GROWTH INHIBITION AND MORTALITY TO LARVAE OF THE BOLLWORM, *Heliothis zea* (Boddie), ON REARING MEDIA TREATED WITH CADMIUM SALTS AND TWO CHLOROPHENOXYTHIAZINE COMPOUNDS

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PREFACE

Chronic cadmium poisoning has been known to produce anemia with a resulting retardation of growth. It has been demonstrated that cadmium interferes with erythrocyte production, and iron and zinc distribution within the body of animals. Subcutaneous injection of cadmium has caused depression of health, loss of appetite, and retardation of growth. Other evidence has indicated that cadmium has a binding effect upon protein. Much work has been performed on mammalian species but little has been done on insects. The need for further investigation of cadmium compounds in relation to insects prompted this study.

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INTRODUCTION

Larvae of the bollworm, Heliothis zea (Boddie), are a serious pest of cotton and other agricultural crops in the southern and western United States. Many attempts have been made at chemical control of this destructive insect. At a time when new spray chemicals are being produced frequently, our attention is turned to a chemical which has been discovered for quite some time but whose insecticidal qualities have been little investigated.

Experiments reported in this thesis were designed to test the growth inhibiting properties of low concentrations of the cadmium salts. The researcher wanted to know how to stop or retard growth of bollworm larvae. This could open a whole new field of scientific investigation if chemicals could be found that, applied in low concentrations, would be relatively harmless to man and animals, yet would inhibit insect growth and reproduction.

REVIEW OF LITERATURE

CADMIUM POISONING.--One of the earliest cadmium poisonings was recorded by Van Hasselt in 1855 when he cited the case of a man who produced nausea and vomiting in himself with one-half grain of cadmium sulfate (Schwartze and Alsberg, 1923). Wheeler in 1876 reported two cases of cadmium bromide poisoning in man from comparatively large doses (250 to 1000 mg.).

Powers (1917) was one of the first to conduct toxicity experiments on animals. He found that concentration of cadmium chloride was not directly correlated with toxicity.

Schwartze and Alsberg (1923) recorded the results of poisoning from continued cadmium feeding experiments. They found that concentrations of 250 or more p.p.m. of cadmium in the diet caused death to animals. The above value was also found to be the mean emetic concentration and recovery followed reduction of that concentration. Although slight loss of appetite, nibbling of food, and occasional vomiting occurred below the mean, no evidence of cumulative systemic action was obtained.

Garber (1946) described the instance in which over 50 of 200 Army enlisted personnel developed nausea, vomiting, retching and abdominal cramps a short time after imbibing a lemonade drink containing cadmium. Some of the men were hospitalized but recovery was prompt.

Hardy and Skinner (1947) assessed the possibility of chronic cadmium poisoning in industrial workers. Experimentally, they obtained

atmospheric cadmium concentrations and cadmium urinalysis data. The clinical records of five men engaged in cadmium-plating work revealed complaints of nausea, vomiting, listlessness, coughing, gastrointestinal symptoms and fatigue.

Princi (1947) presented evidence of chronic cadmium poisoning in 20 industrial workers. The most characteristic finding was a yellow ring on the teeth of those men who had had long cadmium exposure. It was considered the earliest sign of cadmium absorption.

Friberg (1948) reported on finding proteinuria in 18 workmen who had been exposed to cadmium and nickel dust for more than eight years. No proteinuria was found among 19 patients who had been similarly exposed during a period of one to two years. In 14 cases, impaired kidney function was observed. Pathologic changes were found in the blood of eight workers with proteinuria. According to Friberg, these changes implied the possibility of low molecular protein which is not found in healthy persons.

Baader (1952) reported on the symptomatology of chronic cadmium poisoning. Almost all cases were reported to have complained of tickling and soreness of the nose and a deterioration of olfactory function.

Fairhall (1957) remarked that the significant feature of cadmium poisoning by mouth is the rapidity of physiological response. Usually individuals are affected within 15 to 30 minutes after ingestion of food or drink containing toxic quantities of cadmium. DuBois and Geiling (1959) stated that symptoms often occur within 10 minutes following ingestion of cadmium.

METABOLIC CADMIUM.--The earliest and most complete work on the subject of cadmium was conducted by Marme in 1867 (Schwartze and

Alsberg, 1923). He stated that cadmium retards circulation in man and in animals (Prodan, 1932a).

Athanasiu and Langlois, in 1896, reported on experiments with cadmium salts on frogs and on the isolated heart of the turtle (Wilson et al. 1941). These observers noticed that heart action became slower and the force of the heart was diminished after the injection of cadmium sulfate. They also observed that there was a marked prolongation of the pause and a certain periodicity of the rhythm. The effect was the same on the isolated heart of the turtle. They also reported that cadmium differs somewhat in its action on the heart under changed conditions of temperature.

Experiments by Salant and Connet (1920) with the frog showed that cadmium is very toxic to the heart. Very dilute solutions perfused for one or two minutes often produced injurious effects. In one experiment, one p.p.m. cadmium acetate in Ringer's solution perfused for two minutes caused a slight diminution in frequency and a well-marked decrease in heart action. The same concentration caused complete arrest of the heart in about one and one-half minutes in another experiment in which the perfusion time was only one minute. The injurious effect of cadmium acetate was very marked with a concentration of 10 p.p.m. The heart was either completely arrested or its action became very feeble and remained in this condition for 3 or 4 minutes and, sometimes, even longer. In both cases, however, recovery was ultimately observed. But this was not the case with higher concentrations of cadmium acetate. The effect in these experiments was very prompt: the heart action decreased very rapidly and remained at a standstill either permanently or for a long time.

Schwartze and Alsberg (1923) concluded that intravenous injection of cadmium may result in protein precipitation intravascularly. They found that cadmium produced protein precipitation at the site of injection and poor absorption. They also discovered no evidence of storage of cadmium other than in the kidney, liver and spleen. The kidney usually contained the most metal relatively, while the spleen contained the least and sometimes none.

Prodan (1932b) stated that the kidneys, liver and bones of vertebrates retain the major amount of cadmium. He gave the figure of 31% retention in one animal's tissues one month after exposure.

Wilson et al. (1941) cited experiments in which severe anemia occurred in rats eating diets containing 0.0062% of cadmium. Within 2 or 3 months the hemoglobin concentration decreased to 3 or 4 grams per 100 cc. of blood. The more severely poisoned rats had hearts that weighed nearly twice as much as normal, presumably due to the severe anemia. Adrenals, kidneys, and spleen were also heavier than normal.

Granick and Michaelis (1942) reported that cadmium precipitates iron-containing proteins which may cause the low production of hemoglobin. Gilman et al. (1946) reported on the inability of monothiols to reverse heavy metal linkage with sensitive proteins.

Friberg (1952) studied the excretion of cadmium using radioactive tracers. Cadmium sulfate was injected into rabbits in a dose sufficient to cause the appearance of cadmium-protein in the urine after 6 to 8 weeks. Cadmium was excreted in the urine in relatively small amounts during the first 6 to 8 weeks. Also, cadmium was demonstrated to be present in all organs examined, but accumulated most in the liver, kidneys, pancreas and spleen.

Lallier (1955) performed tests on sea-urchin egg development and found that cadmium acetate caused the production of animalized blastulae. He proposed that this action could be due to combination of metal ions with egg proteins.

Turpaev (1956), in experiments carried out on isolated frog ventricles, found that contraction of the cardiac muscle was completely inhibited by an accumulation of 30 to 40 ug of cadmium per gram of tissue.

Margoshes and Vallee (1957) fractionated a low molecular weight naturally-occurring cadmium protein from the horse kidney cortex. Kagi (1960) purified the cadmium protein further to contain 50,000 ug. of cadmium and 11,000 ug. of zinc per gram dry weight, corresponding to 8 grams of metal per mole (molecular weight 13,000). He stated that cadmium occurs in parenchymatous organs of many species of animals.

Maruyama (1957) found that cadmium and other metals increased the enzyme action of sea anemone apyrase.

Ulrychova-Zelinkova (1959) stated that cadmium ions probably cause a disturbance of phosphorus metabolism and interact with zinc ions in nucleoprotein metabolism. Gunn et al. (1962) indicated that cadmium interferes with fecal excretion of Zn^{65}, resulting in an increased retention of radioactive zinc in liver, kidney and pancreas. Supplee (1961) fed turkey poults, from day old on, diets containing added cadmium and these exhibited typical symptoms of zinc deficiency at 2 to 3 weeks of age. All these results seem to indicate that cadmium has a binding effect upon zinc.

Supplee (1963) suggested an antagonistic relationship between dietary cadmium and zinc. He found that growth of chicks decreased, and

specific abnormalities of hocks and feathers increased, when cadmium was added to a zinc-deficient diet. Supplementation of the diet with zinc prevented the adverse effect on hock and feather development and partially offset the effect on growth.

Gunn et al. (1964) proposed that a single injection of cadmium chloride (0.03 m-M/kgm.) to male Wister rats has been shown to be cancerogenic both locally, by the production of pleomorphic sarcomas at the site of injection, and systemically by the production of interstitial cell tumors of the testis.

Hennig and Anke (1964) stated that daily supplement feeding of growing pigs with 160 ug. of cadmium results in an alteration of the iron and zinc distribution within the body. Their findings positively indicated that iron metabolism is more influenced than that of zinc.

Lapanje et al. (1964) found that cadmium ions are bound to pepsin ionically as well as covalently. They also determined the relative amounts of cadmium in either state of binding.

Parizek (1964) hypothesized that cadmium cations cause selective circulatory damage in the testes and in other estrogen-producing organs.

STERILITY.--Kar et al. (1961) suggested that a low dose of cadmium chloride (0.125 mg./100 g. body weight) causes partial degeneration of the rat testes. When the damage is partial, the surviving gametogenic elements proliferate and rebuild the cytoarchitecture of the spermatogenic tubules. Indications are that the accessory genital organs, too, are adversely affected for a short time.

Gunn et al. (1961a) reported that subcutaneous administration of cadmium salt, 0.03 m-M/kgm., to rats produced marked injury to the testis. Testicular injury was characterized by irreversible damage to

the seminiferous tubules resulting in complete loss of fertility. Interstitial tissue was also damaged but later underwent hyperplasia and regained its functional capacity. No pathologic changes were detected in the female reproductive tract or any other major organ.

Gunn et al. (1961b) stated that since testicular injury can be prevented, at least temporarily, by the simultaneous administration of large doses of zinc, it appears that cadmium exerts its toxic effects by virtue of competition for essential sites normally occupied by zinc. The results of their paper show that the capacity of the testis and dorsolateral prostate to take up administered Zn^{65} is depressed by the presence of cadmium.

Allanson and Deansly (1962) stated that cadmium, in a single subcutaneous injection, can destroy spermatogenic and interstitial cells in the rat testis and produce changes in the pituitary. They further stated that the interstitial tissue is restored by ingrowths from the tunica and full androgen secretion returns before there is any regeneration of germinal epithelium. The long-term effects of cadmium on the testes depend on the dose.

Voigt and Skold (1963) found that cadmium acetate administered to mice intramuscularly produces a hemorrhagic necrosis of the testis. Kar and Das (1963) suggested that cadmium is promptly removed from the body by injecting selenium and thereby the testes are spared.

Roe et al. (1964) reported that rats treated with cadmium sulfate or cadmium-precipitated ferritin developed atrophy of the testes and, in many cases, a subsequent hyperplasia of the leydig cells. Castration changes were observed in the pituitaries of these animals.

Chiquoine (1964) reported observations on the early events of

cadmium necrosis of the testis. He stated that earliest alterations are observed in blood vessels suggesting that the site of action of cadmium is upon the endothelium of the vascular bed. Other results suggest the generality that cadmium necrosis is a phenomenon common to species possessing scrotal testes and absent from those possessing abdominal testes. He admitted that the opossum is an exception to that generality.

Experiments conducted by Cameron (1965) showed that cadmium chloride in weak solutions injected directly into the testes of adult male rabbits completely destroyed the seminiferous and leydig cells within a few weeks. Even after periods as long as 333 days there was no evidence of any regeneration of either type of cell, or any recovery in the atrophied accessory glands of reproduction. The accessory glands of the cadmium castrates showed the same regressive changes as those of a parallel series of animals castrated in the normal way.

Abdel-Razig (1966), experimenting with house flies, observed that cadmium compounds tend to delay, reduce or prevent oviposition when fed continuously at different concentrations in the adult food. Higher doses (1.0-5.0%) of Cadminate (cadmium succinate) were observed to prevent oviposition, but a lower dosage (0.5%) decreased fecundity and fertility without causing excessive mortality.

GROWTH INHIBITION AND MORTALITY.--Johns et al. (1923) performed experiments in which various concentrations of cadmium chloride were incorporated in a diet known to be adequate for the normal growth of rats. Very little or no growth occurred and death ensued when the concentration of cadmium was 1000, 500, or 250 p.p.m. When there were 125 p.p.m. of cadmium in the diet, the initial rate of growth was normal.

All of the male rats receiving this concentration of cadmium died in about 50 days, the majority of females survived longer. A concentration of cadmium of 62.5 p.p.m. had no effect on growth, the rate of growth and food intake being normal. The food intake increased as the concentration of cadmium in the diet decreased.

Ginsburg (1934), testing the various insecticidal properties of phosphates, found that cadmium phosphate possessed appreciable toxicity to silk-moth larvae. Preliminary tests in the laboratory revealed that cadmium salts, in general, are toxic to the silk-moth caterpillar. Cadmium oxide and cadmium hydroxide ranked very high in toxicity against three different species of insects: tent caterpillar (Malacosoma americana Fabr.), silk-moth caterpillar (Bombyx mori Linn.), and confused flour beetle (Tribolium confusum Duv.).

Tent caterpillars transferred on apple twigs previously sprayed with $Cd(OH)_2$ in concentrations of 1, 2, 3, and 4 lbs. to 100 gal. of water showed percentage kill after 48 hours at the rate of 70, 90, 100 and 100, respectively. Considerable feeding took place the first day and very little during the last day. About 82% of the silk-moth larvae were dead two days after they were transferred to mulberry leaves, previously dusted with a mixture of 95% talc and 5.0% $Cd(OH)_2$. Confused flour beetles placed in flour containing 15% $Cd(OH)_2$ were dead after 10 days of feeding. Similar results were obtained on these insects with spray and dust mixtures containing CdO.

Ginsburg and Granett (1935), reporting on experiments with several groups of chemicals, stated that, of the 13 inorganic chemicals tested, cadmium oxide and cadmium hydroxide proved highly toxic to silk-moth larvae, tent caterpillars and confused flour beetles but showed no

toxicity to larvae of the Japanese beetle.

Wilson et al. (1941) found that concentrations of 0.0031% or more of cadmium in the diet resulted in decreased rates of growth, the greater effects being obtained with the higher concentration of cadmium. Death occurred progressively earlier with increasing concentration of cadmium. Rats receiving 0.1% cadmium in the diet lived only a few days.

According to Deschiens and Tahiri (1961), cadmium sulfate is selective against mollusks, killing snails at concentrations of 5 to 15 p.p.m. in water. At these concentrations, it has no effect against goldfish (Carassius auratus), frogs (Rana sp.), or crayfish (Cambarus sp.).

Hill et al. (1963) found cadmium to be toxic to chicks at dietary levels of 25 to 400 p.p.m. in a copper- and iron-deficient diet. The toxicity resulted in a reduced growth rate, mortality, anemia, and atony and elongation of the gizzard. The growth depression and gizzard abnormality were corrected by increased dietary zinc. The mortality was reversed by adding copper. Increased dietary iron partially corrected both the mortality and growth depression.

Schroeder et al. (1963), reporting tests on white mice, stated that cadmium had no marked effect on growth, mature weights nor mortalities up to 18 months of age, but after that significantly increased death rates in males when cadmium levels were greater than 1.7 ug./g.

Cameron and Foster (1963) injected cadmium chloride in weak aqueous solutions (9.12 mg./ml.) subcutaneously into male rabbits which produced a marked falling off in condition, loss of appetite, and retardation of growth.

Powell et al. (1964), using eight calves in each of four cafeteria-type experiments, found that the addition of 40 p.p.m. of cadmium (as

$CdCl_2$) did not significantly affect feed consumption. When either 160, 640, or 2560 p.p.m. were added, feed consumption was substantially reduced.

The Geigy experimental insecticides GS 10128 and GS 10133 are closely related compounds which have shown activity as specific stomach insecticides (Geigy, 1965). They appear to be relatively slow acting and have almost no contact action. From 48-72 hours are required for them to cause insect mortality to occur, and the residual effect is considerable. The insects stop eating after ingesting the products but the typical symptoms of poisoning (tremors and shriveling) do not occur. The oral LD_{50} per oz. of GS 10133, on rats, is about one-half that of GS 10128. Sprays of GS 10133 containing approximately ½ lb./ 100 gal. have given good control of the larvae of Prodenia litura, Agrotis C-nigrum, Anthonomus pomorum, Hyponomeuta spp.; and to immature and mature locusts (Locusta migratoria), Colorado beetle (Leptinotarsa decemlineata) and leaf weevils (Phylobius sp.). The same general characteristics were observed for GS 10128 as those reported for GS 10133, however, it seemed less active as a toxicant than GS 10133.

MATERIALS AND METHODS

TEST INSECT.--The bollworm, Heliothis zea (Boddie) was used in this study. Experiments were performed on the larval stage.

Wild bollworm moths were collected from light traps at Stillwater, Oklahoma, in the Fall of 1965. A colony was established and maintained in the Insectary throughout the winter and spring.

Adults and larvae were reared using the procedure of Adams (1966). Larvae were maintained in 1-oz. transparent plastic jelly cups containing approximately ½ oz. of artificial diet developed by Adkisson et al. (1960) and modified by Berger (1963).

CHEMICALS TESTED.--Five chemicals were evaulated for growth inhibition and mortality, including cadmium chloride, cadmium acetate, cadmium sulfate, and two compounds supplied by the Geigy Chemical Company--GS 10128 and GS 10133.[1] The cadmium salts were 99+% pure (Baker's Analyzed) and were in powder form. The two Geigy products were supplied as 50% wettable powders.

TREATMENTS.--Three methods of treating larval diet and one method of dipping larvae under laboratory conditions were employed to evaluate the above-mentioned chemicals. The concentrations of chemicals used, solvents, method of application and laboratory conditions are given for each experiment.

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[1] 7-(4'-chlorophenoxy)-10-chloro-6H-dibenzo[c,e] 1,2-thiazine-5,5-dioxide.

Method I was used in the first 10 experiments listed in this thesis. It consisted of mixing the chemical powder with water at different concentrations. Approximately 2.5 ml. of each concentration were poured on to each of 10 cups of diet. After 5 to 10 minutes, the excess liquid was poured off the diet, leaving approximately 0.5 ml. on each cup. The cups were set upright in experiments 1, 2, and 7 for about one hour to allow the diet surface to dry. For experiments 3, 4, 5, 6, 8, and 9 the cups were left inverted on paper towels for a few hours to dry. In experiment 10, the diet was sliced in half before applying the chemical. Then the halves were removed from the cups and left on paper towels to dry. After drying, two halves from different concentrations were inserted together in a cup or a treated half was placed with an untreated half.

Method II was used in experiments 11 through 19. It consisted of mixing the chemicals with water at different concentrations as in method I. Each concentration was topically applied to cups at the rate of 0.5 ml. using a graduated pipette. The mixtures were evenly distributed over the surface of the medium. After a few hours of standing open, the excess liquid in each cup had either been absorbed into the medium or evaporated.

Method III was used in experiment 20. It consisted of incorporating $CdSO_4$ evenly throughout the diet at the time of formulation and at different concentrations. The diet was then dispensed into the 1-oz. plastic cups as described by Adams (1966).

Method IV was used in experiment 21. It consisted of mixing different concentrations of GS 10128 in water as in methods I and II. Larvae were dipped in these mixtures and placed on cups of fresh diet.

Solvents.--Tap water and distilled water were the two solvents used in the experiments. Tap water was employed to suspend or dissolve the chemicals in experiments 1, 2, 10, 11, and 16. Chemicals of the other tests, except experiment 20, were suspended or dissolved in distilled water. Both distilled and tap water were boiled for 30 minutes for sterilization. Distilled water in experiments 7 and 21 was not boiled.

Concentrations.--The two numbered compounds--GS 10128 and GS 10133--were provided as 50% wettable powders. Their concentrations used in experiments 1, 2, 3, 4 and 11 are listed below. The cadmium salts in experiments 5, 6, 8, 9, 16 and 17 were also applied at the concentrations listed below. They were provided as reagent grade cadmium salts.

Concentration
0.00 mg./in.^{2}
0.02 mg./in.^{2}
0.20 mg./in.^{2}
2.00 mg./in.^{2}

The four concentrations used in experiment 10 are listed below. In the first four treatments listed, one-half of the medium in each cup received no chemical and the other half received one of four concentrations of the GS 10128 compound in water solution. The last two listed were composed of medium in which one-half received a different amount of chemical than the other half.

Left Half	Right Half
UNTREATED	0.02 mg./in.^{2}
UNTREATED	0.08 mg./in.^{2}
UNTREATED	0.20 mg./in.^{2}
UNTREATED	0.33 mg./in.^{2}
0.02 mg./in.^{2}	0.20 mg./in.^{2}
0.08 mg./in.^{2}	0.33 mg./in.^{2}

The following concentrations were employed in experiment 12 for GS 10128 and in experiment 13 for GS 10133. These are also the actual amounts of cadmium salts applied in experiments 18 and 19.

Concentration
0.00 mg./in.^{2}
0.08 mg./in.^{2}
0.20 mg./in.^{2}
0.80 mg./in.^{2}
2.00 mg./in.^{2}

Four concentrations of GS 10128 and of GS 10133 for experiments 14 and 15 were mixed using a percentage weight basis.

Concentration	Per Cent Actual
0.00 mg./in.^{2}	0.00005%
0.01 mg./in.^{2}	0.0005%
0.07 mg./in.^{2}	0.005%
0.70 mg./in.^{2}	0.05%
7.00 mg./in.^{2}	

The amounts of actual GS 10128 mixed with water in experiment 21 are listed below. The entire external surface of each larva was covered when dipped, but it is not known how much was retained on each insect.

Concentration
0.00 mg./ml.
0.06 mg./ml.
0.60 mg./ml.
6.00 mg./ml.

The amounts of $CdSO_4$ which were incorporated into the diet for experiment 20 are listed below. The per cent of actual chemical in each concentration is also given.

Concentration	Actual
0.00 g./kg. medium	0.006%
0.06 g./kg. medium	0.060%
0.60 g./kg. medium	0.600%
6.00 g./kg. medium	

Larval Manipulation.--Larvae were chosen at random and their weights (or instars and lengths in experiment 20) were determined before they were transferred to the treated and untreated medium. The bollworms were then weighed and reweighed at periodical intervals to determine their average rate of growth. Different numbers, sizes, and weights of larvae were observed in experiments 1 through 11, 16, 17 and 21. Only five cups per treatment were apportioned to experiments 12, 13, 14, 15, 18, 19, and 20. Five first instar larvae were transferred to each cup of experiments 1 and 2. Two first instar larvae were added on each cup of experiments 3 and 4, and three larvae to each cup of experiments 5, 6, and 8. All other experiments received only one larva per cup.

Laboratory Conditions.--All test insects were reared under approximately the same conditions of temperature, humidity, etc., in the laboratory at Stillwater. Larvae were transferred to diet treated in the laboratory using either a soft camel hair brush or smooth-nose forceps. Generally, the brush was only used for first instar larvae. These were transferred to souffle cups and weighed correct to the nearest 0.1 mg.

Experiment 20 was performed on wild bollworm larvae at the Irrigation Research Station, Altus, Oklahoma. These larvae were collected from corn plants on the day of treatment. The laboratory in which they were treated and observed had a constant temperature of about 80° F.

At Stillwater, treated larvae and cups were kept on trays (30" x 18") on shelves. Some of the experiments were begun on the same day and

are comparable. The following sets of experiments were conducted at the same time and under identical conditions: 1 and 2, 3 and 4, 5 and 6, 8 and 9, 12 and 13, 14 and 15, and 18 and 19. However, the test insects in these experiments were produced by different pairs of moths. All other experiments were performed on different days, but under approximately the same conditions.

Calculations.--After the larvae of any given treatment were weighed, the weights were totaled and this value was divided by the number of larvae which were weighed at that particular time. For experiment 20, the average length of the larvae was recorded along with the instar of every larva.

RESULTS AND DISCUSSION

A summary of the experimental designs reported in this thesis is given in Table I. It shows the numbers of larvae treated in each experiment which is helpful in the evaluation of the results of each experiment. Heavier or older larvae tended to grow faster. Preventing this discrepancy was a problem because of the difficulty involved with obtaining all larvae that were exactly the same size.

EXPERIMENT 1.--Results from the first experiment, which was conducted on larvae reared on GS 10128 treated medium, show that larval growth was inhibited to some extent at all three concentrations (Table II). Larvae reared on medium containing 2.00 mg./in.^{2} were most inhibited. They weighed only 1/30 as much as those receiving no chemical after 11 days. These same bollworms weighed only 1/16 as much after 16 days. Larvae on 0.20 mg. weighed less than 1/3 as much as those on the check during the entire experiment. Bollworms on the lowest concentration (0.02 mg./in.^{2}) had a growth rate which was less than that of the check larvae, weighing only slightly more than 1/2 the weight of the check larvae after 16 days.

The experiment was concluded after 16 days because the rearing medium began to show decomposition due to microorganisms. Mold or bacteria growing on the artificial diet caused the evolution of odorous gases during the process of decomposition. Bollworms thrived less on medium showing the presence of microorganisms.

TABLE I

A SUMMARY OF THE EXPERIMENTAL DESIGNS

Expt.	Chemical	Trt.	Larvae per Treatment	Inst.	Treatment Dates	Location of Results
1	GS 10128	4	50	1	12/11/65	II., IV.
2	GS 10133	4	50	1	12/11/65	III., IV.
3	GS 10128	4	20	1	03/23/66	1
4	GS 10133	4	20	1	03/23/66	2
5	$CdCl_2$	4	30	1	03/24/66	3
6	$CdSO_4$	4	30	1	03/24/66	4
7	$Cd(C_2H_3O_2)_2$	4	10	2,3,4	02/21/66	5
8	$Cd(C_2H_3O_2)_2$	4	30	1	03/26/66	6
9	$CdSO_4$	4	10	2	03/26/66	7
10	GS 10128	6	10	-	12/18/65	8, V.
11	GS 10128	4	10	-	01/01/66	9
12	GS 10128	5	5	2,3	02/11/66	VI.
13	GS 10133	5	5	2	02/11/66	VII.
14	GS 10128	5	5	2	02/15/66	VIII.
15	GS 10133	5	5	2	02/15/66	IX
16	$CdSO_4$	4	10	-	12/28/65	10
17	$CdSO_4$	4	10	2,3,4,5	01/08/66	X
18	$CdSO_4$	5	5	2	02/13/66	XI.
19	$CdCl_2$	5	5	2	02/13/66	XII.
20	$CdSO_4$	4	5	3,4	06/03/66	
21	GS 10128	4	10	-	03/28/66	XIII.

TABLE II

AVERAGE WEIGHTS IN MG. OF BOLLWORM LARVAE AFTER PLACING THEM ON MEDIUM THAT HAD BEEN TREATED WITH DIFFERENT CONCENTRATIONS OF GS 10128[1]

mg./in.^{2}	Days After Treatment		
	7	11	16
0.00	16.60	165.05	513.85
0.02	15.20	103.00	286.00
0.20	3.80	47.67	89.61
2.00	1.40	5.50	32.27

[1]Data from experiment 1.

TABLE III

AVERAGE WEIGHTS IN MG. OF BOLLWORM LARVAE AFTER PLACING THEM ON MEDIUM THAT HAD BEEN TREATED WITH DIFFERENT CONCENTRATIONS OF GS 10133[1]

mg./in.^{2}	Days After Treatment		
	5	11	16
0.00	4.08	136.82	408.34
0.02	4.64	200.20	399.32
0.20	4.06	70.30	224.80
2.00	1.19	16.00	57.50

[1]Data from experiment 2.

Shortly after 16 days, some of the larvae on the check entered the prepupation period. During this time, larvae became shorter, ceased to feed and ultimately lost weight. This loss of weight may have produced invalid data since larvae on other treatments may still have been actively growing.

EXPERIMENT 2.--The data obtained in experiment 2 for 7-(4-chlorophenoxy)-10-chloro-6H-dibenzo-1,2-thiazine-5,5-dioxide (known as GS 10133) were recorded at the same time as those of experiment 1 (Table III). This compound is closely related to the GS 10128 of experiment 1.

Larvae on the check increased in weight more than 8 times those on the 2.00 mg./in.^{2} treatment after 11 days. By 16 days, larvae on the latter treatment weighed only 1/7 as much as check larvae. The growth of larvae on the 0.02 mg. treatment was about the same as those on the check throughout the experiment. In comparing the results of Tables II and III, it appears that GS 10128 inhibited growth of bollworms more than GS 10133 during the entire period of observation.

The mortalities shown in Table IV indicate a difference in toxicity between the two compounds. Apparently, the GS 10128 produced a higher mortality to bollworms during the five days following treatment. The difference is striking when the totals of dead larvae are compared for the first two observations. This may indicate that GS 10128 is more toxic to earlier stages of bollworms than GS 10133. After 11 days, however, mortalities produced by the two compounds appear to be similar.

TABLE IV

A COMPARISON OF THE MORTALITIES TO BOLLWORM LARVAE PRODUCED BY GS 10128 AND GS 10133 IN EXPERIMENTS 1 AND 2

Chemical	mg./in.^{2}	Bollworm Dead In Days After Treatment			
		3	5	11	16
GS 10128	0.00	6	21	38	40
	0.02	10	31	39	41
	0.20	14	33	43	44
	2.00	44	45	45	47
		74	130	165	172
GS 10133	0.00	6	16	39	40
	0.02	2	7	38	40
	0.20	5	12	40	43
	2.00	15	27	42	45
		28	62	159	168

EXPERIMENT 3.--The third experiment, performed on GS 10128 treated bollworms, shows that growth was inhibited (Figure 1). After 16 days it was noted that the check larvae weighed nearly twice as much as those on the highest concentration. During that period, it was observed that larvae on the 2.00 mg./in.^{2} treated diet ate or bored through the surface layer and reached the untreated diet below. This may explain the more rapid growth rate of larvae on this treatment above those on the other two concentrations. Bollworms on the other treatments appeared to feed more generally over the surface of the medium.

The profuse growth of microorganisms on the diet impelled their transfer to untreated medium after 16 days. In the 12 days of observation following transfer, the rates of growth were similar for all treat-

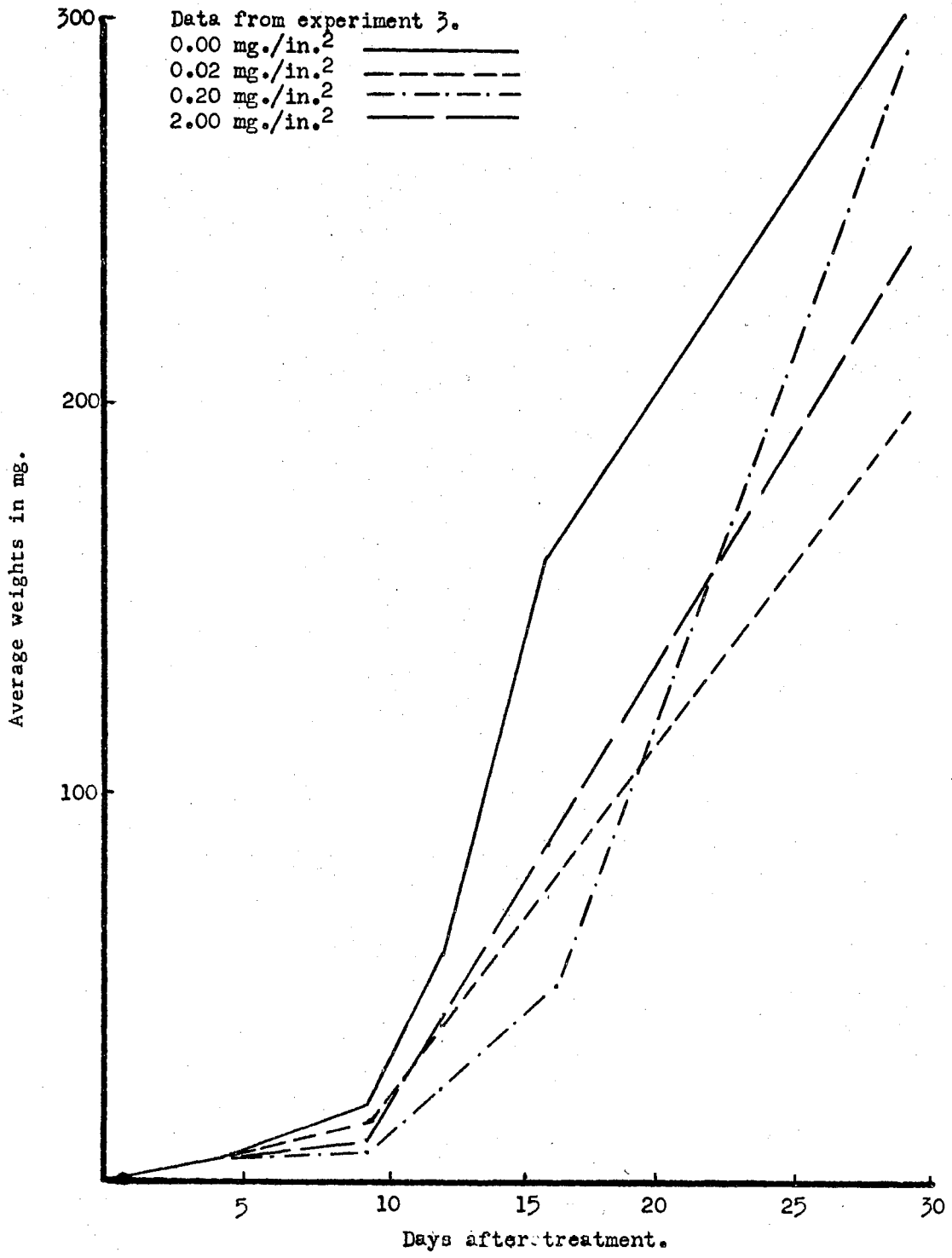

Figure 1. Growth of Bollworm Larvae on GS 10128 Treated Diet.

ments except larvae on the original 0.20 mg./in.^{2} which increased in weight much more. At 28 days, the diet had again decomposed considerably.

EXPERIMENT 4.--Figure 2 shows the results obtained in experiment 4, which was performed on larvae reared on GS 10133 treated medium. As indicated, the results are fairly consistent for the first 12 days following treatment. Then the larvae on 0.02 mg./in.^{2} weighed nearly twice the weight of check larvae by the 16th day. At that time, considerable decomposition had occurred on the diet so larvae were transferred to untreated medium. The rate of growth of check larvae stayed about the same while that of the original 0.20 mg. and 2.00 mg. treatments increased. Apparently growth inhibition occurred on the two highest concentrations until larvae were transferred to the untreated medium. Inhibition was alleviated following transfer.

EXPERIMENT 5.--$CdCl_2$ treated diet was employed in experiment 5 for the rearing of larvae and the results are striking (Figure 3). Larvae on 2.00 mg./in.^{2} of surface were killed after 12 days. At 16 days, larvae on 0.20 mg./in.^{2} weighed less than 1/5 as much as those of the check and 0.02 mg. treated larvae weighed more than 1/2 as much. Decomposition of the rearing medium required that larvae be changed to untreated diet at 16 days. Following the transfer, the check larvae maintained about the same rate of growth while the 0.02 mg. treated larvae decreased and 0.20 mg. treated bollworms increased. The effect at the lowest concentration appears to be similar to that produced in experiment 4. The experiment was concluded after 28 days because of bacterial and mold growth on the diet. Inhibition appears to have been partially alleviated by transferring to untreated medium.

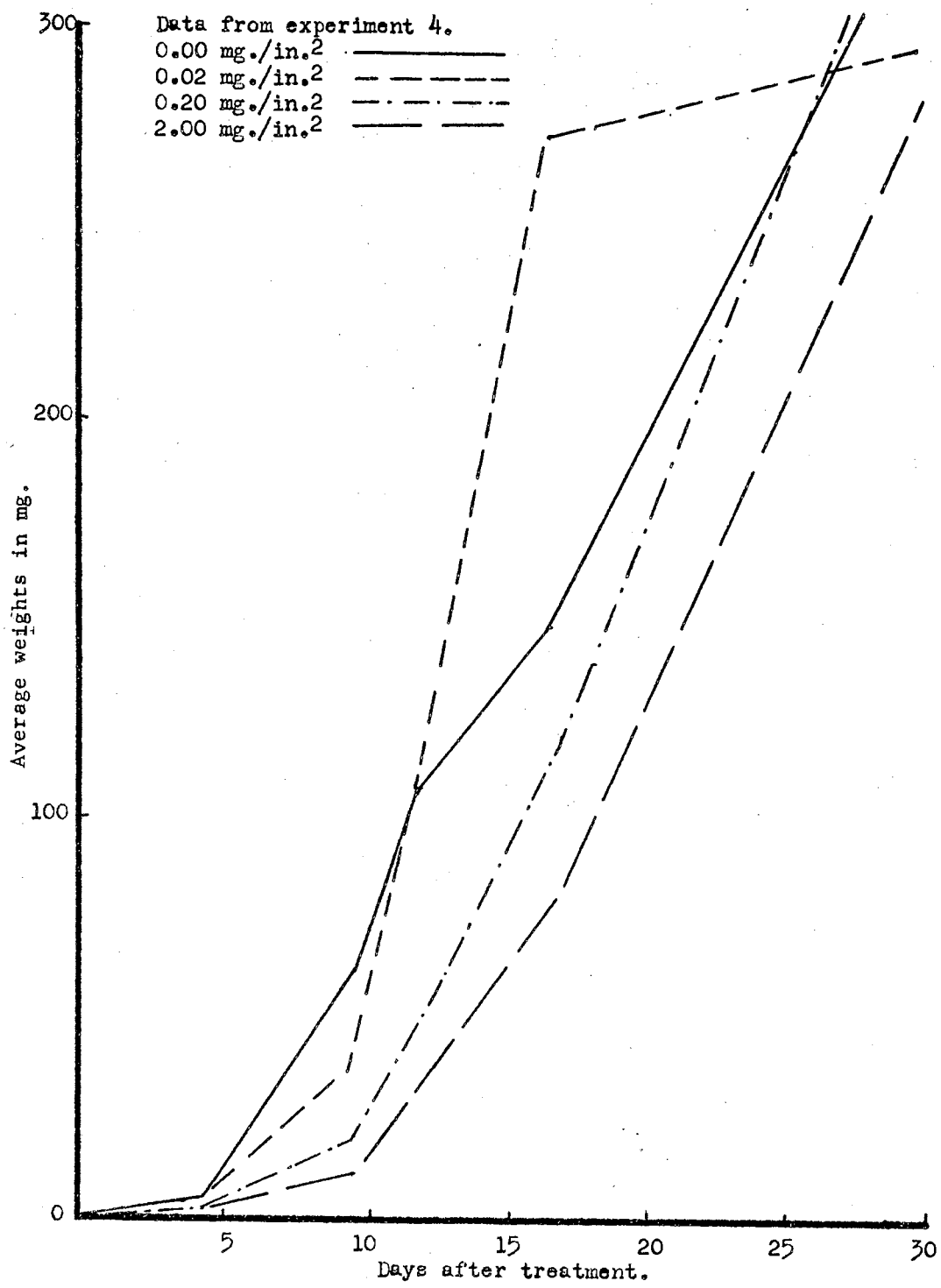

Figure 2. Growth of Bollworm Larvae on GS 10133 Treated Diet.

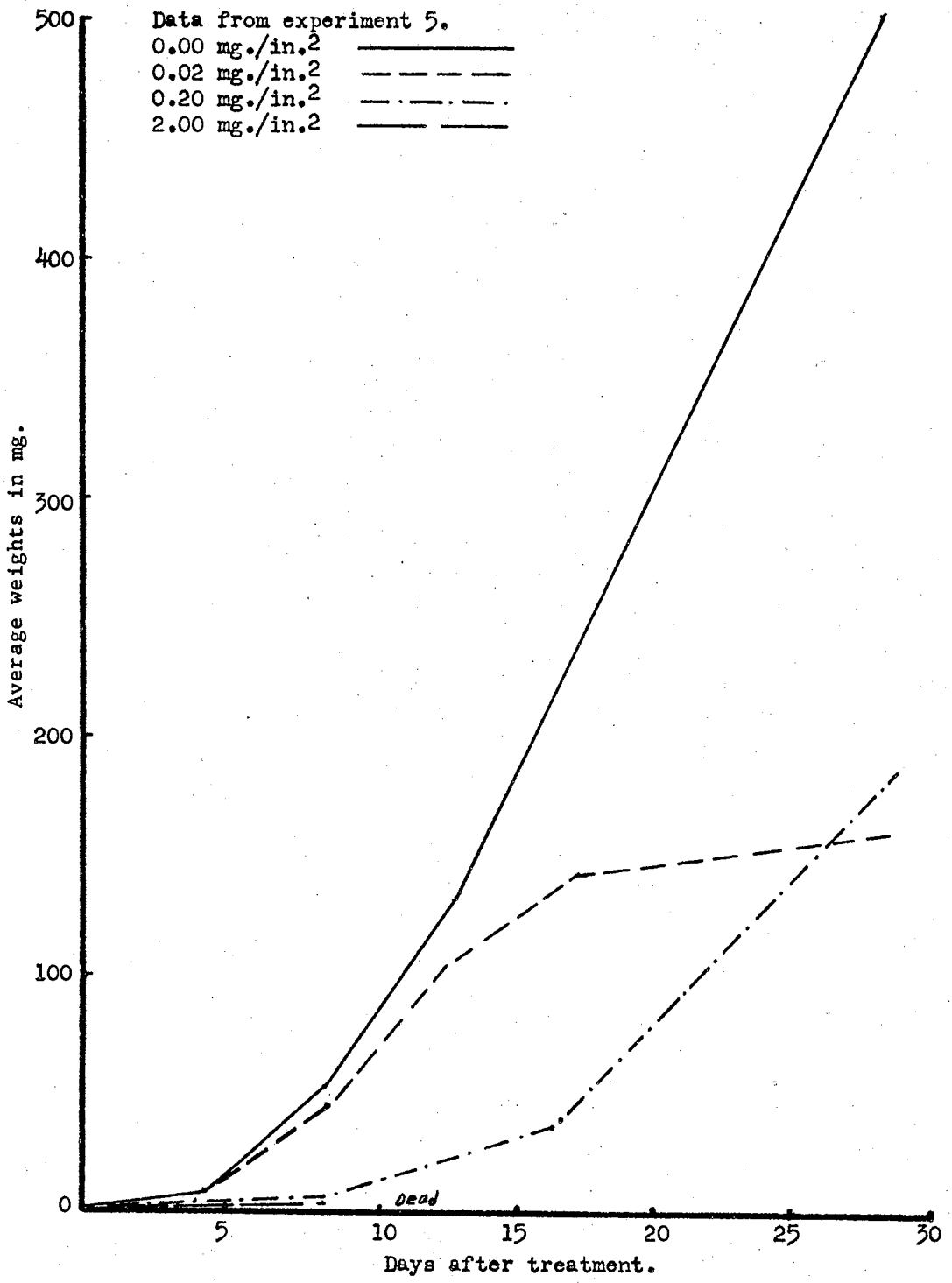

Figure 3. Growth of Bollworm Larvae on $CdCl_2$ Treated Diet For 16 Days Then Transferred to Untreated Medium.

EXPERIMENT 6.--Figure 4 shows the results obtained from experiment 6, in which larvae were reared on $CdSO_4$ treated diet. At 16 days, one larva remaining alive on 2.00 mg. $CdSO_4$ per $in.^2$ weighed less than 1.0 mg. and the bollworms on the 0.20 mg. treatment weighed less than 7.0 mg. The latter value is about 1/14 the weight of check larvae. The growth of 0.02 mg. treated larvae was similar to those of the check. At 16 days, the diet had decomposed considerably so larvae were transferred to untreated medium. Growth inhibition at the two highest concentrations appears to have been only partially alleviated by the transfer. The experiment was concluded after 28 days.

EXPERIMENT 7.--Data from experiment 7 are summarized in Figure 5. Diet was treated with $Cd(C_2H_3O_2)_2$ before weighed larvae were transferred to cups of the material. After 9 days, all of the larvae on the 2.00 $mg./in.^2$ treatment were dead. After 11 days, larvae on 0.20 mg. weighed about 1/3 as much as check larvae and those on 0.02 mg. about 2/3 as much. Larval growth was inhibited at all concentrations of cadmium acetate. There was no mortality in larvae on the lowest concentration after 11 days. The experiment was concluded after 11 days because of decomposition.

EXPERIMENT 8.--Experiment 8 was also conducted using cadmium acetate and the results are summarized in Figure 6. After 4 days, all 1st instar larvae on 2.00 $mg./in.^2$ were dead. Larvae on 0.20 $mg./in.^2$ weighed less than 1/5 as much as check larvae after 10 days. Those on 0.02 mg. weighed almost as much at the 10 day interval. At the latter time, most of the diet in the cups had been decomposed so larvae were transferred to untreated medium. The larval rate of gain for the 0.20 mg. treatment increased greatly during the last 15 days of observation.

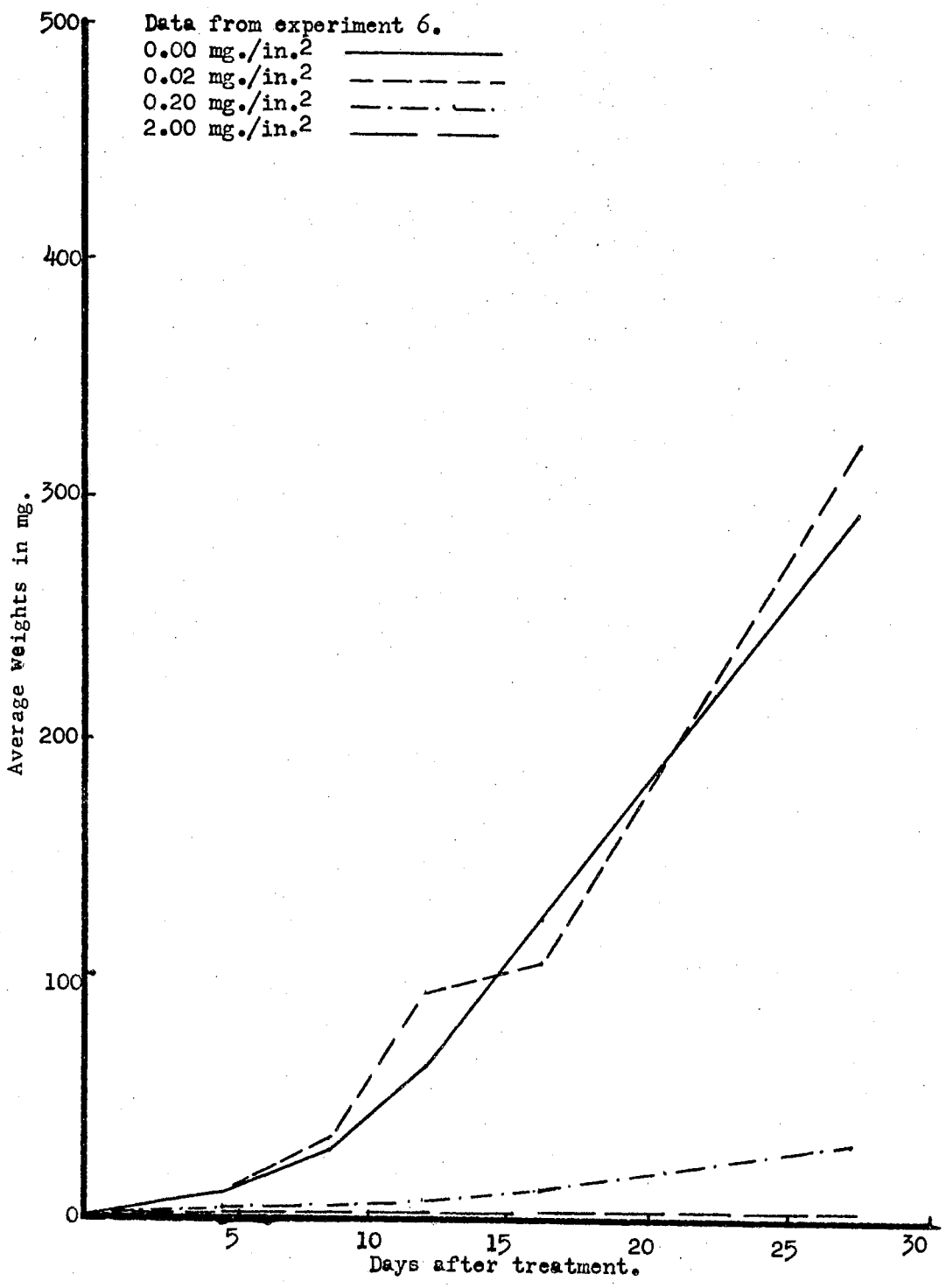

Figure 4. Growth of Bollworm Larvae on $CdSO_4$ Treated Diet For 16 Days Then Transferring to Untreated Medium.

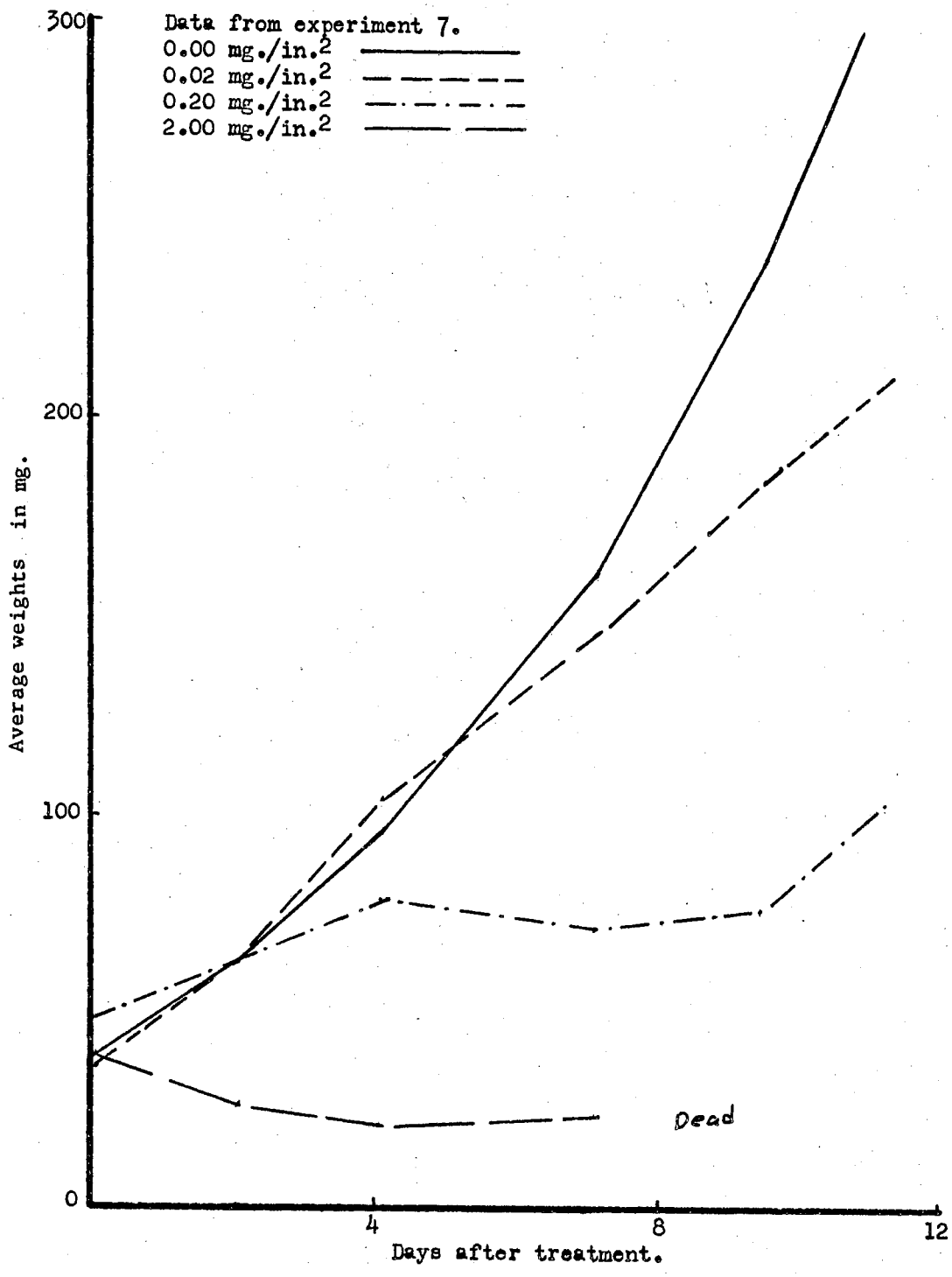

Figure 5. Growth of Bollworm Larvae on $Cd(C_2H_3O_2)_2$ Treated Diet.

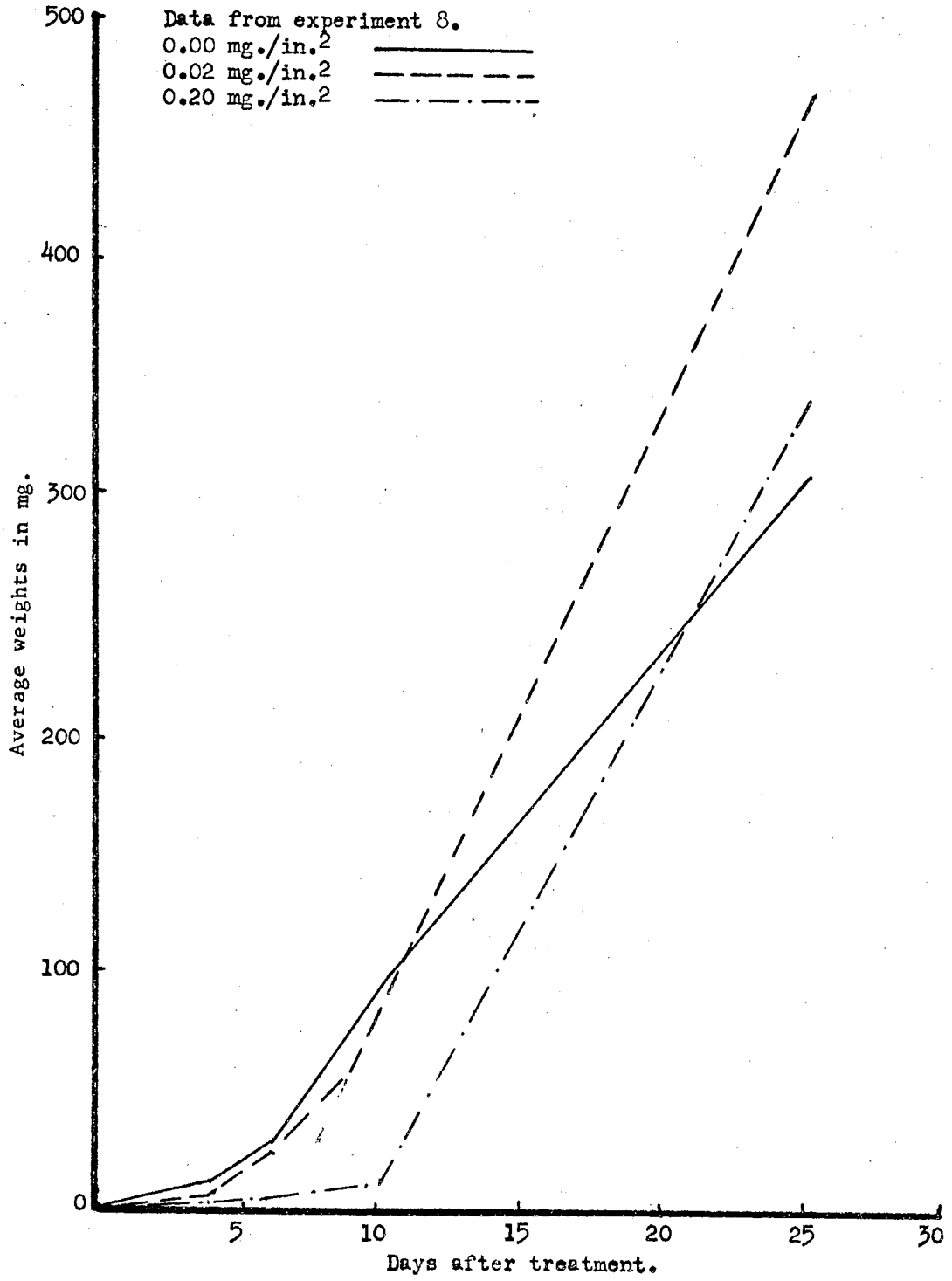

Figure 6. Growth of Bollworm Larvae on $Cd(C_2H_3O_2)_2$ Treated Diet For 10 Days Then Transferring to Untreated Medium.

At the end of 25 days, when the experiment was concluded, the larvae of both treatments weighed more than the check. Growth inhibition at 0.20 mg. was alleviated by transferring larvae from treated to untreated diet.

EXPERIMENT 9.--Results of experiment 9 are summarized in Figure 7. $CdSO_4$ treated diet was used to rear weighed bollworm larvae. Larvae on the check and lowest concentration reached the prepupation period in 11 days. All larvae weighed close to 4.0 mg. at the time of treatment. Larvae on the 2.00 mg./in.^{2} treatment weighed about 1/55 as much as the check larvae after 11 days. At the latter time, considerable diet decomposition had occurred so larvae were transferred to untreated medium. Larvae on the highest concentration increased rapidly in growth after transfer. The pupation period of larvae originally on 0.20 mg./in.^{2} was delayed two weeks behind the check. The curve of the lowest concentration shows that normal growth occurred even to the time of pupation. These data show clearly the growth inhibiting effect produced by $CdSO_4$.

EXPERIMENT 10.--Experiment 10 is included to show the effects produced on bollworms when one side of the diet was treated with one concentration of chemical and the other side was either treated with a different concentration or was left untreated. Figure 8 shows the relative numbers of larvae feeding on the treated and untreated media. The larvae tended to avoid the treated side and fed more on the untreated half. Where more than one concentration was employed, the side with the smallest amount of GS 10128 was preferred. This shows that part of the growth inhibition was due to refusal to eat the treated medium.

Table V shows that larvae on the higher concentrations tended to

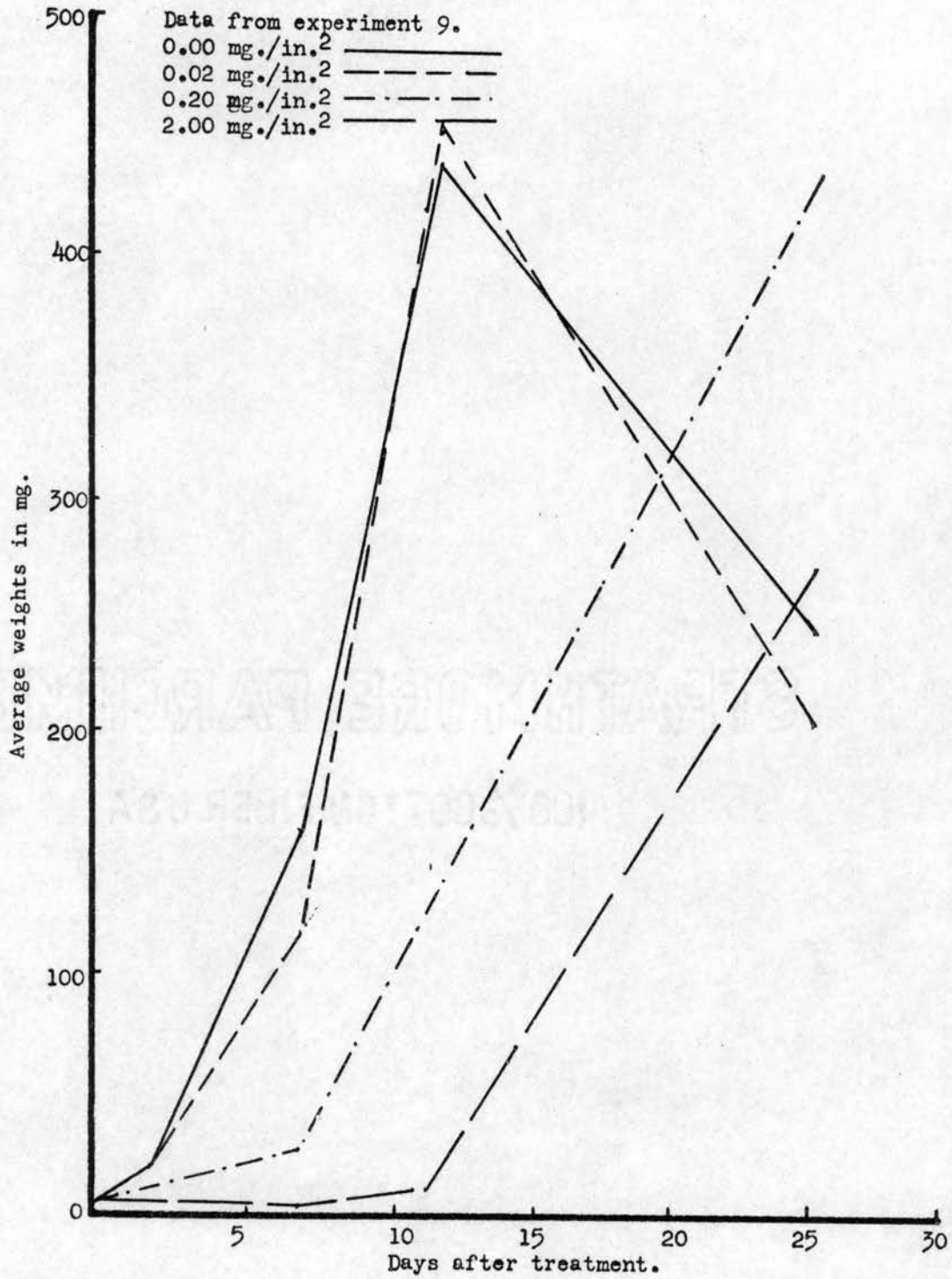

Figure 7. Growth of Bollworm Larvae on $CdSO_4$ Treated Diet For 11 Days Then Transferring to Untreated Medium.

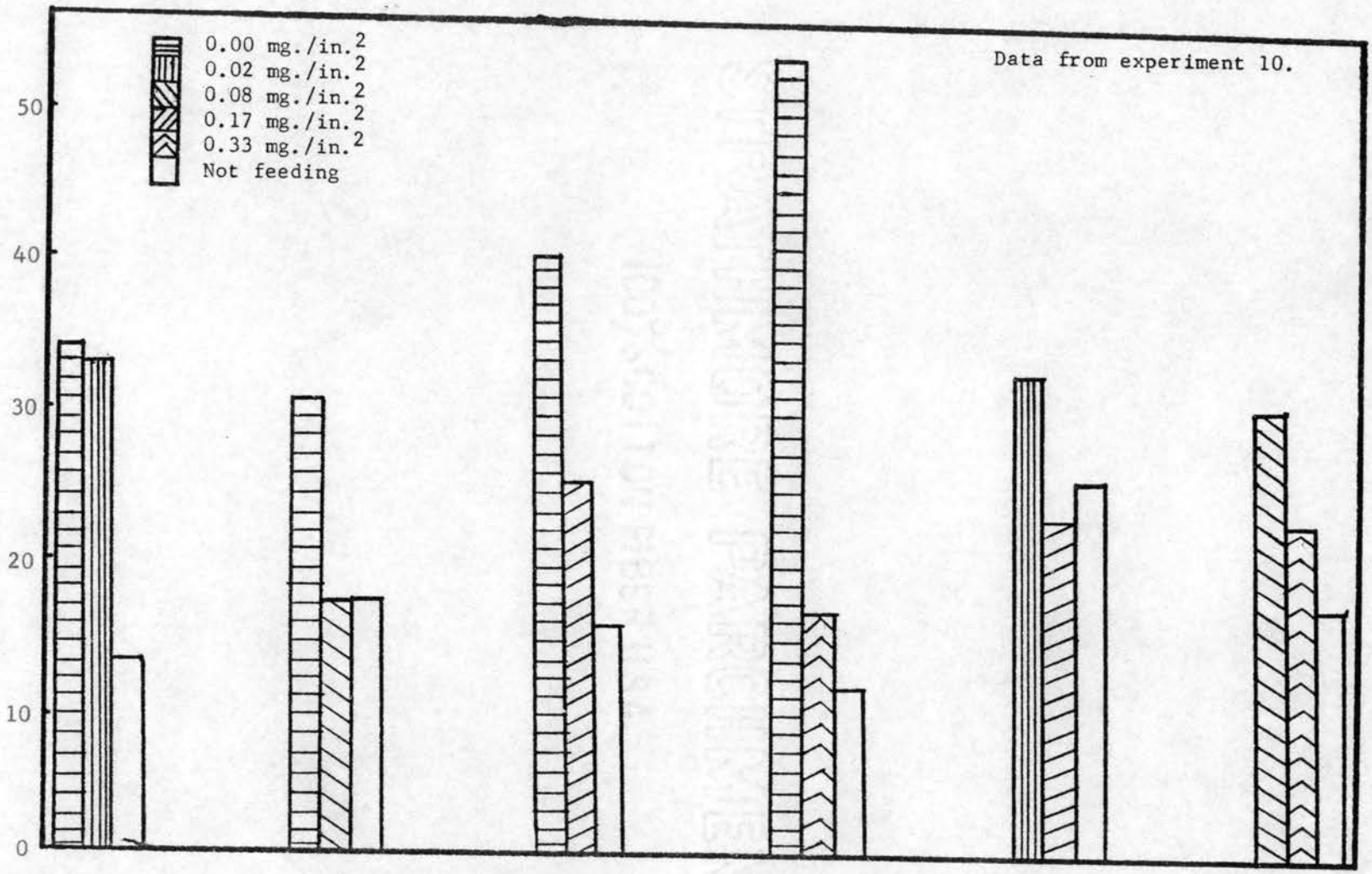

Figure 8. The Numbers of Bollworm Larvae Feeding on GS 10128 Treated Diet and on Untreated Media.

grow less than those on the lower concentrations. Larvae on the highest amount of chemical had a mean gain that was only about 1/2 as much as those on the lowest amount. But, as mentioned previously, part of this inhibition may be due to refusal of the larvae to feed.

TABLE V.

THE EFFECT OF CONCENTRATION OF GS 10128 ON LARVAL GROWTH WHEN OFFERED FREE CHOICE OF TWO LEVELS IN THE REARING CUPS[1]

mg./in.^{2}	Before Treatment	Days After Treatment 9	Mean Gain
0.00/0.02	22.39	555.09	532.70
0.00/0.08	24.90	605.67	580.77
0.00/0.20	22.98	496.32	473.34
0.00/0.33	21.86	512.85	490.99
0.02/0.20	18.36	338.57	320.21
0.08/0.33	21.70	309.43	287.73

[1]Data from experiment 10

EXPERIMENT 11.--Data from the eleventh experiment are summarized in Figure 9. Weighed larvae in this test were reared on GS 10128 treated diet. Those on the highest amount (2.00 mg./in.^{2}) showed a loss of weight at first before any appreciable gain was made. After 5 days, they averaged about 1/3 as much as the check. Most of the check larvae had pupated 16 days following application. Bacterial and mold buildup became prevalent after 11 days but bollworms were not transferred to untreated diet. Evidently, growth was inhibited on all concentrations of the material. No pupation resulted on any of the treated diet.

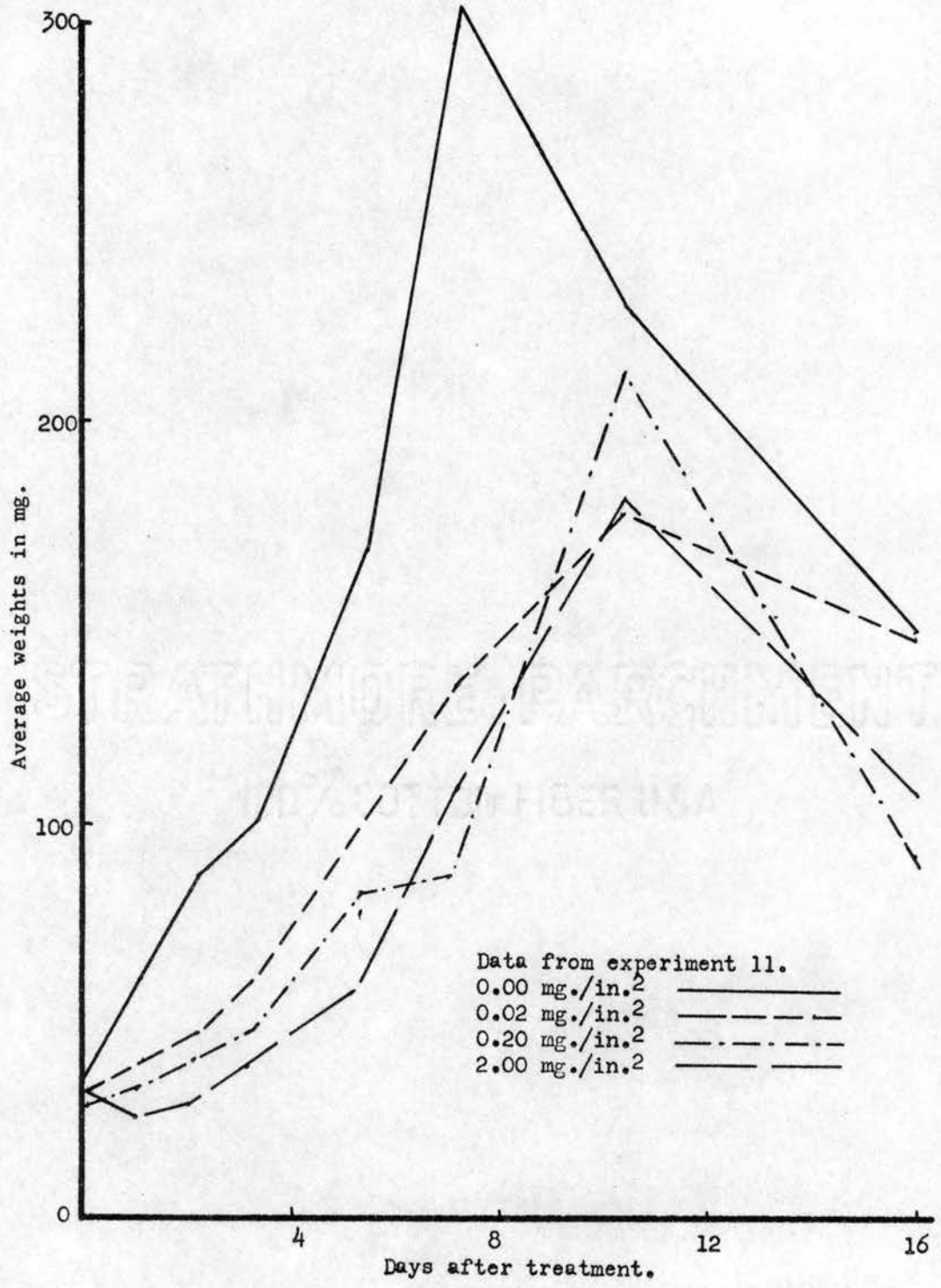

Figure 9. Growth of Bollworm Larvae on GS 10128 Treated Diet.

EXPERIMENT 12.--The results summarized in Table VI for experiment 12 appear to be somewhat erratic. Larvae on the GS 10128 treated medium seemed to grow less than those reared on the check. The compound was originally applied at 0.5 mg./cup of diet using a pipette. The slight amount of excess liquid appeared to accumulate in low areas on the diet surface. Therefore, a greater amount of GS 10128 became deposited in the depressions. Larvae, especially on the higher chemical concentrations, were observed to feed near the cup wall where less of the chemical may have been deposited. Pupation occurred on 2.00 mg./in.^{2}, 0.08 mg./in.^{2} and the check. This method of application does not appear to be as effective in inhibiting larval growth as the first method reported.

EXPERIMENT 13.--The results in Table VII for experiment 13 show inconsistency due to the variation in larval weights before treatment. Check larvae weighed only about 1/2 as much as the other bollworms before application. The pipette method was also used on this test and the inconsistency of results may be due to unevenness of GS 10133 distribution over the diet surface before larval application. Pupation occurred on all treatments including the check.

EXPERIMENT 14.--Experiment 14 data are summarized in Table VIII. Larvae were maintained on GS 10128 treated medium. The 7.00 mg./in.^{2} treatment showed vividly the effects of pipetting the compound on the diet. This concentration was clearly observed to be unevenly distributed over the diet surface. Upon drying, the brown wettable powder could be seen in dark and light areas where unequal accumulation had occurred. A much lighter color persisted near the walls of the cups, indicating a distinct depression in the center of the medium. Larvae were observed to avoid the middle of the cups and feed at the margin

TABLE VI

AVERAGE WEIGHTS IN MG. OF BOLLWORM LARVAE BEFORE AND AFTER PLACING THEM ON MEDIUM THAT HAD BEEN TREATED WITH DIFFERENT CONCENTRATIONS OF GS 10128[1]

mg./in.^{2}	Before Treatment	Days After Treatment				
		2	4	7	10	14
0.00	15.52	27.50	117.46	322.04	654.02	506.30
0.08	14.60	30.61	88.22	170.18	306.16	258.32
0.20	21.90	43.76	86.56	176.18	249.02	380.72
0.80	16.28	29.61	57.52	159.32	163.55	269.42
2.00	17.92	30.76	62.56	250.05	431.05	327.05

[1]Data from experiment 12.

TABLE VII

AVERAGE WEIGHTS IN MG. OF BOLLWORM LARVAE BEFORE AND AFTER PLACING THEM ON MEDIUM THAT HAD BEEN TREATED WITH DIFFERENT CONCENTRATIONS OF GS 10133.[1]

mg./in.^{2}	Before Treatment	Days After Treatment				
		2	4	7	10	14
0.00	6.56	12.86	50.74	155.22	311.38	143.77
0.08	13.02	21.75	64.52	211.85	312.08	241.64
0.20	14.48	30.86	98.16	313.88	439.44	351.52
0.80	13.54	23.30	62.06	198.86	428.22	480.72
2.00	11.68	16.42	56.40	151.78	219.56	224.15

[1]Data from experiment 13.

TABLE VIII

AVERAGE WEIGHTS IN MG. OF BOLLWORM LARVAE BEFORE AND AFTER PLACING THEM ON MEDIUM THAT HAD BEEN TREATED WITH DIFFERENT CONCENTRATIONS OF GS 10128[1]

mg./in.^{2}	Before Treatment	Days After Treatment			
		1	3	6	10
0.00	16.92	28.26	53.08	148.74	287.25
0.01	15.34	25.72	48.52	137.25	197.53
0.07	12.60	20.40	42.92	110.66	120.23
0.70	20.14	26.14	39.04	104.10	249.35
7.00	22.06	27.14	35.36	68.06	256.60

[1]Data from experiment 14.

TABLE IX

AVERAGE WEIGHTS IN MG. OF BOLLWORM LARVAE BEFORE AND AFTER PLACING THEM ON MEDIUM THAT HAD BEEN TREATED WITH DIFFERENT CONCENTRATIONS OF GS 10133.[1]

mg./in.^{2}	Before Treatment	Days After Treatment			
		1	3	6	10
0.00	19.64	21.90	30.70	85.95	93.65
0.01	20.08	25.52	41.40	90.26	68.62
0.07	14.16	22.92	35.62	72.96	139.06
0.70	16.88	18.26	24.17	72.70	246.30
7.00	18.82	22.98	26.23	104.90	341.60

[1]Data from experiment 15.

of the diet. By feeding where a smaller amount of GS 10128 had been deposited, larvae probably obtained unequal amounts of the compound. Table VIII shows considerable inconsistency in weights.

EXPERIMENT 15.--Experiment 15 larvae were treated and observed in an identical manner as those in experiment 14. Table IX shows the results obtained from the GS 10133 treated medium on which larvae were reared. Inconsistency of results also appears in this experiment, probably because of the reasons given for the previous experiment. The recorded weights probably do not give an accurate indication of the growth inhibiting effect of GS 10133. The experiment is included in this thesis so the reader may be able to distinguish between the two different methods of treatment.

EXPERIMENT 16.--Data from experiment 16, in which larvae were maintained on artificial diet treated with $CdSO_4$, are summarized in Figure 10. Larvae on the 2.00 mg./in.^{2} of diet surface grew very little during the two week period following treatment and ultimately died. Bollworms on 0.20 mg./in.^{2} grew to a greater extent but pupation was delayed by a few days. The lowest concentration (0.02 mg./in.^{2}) produced no detectable affect on normal growth or pupation. The prepupation period began after 7 days for the low concentration treated larvae and also those on the check. All of the larvae on 0.02 mg./in.^{2} and on the check pupated but only 50% of those on 0.20 mg./in.^{2} reached pupation. The other half on the latter treatment died.

Four moths emerged from pupae on the 0.02 mg./in.^{2} treatment, three females and one male. Two female moths emerged from the 0.20 mg. pupae. All of these adults appeared to be normally developed. Four other moths, two males and two females, came from the 0.02 mg./in.^{2} pupae whose wings

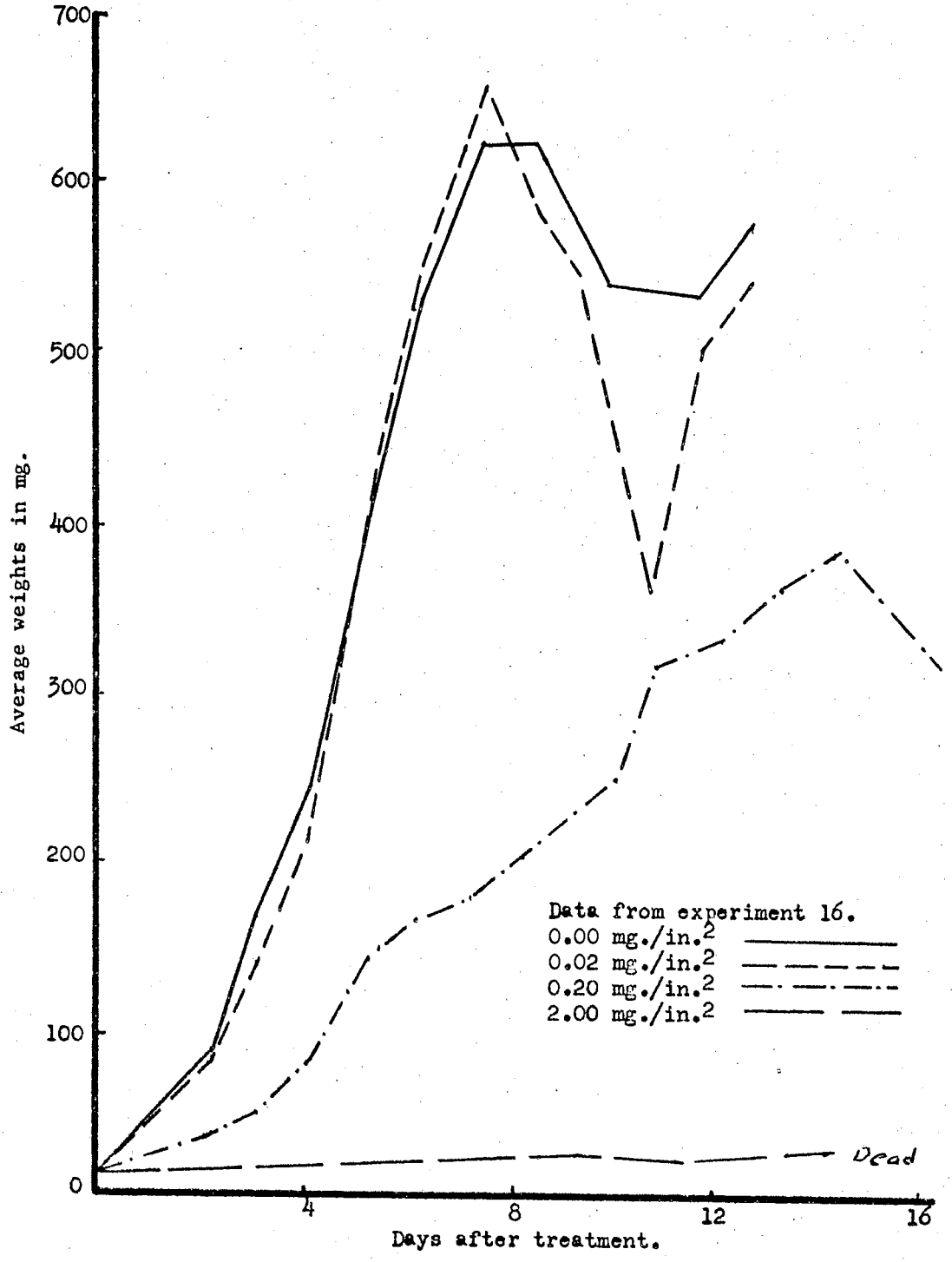

Figure 10. Growth of Bollworm Larvae on $CdSO_4$ Treated Diet.

TABLE XI

AVERAGE WEIGHT IN MG. OF BOLLWORM LARVAE BEFORE AND AFTER PLACING THEM ON MEDIUM THAT HAD BEEN TREATED WITH DIFFERENT CONCENTRATIONS OF $CdSO_4$[1]

mg./in.^{2}	Before Treatment	Days After Treatment				
		1	4	7	11	14
0.00	10.84	14.06	34.06	64.86	99.20	138.40
0.08	12.48	19.40	63.84	169.73	224.50	204.88
0.20	10.80	19.60	52.00	91.90	138.00	139.95
0.80	12.44	11.58	13.10	14.50	Dead	
2.00	12.98	12.72	Dead			

[1]Data from experiment 18.

TABLE XII

AVERAGE WEIGHTS IN MG. OF BOLLWORM LARVAE BEFORE AND AFTER PLACING THEM ON MEDIUM THAT HAD BEEN TREATED WITH DIFFERENT CONCENTRATIONS OF $CdCl_2$[1]

mg./in.^{2}	Before Treatment	Days After Treatment				
		1	4	7	11	14
0.00	11.66	25.02	63.54	168.48	338.02	303.57
0.08	13.18	19.02	46.04	74.72	145.73	206.10
0.20	13.92	14.70	12.32	15.20	Dead	
0.80	12.28	13.00	15.56	23.57	30.75	16.20
2.00	12.36	11.54	Dead			

[1]Data from experiment 19.

were abnormal. Failure of wing expansion may have been the result of cadmium sulfate treatment or it may have been due to some other factor.

The normal adults were mated with moths from untreated larvae. Eggs were produced by most of the pairs but only those from one female, whose larval stage had received 0.02 mg./in.^{2}, laid viable eggs. However, it is not uncommon for moths from both wild and laboratory cultures to lay infertile eggs.

Unlike the previous experiment, the medium surface appeared to be fairly level. An even distribution of $CdSO_4$ over the diet surface appeared to have been obtained.

EXPERIMENT 17.--Data summarized in Table X for experiment 17 indicate the need for uniform larvae and treatment of medium, and the effect of contamination with microorganisms. No clearcut trends were apparent.

TABLE X

AVERAGE WEIGHTS IN MG. OF BOLLWORM LARVAE BEFORE AND AFTER PLACING THEM ON MEDIUM THAT HAD BEEN TREATED WITH DIFFERENT CONCENTRATIONS OF $CdSO_4$[1]

mg./in.^{2}	Before Treatment	Days After Treatment			
		2	6	8	12
0.00	113.68	130.63	159.00	139.01	141.30
0.02	100.66	117.28	169.28	170.61	149.30
0.20	118.90	126.99	144.9.	136.90	124.88
2.00	85.11	88.15	88.15	113.55	106.07

[1]Data from experiment 17.

EXPERIMENT 18.--In experiment 18, the larvae on 2.00 mg./in.^{2} and 0.80 mg./in.^{2} were dead after 4 and 11 days, respectively (Table XI).

Larvae on the other treatments and check did not appear to show consistency of growth inhibition. The pipette method used for this experiment may have produced the erratic results. Apparently the two highest concentrations were toxic to the bollworms. Mortality on the two lowest treatments and check was only 20% after 14 days.

EXPERIMENT 19.--Results of experiment 19, in which larvae were placed on $CdCl_2$ treated diet, are summarized in Table XII. Growth appeared to be inhibited to some extent at all concentrations. At 2.00 mg./in.^{2} and 0.20 mg./in.^{2} of diet surface, $CdCl_2$ appeared to produce 100% mortality after 4 and 11 days, respectively. Only one bollworm remained alive on 0.80 mg./in.^{2} after two weeks. No mortality was recorded in the lowest concentration and check after 7 days, but 20% or more were dead within the two weeks of observation. Pupation occurred only in the check larvae.

EXPERIMENT 20.--Experiment 20 was conducted using larvae which were maintained on medium contained incorporated $CdSO_4$.

All field collected larvae at the time of treatment were in the 3rd and 4th instars of growth and those on 0.6 g./kg. and 6.0 g./kg. of diet did not molt to a new instar at any time during the experiment. Those on 6.0 g. of $CdSO_4$ were dead after 6 days. Larvae on 0.6 g./kg. had not increased in size appreciably after 16 days and, even after 18 days, had not molted. A mortality of 40% occurred after 16 days on 0.6 g./kg. On 0.06 g. and, also on the check, 80% pupation and 20% mortality occurred within 12 days of treatment. These results indicate that $CdSO_4$ definitely inhibits growth of field collected larvae.

EXPERIMENT 21.--The data in Table XIII summarize the growth of larvae dipped in suspensions of GS 10128 in experiment 21. All larvae

appeared to have grown at about the same rate throughout the experiment. Larvae were put on fresh untreated diet directly after dipping. GS 10128, at the concentrations tested, had no affect on the rate or amount of growth, or mortality.

TABLE XIII

AVERAGE WEIGHTS IN MG. OF BOLLWORM LARVAE BEFORE AND AFTER DIPPING THEM IN THREE DIFFERENT CONCENTRATIONS OF GS 10128 SUSPENDED IN WATER[1]

mg./ml.	Before Treatment	Days After Treatment		
		4	9	23
0.00	9.52	67.87	254.82	416.20
0.06	10.05	86.32	317.06	441.93
0.60	9.76	68.15	226.91	452.83
6.00	11.31	71.72	231.40	424.71

[1]Data from experiment 21.

SUMMARY AND CONCLUSIONS

Laboratory experiments were conducted with $CdCl_2$, $CdSO_4$, $Cd(C_2H_3O_2)_2$, 7-(4'-chlorophenoxy)-10-chloro-6H-dibenzo-1,2-thiazine-5,5-dioxide and a closely related compound to determine their effect on growth inhibition, delay of pupation and mortality of the bollworm, Heliothis zea (Boddie).

Experiments were conducted in the laboratory using an artificial diet to which the chemicals were added on the surface only or incorporated in all of the medium. Larvae were also dipped in a suspension of chemical and reared on artificial diet.

Growth rate was determined by weighing the larvae at intervals, comparing time required for pupation, and mortality. Instars of larvae were also recorded to determine if molting was impaired.

$CdCl_2$ was found to inhibit larval growth at all concentrations tested and 100% mortality was produced on 2.00 mg./in.^{2} of diet surface. Larvae on 0.20 mg./in.^{2} gained less than 1/5 as much weight as those on the check in 16 days. Bollworms on 2.00 mg./in.^{2} were killed after 11 days in another experiment while one larva was still alive on 0.80 mg./in.^{2} of diet after two weeks.

$CdSO_4$ produced growth inhibition at 2.00 mg./in.^{2} of diet surface in five experiments. In one of these experiments, a larva on that concentration weighed about 1/60 as much as check larvae after 27 days. In the same experiment, larvae on 0.20 mg./in.^{2} weighed about 1/8 as much as check larvae after 27 days. In another experiment, 2.00

mg. of $CdSO_4$ per $in.^2$ produced little gain in weight until larvae were teansferred to untreated medium. Larvae on 0.20 $mg./in.^2$ weighed 1/3 as much as the check larvae after 10 days. Pupation began at 7 days for larvae on 0.02 $mg./in.^2$ in a third experiment and normal pupation did not appear to be affected by $CdSO_4$ at this concentration. At the same time, pupation was delayed for larvae on 0.20 mg. and 50% mortality was noted on this treatment. A mortality of only 20% was recorded for larvae on 2.00 $mg./in.^2$ after 7 days in the latter experiment and 100% after two weeks.

Cadmium acetate treated larvae showed growth inhibition. In one experiment, larvae on 2.00 mg. produced a net decrease in weight until death ensued after 7 days. Larvae on 0.20 $mg./in.^2$ gained about 1/3 as much and those on 0.02 $mg./in.^2$ weighed 2/3 as much as check larvae after 11 days. Results of another experiment showed that 100% mortality was produced on 2.00 mg. of the compound during 4 days following treatment. Those on 0.20 mg. weighed less than 1/5 those of the check after 10 days. When treated larvae were transferred to untreated diet, the growth increased to more than that of the check.

The GS 10133 (7-(4'-chlorophenoxy)-10-chloro-6H-dibenzo-1,2-thiazine -3,5-dioxide) compound produced inhibition of growth, particularly at 2.00 $mg./in.^2$ but to a lesser degree than cadmium compounds. Larvae on this concentration gained only about 1/7 as much weight as check larvae within 16 days in one experiment and about half as much in another. Bollworms on 0.02 mg. appeared to grow as well as or better than those of the check.

A closely related compound designated as GS 10128, was shown to produce more growth inhibition of larvae than the formerly named

chemical. One experiment showed that bollworms on 2.00 mg./in.^{2} weighed only about half as much as check larvae after 8 days. Even larvae on 0.02 mg. appeared to be inhibited to some extent. Bollworms tended to avoid this compound wherever possible and fed on untreated diet. Results show they remain on the lowest concentration of the compound more than on the higher concentrations when given a choice.

Mortality to early instar larvae was higher for GS 10128 for the first few days following treatment. After 11 days, the mortalities of the two were almost the same. Larvae treated with GS 10133 appeared to grow faster.

The lowest concentration of $CdSO_4$ tested (0.02 mg./in.^{2}) had no detectable effect on normal larval growth or pupation. More than 40% of the adults emerging from pupae whose larvae had been treated with this concentration had abnormally developed wings compared to none in the check. A female from this dosage laid viable eggs which hatched into apparently normal larvae. Other moths from the 0.20 mg. and 0.02 mg./in.^{2} treatments produced non-viable eggs.

When larvae were placed on diet containing incorporated $CdSO_4$, 100% mortality resulted in 6 days on the 0.6% concentration. Virtually all growth ceased at 0.06% but little effect was produced by the 0.006% dosage.

Dipping the larvae in suspensions of GS 10128 produced little or no growth inhibition of the larvae.

From the results of the experiments reported in this thesis, it appears that cadmium salts when topically applied to the diet cause growth inhibition to bollworm larvae. This inhibition appears to be correlated with the dosage applied. The higher concentrations caused

a high mortality to the larvae. When cadmium sulfate was incorporated throughout all of the medium, virtually all growth was stopped at the median concentration and 100% mortality resulted from the highest dosage. The chlorophenoxythiazine compound, GS 10133, and its counterpart, GS 10128, also appeared to produce growth inhibition to bollworms. Indications are that the latter compound was more effective.

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VITA

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