THE INFLUENCE OF CERTAIN RESPIRATORY INHIBITORS AND OTHER CHEMICALS ON THE INTEGRATED METABOLISM OF THE GRAY GARDEN SLUG, DEROCERAS RETICULATUS (MULLER)

by

GRANT MELVIN WHITE

A THESIS

submitted to

OREGON STATE COLLEGE

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

June 1955
APPROVED:

Associate Professor of Entomology
In Charge of Major

Chairman of the Department of Entomology

Chairman of School Graduate Committee

Dean of Graduate School

Date thesis is presented May 11, 1955

Typed by Mary Adams
ACKNOWLEDGMENT

Grateful acknowledgment is made to Dr. Leon C. Terriere for his constructive criticism and helpful guidance during both the experimental work and actual writing of the thesis, and to Dr. Paul O. Ritcher, Chairman of the Department of Entomology, for the graduate research assistantship. I wish to express my thanks to Marvin F. Hill for translating German references and his assistance in collecting specimens.
TABLE OF CONTENTS

Introduction .......................................................... 1
Materials and Methods ............................................... 7
Results and Discussion ................................................ 12
  1. Respiration .................................................... 12
  2. Role of Integument .......................................... 13
Conclusion ............................................................ 23
Summary .................................................................... 24
Bibliography ............................................................. 31

LIST OF FIGURES AND TABLES

Figure

  1. The Effect of Azide and Malonic Acid on Rate of Oxygen Consumption ........................................ 25
  2. The Effect of Arsenite, Fluoride and Urethane on Rate of Oxygen Consumption .......................... 26
  3. The Effect of Metaldehyde on Rate of Oxygen Consumption ....................................................... 27
  4. Scheme to Summarize the Enzyme-Catalysed Steps in the Glycolytic Cycle, Tricarboxylic Acid Cycle, and Cytochrome System, with the Points of Action of the Inhibitory Chemicals .................................................. 28

Table

  1. Toxicity of Parathion to Slugs ............................... 29
  2. Toxicity of Heptachlor to Slugs ............................. 30
THE INFLUENCE OF CERTAIN RESPIRATORY INHIBITORS AND OTHER CHEMICALS ON THE INTEGRATED METABOLISM OF THE GRAY GARDEN SLUG, DEROCERAS RETICULATUS (MULLER)

INTRODUCTION

The gray garden slug, Deroceras reticulatus (Muller), is of growing economic concern in Western Oregon. It is a serious pest to field and truck crops, small fruits, ornamentals, and greenhouse plants, causing considerable losses. The versatile feeding habits, and adaptability of this organism to its environment render it non-susceptible to natural controls. Slugs are one of the most destructive, persistent and difficult to control of all organisms.

The struggle to control slugs undoubtedly commenced when it was first recognized more than two centuries ago in England. Since then, it has increased in economic importance to the present time. As early as 1920, serious studies were undertaken in the hopes of finding an adequate control for the slug. Control measures used were contact materials and irritants such as sulfur and sodium hydroxide; a mixture of carbolic acid, gasoline, and sulfur; nicotine; lead arsenate; paris green; copper sulfate; lemon oil; clove oil; and Bordeaux mixture (23, pp.18-23). Although no worthwhile results were obtained, these observations showed that the Bordeaux mixture possessed repellant qualities. Lovett and Black (23, p.42) recognized the growing importance of this invertebrate and summarized their attempted control as follows: "They have comparatively few natural enemies and are surprisingly resistant to poisons either internal or external. Control practices in common use against
insects and other animal pests are of little avail against them."

In an extensive experimental study of slug toxicants in 1931, (25, pp.390-400) the dusting of copper sulfate, calcium cyanide and calcium carbide upon the slug, resulted in an immediate kill. However, these compounds showed only a temporary control. A short residual life or phytotoxicity were disadvantages that limited their usefulness.

These materials have been gradually discarded for a variety of reasons primarily because of the inadequate control obtained.

Metaldehyde was introduced in slug control about 20 years ago when it was accidentally discovered to be a slug attractant. It seems that a woman residing in South Africa used Meta-fuel (metaldehyde) for heating her curling tongs. Later, after throwing the unused fuel out the window, she observed the assemblage of dead slugs. Upon this discovery, metaldehyde was adopted in England for slug control (14, pp.167-168).

Since then, basic and applied research has evolved around this material. In 1942 metaldehyde was observed by Barnes and Weil (3, pp.56-68) to contribute in some unexplained way to the death of the organism. In 1948, Thomas stated that metaldehyde exhibited three physiological manifestations, "a mortally toxic effect producing a transparency of the gut wall, an anaesthetic effect, and an irritating effect with slime produced" (35, p.207).

Since the discovery of the molluscicidal properties of metaldehyde, many baits have been manufactured which incorporated this
material along with some heavy metal poison, usually arsenic (4, p. 56) (21, pp. 321-322). The latest advance has been the development of metaldehyde for use as a dust or spray (13, p. 370). The high cost, the formulation problems arising from its limited solubility, the short residual life, and most important the inconsistent toxic effects, limit metaldehyde as a reliable molluscicide for large scale operation.

In spite of the many potent materials available in our modern arsenal of insecticides, the control of the slug has not been achieved. The failure of the many toxic materials to possess molluscicidal properties may be due to three factors: the slug's habits and ecology, fundamental biochemical differences, or the possession of some protective feature such as the slime mechanism.

Thus the versatile feeding habits, the ability to be inactive for long periods of time without food, and a prolific reproduction capacity, are characteristics that favor the survival of the slug. In addition, the activity of the slug being greatest during the wet weather would have an important influence on the type of control measures.

A second possibility, given support by the failure of the organic phosphates, the chlorinated hydrocarbons, and other modern insecticides to control the slug, is that this organism, compared to other invertebrates, is different with respect to its metabolic systems. If this were true, its failure to be controlled by current pesticides would be understandable.
Finally, the ability of these animals to cast and discard a slime envelope, whenever irritated, could be a factor for preventing a pesticide from reaching its site of action. If the production of slime is a protective mechanism, it would help explain why current insecticides are inadequate.

The present study was undertaken, from a comparative biochemistry standpoint, to obtain knowledge of the integrated metabolism of the gray garden slug. If this was ascertained to be comparable with other organisms, a study was then to be made to determine whether the integument of the slug includes a defense mechanism.

In recent years workers have emphasized the likelihood that the primary part of drug action upon cells is explained mainly by their effect on cellular enzymes (12, p.74). Accompanying this thought, it is assumed that if a specific enzyme inhibitor effects a complex metabolic process, it can be concluded that the inhibited enzyme takes part in the process (17, pp.191-252).

Knowledge of the integrated metabolism of the slug was obtained in terms of its intermediate metabolism. To accomplish this study, the technique of respiratory inhibition was selected because it has been manifested to be instrumental in identifying specific intermediate enzyme systems. This technique involves the application of various chemicals that are known to interrupt cellular metabolic processes, and studying their effects upon the respiration of the slug. The quantitative measurement of the rate of oxygen consumption is considered to be a reliable index of the total metabolism.
of an organism (6, p.344).

The number of respiratory inhibiting compounds, and the possible modes of action are quite numerous. From a practical standpoint, compounds were selected on the basis of being the most desirable for indicating the presence of the better known enzymatic systems such as the glycolytic, the tricarboxylic acid, and the cytochrome systems.

It is obvious that the interpretation of a comparative biochemical relationship from a comparison of a chemical action on various types of organisms is purely hypothetical. The differences of selective action of chemicals among the same species is common knowledge. The objective of this research is not a study of specific metabolic processes, but to determine whether the gray garden slug is basically different from other organisms in its biochemical structure. It is felt that by studying the integrated metabolism of the gray garden slug, a general picture of its cellular respiratory system will arise.

The rate at which a chemical gains access to its site of action depends largely upon the latent period between administration and onset of action. In many instances the latent period or question of absorption is influenced by the integument. It is therefore conceived that if the integument is a barrier, it will influence the dosage levels of chemicals when they are administered parenterally and topically.

The evaluation of any defense mechanism is based on the percent mortality resulting after a given compound is administered parenterally
and topically. Representative compounds with established toxic doses from the organic phosphate and chlorinated hydrocarbon groups were chosen for this study.

The information so obtained from these studies should be of value to those engaged in developing methods for controlling the gray garden slug.
MATERIALS AND METHODS

In the present study, the manometric method was used for measuring the integrated metabolism of the gray garden slug. This method enables the respiratory rate of the organism to be measured in terms of oxygen consumption. The effect of respiratory inhibitors can then be studied by applying them to the organism and noting the effect on oxygen uptake.

Measurements of oxygen consumption were made with a respirometer of the Warburg constant-volume type described by Umbreit et al (36, pp.1-16). This apparatus consists of a small flask attached in a closed system to a manometer. The slug is placed in the flask and the inhibitory compound is placed in a side arm out of contact with the slug. As the slug consumes the oxygen in the flask the corresponding pressure change is measured by the manometer. Tipping the flask introduces the inhibitor and any effects on oxygen uptake can be noted by the manometer readings.

The specimens were obtained locally from the Entomology farm and were identified as *Deroeceras reticulatus* (Muller). In the laboratory these slugs were kept in small groups in covered jars containing a piece of glass wool saturated with water for maintaining a humid atmosphere. They were stored at a temperature of 15°C. The animals were retained up to 4 days, at which time fresh specimens were obtained. They were starved for 24 hours prior to the beginning of the experiments. The organisms were weighed before the test on a Roller-Smith precision balance. After this, the volume of the slug was calculated
using the water displacement method.

Experiments were conducted in a constant temperature bath adjusted to 18°±0.1° C. This was found to be an ideal temperature for normal slug activity.

The control slugs were exposed to 0.4 ml. of water at the same time that the treated slugs were exposed to the material being tested. This eliminated drowning as a cause of respiratory inhibition. To determine whether the observed results were due to osmotic phenomena, sodium chloride solution, at a concentration equal to that of the most concentrated inhibitory compound, was substituted for the water controls during the studies with sodium fluoride.

The test compounds were dissolved in water and all were used at the same volumes, 0.4 ml. The pH was adjusted to pH 6-7 with sodium hydroxide. Preliminary experiments were conducted to determine the inhibitory concentration of the test compounds. The compounds selected for study and the molar concentrations at which they were used were as follows: malonic acid 0.3 M.; ethyl carbonate (urethane) 0.3 M.; sodium azide 0.1 M.; sodium fluoride 0.1 M.; sodium arsenite 0.1 M., and metaldehyde 0.0001 M.

The solutions were added to the side arms of the flasks before they were connected with the manometers. Exposure of the slugs to the solutions could then be accomplished without opening the system, thereby allowing a constant measurement of oxygen uptake. To assure proper contact with the test media, the slugs were confined on the bottom of the flask by placing a doughnut shaped plastic screen over
them. Exposure consisted of tipping the test material into the bottom of the flask, sixty minutes after the oxygen uptake readings were started. The sixty minute recording of respiration rate prior to exposure to the inhibitors indicated whether the slugs were respiring normally.

A 20 percent potassium hydroxide solution was used for absorbing the expired carbon dioxide, thus neutralizing its effect on the gas pressure within the system.

Readings of the pressure changes were taken every 30 minutes. These figures were then converted to microliters of oxygen as described by Umbreit (36, pp. 57-60).

Each experiment included two controls and four treated slugs. At least two experiments were performed for each of the compounds being studied. At the end of the series of tests, the average normal respiratory rate, based on a unit of weight of untreated slugs, was determined. With the data obtained from preliminary experiments on normal slugs, this average value included data from 50 slugs. The respiration curves of the treated slugs were thus compared with a "normal" respiration obtained from several animals over a 60 day period while each day's run also included controls which indicated whether the apparatus was functioning properly and whether the test animals were normal.

The role of the integument of the slug in its resistance to pesticides was studied by comparing the toxic doses of parathion and heptachlor when administered parentally and topically, respectively.
This work was complicated by the selection of suitable solvents for the materials. A solvent was required which was non-toxic and non-irritating to the slug and which would dissolve sufficient amounts of the toxicants to allow large doses to be given in small volumes. It was concluded after testing several solvents that a common solvent meeting all the specifications was not available. It was found that suspensions suitable for injection could be made by diluting alcoholic solutions of these compounds with water containing a wetting agent. This solvent system was irritating when topically applied, causing immediate production of slime. This reaction was avoided by formulating the parathion as a water suspension and the heptachlor as an n-hexane solution for the topical application. Neither of these solvents caused abnormal slime production.

Dosages were administered on a body-weight basis, using volumes of 0.01 to 0.02 ml. for injections, and 0.01 to 0.03 ml. for the topical treatment. The desired quantity of parathion or heptachlor was injected into the lower pleural abdominal region, half-way between the spiracle and the caudal region.

The injections were made by means of a micrometer-driven standard 0.25 ml. tuberculin syringe, through a 27 gauge needle. Topical treatments were made with a blunt pointed 27 gauge needle. The syringe was calibrated by weighing the mercury delivered.

The desired dosages were given to individual slugs in groups of five. The experiments were repeated two to five times on different days with different organisms. Following the treatments, the
slugs were held in Petri dishes moistened with water to maintain humidity. After 24 hours the percent mortality was calculated.
RESULTS AND DISCUSSION

The ineffectiveness of insecticides and fungicides when administered to the slug, suggest that this invertebrate may utilize a physiological process that is not characteristic of other organisms. If there is a variation in such a fundamental process as cellular oxidation or glycolysis, it should be possible to demonstrate this deviation by the use of chemicals known to effect these processes. The following experiments were designed to determine whether the metabolism of the slug comprises unusual metabolic processes.

Respiration. Five metabolic poisons were selected on the basis of their known action on some part of the glycolytic or terminal oxidative systems of various organisms. The glycolytic system constitutes a universal mechanism by which carbohydrates are degraded to yield energy and pyruvic acid. Likewise, the tricarboxylic acid cycle and the cytochrome system, by which the resultant pyruvic acid is further metabolized to carbon dioxide and water, appear to be present in all living organisms.

The first compound to be tested for its inhibitory action on the respiration of the slug was sodium arsenite. Examples of arsenic toxicity to respiratory metabolism are widespread. Schmitt and Skow (30, pp. 711-719) found that arsenite inhibits the respiration of medullated frog nerves. This compound was also shown to decrease the oxygen consumption of codling moth larvae, cockroaches, and Colorado potato-beetle larvae (9, pp. 275-276).

It is generally thought that the site of action of arsenic is
the tricarboxylic acid cycle, where it interferes with the conversion of ketoglutaric acid into succinic acid (2, p.441). Results obtained by Voegtlin and Dyer (37, p.304) led them to believe that this chemical inhibits the activity of biological systems by reacting with the functional thiol groups on enzymes. This conclusion is substantiated by Stocken and Thompson (33, pp.529-535) and Barron et al (5, pp.221-238).

The experimental data show that when the slug was exposed to a 0.1 molar solution of sodium arsenite, its respiration was inhibited. With the immediate application of arsenic there was a marked decrease in rate of oxygen consumption, stabilizing after 60 minutes with a gradual decrease thereafter (Fig. 2). The average oxygen uptake at the breaking point was 204 μl./gm./hr. The oxygen uptake, 15 minutes after the arsenite exposure, was observed to be 180 μl./gm./hr. The maximum oxygen uptake was 212 μl./gm./hr. before treatment and 34 μl./gm./hr., 150 minutes after treatment.

Considering this inhibitory response in conjunction with the cited evidence, that arsenic acts on the tricarboxylic acid cycle, would indicate that the slug contains such a system.

Further evidence for the presence of the tricarboxylic acid cycle is established by a study of malonic acid. Malonic acid acts as a competitive inhibitor of succinic dehydrogenase thus preventing succinic acid breakdown to fumaric acid (23, pp.693-694). Malonic acid was invaluable in establishing the citric acid cycle theory (19, pp.445-455). Succinic acid has been shown to be an active
metabolite in cells of very diverse types, ranging from bacteria to those of mammals. Malonic acid was found to decrease the cellular metabolism of these animals (27, pp.117-127).

When the slug was exposed to a 0.1 molar solution of malonic acid it underwent a definite decrease in oxygen uptake (Fig. 1). The average respiratory rate at this point was 131 μl./gm./hr. Fifteen minutes after treatment, the rate decreased to 104 μl./gm./hr. The oxygen uptake was 38 μl./gm./hr., 120 minutes after the slug had come into contact with malonic acid, indicating a steady decrease in oxygen consumption.

The malonate inhibition of respiration obtained with the experiments on the slug, would indicate the participation of succinic dehydrogenase in the biological processes occurring in the gray garden slug. Rees (29, pp.478-483) has demonstrated in the snail, a closely related species, a functional tricarboxylic acid cycle.

Attention is now focused on a compound which has been established as an inhibitor of the glycolytic system. Experiments performed by various workers, leave little doubt that sodium fluoride is such a compound. The foundations of our present knowledge of fluoride inhibition of the assimilatory processes were laid by Warburg and Christian who concluded that fluoride inactivates the enzyme, enolase (38, pp.384-421) (39, p.590). This key enzyme is essential in the conversion of enol-phosphopyruvic acid from 2-phosphoglyceric acid. It is thought that fluoride acts on enolase by the displacement of magnesium which is necessary for its action.
The position enolase occupies in the hypothetical metabolic scheme is shown in Fig. 4.

The evidence obtained (Fig. 2) indicates that fluoride is an active inhibitor of slug respiration. The respiratory depression for sodium fluoride was not as marked as the others. The average before treatment was 158 μl./gm./hr. Fifteen minutes after treatment oxygen consumption was 140 μl./gm./hr. and 180 minutes later it was 35 μl./gm./hr. This study may indicate the existence of a glycolytic system within the slug.

A discussion of the following two compounds, urethane and sodium azide, substantiates the probable presence of a cytochrome system or terminal oxidative system. Urethane is classed as a narcotic. Narcotics inhibit the activity of the dehydrogenases and hence, the reduction of oxidized cytochrome (2, pp.176-177). This compound has been demonstrated to be inhibitory to oxidation processes in rat tissue, nerve tissue, heart muscle, grasshopper embryos, and bacteria (8, pp.9-13)(10, pp.159-172)(22, pp.219-239)(26, pp.241-250)(31, pp.851-854).

The effect of urethane when the slug is exposed to a 0.3 molar solution is similar to that obtained with other organisms. Before treatment, the slugs were observed to be respiring at an average of 171 μl./gm./hr. Immediately after the exposure the reduction in respiratory rate was quite sharp, indicating a marked inhibition (Fig. 2). Fifteen minutes after treatment the rate was 43 μl./gm./hr. and 150 minutes after exposure was recorded to be 40 μl./gm./hr.
In direct contrast to the narcotic type of action, sodium azide inhibits the oxidation of the cytochromes by the cytochrome oxidase system in a manner similar to that of potassium cyanide (16, p.165). Since the studies of Keilin, a later contribution suggests that azide dissociates phosphorylation from glycolysis thus preventing cellular utilization of the metabolic energy derived from the glycolytic cycle (32, pp.99-100).

Sodium azide has proven toxic to a great variety of organisms including vertebrates, insect larvae, crustacea, worms, ciliates, moulds, and bacteria. The lethal concentration and time for kill varied from 0.5 percent requiring 3 hours, to 0.1 percent requiring 20 or more hours (17, pp.312-339).

The effect of sodium azide on the slug is indicated in Fig. 1. There was a marked inhibition of respiration almost immediately after exposure to 0.1 molar solution, with a progressive decrease in rate of oxygen consumption occurring over a period of 150 minutes. The oxygen uptake before treatment was an average of 141 μl./gm./hr. The oxygen rate 15 minutes after exposure to azide was 103 μl./gm./hr.

Since urethane and azide inhibit respiratory metabolism by interfering with the cytochrome system, we might conclude that the metabolism of the slug includes this system.

Inasmuch as the compounds discussed thus far are water soluble, it was considered possible that the observed effects were due to a disarrangement of osmotic relationships. This was investigated by treating control slugs with a sodium chloride solution instead of
water. No respiratory effects were noted and it was concluded that osmosis was not a causative factor for the resultant inhibition.

With the establishment of the above evidence, it was considered worthwhile to study the effect of metaldehyde on the respiration of the slug. The administration of a \(1.4 \times 10^{-4}\) molar solution of metaldehyde, which was about 1000 times less concentrated than previously tested compounds, produced a decrease in respiratory rate.

The rate of oxygen consumption decreased immediately after treatment but was not sharp. In some cases the respiratory rate returned to normal in about two hours indicating that the effect was transitory. The respiratory rates of these animals were plotted separately as indicated in Fig. 3. The remaining slugs showed a continual decrease in respiration. It is interesting that despite these differences in respiratory effects, all of the metaldehyde treated organisms died within 12 hours.

At the present time the mechanism and site of action of metaldehyde are not known. Its action has received occasional comment, but no large scale systemic study has developed. Aside from Cragg and Vincent (11, pp.392-406) only two other investigators appear to have studied metaldehyde from a physiological standpoint (7, pp.1-20). Their work with frogs revealed that metaldehyde, injected at a concentration of 1:5000 into the ventral lymph sac, caused a characteristic syndrome, this being in the order of occurrence: hyperexcitability, convulsions, paralysis, and death.

Metaldehyde is practically insoluble in water. It is a
polymer of acetaldehyde (four acetaldehyde molecules) and is depolymerized to acetaldehyde in the presence of strong sulfuric acid. In view of this, it might be suspected that acetaldehyde is the toxic agent.

Acetaldehyde is water soluble. This compound has been manifested to be a normal constituent in cellular metabolism of several types of organisms. It being a decomposition product of pyruvic acid, it is therefore nontoxic (15, pp. 69-34) (20, pp. 550-555) (34, pp. 91-108). This may explain why acetaldehyde was inactive when administered to the slug by Cragg and Vincent (11, pp. 392-406). They administered acetaldehyde in concentrations 100 times that of metaldehyde with no apparent toxic action. In addition, they compared the lethal effects of metaldehyde when administered by the injected, topical, and oral methods. They found that the greatest toxicity to the slug resulted from the topical application of metaldehyde.

The slug has a specific gravity of approximately 1.0360, indicating a probable high water percentage within its tissues. It is conceivable that the comparative insolubility of metaldehyde may cause it to be precipitated within the cells. This would indicate that metaldehyde is not depolymerized when the initial association is with the epidermal cells.

Further study of the problem might profitably be pursued experimentally with greater attention focused on the site of action of metaldehyde.

Role of the Integument. A comparison of the percent mortalities
when parathion and heptachlor are administered parenterally and topically to the slug is given in Tables 1 and 2. It can be seen that parathion injected at a rate of 250 mg./kg. was lethal to all of the slugs. The LD$_{50}$ appears to lie between 150 and 200 mg./kg. This figure is about 50 times that found when parathion is applied to insects and about 20 times the figure usually given for the LD$_{50}$ to vertebrates.

Table 1 also shows that doses as high as 800 mg./kg., applied topically, produce little or no mortality. The results obtained in these experiments were erratic but it is obvious that from a practical standpoint parathion would be useless as a contact poison for slugs.

The low order of toxicity of parathion to the slug is surprising in view of the highly toxic nature of this material to many other animals. There seems to be at least three possible explanations for this lack of toxicity. Metcalf and March (24, pp. 721-728) have shown that organic phosphates which contain the P=O group are more toxic to insects than those containing the P=S group. They postulate that the latter type of compound, as represented by parathion must be converted in vivo into the more toxic form before it can exert its full lethal effect. In the case at hand we might be dealing with an organism which does not possess the enzymes necessary for this conversion.

Parathion is toxic to organisms by virtue of inactivating esterase enzymes that are associated with the chemical transmission of nerve impulses. Essentially, this concept holds that nerve impulses effect responses through the liberation of a chemical
substance that acts as the local exciting agent. This mediator is subsequently destroyed by a specific enzyme.

The high dosage of parathion necessary to elicit a response would indicate that the physiological nervous system possessed by the slug may be different structurally. Namely, the chemical mediator and its antagonistic enzyme may be different with respect to the characteristic ester and esterase type molecules existing in other nervous systems.

Correlated with this reasoning, is the fact that the inhibiting reaction by organic phosphates is proportional to their ease of hydrolysis (1, pp. 473-476). Parathion is effective when given in sufficient concentrations. It is feasible that the slug contains an enzyme which can hydrolyze parathion almost as rapidly as the normal substrates. If this were the case, larger concentrations of parathion would be required for toxicity.

The toxicity of heptachlor to the slug is similar to that obtained when this chemical is administered to other organisms. Table 2 shows that the mortality exceeded 90 percent when the slugs were injected with a dose of 100 mg./kg. The median lethal dose is indicated between 50 and 100 mg./kg. This figure is about five times that found when heptachlor is applied to insects. On the other hand, there is no appreciable difference between the median lethal dose quoted for vertebrates and that found for the slug.

Table 2 also shows that heptachlor applied topically in a dose of 50 mg./kg. resulted in a 30 percent kill. Here again the results
are erratic. There is no appreciable increase in percent mortality as the dose is increased. A dosage eight times that for 30 percent kill, was lethal to only 40 percent of the slugs.

Particularly interesting is the fact that when parathion and heptachlor were injected, the toxicity of parathion was not as great as that of heptachlor. However, experimental evidence indicates a basic correlation between the injection and topical effects of these chemicals when administered to the slug. Neither parathion nor heptachlor was effective in terms of producing a maximum kill when applied topically. The important aspect is that these insecticides once within the body cavity will kill the slug.

The erratic mortality achieved when parathion and heptachlor were applied topically may be related to an inconsistent systemic concentration. There is a possibility that the causative factor for this inconsistency is inadequate absorption due to the formation of slime. Accompanying the topical administration of these foreign materials, there was a copious secretion of thick, white, nonviscous slime. The sliming was severe, especially with the parathion treated slugs. It was observed that injection seemed to reduce any unfavorable reaction, such as sliming.

In support of this observation it was noted that when slugs were treated topically with the pure solvents, hexane, or the water and wetting agent mixture, there was only a slight amount of thin, watery type of slime formed, disappearing shortly. However, with the subsequent application of the solutions containing the active
ingredients, the characteristic thick slime would precipitate immedi-
ately into a firm adherent mass. Once this formed, the slug would
crawl from under it.

These observations in conjunction with the results obtained
with the topical applications would suggest that the slime, the integ-
ument, or both are vital to the welfare of the slug.
CONCLUSION

In conclusion the results obtained in this study indicate that the slug is not essentially different from other organisms in regard to basic metabolic processes. In addition, the integument of the slug appears to constitute a protective barrier, probably by virtue of the slime production.
SUMMARY

The integrated metabolism of the gray garden slug _Doroceras reticulatus_ (Muller), has been studied by utilizing the respiratory inhibition technique. This technique consisted of testing the effects of the metabolic poisons, arsenite, malonic acid, fluoride, urethane and azide upon the rate of oxygen consumption of the slug. In addition, metaldehyde was tested for its effect on respiratory metabolism.

Evidence has been obtained which indicates that the general metabolic processes of the slug compare with those found in other organisms.

Parathion and heptachlor were employed to determine whether the integument of the slug constitutes a defense mechanism. The injection of these two chemicals has been shown to produce toxicity. However, the toxicity of parathion was not as great as that of heptachlor. Both of these compounds have been shown to be relatively ineffective as contact poisons to the slug.

The production of slime has been observed to be correlated with the inadequacy of the topical application.
Fig. 1. The effect of Azide and Malonic Acid on rate of oxygen consumption.
FIG. 3. THE EFFECT OF METALDEHYDE ON RATE OF OXYGEN CONSUMPTION.