LABORATORY EXPERIMENTS ON THE CONTROL OF THE TAPEWORM TRIAENOPHORUS CRASSUS

BY

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LABORATORY EXPERIMENTS ON THE CONTROL OF THE TAPEWORM, *TRIAENOPHORUS CRASSUS*.

A DISSERTATION

SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

FACULTY OF ARTS AND SCIENCE

by

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April, 1951.

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The undersigned hereby certify that they have read and recommend to the School of Graduate Studies for acceptance, a thesis entitled, Laboratory Experiments on the Control of the Tapeworm Triaenophorus Crassus, submitted by Morton Lionel Libin, B.Sc., in partial fulfilment of the requirements for the degree of Master of Science.

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ABSTRACT

The plerocercoids of Triaenophorus crassus found encysted in the flesh of fishes of the genus Leucichthys and of the whitefish Coregonus clupeaformis cause these fishes in many Canadian lakes to be unmarketable.

This study is a search for methods of breaking the life cycle of this cestode by attacking the free living stage of the parasite, using both chemicals and electricity. In order to extend the period of experimentation on the eggs and coracidia, pituitary injections were made on the final host Esox lucius, the pike. The theory underlying these injections is as follows: both the pike and tapeworm release their sexual products during the same period each spring; therefore sexual development of the pike and cestode follow a similar annual cycle of development and regression. It was hoped that by advancing the sexual maturity of the fish with pituitary injections, the tapeworms would become sexually mature, release their eggs and thus permit further chemical and electrical experiments to be conducted.

Dinotro-o-Cyclohexylphenol, Dicyclohexylamine salt, commercially known as Dow K-604 was the only chemical that proved to be effective at a concentration that would justify its use on a large scale. Electricity was effective at voltages varying between 2 and 55 and held for a period of five seconds. The pituitary injections caused a marked advancement in the development of the tapeworms, but no viable eggs were obtained.

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INTRODUCTION

From an economic point of view the cestode Triaenophorus crassus is the most important tapeworm in Canada. The plerocercoids of this parasite appear as conspicuous yellow cysts, chiefly between the muscle segments in the fishes of the family Coregonidae. The whitefish (Coregonus), and the ciscoes (Leucichthys) are particularly affected. The cysts often occur in large numbers and vary greatly both in size and shape. Although harmless to humans they are objectionable from an aesthetic point of view. A substantial percentage of the whitefish production and almost all of the tullibee production is prohibited entry to the United States. During the period from March, 1945 to March, 1946 a total of 402,472 pounds of whitefish from Alberta, Manitoba and Saskatchewan was rejected at the U.S. border. There are many commercially valuable lakes remaining unfished today due to heavy infestations of the whitefish in them.

Life History of Triaenophorus Crassus

Before any control methods can be inaugerated, it is essential to understand the life history. Miller (1943A, 1943B, 1945C, 1945D, 1946) has thoroughly investigated the life cycle of Triaenophorus crassus and cleared up many previously disputed points. The life history is as follows: In Alberta the adult tapeworm lives only in the intestine of

the pike, Esox lucius. The scolex is firmly imbedded in the gut wall just below the pyloric sphincter. The adult tapeworm gradually matures during the winter months and reaches full sexual maturity in the early spring. The tapeworm sheds its eggs during the pike spawning period, which varies slightly from year to year, occurring usually at the end of April and early part of May. Once the eggs are released the worm dies and passes out of the gut into the water.

The eggs, being slightly denser than water, slowly sink to the bottom. When first released they are white but after about 45 minutes change to a brown color. Each ripe egg contains an onchosphere, bearing six hooks. Movements of the onchospheres can be clearly detected about two days prior to hatching as the egg is transparent. These movements gradually become more violent and finally result in the knocking open of the operculum at the small end of the egg. The embryo, once escaped from the egg, is termed a coracidium. The coracidia at first are the same size as the egg, but one hour after hatching enlarge to twice the size, due to the swelling of the ciliated jelly-like layer surrounding them. The coracidia lead a free swimming existence, swimming aimlessly about by means of their cilia for 24 to 48 hours. If the next host is not available within 48 hours, the coracidia die.

The first intermediate host is the copepod, Cyclops bicuspidatus which swallows the coracidia. Miller (1943B)

has observed anywhere from 1 to 32 parasites in a single Cyclops. When the coracidia are swallowed, they crawl out of their ciliated envelope, and by means of their hooks dig their way through the stomach wall. Once they are in the body cavity of the Cyclops they are referred to as procercoids. Within four days the procercoids double in size, and by the eighth day reach mature size, roughly six times that of the coracidia. The procercoid is considered mature when the cercomere (caudal appendage bearing the hooks) is pinched off. Once the cercomere is established the procercoid either grows very slowly or stops growing altogether, and can go on living for thirty days with no apparent change in appearance.

The second intermediate hosts are the coregonine fishes, i.e. tullibee, whitefish, and Rocky Mountain whitefish. Miller (1944, 1945) has shown that the tullibee is probably the natural host and that the whitefishes are incidental hosts. When an infected copepod is swallowed by the second intermediate host, the procercoid is liberated by the digestive juices. It is thought that the procercoid then proceeds to penetrate a pyloric caecum by means of its frontal gland, after which the cercomere drops off. It then crosses the body cavity and enters the flesh. Once in the flesh it acquires the characteristics of the plerocercoid, and becomes enclosed in a cyst formed by the connective tissue of the host. This penetration and encystment takes place during July, requiring generally one month or more. The cysts live three to four years and then die, the

greatest infestations being noted in tullibee, three to six years old. The plerocercoids resemble the adult worm closely, differing only by the lack of sex organs.

The completion of the life cycle from plerocercoid to the adult is accomplished by a pike swallowing an infested tullibee or other second intermediate host. The cyst is digested away within a few hours, releasing the plerocercoid, which by the end of three days is quite firmly attached to the gut wall. New infestations are picked up by the pike, starting in the late summer.

Possible Methods of Control

On examining the life history of *T. crassus* the most logical approach to a method of control is some way of breaking the life cycle. There are four possibilities:

1. Elimination of the first intermediate host, *Cyclops bicuspidatus*.
2. Elimination or reduction of the second intermediate hosts, the coregonine fishes.
3. Extermination or reduction of the definitive host, *Esox lucius*.
4. Control of the coracidia.

Control of the First Intermediate Host.

Owing to the cosmopolitan nature of *Cyclops bicuspidatus*, this line of attack seems highly improbable.

Miller (1944) has found this species of copepod in all of the numerous Alberta lakes that he has investigated. It is also known to occur across Canada and Europe. Even if a successful "clean-up" were possible, it would be impossible to prevent the rapid re-entry of this copepod into the bodies of water treated.

Control of the Second Intermediate Host.

The removal of tullibee, the second natural intermediate host, would serve as a method of breaking the life cycle. By allowing year around fishing with no limits, tullibee numbers could be reduced sufficiently to result in a reduction of the plerocercoids in whitefish. The Alberta Government has adopted this policy at Lesser Slave Lake. Miller (1948) has shown that during the period 1944 to 1947 the number of Triaenophorus cysts in whitefish at Lesser Slave Lake decreased from 265 per 100 fish to 26 per 100 fish. This method of cyst reduction is applicable where there is a demand for tullibee, i.e. mink feed, thus maintaining fishing pressure. It would be preferable to find some other control method if at all possible, as tullibee, an excellent smoking fish, would find a ready market if free of cysts.

Control of the Definitive Host

Overfishing, poisoning, electrocution, and the use of dams are some of the methods that could be used to reduce the jackfish population. The Central Station of the Fisheries Research Board is attacking the pike-whitefish parasite problem in Manitoba from this angle. Netting has been tried and is successful in the removal of larger pike, but many of the smaller ones escape and continue to restock the lakes. Fish tox, a rotenone poison has been used in Manitoba and also by Miller (1948, 1950) in Alberta. The results of poisoning pike do not justify the expense, and could hardly be extended to large, commercially fished lakes.

Coracidia Control.

The coracidia generally occur for a brief period, in shallow water near the shore. It is hoped that by means of chemical or electrical treatment of the water at appropriate times, the coracidia will be killed. This phase of control has been the object of this work and will later be dealt with in detail.

Previous Chemical Research on Egg and Coracidia Control

The earliest research on coracidia control was carried out by Miller (1944), who discovered that by increasing the

acidity of cultures to a pH of 5.0, the coracidia are killed.

An attempt was made in May, 1945 at Baptiste Lake, Alberta to kill the coracidia by lowering the pH to 5.0 (Miller and Watkins 1946). Twenty tons of commercial sulphuric acid were introduced into the pike spawning areas, over a period of ten days. This should have been sufficient acid to reduce the pH to 5.0. The experiment proved to be a failure, as the pH was reduced only for a short period of time. The presence of carbonates in the bottom mud probably neutralized the acid within a few hours.

Miller and Huston (unpublished) next carried out a number of laboratory experiments, testing the effect of various chemicals on the eggs and coracidia. A large series of chemicals was tested and recorded. The following is a brief summary of their findings:

Certain chemicals were effective in killing the coracidia; these are:

Gentian Violet at 1,000 p.p.m., but not at lower concentrations.

Malachite Green at 10 p.p.m.

Auramine at 2 p.p.m.

Phenol shows variable results, though it appears effective at 1 p.p.m.

Betanaphthol at 100 p.p.m.

Hexyl resorcinol at 5 p.p.m.

Lysol at 0.1 p.p.m.

Borax at 1,000 p.p.m.

Alum at 1,000 p.p.m.

Rotenone is very effective but is a potent fish poison.

Zinc Carbonate at maximum solubility.

Bismuth Carbonate at maximum solubility.

Lead Carbonate at maximum solubility.

Calcium Propionate at 100 p.p.m.

Merthiolate at 1 p.p.m.

Copper Sulphate at 1 p.p.m.

Phenyl Mercuric Chloride at 10 p.p.m.

Zephiran Chloride at 10 p.p.m.

Formalin at 100 p.p.m.

Benzyl Benzoate at maximum solubility.

Carbon Tetrachloride at 800 p.p.m.

Kerosine is effective but impractical.

A number of chemicals showed reactions that were classed as doubtful, and require retesting; these are: methenamine, salol, quinoline and barium carbonate.

A large number of chemicals had very little effect on the coracidia; these are: cresol, resorcinol, pyrocatechol, mulcide, hexachlorethane, phenothiazine, sodium iodide, aerosol, d.d.t., sodium benzoate, magnesium sulphate, potassium permanganate, potassium dichromate, lauryl pyridinium chloride, ethyl mercuric phosphate, chloramine, and picric acid.

It was found that the eggs were more resistant than the coracidia and only a few of the previously mentioned chemicals had any effect on hatching. The chemicals that prevented

hatching to a certain degree are:

Gentian Violet at 1,000 p.p.m. prevents hatching.

Auramine at 5 p.p.m. is effective.

Hexyl resorcinol has some effect at 5 p.p.m.

Lysol at 100 p.p.m. prevents hatching.

Formalin at 100 p.p.m. is effective.

Carbon Tetrachloride is effective at 800 p.p.m.

Of the numerous chemicals tested lysol appears to be the most promising and is also the cheapest. (In the spring of 1949 I thoroughly retested lysol and found it to be effective in killing coracidia at 5 p.p.m. At such a high concentration, the cost of treating a lake would be too large to be practical).

In this study the work of Miller and Huston (unpublished) on testing chemicals for their effect on eggs and coracidia has been continued. The effect of electricity has also been given preliminary exploration. A difficulty in this work has been that the period when eggs are obtainable is limited to a few weeks in the spring. It would be desirable to find some way of obtaining eggs over a longer period of the year. Accordingly, some experiments have been carried out to test the response of the worms to pituitary injections of the definitive host, the pike. The coracidial work is presented here as Part 1 and the pituitary work as Part 11.

PART 1

ON THE CONTROL OF EGGS AND CORACIDIA OF TRIAENOPHORUS CRASSUS

The purpose of this laboratory work was to find some method that will prevent hatching of the eggs, or kill the coracidia of T. crassus. The method must be such that it would be economically possible to treat large infested bodies of water. Both chemicals and electricity were used for experimentation.

Chemical Attack

With the work of Miller and Huston serving as a guide, a number of chemicals were chosen to be tested. I had no idea what type of chemical would be most effective, so a variety of disinfectants, insecticides and generally cheap chemicals were chosen. The ideal chemical would be one cheap enough to use on a large scale, effective at low concentrations, non toxic to fish and other water-dwelling or using animals, sufficiently water soluble to reach the effective concentration and not neutralized, destroyed or precipitated under natural conditions.

Procedure

Towards the end of April worms were removed from the

intestines of jackfish, identified and placed in petri dishes filled with water. If the eggs are sufficiently developed they are released when the worms contact the water. In some cases when the eggs are not extruded spontaneously, the worms may be induced to release them by pulling the worm between the thumb and finger. Worms were obtained by having pike intestines sent in from Fishery Officers and by making trips to the lakes; the best results being obtained by the latter means. Once the eggs were collected they were kept in 50 c.c. bottles at refrigerator temperatures.

The various chemicals to be tested were made up in distilled water to serve as concentrates. With the appearance of coracidia in the egg cultures, experimental cultures having a volume of 50 c.c. were made up in petri dishes. Sufficient amounts of concentrated chemical and distilled water were added to petri dishes to produce the required experimental strength. This was followed by the addition of 10 c.c. of egg culture. Each chemical tested was mixed in three different concentrations, generally 100, 10 and 2 p.p.m. There were also two controls run for each chemical, consisting of 10 c.c. of egg culture plus 40 c.c. distilled water. All cultures were kept at refrigerator temperatures of about 7 to 8 degrees Centigrade.

Each day the experimental cultures were aerated by means of an eye-dropper and one drop of culture removed to be examined under the microscope. The readings were carried out for 6 to 7 days for each culture and were made to

determine the ratio of hatched to unhatched eggs and of live to dead coracidia. The eggs being transparent enabled easy diagnosis of the hatched and unhatched condition; as the latter are seen to contain the embryo while the former are empty. Motility served as the method of determining live from dead coracidia.

Chemicals Tested in 1949

During the spring of 1949 ten chemicals were tested with egg cultures. Several other chemicals were available for testing but the egg supply failed. The records of these experiments may be found in the appendix, tables V11 to XV1. The following are the chemicals at the concentrations tested:

L-F disinfectant #17H - 14 at 100, 10 and 2 p.p.m. Even at 100 p.p.m. this disinfectant proved impotent as it did not affect hatching of the eggs and scarcely reduced the percentage of live coracidia.

Sodium ethylene bis-dithiocarbonate at 100, 10 and 2 p.p.m. This chemical at 10 p.p.m. appears to be quite effective in killing the coracidia and in preventing hatching of the eggs.

CCl_4 triton x at 100, 10 and 2 p.p.m. The viability of the coracidia was reduced considerably at 10 p.p.m. while at this same concentration no effect on hatching was observed.

Y-77 at 20, 10 and 2 p.p.m. While there was no apparent effect on the eggs at 20 p.p.m; all the coracidia were killed; at 10 p.p.m. the coracidia were reduced by approximately 60 per cent.

Lysol at 10, 5 and 2 p.p.m. Close to 100 per cent of the coracidia were killed at 10 and 5 p.p.m; there was little effect on hatching.

Copper Sulfate at 100, 10 and 2 p.p.m. This chemical was 100 per cent effective in killing the coracidia and completely inhibited hatching at 100 p.p.m. At 10 p.p.m. it was ineffective.

Toxaphene at 8, 5 and 2 p.p.m. There were no noticeable effects oneither the eggs or the coracidia at all three concentrations.

Mulcide B at 100, 10 and 2 p.p.m. The coracidia were effectively reduced, though hatching was not inhibited at 100 p.p.m.

Sodium Caprylate at 100, 10 and 2 p.p.m. This chemical did not prevent hatching, but did substantially reduce the coracidia at 100 p.p.m.

Dowklor at 100, 10 and 2 p.p.m. At 100 p.p.m. the coracidia were nearly completely killed off; while hatching was not affected.

The following three chemicals out of the ten tested were considered to possess a desirable degree of potency: Sodium ethylene bis-dithiocarbonate, ccl_4 triton x and lysol. But the cost of using any of these three to treat an infested lake would be too great to be practical, so other chemicals were chosen to be tested in 1950.

Chemicals Tested In 1950

The eggs commenced hatching about two weeks later than those of the previous year, the first coracidia being obtained May 11, 1950. Several field trips were made and a large supply of eggs obtained. I had anticipated running a large series of experiments, but due to bacterial and protozoal invasions, only twenty per cent of the egg cultures were suitable for experimental purposes. Plans were made to continue the chemical experiments well into July, using two different species of Triaenophorus eggs that normally hatch later than those of T. crassus. The first of these, Triaenophorus nodulosus, spends its adult life in the intestine of the pike, releasing its eggs during the latter part of May to the middle of June. This is approximately one month later than T. crassus (Miller, 1943A). The second species of Triaenophorus, T. stizostedionis, occurs as the adult in the intestine of the pike-perch, Stizostedion vitreum. This species of Triaenophorus spawns during the first part of June (Miller 1945D). A trip was made to Lesser Slave Lake early in June to collect eggs of either T. nodulosus or T. stizostedionis. Over one hundred fish were examined and no adult worms were found attached. There were signs of previous infestations and a few worms were found passing out of the intestine. It appears that where T. crassus was late in spawning, both T. nodulosus and T. stizostidionis were earlier than usual.

Owing to the shortage of satisfactory egg supplies only a few chemicals were tested. These are:

Antimony Tartrate at 100, 10 and 2 p.p.m. There was no great visible effect to either the coracidia or the hatching of the eggs.

Chloromycetin at 10, 5 and 1 p.p.m. This antibiotic was impotent.

Two per cent Tyrothricin at 200, 100 and 20 p.p.m. There was no observable effect to either the eggs or the coracidia.

Toxaphene at 100, 10 and 2 p.p.m. This insecticide proved quite effective in killing the coracidia at 100 p.p.m., but had no effect on hatching of the eggs. At 10 p.p.m. it reduced the numbers of live coracidia by roughly 65 per cent.

Dow K-604 at 20,10,2.5,2,1,0.5,0.35,0.25, and 0.05 p.p.m. The results from this chemical look very promising, at 0.35 p.p.m. it was 100 per cent effective in killing coracidia, while at 0.5 p.p.m. as well as killing all of the coracidia it reduced hatching by about 35 per cent. Further statistical details may be obtained by referring to the tables in the appendix.

Information on Dow K-604

Dow K-604 is a highly toxic, yellow compound, composed of Dinitro-o-Cyclohexylphenol Dicyclohexylamine salt, and is manufactured by the Dow Chemical Company at Midland Michigan U.S.A. It is very insoluble at 1000 p.p.m., but at 25

p.p.m. goes nearly completely into solution. A more soluble form is available commercially for spraying plant mites and is termed Dow DN-111. Dow DN-111 contains 20 per cent K-604; the remainder being made up of an inert soluble powder. James W. Ingalls Jr. (letter on file) has fed Dow DN-111 at 100 p.p.m. to mice and guinea pigs as their only water source for one month with no apparent ill effects to the animals. It appears that this chemical is perfectly safe to mammals at a much higher concentration than that necessary to kill the coracidia, and inhibit hatching.

Cost of Using Dow K-604 in Lakes.

The following are prices quoted June 30, 1950 by the Dow Chemical Company:

Dow K-604 - $1.00 per pound, plus duty and exchange.

Dow DN-111 - $0.52 per pound packed in 3¾ pound bags (12 per case), freight allowed on orders of 100 pounds net or more. Dow K-604 can be supplied only from Midland Michigan, while DN-111 is available at Sarnia, Ontario; or Michigan. The Dow Company strongly advises the use of DN-111 which can be easily compounded.

On the basis of these prices, it would be possible to have a concentration of 0.35 p.p.m. Dinitro-o-Cyclohexylphenol Dicyclohexylamine at $0.95 per acre foot using Dow K-604, and $2.48 using DN-111.

In order to treat Square Lake having a total area of

2.5 square miles and requiring treatment of 2,000 acre feet, it would cost $1,900.00 using Dow K-604 and $4,960.00 using DN-111 to give a concentration of 0.35 p.p.m. K-604.

Gold Fish Experiments

To determine the effect of Dow K-604 on fish, three gold fish were placed in a bowl containing the chemical at the desired concentration. A control was also run, consisting of three gold fish in tap water.

At 0.35 p.p.m. Dow K-604 seemed to cause a loss of colour in the gold fish, but aside from this they appeared quite normal. At the end of two days, the experimental gold fish were placed in 0.5 p.p.m. K-604. They appeared to be quite normal for the first few hours, but by morning all three fish were dead. The next day one of the controls was placed in 0.4 p.p.m. K-604 and after 24 hours appeared to have a loss of colour, sluggish behavior and the development of a white filamentous coating over the eyes. After 72 hours this fish died. The two remaining controls were still alive after two months had passed. It appears that K604 is quite toxic to fish at concentrations over 0.35 p.p.m.

Invasive Power of Chemically Treated Coracidia

During the spring of 1949 chemically treated coracidia were fed to Cyclops bicuspidatus, the first intermediate host. The purpose of this experiment was to determine if the chemical, while not killing the coracidia, may weaken them to

such an extent that they were unable to infest the host.

Procedure

Cultures of eggs in distilled water were examined daily to determine the numbers and viability of the coracidia. Those cultures containing active coracidia were then mixed with the chemical to be tested to produce a concentration of 2 p.p.m. of the chemical. A few c.c. of this culture were placed in a syracuse watch glass and a number of Cyclops bicuspidatus then added. At the same time a control was run containing coracidia and Cyclops but no chemical. The following day a number of Cyclops were removed by means of an eye-dropper and examined under the microscope to determine if they had been invaded by coracidia.

Results

In the controls an average of 65 per cent of the Cyclops contained one or more coracidia; the experimentals were about 60 per cent infested with coracidia. The chemicals tested and the results obtained are shown in Table 1.

Table 1

THE EFFECT OF CHEMICALS ON THE INVASIVE POWER OF CORACIDIA OF T. CRASSUS

DATE	CONTROLS		
	NO. OF CYCLOPS EXAMINED	NO. OF CYCLOPS INFESTED	PERCENT OF CYCLOPS INFESTED
28-5-50	5	2	40
30-5-50	6	2	33.3
31-5-50	12	9	75
1-6-50	7	5	71.4
1-6-50	20	15	75
9-6-50	12	9	75

DATE	EXPERIMENTALS			
	CHEMICAL TESTED AT 2 p.p.m.	NO. OF CYCLOPS EXAMINED.	NO. OF CYCLOPS INFESTED	PER CENT INFESTED
28-5-50	Lysol	3	2	66.7
30-5-50	L-F Disinfectant	17	9	52.9
31-5-50	Y-77	12	8	66.7
1-6-50	CCl_4 Triton X	6	5	83.3
1-6-50	Copper Sulfate	9	0	0
9-6-50	Mulcide-B	9	7	77.8

It is quite clear that, with the exception of copper sulphate, all of the chemicals, are ineffective. Copper sulphate is effective in killing the coracidia at 100 p.p.m. (Table XII appendix) and in weakening them at 2 p.p.m. to such an extent that they are unable to infest the first

intermediate host. Copper sulphate has the disadvantage of being precipitated by the carbonates in the lake water, and thus rapidly becoming unavailable.

Electrical Experiments on the Eggs and Coracidia of T. Crassus

Many investigators have noticed the lethal effect of alternating current on insect larvae and fish (Smith, G.F.M. and P.F. Elson, 1949). It was thought that an A.C. apparatus similar to the type designed for fish population surveys could be used in the pike spawning areas in an attempt to kill the coracidia of T. crassus. To build the necessary apparatus would require considerable expense, so preliminary experiments were run in a specially designed and constructed laboratory set-up.

Apparatus

The apparatus (fig. 1) was designed by Professor Harle, to whom we are grateful, and built in the University Machine Shop. The current is taken from an A.C. wall outlet and the voltage regulated by means of a variac. The voltages obtained varied from a minimum of two, to a maximum of one hundred and twenty. The specially designed cell is constructed from lucite; it is $4\frac{3}{4}$ inches long by 5 inches wide and made in easily removable sections to facilitate cleaning. The two electrodes are of 1/16 inch stainless steel measuring

$\frac{7}{8}$ x $\frac{7}{8}$ inches and are situated $3\frac{3}{4}$ inches apart. The electrodes are scavenged by water passing through built-in tubes. The centre of the cell is occupied by a small chamber $\frac{1}{4}$ x 1 x $\frac{3}{4}$ inches in size and separated from the cell proper by vegetable parchment diaphragms. The cell's design enables the voltage gradient between the two electrodes to be readily calculated.

Experimental Procedure

Cultures of eggs and active coracidia were chosen and the percentages of live to dead coracidia and hatched to unhatched eggs determined. Then by means of an eye-dropper 2 c.c. of culture were placed in the centre chamber of the cell. The voltage was then set by means of a calibrated variac and the current turned on for the desired period of time. Following electrocution the 2 c.c. of culture were withdrawn from the centre chamber and examined to determine percentages, as was done prior to the experiment. The experimental culture along with a control was then stored in a refrigerator for two or more days after which another series of counts was made. The centre chamber was washed out with distilled water following each experiment.

Results

A number of experiments were run, the shortest time used was five seconds with voltages of 2,5,10,15,20,25,26,

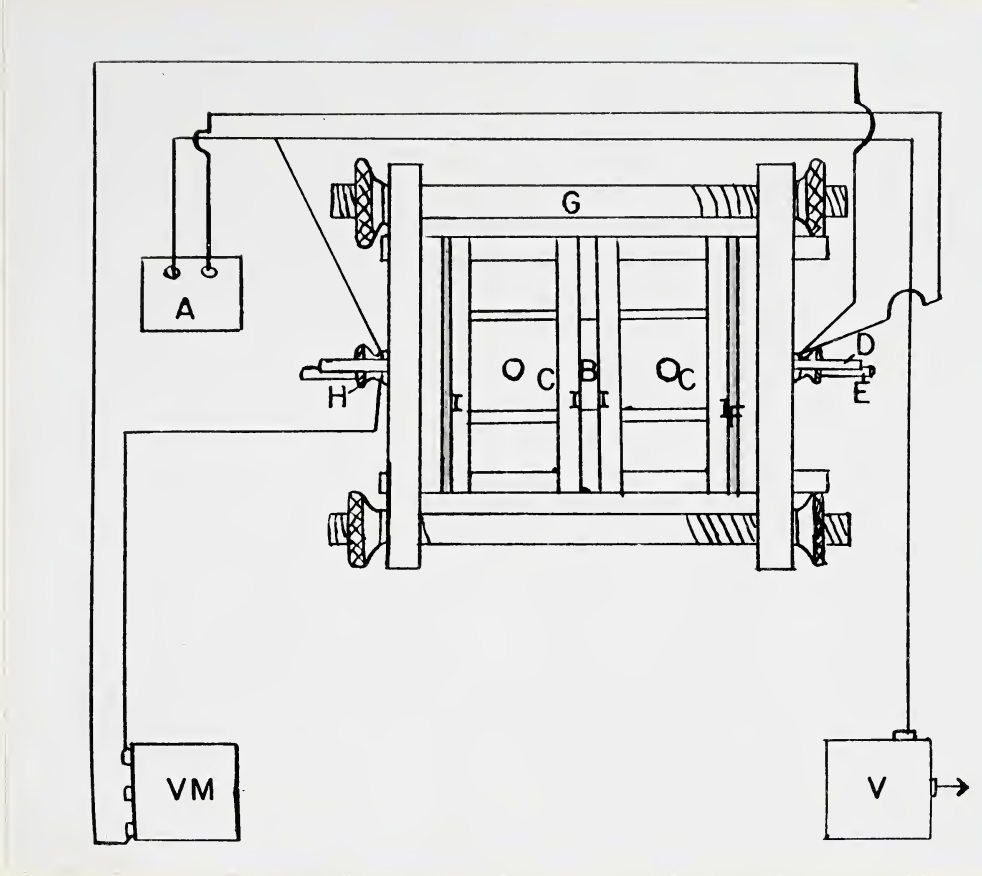

Figure 1

ELECTRICAL APPARATUS USED IN CORACIDIAL CONTROL EXPERIMENTS.

Scale - $\frac{1}{2}$ inch is equal to 1 inch.

KEY

A - Ammeter
B - Chamber for holding eggs and coracidia
C - Opening to electrode chamber
D - Water inlet pipe
E - Water outlet pipe
F - Rubber gasket
G - Alignment bar
H - Post connected to electrode
I - Vegetable parchment diaphragm
V - Variac
VM- Voltmeter

and 55. At fifteen seconds duration voltages of 45,50,52, 54, 55 and 110 were used, while at 20 seconds 2,5,10,15,20, 25,30,35,40,45,50,55 and 110 were used. Voltages of 110 and 120 were tried for a period of thirty seconds. Records of the experiment may be found in Table XXIV appendix.

Those voltages used for a five second duration were successful in killing the coracidia nearly one hundred per cent, but due to the original cultures not being too active this set of results should not be relied upon. At 15 seconds the voltages used showed no great effect on either the eggs or the coracidia, while at twenty seconds the percentage of dead coracidia was increased by about twenty-five per cent at voltages between two and forty. The current at 110 and 120 volts held for thirty seconds proved ineffective. Immediately following electrocution, in the majority of cases the coracidia appeared extremely active as though under stimulation. Also in those cultures infested with protozoa, the protozoa appeared quite normal after electrocution. In most cases, there were slight increases in the percentages of hatched eggs and live coracidia when read on the second day following experimentation.

SUMMARY OF PART 1

The most desirable way to control the cestode Triaenophorus crassus would be some method of killing the free living stage of the tapeworm. Both chemical and electrical attacks were made on the eggs and coracidia. The purpose was to prevent hatching of the eggs and to kill the coracidia, or weaken them to such an extent that they would be unable to infest their first host Cyclops bicuspidatus.

A total of fifteen chemicals was tested with favorable results being obtained in the following: sodium ethylene bis-dithiocarbonate, CCl_4 triton x, lysol and Dow K-604 (Dinitro-o-Cyclohexylphenol Dicyclohexylamine salt). By balancing potency against cost, Dow K-604 is the only chemical tested that would be economically feasible to use on a large scale. At 0.35 p.p.m. it is one hundred per cent effective in killing the coracidia. It is very unlikely that a more potent chemical could be found, but it would be desirable to find a chemical as potent and considerably cheaper. The final test of K-604 would be to run an experiment on a small infested lake.

Seven chemicals were tested to see if they could weaken the coracidia to such an extent that they would be unable to infest the first intermediate host. Copper sulphate was the only effective chemical and it completely prevented infestation at 2 p.p.m. The main disadvantage of copper sulphate is that it is precipitated by the carbonates in the lake.

The effect of electricity on the eggs and coracidia was tested by means of a specially built laboratory set up. Voltages ranging from 2 to 120 were tested for varying periods of time. Those voltages varying from 2 to 55 and held for a period of five seconds were one hundred per cent effective in killing the coracidia. The voltages used for longer periods of time had no great effect. On the basis of this experiment it appears that electricity could be used to control the coracidia. Owing to the poor condition of the cultures tested it would be desirable to run further series of electrical experiments with more active cultures, to verify and expand this work.

PART 2

OBSERVATIONS ON THE EFFECT OF PITUITARY SUSPENSIONS AND EXTRACTS ON TRIAENOPHORUS CRASSUS AND ITS HOST, ESOX LUCIUS

INTRODUCTION

Experimentation on the free living stage of Triaenophorus crassus is limited to a few weeks of each year, the period being determined by the availability of the tapeworm eggs. The object of this work was to obtain egg supplies over various seasons of the year and thus enable a year around attack on the coracidia.

Both T. crassus and its host, the pike, Esox lucius, release their sexual products at approximately the same date. There is no set time for spawning; it fluctuates by a week or two from year to year, occurring shortly after the ice breaks, either in the latter part of April or early May. The fluctuations in spawning dates of the pike may be due to the seasonal rise in temperature of the water. This was pointed out by Turner (1919) fide Craig-Bennett (1931). This coinciding of spawning times is not a mere chance occurence, but has been observed over several years and indicates that there must be some controlling force. It seems reasonable to hypothesize that the host exerts a controlling effect

over the cestodes, causing them to reach full maturity during the pike spawning period. This controlling force or mechanism could be the pituitary gland, whose secretions are the chief regulatory influences operating on the gonads. If this were the case, we would expect the ripening of the cestode to coincide with the advancement of oogenesis and spermatogenesis in the pike. This is precisely what occurs.

Moore (1942) reports that spermatogenesis, whether of the seasonal or continuous variety, appears to depend upon pituitary activity. In those animals having a strictly seasonal period of spermatogenetic activity, gonadotropic treatments will stimulate the testicle to activity and spermatozoa will appear at unusual periods. There have been several attempts to induce premature ripening of the gonads with the aid of pituitary glands. Craig-Bennett (1931) injected extract of the anterior lobe of the pituitary into Gasterosteus aculeatus, causing redevelopment of the secondary sexual characters in five cases out of eight. Hasler, Meyer, and Field (1939) obtained both mature eggs and sperm from rainbow trout six to seven weeks in advance of the onset of the normal spawning period. This was accomplished by means of intraperitoneal injections of fresh and acetone-dried pituitary glands of the carp. Pregnant mare serum and the follicle stimulating fraction prepared from sheep pituitary failed to induce spawning prematurely in trout. The above mentioned three authors (1940) also treated captive muskellunge with pituitary hormones of the carp and

induced spawning three to six days following injection. The eggs were fertilized and a normal hatch occured. There was no spawning among the controls.

On the basis of the previous mentioned hypothesis and the success obtained by other workers in advancing spawning times, the following research program was carried out. Pike were kept in captivity and injected with various dosages of pituitary suspensions and extracts. It was hoped that this would advance spermatogenesis and oogenesis in the fish and at the same time cause the tapeworms to reach full adult maturity.

EQUIPMENT, MATERIALS AND SPECIMENS

Preliminary to experimentation it was necessary to procure and prepare various specimens, materials and equipment.

Tank and Aerator

In order to house the pike over fairly lengthy periods of time, a special tank and aerator were secured (figures 2 and 3). The tank consisted of a small concrete pond measuring 98 by 75 inches, with a depth of five inches for the first thirty inches of its width and then gradually increasing to a maximum depth of eighteen inches. This tank was originally designed for the storage of frogs and turtles and did not prove to be favorable for keeping a dozen or more

Figure 2

TOP VIEW OF FILTER

Figure 3

VIEW OF TANK, AERATOR AND FILTER

medium-sized pike in captivity at one time.

It was necessary to fill the tank with lake and river water, as pike will not survive in Edmonton's chlorinated water. To provide the necessary oxygen content and to keep the water relatively clean a filter and spray were installed. The filter consisted of a metal box measuring 49 by 36 inches and having an overall depth of 25.5 inches. Lining the bottom was a three inch layer of gravel made up of stones measuring one half inch or over in diameter. Above this lay six inches of gravel made up of stones less than one half inch in diameter. The filter was mounted above the tank (fig. 3) and a pipe containing several small holes carried the filtered water in the form of a spray into the tank. A 1.15 horsepower A.C. motor pumped the water from the tank into the filter.

Pituitary Glands

The entire pituitary glands from six hundred pike were removed and stored in small vials of acetone in the refrigerator. Forty-seven frog pituitaries were also extracted and stored in a similar manner. In a few cases pituitaries were obtained from freshly killed pike and utilized immediately.

Preparation of Pituitary Suspensions

About one hour prior to injection the desired number of whole pituitary glands was chosen and the acetone filtered

off. The pituitaries were allowed to dry and then ground to a fine powder using a mortar and pestle. The final step was the addition of ten c.c. of distilled water whereupon the suspension was ready to be used.

Preparation of Pituitary Extracts

Pituitary extracts proved to be more suitable than suspensions, as the latter had a tendancy to clog the hypodermic needle and thus interfere with injection. Preparation of the extract consisted of filtering off the acetone, drying and grinding the pituitaries in the same manner as for the suspensions. Next, three c.c. of distilled water were added and the mixture further ground and the particles allowed to settle. The extract was then decanted and two more c.c. of distilled water added and the grinding, settling and decanting repeated. The second portion of extract was combined with the first and then used for injection.

Pike

The pike were secured during the fall and winter months from Baptiste Lake, Alberta. During the period November 4, 1949 to January 28, 1950, four trips were made to the lake and a total of 53 pike were brought to the tank. From September 26 to October 31, 1950, three trips resulted in 32 pike being procured in good shape for experimental purposes. The fish were captured with gill nets and experience proved

that fish obtained from nets set and run every hour or two, were more healthy and had a greater survival rate than those fish procured in overnight sets. A trammel net was set in October, 1950, but no fish were caught. The fish were transported to Edmonton in a truck equipped with a tank specially built for this purpose.

EXPERIMENTAL PROCEDURE

The experimental procedure consisted of injecting suspensions or extracts of whole pituitary glands into marked pike. Two or three days following injection certain experimental and control fish were killed and examined to determine the condition of their gonads and tapeworms. The worms and gonads were then fixed and prepared for histological study.

Injection and Marking of the Pike

Two people were required for this operation, one to catch and hold the fish and the other to inject and mark it. The injections were all intraperitoneal, being made on the left ventro-lateral surface just anterior to the pelvic girdle, so as to avoid damage to any of the underlying organs. It was a matter of sheer guess work regarding dosages used, both large and small doses of pituitaries were given, with the more favorable results being obtained with small doses repeated at two or three day intervals. Various fin combin-

ations were clipped to facilitate future identification and the fish were then returned to the tank. The dosages used and fins cut were then recorded, the whole operation taking less than two minutes.

Examination of the Pike

Examinations were conducted twice daily and any dead fish removed for detailed study. The pike were watched closely for colour changes, evidence of spawning, and worm ejection. A few days following injection certain experimental and control fish were killed and examined to determine the condition of their gonads and tapeworms. Ripe males were readily identified, as any slight pressure exerted on their abdomens resulted in the exudation of milt. Very little could be told from the appearance of the ovaries in the females. The worms were removed, identified and placed in water to see if they could be induced to shed eggs. Portions of the gonads were fixed in Bouin's solution, while the worms were fixed either in ten per cent formalin or in A.F.A., a special cestode fixative of the following composition:

46 per cent distilled water
24 per cent ethyl alcohol
15 per cent commercial formalin
10 per cent glycerine
5 per cent glacial acetic acid.

Sections of the gonads were made and stained either with iron haematoxylin, Delafield's haematoxylin or Mallory's

triple stain, the best results being obtained with iron haematoxylin. Both sections and whole mounts were made of the tapeworms and stained with iron haematoxylin stain. Detailed microscopic examinations were made of all slides and the results tabulated.

MICROSCOPIC STRUCTURE OF PIKE GONADS

The microscopic examinations of the gonads were made with two objects in view, to determine the effect of the injections and to correlate the condition of the gonads with the stages of tapeworm development.

Testis Structure

The basis of testis structure is connective tissue which forms the thin covering of the testis and fills the intertubular spaces. There is no muscular tissue in the testis and the discharge of spermatozoa is brought about chiefly by the elastic contraction of the connective tissue (Craig-Bennett 1931). The germ cells are contained within numerous tubules which reach their maximum size when filled with spermatozoa. The tubules contain no permanent germinal epithelium and are therefore not strictly comparable to mammalian seminiferous tubules.

The germ cells of the testis undergo spermatogenesis,

the stage of least development being shortly after spawning and that of maximum development sometime during April. In the numerous slides examined the testes appeared fully developed as early as February. Craig-Bennett (1931) reports that functional spermatozoa may be present in the testes of Gasterosteus aculeatus one to two months prior to the breeding season.

The first stage of germ cell development is division of the spermatogonia. Not one of the testes examined showed this stage exclusively. Following this the spermatogonia are transformed into primary spermatocytes contained in thin walled cysts; these are the largest cells observed in the testis. The primary spermatocytes undergo the first maturation division resulting in the formation of secondary spermatocytes which are considerably smaller than the primary spermatocytes. The second maturation division results in spermatids and these metamorphose into spermatozoa.

A ripe testis contains tubules packed with spermatozoa and very few spermatocytes are detectable (fig. 8). In a less developed testis, even though the tubules may be full of spermatozoa, cysts of spermatocytes are plentiful and easily observed (fig. 9).

Coinciding with the maturation of the sex cells is the development of the interstitium. The interstitial, connective and vascular tissues of the testis undergo an annual development and regression. The interstitium increases in volume as spermatogenesis nears completion. The interstitial cells

are large nucleated cells with a granular cytoplasm and generally reach their maximum development just prior to the breeding season (figs. 8 and 10). Bouin and Ancel (1903 fide Craig-Bennett 1931) hypothesized that the interstitial tissue of the testis is the source of the hormone which causes the development of secondary sexual characteristics of the male, in mammals and probably other vertebrates. Bennett (1931) concluded that the interstitial tissue of Gasterosteus is an endocrine gland.

Ovary Structure

The cavity of the ovary is divided by a number of transverse lamellae originating from the dorsal wall. The growing oocytes are embedded in these lamellae, surrounded by a fine follicular epithelium. The ovary undergoes an annual cycle of development. Oogonial division occurs shortly after spawning and maturation divisions occur over the year resulting in mature ova at the breeding season. The interstitium of the ovary is always thin and there are no interstitial cells.

In this study several ovaries were sectioned and stained. No details could be observed of the various stages of oogenesis and as a result detailed histological examinations were confined to the testes.

DEVELOPMENT OF GENITALIA IN TRIAENOPHORUS CRASSUS

In order to correlate the development of the cestode genitalia with the pituitary injections in the pike, it is essential to have an understanding of these organs and of their sequence of development. Each mature proglottis contains a functional set of both male and female sex organs. The genital pores are marginal and alternate irregularly. Every set of male genitalia consists of the following: numerous testes, vasa efferentia, vas deferens, vesicula seminalis, cirrus and cirrus sac. Situated among the male organs are the following female sex organs: a bilobed ovary, vitellaria, vitelline ducts, yolk resevoir, oviduct, shell gland, uterine sac, uterus and vagina.

Normally the anlagen of the genitalia appear in November; the first eggs appear in the uteri in January (Miller 1943). The uteri are distended with eggs early in February, but the eggs do not contain recognizable onchospheres until March and April. These various stages are readily differentiated with the aid of a microscope and a ripe worm may be easily recognized with the naked eye.

The various sex organs exhibit a stereotyped pattern of development. When first differentiated the genitalia appear as solid structures; they later acquire lumina and become functional organs. The following pattern of development was observed in a large series of whole mounts. The first structure to appear is the shell gland and following this is the

KEY TO FIGURES 4,5,6 and 7

c - cirrus sac

e - egg

m - marginal pore

o - ovary

s - shell gland

u - uterus

us - uterus sac

v - vagina

Magnifications indicated in figures 4,5,6 and 7 are those for the original drawing.

Magnifications produced by photographic reduction are X 50.

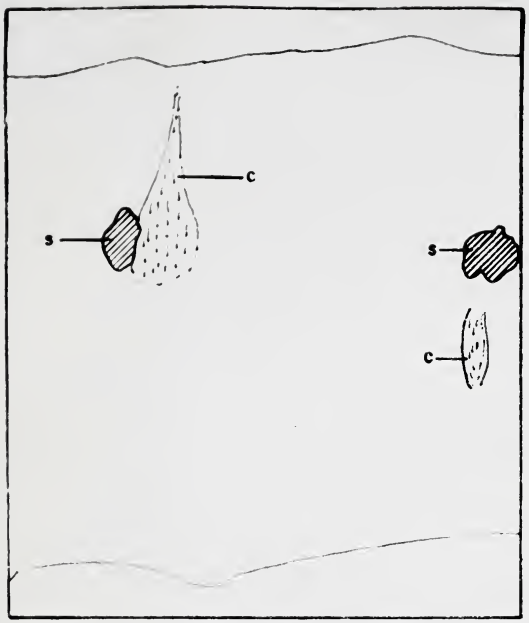

FIG.4. X80.

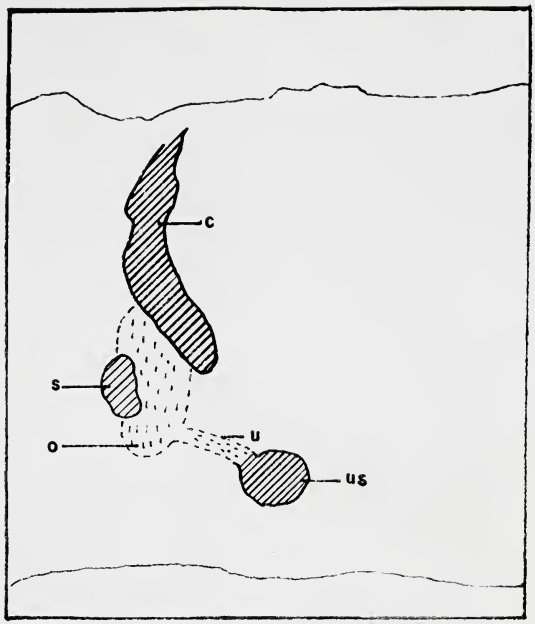

FIG.5. X80.

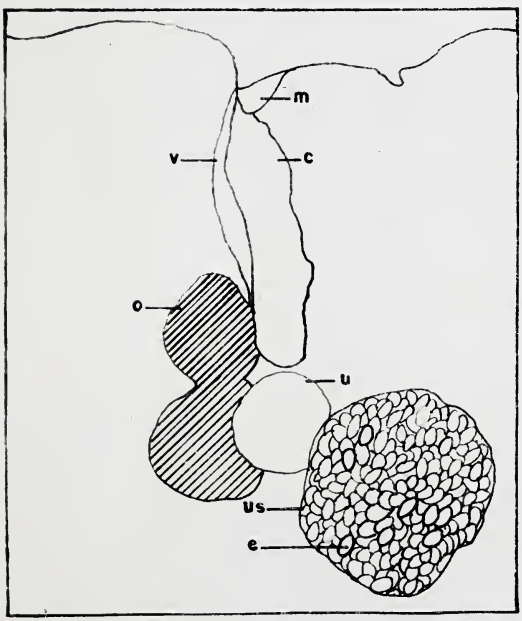

FIG.6. X80.

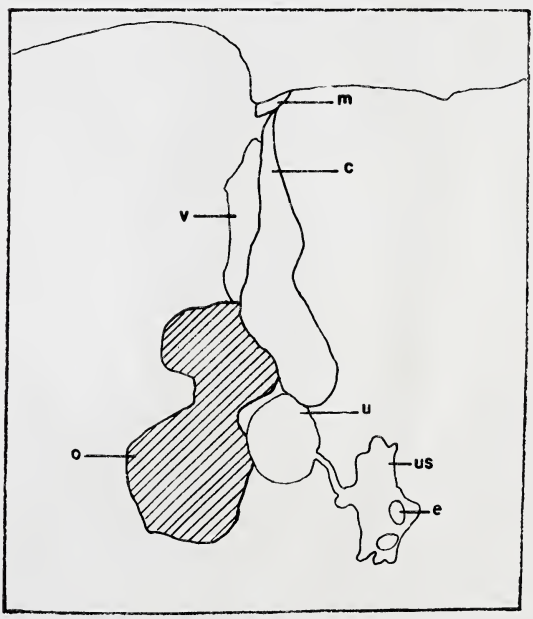

FIG.7. X80.

DEVELOPMENT OF GENITALIA.

cirrus sac (fig. 4). Both the above mentioned structures were seen in worms taken during October. The next stage of development is shown in figure 5, where, in addition to a distinct shell gland and solid cirrus sac, the uterine sac is present as a solid structure and the ovary and uterus are beginning to form. The development of functional sex organs follows this and may be seen in figure 6 which is a camera lucida sketch from a worm taken January 28, 1950. The sex organs are seen to be well differentiated. The uterine sac is distended with eggs without onchospheres and the uterus is greatly enlarged. The ovary and oviduct are distinct as is the cirrus sac. The shell gland is not observable, as it probably collapsed after serving its function. Figure 7 depicts a proglottis in the post spawning condition, showing well developed sex organs and the uterine sac collapsed as a result of releasing the eggs.

By a comparison of the gonads of experimental and control tapeworms, any effects due to the pituitary injections may be readily seen.

EXPERIMENTAL INVESTIGATION

Seven trips were made to Baptiste Lake, with the pike from each trip serving as separate experimental lots. Before actual experimentation commenced, the fish were allowed to adapt themselves to tank conditions for two days. The

first two experiments provided the necessary experience and technique in housing and handling of the fish and following these more satisfactory results were obtained.

Experiment One

On November 5, 1949 twenty large pike were placed in the tank, which had been previously filled with lake and city water. At this time the filter box contained no gravel and functioned only as an aerator. All of the fish were found dead on the morning of November 8. There are several explanations that could account for this sudden and complete killing, such as overcrowding, city water being added, new fittings on the pump and filter may have given off lead; or the accumulation and decomposition of partially digested tullibee, regurgitated by the pike, may have poisoned the water. Another factor that may have played an important role in the sudden fish mortality was their poor physical condition which resulted from their being caught in overnight sets and damaging themselves by attempting to escape. One might conclude that the fish died due to the cumulative effect of all these factors.

Experiment Two

Prior to obtaining the fish the filter was filled with gravel and the tank with river water. Ten healthy medium sized pike were added to the tank on November 11, 1949. In an attempt to determine the effect of city water, six healthy

pike were placed in a second tank filled with tap water. The tank of city water had a constant supply of tap water entering and thus kept the water relatively clean and oxygenated. Within four hours three of the fish in the city water were dead and by morning the remaining three had died. The ten fish in lake and river water were all active, therefore indicating that chemically treated water from the city is lethal to pike. Three pike were found dead on the evening of November 13. The remaining seven fish served as experimentals and controls being numbered, marked and injected with suspensions of pituitary as indicated in table 11.

Table 11

RECORDS OF INJECTED AND CONTROL FISH FOR EXPERIMENT TWO

FISH NO.	SEX	FIN CUT	INJECTION DATE	NO. OF PITUITARIES INJECTED	DATE OF DEATH
1	♂	R. Pect.	14-11-49	9	19-11-49
2	♀	R. Pelvic	14-11-49	9	18-11-49
3	♂	L. Pect.	14-11-49	13	18-11-49
4	♂	L. Pelvic	15-11-49	30	15-11-49
5	♂	R. Pelvic	15-11-49	48	19-11-49
6	♀	---	---	--	15-11-49
7	♂	---	---	--	19-11-49

The first observable effect due to the pituitary injections was the marked loss of colour in all of the experimental fish. Their normally dark green backs had faded to a very pale shade of yellow green.

Condition of the Pike and Their Parasites at Time of Death

The pike appeared to be perfectly normal aside from the loss of colour exhibited by the injected fish. Three out of the four injected male pike were brought into a breeding condition as shown by the active sperm obtained. Fish number four was the experimental male failing to reach the ripe condition, this being due to its death shortly following injection and before enough time had elapsed for the pituitaries to cause any noticeable changes. Fish number seven served as the male control and was not ripe. In both the injected female and the control the ovaries were in similar unripe condition.

The tapeworms were firmly attached in all of the fish and when placed in water failed to release their eggs.

Histological Examination of the Pike Gonads

Fish numbers, one, three, five and seven were chosen for detailed study of the testes. The testes of the first three of these were ripe and all showed a similar state of development. They all had tubules crammed with spermatozoa and only one or two tubules contained stages of spermatogenesis. The intertubular spaces were filled with well developed connective tissue, interstitial cells and small amounts of spermatozoa from broken tubules. Figure 8 shows these details as they appeared in fish number one.

The control testis (fig. 9) differed considerably from

the experimentals. The tubules were filled with spermatozoa but there was also an abundance of thin walled cysts filled with the various stages of spermatogenesis. The connective tissue was not too well developed and interstitial cells were scarce.

Histological Examination of the Tapeworms

Cross sections were made of worms removed from fish one, three, five and seven and whole mounts of those from fish one and seven. The cross sections were of no value as no correlation could be made between the condition of the sex organs of the various worms. The whole mounts revealed various differences in degree of development of the genitalia; these were compared to the camera lucida drawings (figures 4,5,6,7). Both the worms from the experimental and control fish were in a similar state of development, a stage lying between that shown in figures 4 and 5.

Experiment Three

Thirteen pike were added to the tank on November 19, 1949. The next day found five fish dead from which the pituitary bodies were dissected and an extract made. Following the death of one more fish on November 21, seven were left for experimentation with pituitary extracts. The experimental data may be found in table III.

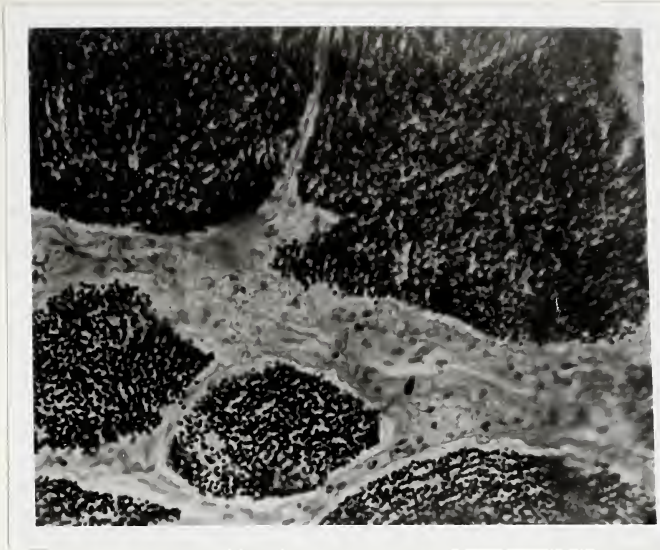

Figure 8

PHOTOMICROGRAPH OF THE TESTIS FROM FISH #1

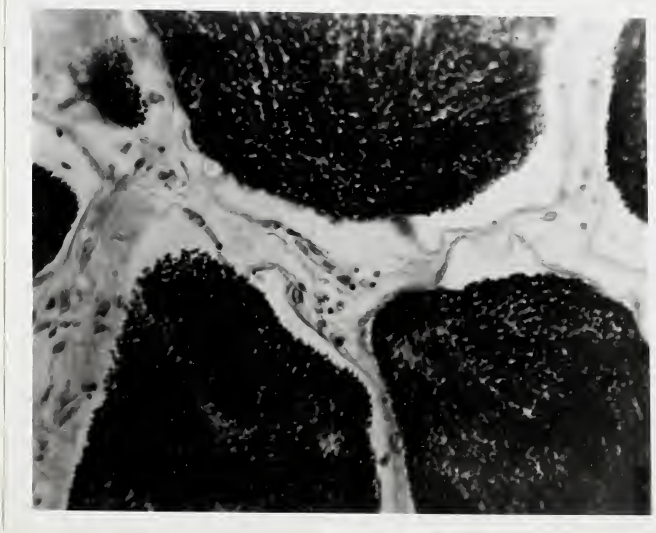

Figure 9

PHOTOMICROGRAPH OF THE TESTIS FROM FISH #7

TABLE 111

RECORD OF EXPERIMENTAL AND CONTROL FISH FOR EXPERIMENT THREE

FISH NO.	SEX	FIN CUT	INJECTION DATE	NO. OF PITUITARIES INJECTED	DATE OF DEATH
8	♂	R. Pect.	21-11-49	8 (Fresh)	21-11-49
9	♂	L. Pect.	21-11-49	17 (Frog)	21-11-49
10	♂	L. Pelvic	21-11-49	5	21-11-49
11	♀	L. Pelvic	21-11-49	5	21-11-49
12	♀	R. Pelvic	24-11-49	5	24-11-49
13	♂	--	--	--	21-11-49
14	♀	--	--	--	21-11-49

Examination

The experimental fish died shortly after injection and on examination showed no further sexual advancement than the controls. The worms in both the experimentals and controls were all firmly imbedded and failed to release their eggs when placed in water. The forementioned results were to be expected as not enough time had elapsed for the injections to take any noticeable effect. For this reason no histological examination was conducted.

Experiment Four

After three days of fishing on Baptiste Lake, ten fish were obtained for this experiment. The fish all appeared to be in a healthy condition, probably because the nets were run every two or three hours and thus excess damage to the fish was avoided. The fish were placed in the tank on January 30, 1950 and treated as indicated in table IV.

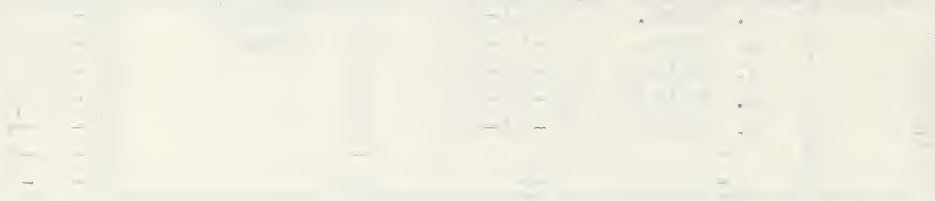

TABLE IV

RECORD OF EXPERIMENTAL AND CONTROL FISH FOR EXPERIMENT FOUR

FISH NO.	SEX	FINS CUT	DOSAGES IN WHOLE PITIUITARIES 30-1-50	3-2-50	7-2-50	13-2-50	DATE OF DEATH
15	♀	R. Pect.	2	2	--	--	13-2-50
16	♂	R. Pelvic	3	3	3	--	8-2-50
17	♂	L. Pect.	4	4	--	--	6-2-50
18	♀	L. Pelvic	5	--	--	--	9-2-50
19	♂	Both Pect.	10	--	--	--	11-2-50
20	♀	R. Pelvic	--	--	--	3	16-2-50
21	♂	--	--	--	--	--	6-2-50
22	♀	--	--	--	--	--	9-2-50
23	♀	--	--	--	--	--	11-2-50
24	♂	--	--	--	--	--	16-2-50

This lot of fish was exceptionally vigorous, jumping and thrashing the water continuously. The injected pike appeared to lose their dark color and considerable contrast could be observed between experimentals and controls. Seven days following the first injections a number of loose worms in the spent condition were found in the water. Several eggs slightly smaller than normal were obtained from these worms and were found to contain well formed embryos. The eggs were stored in the refrigerator but hatching did not occur.

Condition of the Pike and Their Parasites At Time of Death

Apart from a loss of colour in the experimental fish the pike all appeared perfectly normal. When stripped every injected male fish released milt containing active sperms, while among the male controls milt was unobtainable in all

cases but one. Thus, one control was possibly in a mature condition; the high temperature of the tank water probably provided the necessary stimulus for maturation. The gonads of the female experimental and control pike were in a similar condition and stripping did not cause the eggs to be released.

Fish numbers 16,17 and 19 which were experimental males showed signs of heavy infestations, but only a few scolices were found imbedded near the pyloric sphincter region and a number of loose worm fragments were located in the ileum. This same situation was found in fish number eighteen, a female experimental. The worm fragments found in the ileum released eggs containing well formed onchospheres. Both female experimentals 15 and 20 had worms loosely attached as well as some passing down the intestine; all worms were in the spent condition.

Among the controls, fish numbers 22 and 24, female and male respectively, showed no signs of previous infestation. Both number 21, a ripe male and number 23, a female, had many Triaenophorus crassus firmly attached, which on contact with water released eggs that contained no embryos.

Histological Examination of the Pike Testes

The testes of experimental fish 17 and 19 and controls 21 and 24 were examined. The two experimental fish were in approximately the same stage of sexual development (fig. 10), both having well developed interstitium and interstitial cells. The tubules were large and many were empty, having

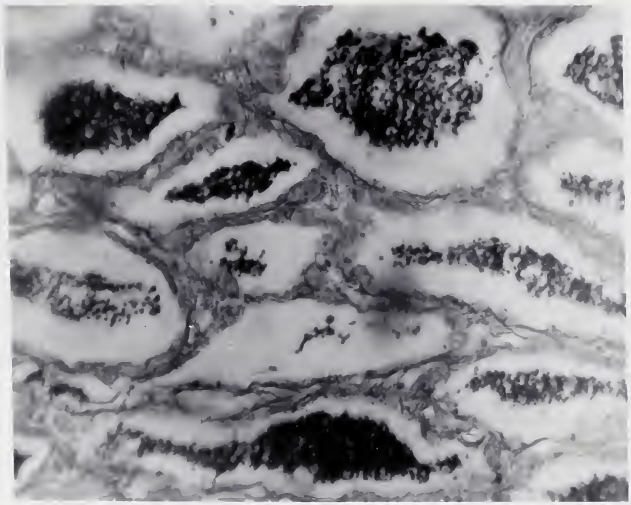

Figure 10

PHOTOMICROGRAPH OF THE TESTIS FROM FISH #19

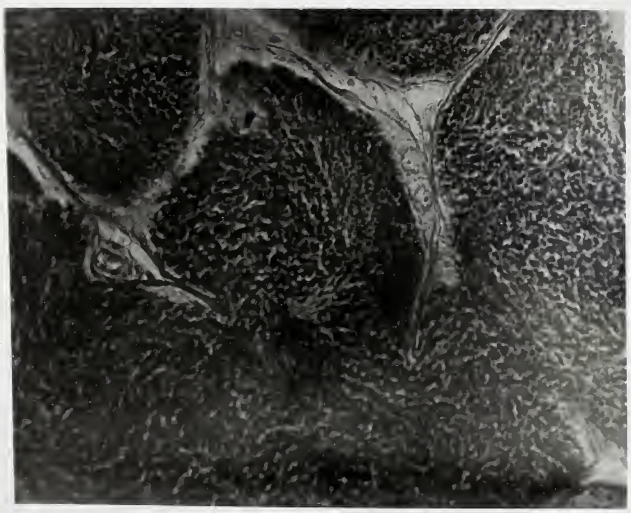

Figure 11

PHOTOMICROGRAPH OF THE TESTIS FROM FISH #24

previously released their spermatozoa. Occasional groups of spermatocytes were observed. The two control fish were at similar stages of development (fig. 11). Both had tubules packed with spermatozoa, but very little tubule breakdown could be detected. The interstitium was well developed and cysts containing spermatocytes were easily seen.

Histological Examination of the Tapeworms

Whole mounts were made of the Triaenophorus found in fish numbers 17, 18 and 19. The cestodes from numbers 17 and 19 were immature; as they were taken from the ileum they are probably anterior fragments of ripe worms that had passed out. They were developed to a stage corresponding to that shown in figure 5. In experimental fish 18 the tapeworms were also loose but none had passed out from the alimentary canal. These were all in a spent condition similar to that shown in figure 7.

Worms from fish 21 were chosen to serve as controls, they were found to be developed to the state shown in figures 6 and 7; all were well developed and a few appeared spent.

Experiment Five

On October 21, 1950 sixteen pike were obtained in good condition by setting and running the nets every two hours. When placed in the tank they were fairly active, but like the fish in all previous experiments they regurgitated a

number of partially digested tullibee and refused to eat any food offered them. The first extract injections were made October 23, 1950, the details of which may be found in table V.

TABLE V

RECORD OF EXPERIMENTAL AND CONTROL FISH FOR EXPERIMENT FIVE

FISH NO.	SEX	FINS CUT	INJECTION DATE	NO. OF WHOLE PITUITARIES	DATE OF DEATH
25	♀	L. Pect.	23-10-50	2	27-10-50
26	♀	Both Pect.	23-10-50	3	25-10-50
27	♀	R. Pelvic	23-10-50	4	27-10-50
28	♂	L. Pelvic	24-10-50	1	27-10-50
29	♀	Both Pelv.	24-10-50	2	27-10-50
30	♀	L. Pelvic L. Pect.	24-10-50	5	25-10-50
31	♂	--	--	--	25-10-50
32-36	♂	--	--	--	27-10-50
37-38	♀	--	--	--	27-10-50

This experiment did not prove too successful, as all of the pike but one were dead by October 27. A number of the dead fish were found firmly wedged between the overflow pipe and side of the tank, apparently having been caught here and died in their attempts to free themselves.

Condition of the Pike and Their Parasites at Time of Death

Of the six injected fish only number 28 proved to be a male, this fish had received the extract of one whole pituitary gland. The injected male was not ripe and appeared no different than the numerous male controls examined. The

five female experimentals appeared to be in the same stage of immaturity as the controls.

In both the control and experimental fish the cestodes were firmly attached and failed to release their eggs when submerged in water. In the smaller pike the worms were found to be Triaenophorus nodulosus, while those in the larger fish were T. crassus.

The fish appeared to be perfectly normal and while there was a certain amount of colour loss in the experimentals it was not nearly as marked as in earlier experiments.

Histological Examination of the Testes

The testes from fish 28 showed tubules filled with spermatozoa and a poorly developed interstitium. Stages of spermatogenesis were present but very hard to detect. Sections from control testes revealed a similar condition.

Histological Examination of the Tapeworms

The cestodes used in this examination had been previously fixed in A.F.A. cestode fixative and proved far easier to handle, stain and mount than those of the previous experiments fixed in formalin. Worms from fish 26 and 30 were chosen as typical experimentals. The sex organs of these worms were distinct but had not acquired lumina. Both were in similar stages of development, identical to that shown in figure 5, which was drawn from a worm secured from fish thirty.

Worms from fish 31 and 32 served as controls and were all in a similar stage of development, not quite as advanced as that shown by the experimentals. The ovaries were not seen and the cirrus sacs not complete; the stage of development was somewhat between that shown in figures 4 and 5.

Experiment Six

Seven pike were injected with extracts of pituitary glands, while eight served as controls. Details of the injections may be found in table V1 which follows.

TABLE V1

RECORD OF EXPERIMENTAL AND CONTROL FISH FOR EXPERIMENT SIX

FISH NO.	SEX	FIN CUT	NO. OF WHOLE PITUITARIES INJECTED				DATE OF DEATH
			2-11-50	7-11-50	8-11-50	15-11-50	
39	♂	L. Pect.	2	2	--	--	13-11-50
40	♂	R. Pect.	3	3	--	--	9-11-50
41	♂	R. Pelv.	4	4	--	--	13-11-50
42	♂	Both Pelvics	--	--	5	--	20-11-50
43	♀	Both Pectorals	--	--	10	--	20-11-50
44	♀	L. Pelv.	--	--	--	1	20-11-50
45	♀	R. Pelv.	--	--	--	1	20-11-50
46	♀	----	--	--	--	--	13-11-50
47	♂	----	--	--	--	--	15-11-50
48 & 49	♂	----	--	--	--	--	20-11-50
50 & 51	♀	----	--	--	--	--	20-11-50
52	♀	----	--	--	--	--	21-11-50
53	♂	----	--	--	--	--	21-11-50

Condition of the Pike and Their Parasites

At time of Death

The fish all appeared normal and very little colour

change was observed.

Three out of the four injected males exuded milt upon stripping. The immature male was fish 42 and had received one injection of five pituitaries twelve days prior to his death. Not one of the male controls was in a ripe condition. There was no visible difference between the ovaries of the female experimentals and controls.

The condition of the cestodes varied; worms were found loose in the ileum or trailing from the vent in fish numbers 39, 40 and 41, while those in fish 42, 43, 44 and 45 were all firmly attached. The loose worms when placed in water released eggs that did not contain embryos, while the attached worms could not be induced to shed their eggs. This would indicate that smaller and more frequent injections are more effective than large single ones, as only those fish receiving two small injections contained worms that released eggs in water.

In all of the controls the worms were found to be firmly anchored. A few worms were also found loose in the tank.

Histological Examination of the Testes

Experimental fish 39, 41 and 42 were chosen for detailed microscopic examination. Both 39 and 41 were ripe fish with gonads in about the same condition. The tubules were jammed with spermatozoa and many tubules showed the walls breaking and sperm being released. The interstitium was thick and interstitial cells plentiful, while stages of

spermatogenesis were not common. The testis from fish 42 did not exrete milt; on microscopic examination it appeared quite similar to testes of 39 and 41, except that there was a lack of tubule wall breakdown and more tubules showing spermatogenesis.

The control testes resembled the testis of fish 42, in that they appeared ripe, but no breakage in the tubule walls could be detected and cysts of spermatocytes were abundant.

Histological Examination of the Tapeworms

Tapeworms were examined from experimental fish 39, 40 and 41. Worms from fish 39 were mature with collapsed uteri, indicating egg release and in general appeared similar to figure 7. The worms from fish 40 were not nearly as well developed as those from 39. This may be due to the worms from fish 40 being loose in the ileum and therefore remnants of ripe worms that had previously passed into the tank. The genitalia of the worm fragments were in the process of acquiring lumina and therefore somewhat between the stages depicted in figures 5 and 6. Fish 41 had worms developed similar to that of figure 6, that is all of the sex organs developed and the uteri filled with eggs.

Those worms found in the tank were in a spent condition, with the uteri collapsed in the majority of proglottides much like that in figure 7.

Cestodes from fish 46, 48 and 50 served as controls. In all of the controls the genitalia of the tapeworms showed

a significant difference from those of the experimentals; they were not as far advanced. The cirrus sac and uteri were beginning to acquire lumina and the ovaries, just forming, were in a developmental stage between figures 5 and 6.

This difference observed in the tapeworms in experiment six is sufficient to prove that the factors responsible for gonadal development in the pike also control the development of the parasite Triaenophorus crassus.

SUMMARY AND CONCLUSIONS OF PART II

1. This study was conducted primarily for the purpose of obtaining Triaenophorus eggs over various seasons of the year. It was thought that pituitary injections into the host, Esox lucius, would result in maturation of their tapeworms and thus enable eggs to be procured when desired. If this were the case, chemical experiments on the control of the eggs and coracidia could be conducted over greater periods of time each year.

2. Although viable cestode eggs were not obtained, a large amount of interesting data were recorded regarding the effects of pituitary on the pike, Esox lucius, and its parasite Triaenophorus crassus.

3. A high rate of mortality among the pike interfered with the early experiments. This may possibly be attributed to the method of capture; those fish caught in nets set overnight had a much lower survival rate than those taken in short sets.

4. Pike are unable to survive in Edmonton's city water for periods longer than 24 hours. As a result water for the tank was taken from the lake and river.

5. Extracts of pituitary are more suitable for injection than are suspensions.

6. Small doses of pituitary extract at two or three day intervals are more effective than large single doses.

7. Loss of colour is the first noticeable change caused by pituitary injections.

8. The injections resulted in the male sex organs of the pike maturing to the extent that viable spermatozoa were obtained as early as four days following injection.

9. No advancement could be detected in injected female pike.

10. Histologically a ripe testis differs from an immature one, by the abundance of interstitial tissue and cells, tubules full of spermatozoa and a reduction in all stages of spermatogenesis.

11. The injections into the pike resulted in their tapeworms developing to a more mature condition than those found in control fish. Some worms were advanced to the stage where they released their undeveloped eggs upon contact with water.

12. The nearer the dates of injection to the natural spawning period, the easier it is to advance both the sex organs of the fish and those of their parasite T. crassus.

13. The higher temperature of the tank water as compared to that in the lake probably advances spermatogenesis to a certain extent.

14. There is a definite relationship between maturation of the pike gonads and the parasite T. crassus. The controlling factors are the hormones secreted by the pituitary gland of the host Esox lucius.

15. Viable eggs and coracidia were not obtained, although this condition was nearly attained when certain experimental worms released eggs which contained well formed onchospheres. A possible explanation as to why ripe eggs were not shed is that the dosages of pituitaries were too large and the time intervals between injections too brief. This resulted in the worms receiving large amounts of hormonal stimulus which either simulated or forced them to release themselves from the intestinal wall before their eggs had time to mature.

16. More desirable results would probably be obtained if the pike could be kept alive over longer periods of time and the number of whole pituitaries reduced and injected at lengthy intervals.

ACKNOWLEDGMENTS

I wish to express my gratitude to both the Fisheries Research Board of Canada and the Fisheries Branch of the Government of the Province of Alberta, whose interest and financial support has made this study possible.

I am deeply indebted to Dr. R.B. Miller of the Department of Zoology for his constant interest, advice and patient reading of this paper.

My thanks are also extended to Dr. M.J. Huston of the Department of Pharmacy for his guidance and provision of materials, apparatus and laboratory space required to conduct the chemical experiments; to Professor J.A. Harle of the Department of Electrical Engineering for designing the cell used in the electrical experiments; to Messrs. H.B. Watkins, W.H. MacDonald, L. Silver, C. Scott and M. Heron of the Fisheries Branch, for their help in obtaining fish; to my wife for her aid in typing the manuscript, and to Mr. R. Lister for his willing help and advice in the preparation of the slides used in the microscopic studies.

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APPENDIX

TABLES V11 - XX1V

TABLE V11

COUNTS FROM CONTROL AND EXPERIMENTAL CULTURES USED TO TEST LF DISINFECTANT #17H-14

DATE	HATCHED EGGS		UNHATCHED EGGS		DEAD CORACIDIA		LIVE CORACIDIA	
	NO.	%	NO.	%	NO.	%	NO.	%
CONTROL 1A								
3-5-49	190	44	241	56	30	45	36	55
4-5-49	135	22	475	78	15	18	68	82
5-5-49	130	25	387	75	17	35	32	65
6-5-49	83	28	216	72	37	61	24	39
7-5-49	93	25	273	75	62	74	22	26
9-5-49	130	26	375	74	24	41	34	59
10-5-49	63	25	186	75	26	58	19	42
CONTROL 1B								
3-5-49	172	20	709	80	32	27	89	73
4-5-49	102	26	296	74	33	46	38	54
5-5-49	87	27	234	73	24	38	39	62
6-5-49	75	22	265	78	28	64	16	36
7-5-49	75	28	198	72	41	84	8	16
9-5-49	82	31	182	69	30	73	11	27
10-5-49	144	28	367	72	37	68	17	32
CULTURE #1 - 100 P.P.M. LF DISINFECTANT #17H-14								
3-5-49	182	24	588	76	40	95	2	5
4-5-49	134	26	385	74	33	61	21	39
5-5-49	162	21	597	79	17	51	16	49
6-5-49	75	23	252	77	20	87	3	13
7-5-49	68	25	207	75	22	92	2	8
9-5-49	115	23	384	77	62	89	8	11
10-5-49	88	24	286	76	61	98	1	2
CULTURE #1 - 10 P.P.M. LF DISINFECTANT #17H-14								
3-5-49	247	23	820	77	98	42	137	58
4-5-49	150	28	379	72	33	42	46	58
5-5-49	141	28	367	72	50	60	33	40
6-5-49	84	29	207	71	34	79	9	21
7-5-49	90	25	273	75	51	88	7	12
9-5-49	134	31	294	69	85	83	17	17
10-5-49	126	26	356	74	68	92	6	8
CULTURE #1 - 2 P.P.M. LF DISINFECTANT #17H-14								
3-5-49	152	23	501	77	19	36	33	64
4-5-49	110	32	229	68	78	40	119	60
5-5-49	103	22	373	78	55	66	28	34
6-5-49	76	25	229	75	34	42	47	58
7-5-49	103	27	272	73	46	90	5	10
9-5-49	150	28	388	72	55	83	11	17
10-5-49	155	32	327	67	74	95	4	5

TABLE V111

COUNTS FROM CONTROL AND EXPERIMENTAL CULTURES USED TO TEST SODIUM ETHYLENE BIS-DITHIOCARBONATE

DATE	HATCHED EGGS		UNHATCHED EGGS		DEAD CORACIDIA		LIVE CORACIDIA	
	NO.	%	NO.	%	NO.	%	NO.	%
			CONTROL #2A					
5-5-49	69	15	382	85	28	67	14	33
6-5-49	25	21	94	79	11	46	13	54
7-5-49	80	38	132	62	13	27	35	73
9-5-49	92	45	111	55	34	45	42	55
10-5-49	251	57	187	43	86	53	75	47
11-5-49	254	61	165	39	95	53	83	47
12-5-49	180	60	120	40	83	77	25	23
13-5-49	226	68	108	32	132	88	18	12
			CONTROL #2B					
5-5-49	61	22	214	78	32	68	15	32
6-5-49	18	19	75	81	22	63	13	37
7-5-49	55	36	98	64	9	29	22	71
9-5-49	77	42	105	58	29	48	31	52
10-5-49	198	54	170	46	56	56	44	44
11-5-49	83	59	59	41	53	64	30	36
12-5-49	71	59	49	41	61	86	10	14
13-5-49	199	68	92	32	153	92	14	8
			CULTURE #2 100 P.P.M. SODIUM ETHYLENE BIS-DITHIOCARBONATE					
5-5-49	40	13	272	87	28	100	0	0
6-5-49	23	10	217	90	34	90	4	10
7-5-49	22	11	175	89	26	90	3	10
9-5-49	21	11	171	89	24	100	0	0
10-5-49	54	13	379	87	26	81	6	19
11-5-49	38	14	239	86	53	100	0	0
12-5-49	17	8	211	92	54	100	0	0
13-5-49	18	10	161	90	44	100	0	0
			CULTURE #2 10 P.P.M. SODIUM ETHYLENE BIS-DITHIOCARBONATE					
5-5-49	147	27	392	73	94	95	5	5
6-5-49	36	20	143	80	24	67	12	33
7-5-49	13	8	160	92	25	89	3	11
9-5-49	39	12	296	88	47	94	3	6
10-5-49	52	16	277	84	59	94	4	6
11-5-49	47	15	267	85	38	95	2	5
12-5-49	31	17	147	83	20	100	0	0
13-5-49	76	15	435	85	71	100	0	0
			CULTURE #2 2 P.P.M. SODIUM ETHYLENE BIS-DITHIOCARBONATE					
5-5-49	76	20	312	80	29	74	10	26
6-5-49	32	28	82	72	15	58	11	42
7-5-49	33	27	90	73	9	41	13	59
9-5-49	71	37	123	63	33	44	42	56
10-5-49	120	46	139	54	72	67	36	33
11-5-49	107	56	83	44	80	69	36	31
12-5-49	91	63	54	37	58	73	22	27
13-5-49	99	66	50	34	46	79	12	21

TABLE IX

COUNTS FROM CONTROL AND EXPERIMENTAL CULTURES USED TO TEST TRITON X

DATE	HATCHED EGGS		UNHATCHED EGGS		DEAD CORACIDIA		LIVE CORACIDIA	
	NO.	%	NO.	%	NO.	%	NO.	%
CONTROL 3A								
7-5-50	13	5	235	95	1	25	3	75
9-5-50	25	8	298	92	9	69	4	31
10-5-50	16	5	281	95	27	87	4	13
11-5-50	15	6	243	94	14	87	2	13
12-5-50	18	6	296	94	24	92	2	8
13-5-50	16	5	299	95	74	97	2	3
14-5-50	20	10	181	90	86	98	2	2
16-5-50	30	9	311	91	169	99.4	1	0.6
CONTROL 3B								
7-5-50	18	5	344	95	6	67	3	33
9-5-50	15	5	288	95	6	40	9	60
10-5-50	42	7	573	93	36	84	7	16
11-5-50	29	7	370	93	24	89	3	11
12-5-50	27	6	406	94	29	83	6	17
13-5-50	14	5	273	95	76	96	3	4
14-5-50	10	5	201	95	81	98	2	2
16-5-50	17	6	261	94	139	99.3	1	0.7
CULTURE #3 - 100 P.P.M. CCL4-TRITON X								
7-5-50	17	6	283	94	7	70	3	30
9-5-50	15	4	326	96	4	67	2	33
10-5-50	29	7	397	93	23	92	2	8
11-5-50	24	6	398	94	29	100	0	0
12-5-50	35	8	433	92	51	100	0	0
13-5-50	22	10	204	90	50	100	0	0
14-5-50	7	8	82	92	18	100	0	0
16-5-50	9	7	130	93	59	100	0	0
CULTURE #3 - 10 P.P.M. CCL4-TRITON X								
7-5-50	1	2	40	98	1	50	1	50
9-5-50	2	4	43	96	5	83	1	17
10-5-50	3	7	42	93	9	100	0	0
11-5-50	6	7	85	93	18	90	2	10
12-5-50	9	10	84	90	21	95	1	5
13-5-50	1	6	15	94	13	100	0	0
14-5-50	2	14	12	86	31	100	0	0
16-5-50	3	9	30	91	64	100	0	0
CULTURE #3 - 2 P.P.M. CCL4-TRITON X								
7-5-50	12	3	377	97	3	75	1	25
9-5-50	9	8	100	92	7	58	5	42
10-5-50	41	6	610	94	23	72	9	28
11-5-50	17	6	280	94	29	93	2	7
12-5-50	11	3	341	97	23	88	3	12
13-5-50	13	6	217	94	45	100	0	0
14-5-50	24	9	254	91	51	100	0	0
16-5-50	22	7	290	93	143	99	2	1

TABLE X

COUNTS FROM CONTROL AND EXPERIMENTAL CULTURES USED TO TEST Y-77

DATE	HATCHED EGGS		UNHATCHED EGGS		DEAD CORACIDIA		LIVE CORACIDIA	
	NO.	%	NO.	%	NO.	%	NO.	%
CONTROL 4A								
10-5-49	183	39	286	61	81	48	88	52
11-5-49	224	44	286	56	106	53	94	47
12-5-49	117	36	205	64	73	68	35	32
13-5-49	70	42	98	58	41	67	20	33
14-5-49	88	40	132	60	86	90	10	10
16-5-49	162	58	117	42	108	86	17	14
17-5-49	140	54	118	46	161	86	26	14
CONTROL 4B								
10-5-49	212	45	260	55	80	67	40	33
11-5-49	203	46	236	54	77	60	51	40
12-5-49	251	45	309	55	109	60	72	40
13-5-49	108	42	147	58	63	78	18	22
14-5-49	86	40	129	60	67	84	13	16
16-5-49	94	53	83	47	76	89	9	11
17-5-49	308	56	246	44	252	94	15	6
CULTURE #4 - 20 P.P.M. Y-77								
10-5-49	200	45	242	55	220	100	0	0
11-5-49	198	45	246	55	258	100	0	0
12-5-49	192	49	198	51	198	100	0	0
13-5-49	156	50	155	50	174	100	0	0
14-5-49	125	47	141	53	201	100	0	0
16-5-49	284	52	263	48	344	100	0	0
17-5-49	208	54	178	46	410	100	0	0
CULTURE #4 - 10 P.P.M. Y-77								
10-5-49	180	47	203	53	134	82	29	18
11-5-49	231	51	221	49	157	94	11	6
12-5-49	142	43	191	57	120	95	7	5
13-5-49	150	49	153	51	142	94	9	6
14-5-49	113	52	103	48	80	94	5	6
16-5-49	124	55	100	45	121	97	4	3
17-5-49	135	49	139	51	173	97	5	3
CULTURE #4 - 2 P.P.M. Y-77								
10-5-49	187	49	193	51	86	76	27	24
11-5-49	156	49	162	51	55	60	36	40
12-5-49	238	45	295	55	128	96	5	4
13-5-49	117	40	173	60	78	82	17	18
14-5-49	103	46	123	54	64	79	17	21
16-5-49	151	56	118	44	99	88	13	12
17-5-49	263	63	153	37	165	92	14	8

TABLE XI

COUNTS FROM CONTROL AND EXPERIMENTAL CULTURES USED TO TEST LYSOL

DATE	HATCHED EGGS		UNHATCHED EGGS		DEAD CORACIDIA		LIVE CORACIDIA	
	NO.	%	NO.	%	NO.	%	NO.	%
			CONTROL #5A					
11-5-49	313	29	756	71	100	49	103	51
12-5-49	216	29	538	71	88	40	134	60
13-5-49	146	29	352	71	94	50	94	50
14-5-49	101	35	184	65	52	48	56	52
16-5-49	213	40	324	60	129	70	56	30
17-5-49	388	40	583	60	236	70	103	30
19-5-49	320	52	300	48	155	85	28	15
			CONTROL #5B					
11-5-49	146	29	355	71	61	45	75	55
12-5-49	301	29	745	71	166	50	167	50
13-5-49	245	32	528	68	99	50	100	50
14-5-49	187	39	290	61	97	65	51	35
16-5-49	267	41	384	59	192	71	77	29
17-5-49	512	45	618	55	344	76	106	24
19-5-49	464	50	472	50	303	78	85	22
			CULTURE #5 - 10 P.P.M. LYSOL					
11-5-49	97	27	267	73	91	100	0	0
12-5-49	96	19	409	81	136	98	3	2
13-5-49	241	24	748	76	354	99	3	1
14-5-49	122	20	474	80	210	99	1	1
16-5-49	249	25	744	75	268	100	0	0
17-5-49	205	27	545	73	318	100	0	0
19-5-49	182	30	417	70	243	100	0	0
			CULTURE #5 - 5 P.P.M. LYSOL					
11-5-49	247	27	668	73	242	84	45	16
12-5-49	253	29	610	71	274	93	21	7
13-5-49	154	31	349	69	193	98	5	2
14-5-49	121	25	362	75	137	99.3	1	.7
16-5-49	224	36	390	64	302	99.7	1	.3
17-5-49	173	37	300	63	258	100	0	0
19-5-49	331	38	537	62	326	100	0	0
			CULTURE #5 - 2 P.P.M. LYSOL					
11-5-49	163	30	380	70	67	51	63	49
12-5-49	191	30	456	70	84	53	74	47
13-5-49	167	30	383	70	109	63	64	37
14-5-49	167	33	332	67	99	72	39	28
16-5-49	182	35	339	65	146	91	15	9
17-5-49	346	40	511	60	316	97	8	3
19-5-49	312	47	357	53	262	97	7	3

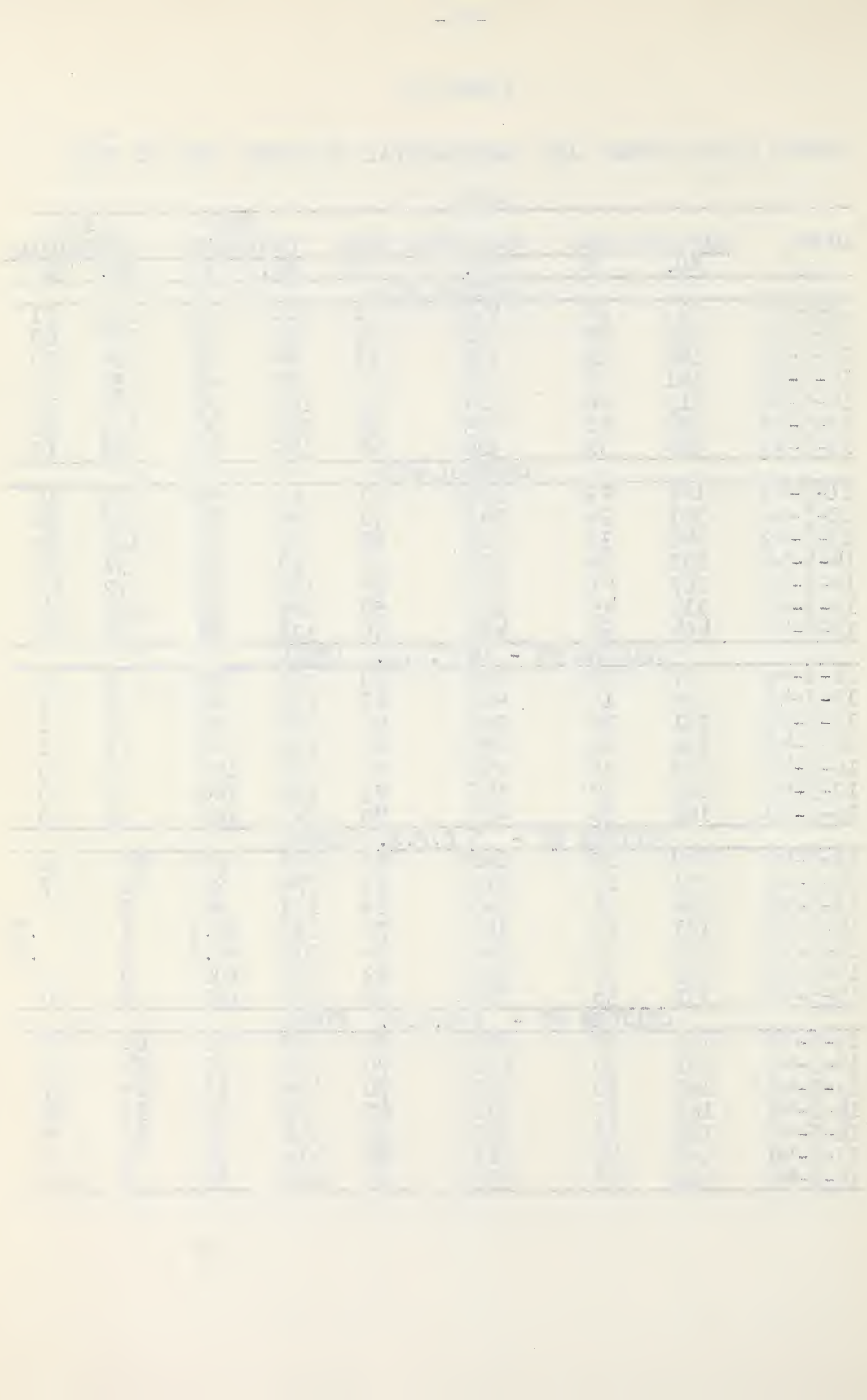

TABLE XII

COUNTS FROM CONTROL AND EXPERIMENTAL CULTURES USED TO TEST COPPER SULFATE

DATE	HATCHED EGGS		UNHATCHED EGGS		DEAD CORACIDIA		LIVE CORACIDIA	
	NO.	%	NO.	%	NO.	%	NO.	%
			CONTROL #6A					
16-5-49	92	15	523	85	45	62	28	38
17-5-49	129	20	529	80	81	76	26	24
19-5-49	175	24	555	76	155	88	22	12
20-5-49	117	23	397	77	150	97	5	3
21-5-49	215	31	477	69	203	94	13	6
25-5-49	476	52	445	48	387	90	44	10
26-5-49	615	54	516	46	431	89	55	11
			CONTROL #6B					
16-5-49	72	16	383	84	39	64	22	36
17-5-49	162	18	734	82	154	78	44	22
19-5-49	168	24	525	76	152	96	6	4
20-5-49	144	26	410	74	114	98	2	2
21-5-49	180	31	392	69	162	91	15	9
25-5-49	183	47	204	53	153	88	21	12
26-5-49	286	56	228	44	226	90	26	10
	CULTURE #6 - 100 P.P.M. COPPER SULFATE							
16-5-49	74	12	531	88	84	100	0	0
17-5-49	89	14	554	86	302	99.3	2	0.7
19-5-49	91	15	495	85	189	99.5	1	0.5
20-5-49	92	12	654	88	364	100	0	0
21-5-49	122	11	960	89	505	100	0	0
25-5-49	61	15	341	85	176	100	0	0
26-5-49	119	14	733	86	325	100	0	0
	CULTURE #6 - 10 P.P.M. COPPER SULFATE							
16-5-49	84	14	509	86	38	64	21	36
17-5-49	90	16	479	84	28	55	23	45
19-5-49	163	23	553	77	114	69	51	31
20-5-49	214	28	558	72	293	84	57	16
21-5-49	242	29	601	71	291	87	42	13
25-5-49	278	42	382	58	288	94	19	6
26-5-49	485	45	591	55	561	98	10	2
	CULTURE #6 - 2 P.P.M. COPPER SULFATE							
16-5-49	104	18	484	82	64	68	30	32
17-5-49	133	16	719	84	189	79	49	21
19-5-49	52	25	159	75	138	91	13	9
20-5-49	197	27	541	73	217	92	19	8
21-5-49	221	30	526	70	248	98	5	2
25-5-49	315	40	465	60	475	98	11	2
26-5-49	344	42	474	58	398	98	8	2

TABLE XIII

COUNTS FROM CONTROL AND EXPERIMENTAL CULTURES USED TO TEST TOXAPHENE

DATE	HATCHED EGGS		UNHATCHED EGGS		DEAD CORACIDIA		LIVE CORACIDIA	
	NO.	%	NO.	%	NO.	%	NO.	%
CONTROL #7A								
20-5-49	58	11	474	89	71	91	7	9
21-5-49	58	11	452	89	64	85	11	15
25-5-49	128	39	198	61	119	73	44	27
26-5-49	210	39	324	61	195	81	45	19
27-5-49	175	46	201	54	268	85	46	15
28-5-49	239	58	174	42	262	83	53	17
29-5-49	272	68	129	32	406	86	65	14
CONTROL #7B								
20-5-49	36	10	318	90	30	91	3	9
21-5-49	35	12	248	88	31	86	5	14
25-5-49	94	43	124	57	67	78	19	22
26-5-49	130	49	137	51	78	74	27	26
27-5-49	238	54	201	46	224	81	53	19
28-5-49	191	61	120	39	191	84	35	16
29-5-49	257	67	127	33	236	89	30	11
CULTURE #7 - 8 P.P.M.- TOXAPHENE 25% WETTABLE								
20-5-49	35	7	478	93	69	90	8	10
21-5-49	55	12	417	88	64	89	8	11
25-5-49	142	36	255	64	168	91	16	9
26-5-49	163	38	264	62	199	93	16	7
27-5-49	186	43	249	57	254	88	34	12
28-5-49	334	53	292	47	323	93	25	7
29-5-49	190	58	138	42	276	91	28	9
CULTURE #7 - 5 P.P.M.- TOXAPHENE 25% WETTABLE								
20-5-49	28	8	321	92	68	93	5	7
21-5-49	31	12	238	88	36	95	2	5
25-5-49	113	29	272	71	93	71	39	29
26-5-49	114	36	202	64	109	76	34	24
27-5-49	120	41	170	59	145	82	32	18
28-5-49	87	47	99	53	157	84	29	16
29-5-49	184	49	191	51	133	76	43	24
CULTURE #7 - 2 P.P.M. TOXAPHENE 25% WETTABLE								
20-5-49	59	9	602	91	77	94	5	6
21-5-49	43	10	402	90	58	92	5	8
25-5-49	96	33	196	67	123	80	31	20
26-5-49	155	35	285	65	135	88	19	12
27-5-49	223	57	169	43	219	82	49	18
28-5-49	211	44	271	56	427	92	36	8
29-5-49	247	55	206	45	249	79	67	21

TABLE XlV

COUNTS FROM CONTROL AND EXPERIMENTAL CULTURES USED TO TEST MULCIDE B

DATE	HATCHED EGGS		UNHATCHED EGGS		DEAD CORACIDIA		LIVE CORACIDIA	
	NO.	%	NO.	%	NO.	%	NO.	%
			CONTROL #8A					
20-5-49	18	10	157	90	45	94	3	6
25-5-49	139	46	160	54	241	93	17	7
26-5-49	167	47	188	53	307	94	20	6
27-5-49	132	47	147	53	336	93	24	7
28-5-49	238	56	187	44	617	94	40	6
29-5-49	180	60	119	40	427	92	37	8
30-5-49	110	70	48	30	127	90	14	10
			CONTROL #8B					
20-5-49	29	13	194	87	63	95	3	5
25-5-49	127	37	217	63	230	91	23	9
26-5-49	150	45	183	55	278	89	34	11
27-5-49	84	45	104	55	341	95	16	5
28-5-49	153	62	95	38	393	93	30	7
29-5-49	195	70	85	30	335	94	23	6
30-5-49	120	73	44	27	334	95	17	5
	CULTURE #8 - 100 P.P.M. MULCIDE B							
20-5-49	33	9	355	91	61	100	0	0
25-5-49	110	30	254	70	167	100	0	0
26-5-49	193	37	322	63	265	99.3	2	0.7
27-5-49	166	47	185	53	296	99	3	1
28-5-49	161	45	198	55	242	98	2	2
29-5-49	146	54	125	46	229	99	3	1
30-5-49	154	59	109	41	227	100	0	0
	CULTURE #8 - 10 P.P.M. MULCIDE B							
20-5-49	36	12	269	88	93	98	2	2
25-5-49	89	30	211	70	138	98	3	2
26-5-49	122	41	179	59	177	93	13	7
27-5-49	200	49	205	51	256	97	9	3
28-5-49	77	42	106	58	150	94	9	6
29-5-49	98	56	78	44	190	93	14	7
30-5-49	129	63	75	37	216	92	20	8
	CULTURE #8 - 2 P.P.M. MULCIDE B							
20-5-49	24	11	203	89	55	92	5	8
25-5-49	149	35	274	65	147	85	26	15
26-5-49	102	36	184	64	223	89	27	11
27-5-49	148	41	211	59	276	93	22	7
28-5-49	56	50	57	50	167	92	15	8
29-5-49	134	59	95	41	423	96	18	4
30-5-49	130	64	72	36	284	94	19	6

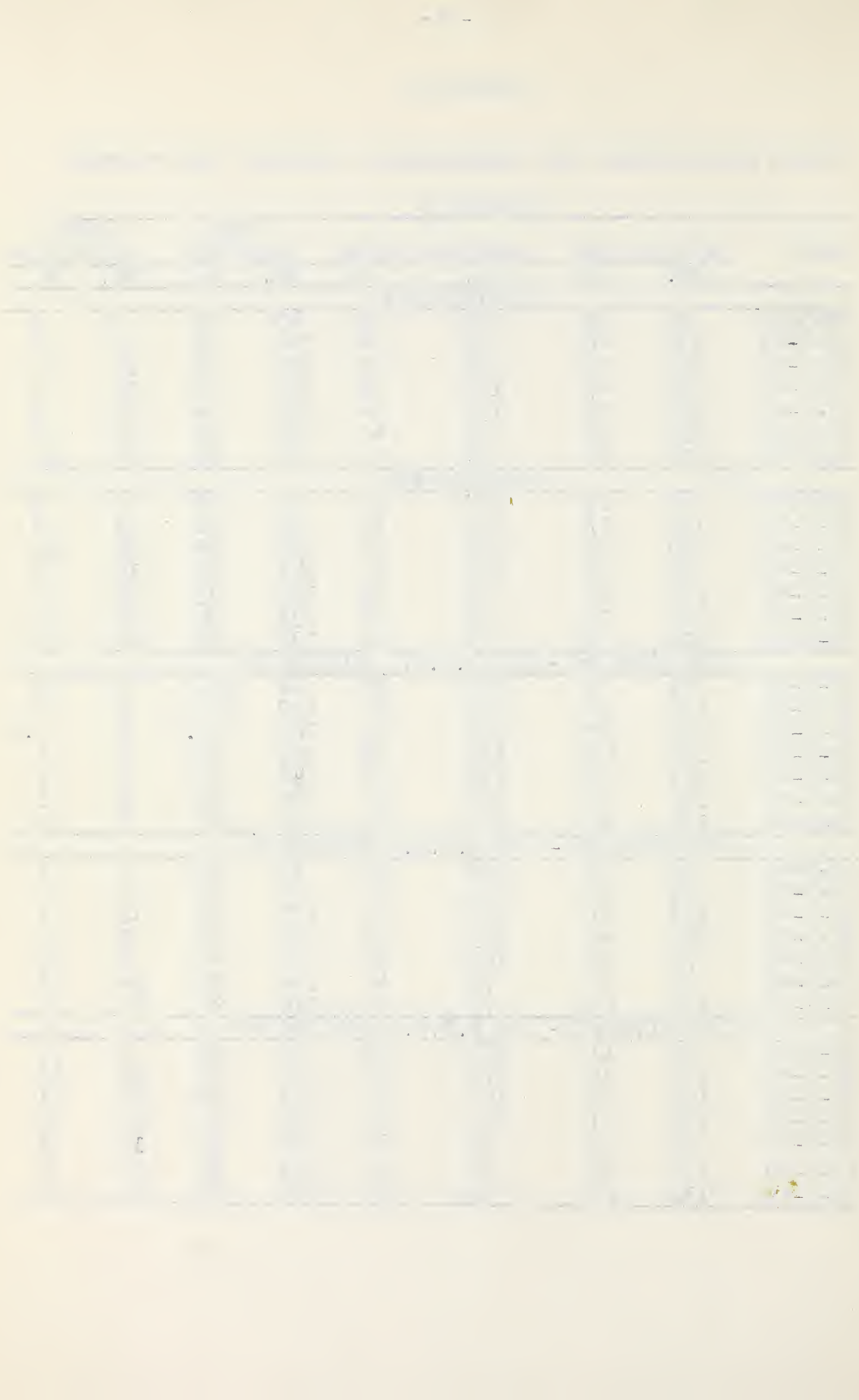

TABLE XV

COUNTS FROM CONTROL AND EXPERIMENTAL CULTURES USED TO TEST SODIUM CAPRYLATE

DATE	HATCHED EGGS		UNHATCHED EGGS		DEAD CORACIDIA		LIVE CORACIDIA	
	NO.	%	NO.	%	NO.	%	NO.	%
			CONTROL #9A					
26-5-49	73	15	415	85	398	99	6	1
27-5-49	37	20	148	80	207	99	2	1
28-5-49	75	21	283	79	315	98	5	2
29-5-49	33	20	131	80	114	96	5	4
30-5-49	93	30	217	70	292	97	10	3
31-5-49	71	26	202	74	244	95	12	5
1-6-49	79	31	179	69	234	96	9	4
			CONTROL #9B					
26-5-49	53	12	379	88	281	98	7	2
27-5-49	52	16	271	84	259	98	4	2
28-5-49	49	18	229	82	236	99.6	1	0.4
29-5-49	61	25	181	75	227	97	6	3
30-5-49	58	21	216	79	199	96	9	4
31-5-49	58	25	175	75	131	96	6	4
1-6-49	72	33	145	67	124	96	5	4
	CULTURE #9 - 100 P.P.M. SODIUM CAPRYLATE							
26-5-49	50	15	285	85	268	100	0	0
27-5-49	62	14	379	86	739	99.9	1	0.1
28-5-49	46	17	232	83	469	100	0	0
29-5-49	37	18	174	82	362	100	0	0
30-5-49	37	22	133	78	202	100	0	0
31-5-49	75	24	237	76	298	99	4	1
1-6-49	96	26	270	74	322	98	6	2
	CULTURE #9 - 10 P.P.M. SODIUM CAPRYLATE							
26-5-49	56	14	352	86	342	99	5	1
27-5-49	57	15	327	85	375	98	6	2
28-5-49	49	14	302	86	356	99	2	1
29-5-49	32	18	150	82	196	98	4	2
30-5-49	38	17	191	83	252	97	7	3
31-5-49	39	32	83	68	102	97	3	3
1-6-49	111	35	209	65	291	95	16	5
	CULTURE #9 - 2 P.P.M. SODIUM CAPRYLATE							
26-5-49	70	17	330	83	274	99	4	1
27-5-49	54	15	302	85	270	99.6	1	0.4
28-5-49	69	19	287	81	260	98	6	2
29-5-49	44	23	149	77	130	99	2	1
30-5-49	85	27	226	73	213	95	12	5
31-5-49	52	27	144	73	143	99	2	1
1-6-49	55	28	145	72	107	95	6	5

TABLE XV1

COUNTS FROM CONTROL AND EXPERIMENTAL CULTURES USED TO TEST DOWKLOR

DATE	HATCHED EGGS		UNHATCHED EGGS		DEAD CORACIDIA		LIVE CORACIDIA	
	NO.	%	NO.	%	NO.	%	NO.	%
CONTROL #10A								
1-6-49	40	10	342	90	88	97	3	3
2-6-49	28	10	241	90	78	91	8	9
3-6-49	29	19	125	81	46	92	4	8
7-6-49	54	21	208	79	85	83	18	17
8-6-49	86	20	345	80	123	84	24	16
9-6-49	182	29	439	71	153	82	33	18
10-6-49	96	35	176	65	119	87	18	13
CONTROL #10B								
1-6-49	51	6	735	94	123	99	1	1
2-6-49	30	7	430	93	91	95	5	5
3-6-49	59	13	402	87	146	94	10	6
7-6-49	157	35	291	65	124	81	30	19
8-6-49	161	39	250	61	118	81	28	19
9-6-49	166	46	191	54	106	84	20	16
10-6-49	203	54	175	46	121	88	16	12
CULTURE #10 - 100 P.P.M. DOWKLOR								
1-6-49	35	7	445	93	141	100	0	0
2-6-49	28	7	348	93	148	99.3	1	0.7
3-6-49	72	13	484	87	282	100	0	0
7-6-49	86	24	273	76	155	100	0	0
8-6-49	67	26	191	74	169	100	0	0
9-6-49	112	30	267	70	200	100	0	0
10-6-49	88	29	213	71	205	99.5	1	0.5
CULTURE #10 - 10 P.P.M. DOWKLOR								
1-6-49	26	6	381	94	92	96	4	4
2-6-49	33	8	358	92	111	96	5	4
3-6-49	100	12	727	88	259	96	12	4
7-6-49	98	29	235	71	61	75	20	25
8-6-49	133	35	248	65	123	85	21	15
9-6-49	145	41	207	59	71	82	16	18
10-6-49	195	47	224	53	100	83	21	17
CULTURE #10 - 2 P.P.M. DOWKLOR								
1-6-49	35	9	355	91	111	98	2	2
2-6-49	46	10	404	90	126	98	2	2
3-6-49	48	10	412	90	113	96	5	4
7-6-49	98	29	245	71	118	97	3	3
8-6-49	145	39	231	61	159	99.4	1	0.6
9-6-49	194	40	289	60	235	97	6	3
10-6-49	191	44	242	56	251	98	5	2

TABLE XV11

COUNTS FROM CONTROL AND EXPERIMENTAL CULTURES USED TO TEST ANTIMONY TARTRATE

DATE	HATCHED EGGS		UNHATCHED EGGS		DEAD CORACIDIA		LIVE CORACIDIA	
	NO.	%	NO.	%	NO.	%	NO.	%
			CONTROL 11A					
12-5-50	3	4.16	69	95.8	0	0	3	100
13-5-50	13	13.7	82	86.3	1	33.3	2	66.7
15-5-50	11	16.7	55	83.3	1	20	4	80
16-5-50	11	16.4	56	83.6	0	0	2	100
17-5-50	14	14.1	85	85.9	1	20	4	80
18-5-50	35	28	90	72.0	5	33.3	10	66.7
19-5-50	52	36.4	91	63.6	9	64.3	5	35.7
22-5-50	77	53	67	47	9	47.4	10	52.6
			CONTROL 11B					
12-5-50	7	7.86	82	92.1	1	25	3	75
13-5-50	5	8.8	52	91.2	0	0	1	100
15-5-50	12	7.5	148	92.5	0	0	1	100
16-5-50	21	12.7	144	87.3	0	0	2	100
17-5-50	4	8.9	41	91.1	1	25	3	75
18-5-50	18	18.0	82	82.0	3	37.5	5	62.5
19-5-50	44	24.7	134	75.3	4	50	4	50
22-5-50	45	39.8	68	60.2	12	52.2	11	47.8
			CULTURE #11 - 100 P.P.M. ANTIMONY TARTRATE					
12-5-50	11	6.96	147	93	5	83.3	1	16.6
13-5-50	9	13.2	59	86.8	0	0	4	100
15-5-50	21	11.2	166	88.8	13	77	4	23.5
16-5-50	4	5.7	66	94.3	4	66.7	2	33.3
17-5-50	24	16.8	119	83.2	9	100	0	0
18-5-50	25	14.2	151	85.8	8	61.5	5	38.5
19-5-50	31	17.1	150	82.9	12	92.3	1	7.7
22-5-50	33	41.8	46	58.2	8	100	0	0
			CULTURE #11 - 10 P.P.M. ANTIMONY TARTRATE					
12-5-50	4	9.75	37	90.2	3	75	1	25
13-5-50	15	11.3	118	88.7	4	57.1	3	42.9
15-5-50	16	16.1	83	83.9	1	25	3	75
16-5-50	5	7.3	63	92.7	1	50	1	50
17-5-50	9	7.3	113	92.7	2	25	6	75
18-5-50	15	15.8	80	84.2	1	25	3	75
19-5-50	21	21.2	78	78.8	4	44.5	5	55.5
22-5-50	23	31.1	51	68.9	7	77.8	2	22.2
			CULTURE #11 - 2 P.P.M. ANTIMONY TARTRATE					
12-5-50	4	5.5	69	95	1	50	1	50
13-5-50	3	4.1	70	95.9	3	75	1	25
15-5-50	6	6.5	87	94	3	50	3	50
16-5-50	9	11.3	72	88.7	2	100	0	0
17-5-50	15	12.9	102	87.1	0	0	2	100
18-5-50	9	12.7	62	87.3	0	0	3	100
19-5-50	18	15.0	102	85.0	0	0	8	100
22-5-50	18	34.0	35	66.0	1	14.3	6	85.7

TABLE XVlll

COUNTS FROM CONTROL AND EXPERIMENTAL CULTURES USED TO TEST CHLOROMYCETIN

DATE	HATCHED EGGS		UNHATCHED EGGS		DEAD CORACIDIA		LIVE CORACIDIA	
	NO.	%	NO.	%	NO.	%	NO.	%
			CONTROL 13A					
29-5-50	40	30.8	90	69.2	11	50	11	50
30-5-50	27	36.9	46	63.1	3	25	9	75
31-5-50	13	38.2	21	61.8	2	40	3	60
1-6-50	55	50.4	54	49.6	13	56.5	10	43.5
2-6-50	35	62.5	21	37.5	5	45.4	6	54.6
3-6-50	60	70.6	25	29.4	10	71.4	4	28.6
5-6-50	93	60.8	60	39.2	14	70	6	30
			CONTROL 13 B					
29-5-50	33	18.4	146	81.6	2	16.7	10	83.3
30-5-50	43	23.9	137	76.1	14	44	11	56
31-5-50	46	48.9	48	51.1	14	40	21	60
1-6-50	55	51.9	51	48.1	13	61.9	8	38.1
2-6-50	84	65.6	44	34.4	20	54	17	46
3-6-50	26	39.4	40	60.6	15	65.2	8	34.8
5-6-50	107	59.8	72	40.2	26	86.7	4	23.3
	CULTURE #13 - 10 P.P.M. CHLOROMYCETIN							
29-5-50	18	17.5	85	82.5	15	75	5	25
30-5-50	84	37.8	138	62.2	18	48.6	19	51.4
31-5-50	90	41.5	127	58.5	12	40	18	60
1-6-50	74	43.5	96	56.5	10	40	15	60
2-6-50	99	49.1	103	50.9	15	68.2	7	31.8
3-6-50	71	44.6	88	55.4	14	66.7	7	33.3
5-6-50	85	58.6	60	41.4	10	62.5	6	37.5
	CULTURE #13 - 5 P.P.M. CHLOROMYCETIN							
29-5-50	51	25.6	148	74.4	16	48.5	17	51.5
30-5-50	38	42.2	52	57.8	6	42.8	8	57.2
31-5-50	44	50	44	50	7	36.9	12	63.1
1-6-50	77	48.1	83	51.9	16	40	24	60
2-6-50	33	45.8	39	54.2	10	45.5	12	54.5
3-6-50	94	57.7	69	42.3	7	41.2	10	58.8
5-6-50	196	65.3	104	34.7	24	72.7	9	27.3
	CULTURE #13 - 1 P.P.M. CHLOROMYCETIN							
29-5-50	75	28.6	187	71.4	9	37.5	15	62.5
30-5-50	34	52.3	31	47.7	7	41.2	10	58.8
31-5-50	32	38.5	51	61.5	6	37.5	10	62.5
1-6-50	42	43.7	54	56.3	7	38.9	11	61.1
2-6-50	49	50.5	48	49.5	21	65.6	11	34.4
3-6-50	59	52.2	54	47.8	13	56.5	10	43.5
5-6-50	23	47.9	25	52.1	8	80	2	20

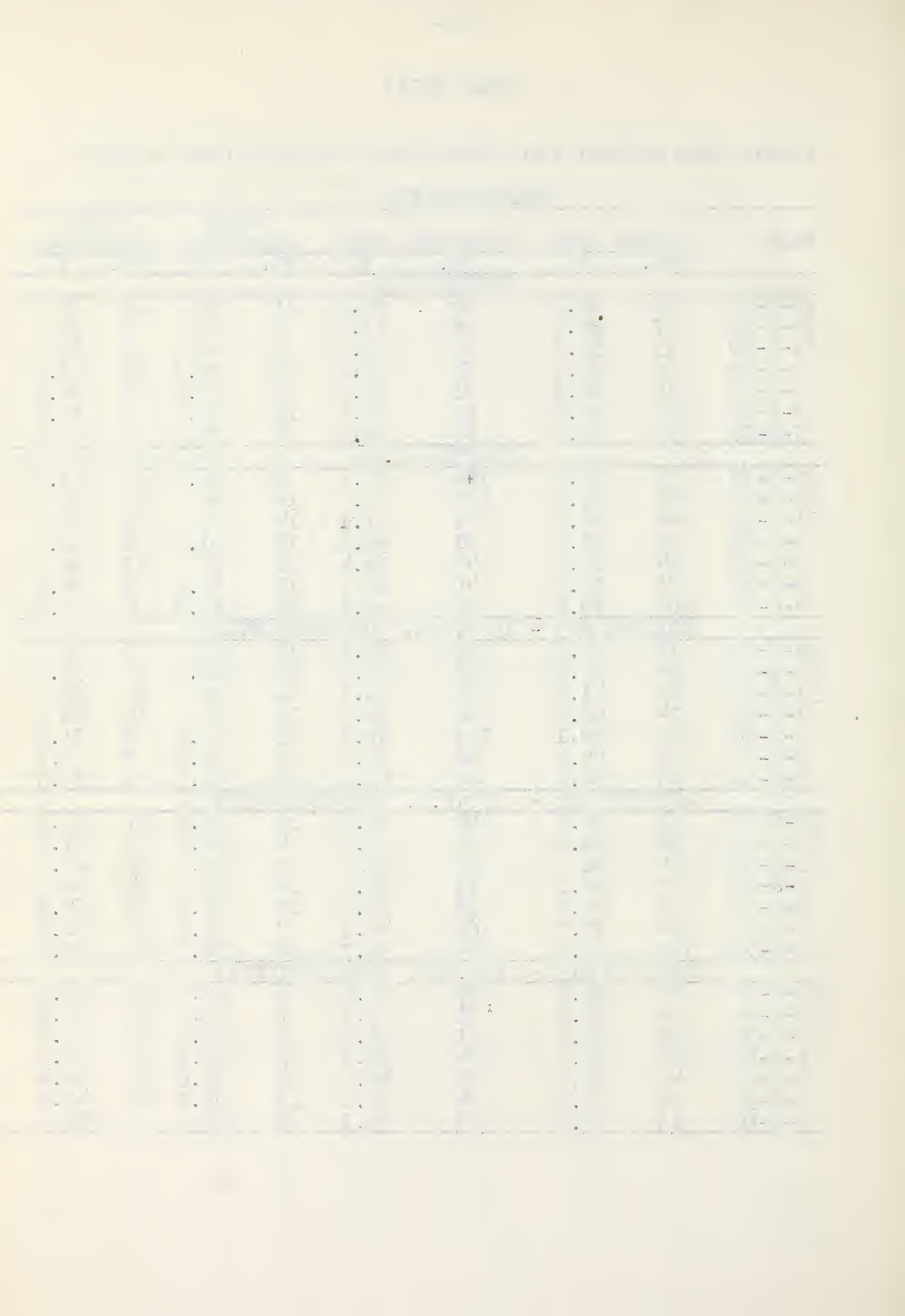

TABLE XIX

COUNTS FROM CONTROL AND EXPERIMENTAL CULTURES USED TO TEST DOW K-604

DATE	HATCHED EGGS		UNHATCHED EGGS		DEAD CORACIDIA		LIVE CORACIDIA	
	NO.	%	NO.	%	NO.	%	NO.	%
			CONTROL 12A					
20-5-50	27	9.9	246	90.1	5	83.3	1	16.7
22-5-50	37	15.1	208	84.9	3	16.7	15	83.3
23-5-50	83	33.2	167	66.8	6	20.7	23	79.3
25-5-50	35	41.7	49	58.3	4	16.7	20	83.3
27-5-50	120	44.4	150	55.6	5	22.7	17	77.3
29-5-50	44	62.8	26	37.2	1	14.3	6	85.7
30-5-50	89	66.9	44	33.1	3	18.8	13	81.2
			CONTROL 12B					
20-5-50	15	10.5	128	89.5	4	36.4	7	63.6
22-5-50	30	26.8	82	73.2	1	9.1	10	90.9
23-5-50	70	31.4	153	68.6	10	31.2	22	68.8
25-5-50	102	49.5	104	50.5	11	30.6	25	69.4
27-5-50	162	60.0	108	40.0	8	36.4	14	63.6
29-5-50	33	55	27	45	1	16.7	5	83.3
30-5-50	44	56.4	34	43.6	1	50	1	50
	CULTURE #12 - 20 P.P.M. DOW K-604							
20-5-50	18	8.7	188	91.3	10	100	0	0
22-5-50	14	11.5	108	88.5	11	100	0	0
23-5-50	21	9.7	196	90.3	8	100	0	0
25-5-50	20	11.9	157	88.7	13	100	0	0
27-5-50	18	13.1	119	86.9	6	100	0	0
29-5-50	12	12.8	82	87.2	5	100	0	0
30-5-50	15	15.8	80	84.2	8	100	0	0
	CULTURE #12 - 10 P.P.M. DOW K-604							
20-5-50	11	4.7	224	95.3	5	100	0	0
22-5-50	21	10.6	178	89.4	12	100	0	0
23-5-50	15	15.8	80	84.2	4	100	0	0
25-5-50	20	19.8	81	80.2	19	100	0	0
27-5-50	18	15.9	76	84.1	5	100	0	0
29-5-50	13	13.8	81	86.2	9	100	0	0
30-5-50	30	15.2	168	84.8	8	100	0	0
	CULTURE #12 - 2.5 P.P.M. DOW K-604							
20-5-50	13	3.7	336	96.3	7	77.8	2	22.2
22-5-50	23	13.3	150	86.7	13	92.9	1	7.1
23-5-50	40	11.2	317	88.8	28	100	0	0
25-5-50	19	12.0	140	88.0	3	100	0	0
27-5-50	37	10.0	332	90.0	8	100	0	0
29-5-50	12	10.5	102	89.5	6	100	0	0
30-5-50	10	23.8	32	76.2	12	100	0	0

TABLE XX

COUNTS FROM CONTROL AND EXPERIMENTAL CULTURES USED TO TEST DOW K-604

DATE	HATCHED EGGS		UNHATCHED EGGS		DEAD CORACIDIA		LIVE CORACIDIA	
	NO.	%	NO.	%	NO.	%	NO.	%
CONTROL 14A								
29-5-50	23	35.9	41	64.1	14	73.6	5	26.4
30-5-50	30	43.4	39	56.5	10	58.8	7	41.2
31-5-50	43	48.3	46	51.7	22	75.9	7	24.1
1-6-50	99	49.1	103	50.9	15	50	15	50
2-6-50	96	64	54	36	17	58.6	12	41.4
3-6-50	64	60.4	42	39.6	8	57.1	6	42.9
5-6-50	57	71.2	23	28.8	8	66.7	4	33.3
6-6-50	29	61.7	18	38.3	2	50	2	50
CONTROL 14B								
29-5-50	10	27.1	27	72.9	13	92.8	1	7.2
30-5-50	34	53.1	30	46.9	12	70.6	5	29.4
31-5-50	51	50	51	50	16	66.7	8	33.3
1-6-50	40	56.3	31	43.7	3	30	7	70
2-6-50	39	54.9	32	45.1	6	50	6	50
3-6-50	180	65.2	96	34.8	10	50	10	50
5-6-50	128	73.1	47	26.9	13	65	7	35
6-6-50	238	73.7	85	26.3	8		4	
CULTURE #14 - 2 P.P.M. DOW K-604								
29-5-50	24	22.2	84	77.8	30	100	0	0
30-5-50	18	37.5	30	62.5	18	100	0	0
31-5-50	20	29.4	48	70.6	8	100	0	0
1-6-50	29	33.3	58	66.7	7	100	0	0
2-6-50	18	33.3	36	66.7	7	100	0	0
3-6-50	20	45.5	24	54.5	3	100	0	0
5-6-50	64	33	130	67	9	100	0	0
6-6-50	9	39.1	14	60.9	3	100	0	0
CULTURE #14 - 1 P.P.M. DOW K-604								
29-5-50	22	29.3	53	70.7	11	100	0	0
30-5-50	8	23.5	26	76.5	13	100	0	0
31-5-50	26	43.3	34	56.7	9	100	0	0
1-6-50	41	38.7	65	61.3	12	100	0	0
2-6-50	11	32.3	23	67.7	4	100	0	0
3-6-50	34	48.6	36	51.4	4	100	0	0
5-6-50	30	49.2	31	50.8	5	100	0	0
6-6-50	29	41.4	41	58.6	3	100	0	0
CULTURE #14 -0.5 P.P.M. DOW K-604								
29-5-50	21	36.9	36	63.1	8	100	0	0
30-5-50	53	51.9	49	48.1	20	96.9	3-very	3.1
31-5-50	43	43	57	57	14	100	0 weak	0
1-6-50	26	36.1	46	63.9	7	100	0	0
2-6-50	53	46.5	61	53.5	7	100	0	0
3-6-50	19	28.8	47	71.2	6	100	0	0
5-6-50	90	42.5	122	57.5	5	100	0	0
6-6-50	36	43.4	47	56.5	5	100	0	0

Examination of cultures on the 29-5-50 was carried out 10 minutes after mixing.

TABLE XX1

COUNTS FROM CONTROL AND EXPERIMENTAL CULTURES USED TO TEST DOW K-604

DATE	HATCHED EGGS		UNHATCHED EGGS		DEAD CORACIDIA		LIVE CORACIDIA	
	NO.	%	NO.	%	NO.	%	NO.	%
			CONTROL 16A					
28-6-50	154	68.1	72	31.9	3	30	7	70
29-6-50	400	67.8	190	32.2	13	56.5	10	43.5
30-6-50	132	76.7	40	23.3	8	61.5	5	38.5
3-7-50	------- NO LIVE CORACIDIA ----------							
			CONTROL 16B					
28-6-50	123	65.8	64	33.2	2	25	6	75
29-6-50	118	77.6	34	22.4	3	20	12	80
30-6-50	84	78.5	23	21.5	1	16.7	5	83.3
3-7-50	------- NO LIVE CORACIDIA ----------							
	CULTURE #16 0.35 P.P.M. DOW K-604							
28-6-50	109	66.5	55	33.5	4	100	0	0
29-6-50	130	67.3	63	32.7	4	100	0	0
30-6-50	87	64.9	47	35.1	2	100	0	0
3-7-50	--------NO LIVE CORACIDIA ----------							
	CULTURE #16 0.25 P.P.M. DOW K-604							
28-6-50	100	71.9	39	28.1	1	25	3	75
29-6-50	159	68.2	74	21.8	6	75	2	25
30-6-50	54	72	21	28	3	100	0	0
3-7-50	------- NO LIVE CORACIDIA ----------							
	CULTURE #16 0.05 P.P.M. DOW K-604							
28-6-50	53	68.8	24	31.2	0	0	5	100
29-6-50	88	78.6	24	21.4	3	37.5	5	62.5
30-6-50	134	68.4	62	31.6	7	58.3	5	41.7
3-7-50	------- NO LIVE CORACIDIA ----------							

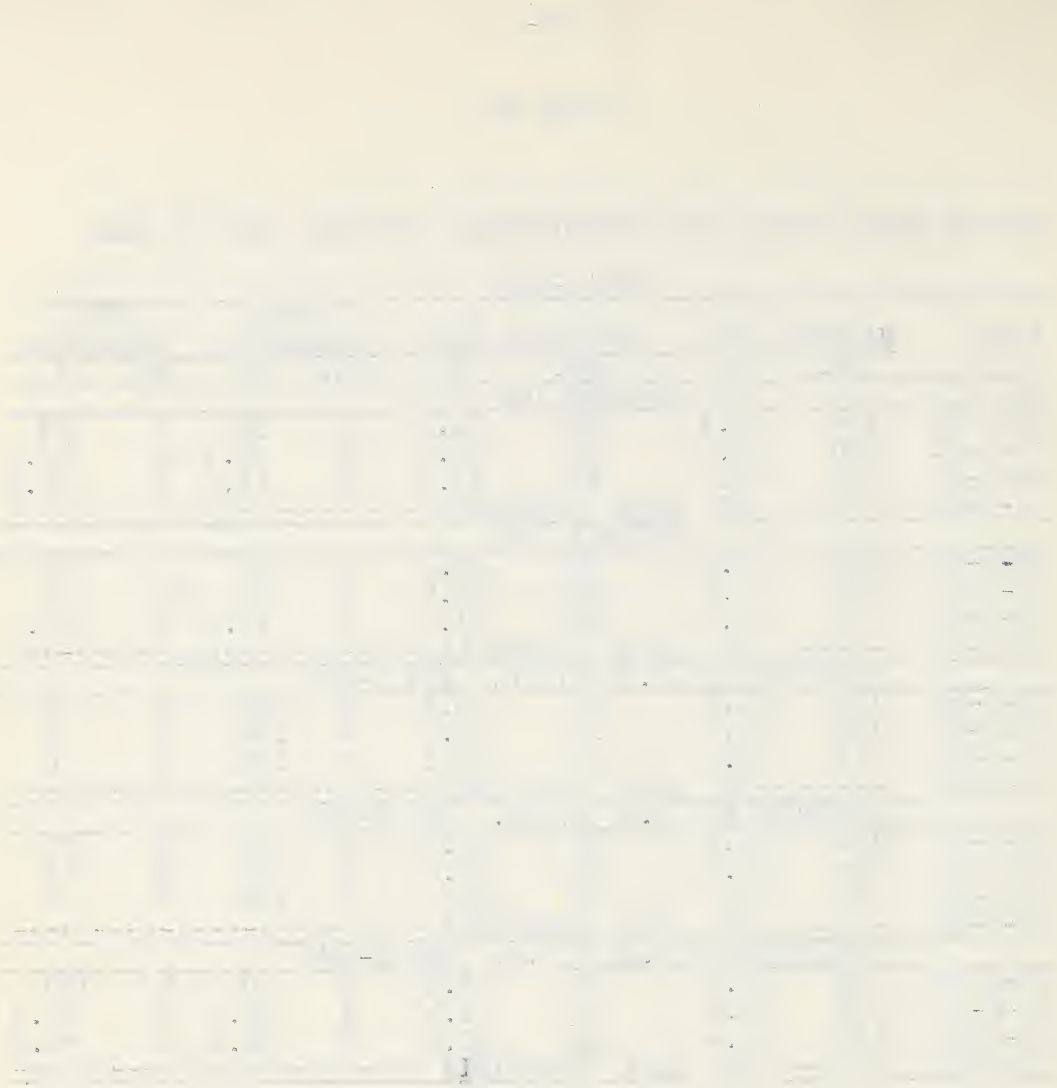

TABLE XX11

COUNTS FROM CONTROL AND EXPERIMENTAL CULTURES USED TO TEST 2% TYROTHRICIN

DATE	HATCHED EGGS		UNHATCHED EGGS		DEAD CORACIDIA		LIVE CORACIDIA	
	NO.	%	NO.	%	NO.	%	NO.	%
CONTROL 15A								
30-5-50	7	28	18	72	3	75	1	25
31-5-50	34	35.4	62	64.6	6	54.5	5	45.5
1-6-50	17	41.5	24	58.5	6	66.7	3	33.3
2-6-50	17	36.2	30	63.8	2	40	3	60
3-6-50	15	24.2	47	75.8	5	55.5	4	44.5
5-6-50	15	50	15	50	7	77.8	2	22.2
6-6-50	16	41	23	59	5	83.3	1	16.7
CONTROL 15B								
30-5-50	24	32.4	50	67.6	10	83.3	2	16.7
31-5-50	26	35.1	48	64.9	9	56.2	7	43.8
1-6-50	27	40.3	40	59.7	5	55.5	4	44.5
2-6-50	15	25.9	43	74.1	2	40	3	60
3-6-50	35	36.8	60	63.2	8	66.7	4	33.3
5-6-50	24	64.9	13	35.1	0	0	3	100
6-6-50	22	40.7	32	59.3	6	75	2	25
CULTURE #15(2% TYROTHRICIN)-200 P.P.M.								
30-5-50	10	34.5	19	65.5	6	75	2	25
31-5-50	21	45.6	25	54.4	9	81.8	2	18.2
1-6-50	18	26.1	51	73.9	3	75	1	25
2-6-50	29	42.6	39	57.4	5	62.5	3	37.5
3-6-50	26	32.9	53	67.1	4	66.7	2	33.3
5-6-50	28	52.8	25	47.2	4	50	4	50
6-6-50	22	45.8	26	54.2	6	75	2	25
CULTURE #15-100 P.P.M. (2% TYROTHRICIN)								
30-5-50	12	34.3	23	65.7	12	100	0	0
31-5-50	17	27.9	44	72.1	6	100	0	0
1-6-50	6	33.3	12	66.7	3	75	1	25
2-6-50	21	34.4	40	65.6	6	66.7	3	33.3
3-6-50	12	25	36	75	3	60	2	40
5-6-50	45	50	45	50	8	80	2	20
6-6-50	4	26.7	11	73.3	5	71.4	2	28.6
CULTURE #15- 20 P.P.M. (2% TYROTHRICIN)								
30-5-50	11	29.7	26	70.3	6	66.7	3	33.3
31-5-50	10	37.1	17	62.9	4	66.7	2	33.3
1-6-50	10	35.7	18	64.3	9	81.8	2	18.2
2-6-50	24	38.1	39	61.9	4	66.7	2	33.3
3-6-50	19	27	51	73	5	83.3	1	16.7
5-6-50	49	47.6	54	52.4	14	70	6	30
6-6-50	28	52.8	23	47.2	2	25	6	75

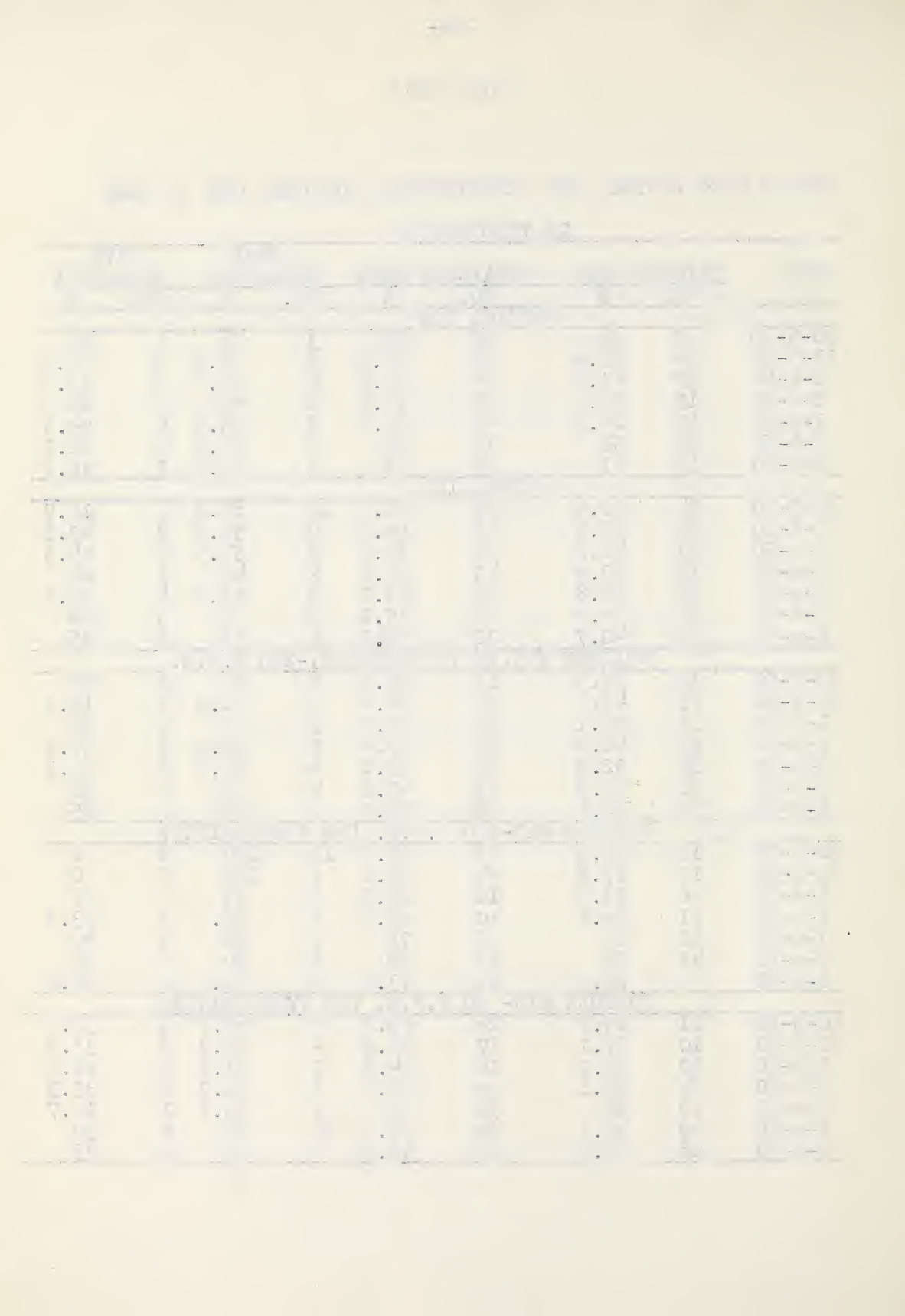

TABLE XXIII

COUNTS FROM CONTROL AND EXPERIMENTAL CULTURES USED TO TEST TOXAPHENE

DATE	HATCHED EGGS		UNHATCHED EGGS		DEAD CORACIDIA		LIVE CORACIDIA	
	NO.	%	NO.	%	NO.	%	NO.	%
CONTROL 17A								
29-6-50	51	35.9	91	64.1	2	16.7	10	83.3
30-6-50	201	63.6	115	36.4	15	33.3	30	66.7
4-7-50	112	88.2	15	11.8	4	36.4	7	63.6
5-7-50	88	83	18	17	2	40	3	60
CONTROL 17B								
29-6-50	26	59.1	18	40.9	6	37.5	10	62.5
30-6-50	68	67.3	33	32.7	2	10	18	90
4-7-50	103	84.4	19	15.6	2	22.2	7	77.8
5-7-50	153	81.8	34	18.2	3	37.5	5	62.5
6-7-50	96	88.9	12	11.1	6	75	2	25
CULTURE #17 100 P.P.M. TOXAPHENE								
29-6-50	49	54.4	41	45.6	21	75	7	25
30-6-50	57	68.7	26	31.3	13	92.8	1	7.2
4-7-50	35	83.3	7	16.7	2	100	0	0
5-7-50	118	80.8	28	19.2	6	100	0	0
6-7-50	98	89.1	12	10.9	8	100	0	0
CULTURE #17 10 P.P.M. TOXAPHENE								
29-6-50	42	52.5	38	47.5	8	72.8	3	27.2
30-6-50	96	66.7	48	33.3	19	82.6	4	17.4
4-7-50	87	92.5	7	7.5	5	83.3	1	16.7
5-7-50	107	88.4	14	11.6	5	100	0	0
6-7-50	110	85.9	18	14.1	5	100	0	0
CULTURE #17 2 P.P.M. TOXAPHENE								
29-6-50	90	48.9	94	51.1	7	50	7	50
30-6-50	90	68.7	41	31.3	11	26.8	30	73.2
4-7-50	117	87.3	17	12.7	7	58.3	5	41.7
5-7-50	66	94.3	4	5.7	6	100	0	0
6-7-50	133	88.6	17	11.4	8	100	0	0

TABLE

COUNTS FROM CONTROL AND EXPERIMENTAL CULTURES

DATE	EXPERIMENT NO.	TIME IN SEC.	VOLTS	DEAD COR.	% DEAD	LIVE COR.	% LIVE	HATCHED EGGS	% HATCHED	UNHATCHED EGGS
30-5-50	1	5	2	3	100	0	0	11	16	59
30-5-50	2	5	5	14	100	0	0	24	10	210
30-5-50	3	5	10	3	100	0	0	18	12	137
30-5-50	4	5	15	4	100	0	0	27	18	120
30-5-50	5	5	20	19	95	1	5	48	22	167
30-5-50	6	5	25	4	100	0	0	15	8	172
30-5-50	7	5	26	5	100	0	0	49	26	137
30-5-50	C.#1	-	--	5	71	2	29	20	29	70
30-5-50	C.#2	-	--	8	73	3	27	40	11	321
30-5-50	C.#3	-	--	5	71	2	29	10	16	51
30-5-50	8	5	55	27	63	16	37	115	19	497
1-6-50	9	15	45	9	82	2	18	27	17	134
1-6-50	10	15	50	4	80	1	20	15	19	65
1-6-50	11	15	52	10	91	1	9	41	19	179
1-6-50	12	15	54	8	100	0	0	43	18	201
1-6-50	13	15	55	13	100	0	0	33	20	129
1-6-50	C.#4	--	--	7	78	2	22	14	19	58
1-6-50	C.#5	--	--	9	90	1	10	42	18	197
5-6-50	14	20	2	21	87	3	13	48	42	65
5-6-50	15	20	5	12	92	1	8	37	47	41
5-6-50	16	20	10	6	75	2	25	28	34	54
5-6-50	17	20	15	10	91	1	9	47	59	32
5-6-50	18	20	20	12	80	3	20	41	41	59
5-6-50	19	20	25	24	83	5	17	81	37	135
5-6-50	20	20	30	19	79	5	21	48	44	61
5-6-50	21	20	35	21	78	6	22	58	36	102
5-6-50	22	20	40	12	86	2	14	36	41	52
5-6-50	23	20	45	9	69	4	31	23	38	38
5-6-50	24	20	50	14	64	8	36	88	45	104
5-6-50	25	20	55	35	69	16	31	104	44	133
5-6-50	C.#6	--	--	12	52	11	48	37	34	71
5-6-50	C.#7	--	--	20	83	4	17	39	36	69
9-6-50	26	15	110	6	86	1	14	38	72	15
9-6-50	27	20	110	9	64	5	36	75	65	40
9-6-50	28	30	110	6	75	2	25	62	66	32
9-6-50	29	30	120	1	33	2	67	10	71	4
9-6-50	C.#8	--	--	6	75	2	25	50	64	28
9-6-50	C.#9	--	--	3	60	2	40	40	63	23

KEY

Cor. - Coracidia.
C. - Control.

XXIV

USED TO TEST THE EFFECT OF ELECTRICITY

% UN-HATCHED	DATE REREAD	DEAD COR.	% DEAD	LIVE COR.	% LIVE	HATCHED EGGS	% HATCHED	UNHATCHED EGGS	% UNHATCHED
84	2-6-50	3	60	2	40	15	17	73	83
90	2-6-50	4	50	4	50	13	12	91	88
88	2-6-50	3	43	4	57	15	18	68	82
82	2-6-50	6	46	7	54	22	18	98	82
78	2-6-50	11	55	9	45	68	26	199	74
92	2-6-50	5	62	3	38	26	22	91	78
74	2-6-50	11	79	3	21	61	50	60	50
71	2-6-50	9	60	6	40	46	29	115	71
89	2-6-50	6	40	9	60	28	25	85	75
84	2-6-50	10	77	3	23	70	60	46	40
81	2-6-50	33	92	3	8	140	33	281	67
83	3-6-50	8	61	5	39	44	29	107	71
81	3-6-50	11	92	1	8	21	28	55	72
81	3-6-50	4	57	3	43	16	48	33	52
82	3-6-50	3	75	1	25	15	30	35	70
80	3-6-50	12	86	2	14	24	23	79	77
81	3-6-50	14	70	6	30	50	25	147	75
82	3-6-50	9	90	1	10	42	18	197	82
48	8-6-50	13	72	5	28	42	61	27	39
53	8-6-50	23	66	12	34	91	59	63	41
66	8-6-50	5	83	1	17	34	62	21	38
41	8-6-50	10	71	4	29	44	57	33	43
59	8-6-50	26	87	4	13	69	55	56	45
63	8-6-50	8	73	3	27	45	65	24	35
56	8-6-50	11	52	10	48	33	58	24	42
64	8-6-50	7	58	5	42	60	74	21	26
59	8-6-50	11	48	12	52	116	66	59	34
62	8-6-50	4	40	6	60	46	57	35	43
55	8-6-50	20	71	8	29	83	61	52	39
56	8-6-50	5	36	9	64	36	56	28	44
66	8-6-50	13	48	14	52	60	61	38	39
64	8-6-50	6	60	4	40	33	62	20	38
28									
35									
34									
29									
36									
37									