acting from the water towards the blood is suggested as the first step. In time the liver can not detoxify at a rate equal to the uptake and the blood copper concentration increases. The result is an eventual decrease in copper uptake rate due to a decrease in the concentration gradient. Thus, more copper ions remain in contact with the mucous coating of the gills; precipitation follows with resulting blockage of oxygen transfer and finally, asphyxiation. However, during the process of gradient equilization various organs become sites of copper deposition. At high concentrations this equalization is attained more rapidly than at lower concentrations. This results in a more rapid blockage of the gills which directly decreases the number of
organs affected, and the amount of copper deposited per organ. At low concentrations the reverse is true.

In the case of aquarium tank conditions, the process of equalization would be accelerated due to the rather "immobile" copper ions in the quiet water. In flowing water, however, the rate of equilization between environmental copper and blood copper is delayed since the current carries the ions away from the gill. Thus, a normally lethal concentration in quiet water would not necessarily have the same effect in flowing water. However, the amount of copper uptake would be more in the flowing situation than in the aquarium situation although both were treated with the same concentration of copper sulphate. Please refer to Table 9, runs 2 and 4. This same inference can be applied to the latent oxygen phenomena of Phase II. The expected gradual decrease of oxygen consumption would not occur if the gill filaments remained relatively free from precipitated mucous over a period of time. One other point to consider is the increased deposition of copper in flowing water. Since the oxygen consumption does not markedly decrease up to the point of death, asphyxiation does not seem to be the main cause of death. However, if one considers the marked increase of copper (Table 9, run 4) under flowing water conditions it is
possible that disturbance of metabolic functions by the copper ion may play an important part in the final effect on the specimen.

It is felt that the physiological effect of copper on various organ systems warrants further investigation since the experimental data show a definite uptake of substantial concentrations of the metal.
CONCLUSIONS

1. The experimental data obtained on the small mouth black bass, Micropterus d. dolomieu, showed that there is a definite proportionate correlation between the lethal dosage of copper sulphate and the methyl orange alkalinity of the water.

2. The formation of an index curve based on this proportional relationship seems possible but verification through future experimental work is required.

3. The index curve is derived by calculating the regression curve which is the average curve that represents the experimental relationship of minimum lethal dosage to the alkalinity of the water.

4. An index curve based on experimental data needs to be formulated for each species.

5. That theoretical values may be calculated for experimental results by the calculation of the average minimum lethal dosage for the average methyl orange alkalinity of the water. The resultant values may be used as a check of the lethal values indicated by the index (regression) curve.

6. The probability that the apparent proportionality of the index curve was not due to chance was found to fall well within the 1 per cent confidence level based on the F-population statistic.
7. Future experimental data for a species should be acquired at the normally warm temperature season of the test region in order to ensure that the index values are useful at lower temperatures.

8. The presence of antagonistic compounds in water should not adversely affect the index values in terms of practical usage in the treatment of waters.

9. The presence of synergistic compounds in the water would affect the index values. It is recommended that future experimental study be done on such compounds in an attempt to determine correction factors to be applied to index values.

10. The effect of copper sulphate on oxygen consumption is inconclusive and warrants further experimental work.

11. The absorption of copper over the gills of a fish does occur. In general, the amount of copper absorbed during a 12 hour period is inversely related to the concentration of copper sulphate in the water.

12. The number of sites of internal deposition of absorbed copper increases as the concentration of copper sulphate in the water decreases.

13. The amount of copper absorbed is greater in flowing water than in quiet water.

14. The excretion rate of copper deposited in the kidney is negligible over a 9 hour test period.
15. The possibility of the occurrence of internal toxic reactions to copper sulphate by fish, at below lethal concentrations, seemed evident.
MISCELLANEOUS OBSERVATIONS

In the course of testing various species with copper sulphate it was noticed that many individuals of the small mouth black bass, Micropterus d. dolomieu, and the goldfish, Carassius auratus, showed evidence of melanin disturbances. This is of particular interest since the hormone intermedin, which is involved in melanin production, is described as a copper-complex protein produced in the pituitary region of the fish brain.

All those specimens that were observed to exhibit this melanin disturbance had a history of being present at the time of disturbance in water containing some source of copper sulphate. The following case history is an example of such a disturbance.

The specimen was a four inch goldfish. The snout was extremely black, this being the only dark area present on the body. Upon treatment with 5 ppm copper sulphate a large dark blotch appeared just anterior to the dorsal fin. The specimen was then removed from the copper sulphate water and placed in fresh water. The dark blotch increased in intensity upon excitation of the specimen, and would fade to a light grey during periods of calm. In about two weeks the blotch migrated slowly downward, and at an oblique angle, to the opercular region. From this point on it began to fade; disappearing in about four weeks. At this
point the original dark area of the snout began to fade. In approximately two and a half weeks it had disappeared. The specimen was completely gold in color and remained so from this point on even though reexposed to various concentrations of copper sulphate. The specimen remained in a healthy condition for approximately 10 months. At this time it expired from a fungus which had contaminated the tank.

No explanation of this phenomenon is attempted. It has been described merely as a point of interest. In this view one should also note the amounts of copper-64 detected in the brain during the radiation phase of the project, especially in the continuous-flow split chamber experiment (Table 9).
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AUTobiography

I, Robert F. Normandin, was born in Laconia, New Hampshire, July 22, 1927. I received my secondary education in the parochial and public schools of Laconia, New Hampshire, and from St. John's Preparatory School, Danvers, Massachusetts. My undergraduate training was at St. Anselm's College, Manchester, New Hampshire, which granted me the Bachelor of Arts degree in 1950. I received my Master of Science degree from the University of New Hampshire in 1953. I came to the Department of Zoology and Entomology, The Ohio State University, in 1956. While completing the requirements for the degree of Doctor of Philosophy, I was employed for three years as Curator of the Museum of Pathology, Department of Pathology, The Ohio State University.