

# THE EXCRETION OF ARSENIC BY THE MALPIGHIAN TUBES OF GALLERIA MELLONELLA, TENEBRIO MOLITOR, AND RHODOPHORA FLORIDA<sup>1</sup>

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## INTRODUCTION

The work herein presented formed part of a program of research dealing with the physiological factors in insect control with insecticidal materials. The ultimate goal of this program is the determination and estimation of basic physiological factors and the improvement of control practice according to the factors involved. As a starting point the elimination of arsenious ion by the organs of excretion was investigated. The rates of absorption of a solution of sodium arsenite of lethal concentration in normal saline were determined and the effects of the arsenious ion upon the activity of certain enzyme systems were assayed.

The specific purpose of the investigation was to estimate the part played by the excretory system of insects of different degrees of susceptibility in the elimination of arsenic.

## PREVIOUS WORK

Several attempts have been made to determine how arsenic acts on insects and why some insects are resistant to it. Fink (3)<sup>2</sup> measured the respiratory rates and the respiratory quotients of potato beetles and wireworms which had been fed various arsenicals, and found that in general the oxygen intake was reduced and the respiratory quotient was increased. Following this study Fink (4) determined the effects of arsenic upon the glutathione content of a series of insects. He found the content reduced with arsenic poisoning, but observed that in *Malacosoma americana*, his most resistant species, the initial glutathione content was the lowest. In a third series of investigations, Fink (5) studied the activities of arsenic upon amylase, lactase, maltase, invertase, lipase, and protease from potato beetles. No significant effects were observed in the presence of paris green and lead arsenate. Parfentjev and Devrient (9) observed no significant changes in the respiratory quotient of cockroaches poisoned with arsenic, and the respiration of tissues (muscle, midgut, and nerve tissue) dipped in a solution containing a lethal concentration of arsenic showed no significant reduction. Voskresenskaya (12) described the effects of sodium arsenite upon the rate of passage of food through the alimentary canal, its periodic concentration within the tissues of the insect, and the effects of arsenic upon the anterior sphincter of the midgut. She concluded that resistance depends largely upon the

<sup>1</sup> Received for publication November 11, 1942.

<sup>2</sup> Italic numbers in parenthesis refer to Literature Cited, p. 414.

ability of the insect to regurgitate the dose that has been taken in with the food and to maintain a constant relation between the rate of absorption and the rate of elimination of the material from the blood.

### MATERIALS AND METHODS

Three species of insects were used in this study, namely, last instar larvae of *Galleria mellonella* L.; late instar larvae of *Tenebrio molitor* L., both of these from laboratory cultures; and last instar larvae of *Rhodophora florida* Gn., a noctuid collected from its host plant, the evening primrose.

The median lethal dose (M. L. D.) of the arsenious ion was determined for *Galleria mellonella* and *Tenebrio molitor* by buccal injection of measured doses. The solution used was prepared from reagent grade (Baker's) arsenious oxide and sodium hydroxide. The pH of the final product was adjusted to neutrality. The sigmoid mortality curve derived from these measurements was rectified by the method of probits described by Bliss (1). Unfavorable weather conditions prevented the collection of sufficient numbers of *Rhodophora florida* to make possible the construction of mortality curves.

The rate-of-absorption measurements were made by the method described by Patton and Craig (10). In order to eliminate the factor of individual variation, two absorption gages were used simultaneously with each insect, one containing normal saline and a loop of Malpighian tube and the other containing a symmetrical loop from the same larva and a saline made 0.01 N with sodium arsenite.<sup>3</sup> The normal saline was prepared in a stock solution by dissolving 1.95 gm. of NaCl, 1.52 gm. of KCl, and 1.00 gm. of CaCl<sub>2</sub> in distilled water and making up to a volume of 100 ml. at 20° C. The pH was adjusted to neutrality by adding sodium bicarbonate or hydrochloric acid. This stock solution was adjusted to an osmotic pressure nearly corresponding to that of the normal blood of the insect under investigation. The osmotic pressure values were determined by the capillary method of Halket (6) for the lepidopterous larvae and the values were taken from previous work (Patton and Craig (10)) for the mealworm. The osmotic pressure of the saline was adjusted by dilution from a curve prepared by determining the freezing-point depression of several known dilutions of the stock saline.

Data on selective absorption were obtained by measuring the electrical conductivity of the solution in the bulb of the gage at regular intervals over a period of 24 hours. Measurements were made by means of a conductivity bridge.

The recovery of arsenic in the hind-gut was determined by inserting a short section of No. 20 white cotton thread incased in a finely drawn glass tube into the hind-gut through the anal opening of the insect during an absorption measurement. Analysis was subsequently made on the thread by a micro Gutzeit method essentially the same as that of Howe (8).

Estimation of the presence of certain of the respiratory enzyme systems (monophenol, polyphenol, and indophenol oxidase, peroxidase, and glutathione), and of the autolytic activity of a protease was

<sup>3</sup> This constitutes a calculated lethal concentration based upon figures published by Yeager and Tauber (13) on the blood volume of *Periplaneta fuliginosa*, and by Fay (2) on lethal arsenic content of the blood of *P. americana*.

made by using well-known colorimetric tests such as are enumerated by Hawk and Bergeim (7). The protease activity of the tissues comprising the tubes was estimated from the free amino acid content of an incubated brei by the Danielson modification of the method of Folin (Hawk and Bergeim (7)).

### RESULTS

As a result of a series of measurements of the rates of absorption, it was demonstrated that in all species upon which observations were made, arsenic was readily taken up by the Malpighian tubes. In *Galleria mellonella*, the more susceptible species (M. L. D.=97 (0.009 mg.) As<sup>+++</sup> per gram live weight), it was possible to show a slight decrease in rate of absorption in the presence of arsenic. When these data were evaluated statistically by the method of individual comparison according to Snedecor (11), the Z chart test showed that the significance of the reduction in rate was barely within the 5 percent level. (Mean difference=0.042, standard deviation=0.149, and the number of pairs=48.) Significance could not be established between differences found in the rates of absorption of normal and arsenic-bearing saline with either *Tenebrio molitor* or *Rhodophora florida*. (M. L. D. for *T. molitor*=12 $\gamma$  (0.012 mg.) As<sup>+++</sup> per gram; for *R. florida* the M. L. D. was undetermined but is known to have been less than 15 $\gamma$  (0.015 mg.) As<sup>+++</sup> per gram.)

The qualitative survey of the effects of arsenic upon respiratory enzyme activity showed no appreciable change in the activity of the enzymes demonstrated to be present in the normal Malpighian tissues. No monophenol oxidase, polyphenol oxidase, or glutathione could be demonstrated in any case. The presence of arsenic appeared to have no effect upon the activity of indophenol oxidase, which gave a weak test with Nadi<sup>4</sup> reagent in both treated and untreated samples, or upon the activity of the peroxidase present.

A comparison of the relative concentrations of free amino acid in salines containing nearly equivalent amounts of Malpighian tissue was made after an incubation period of 3 hours. The tubes of *Galleria mellonella* and *Tenebrio molitor* showed nearly equal concentrations of free amino acids in both samples. Apparently protein autolysis is not significantly inhibited by the presence of As<sup>+++</sup>.

That the absorption of arsenic by the tubes is not selective in *Galleria mellonella* and *Tenebrio molitor* was demonstrated by the fact that the electrical conductivity of the saline bathing the tubes was unchanged over a period of 24 hours. During this time an average total volume of 4.9 $\lambda$  (cubic millimeters) of solution was absorbed.

Analysis of the threads which had been inserted into the hind-gut of the larvae of *Galleria mellonella* showed an average recovery of 38 percent of the (calculated) arsenic absorbed by the tubes. Difficulty in collecting the samples made quantitative recovery impossible. Anatomical differences precluded comparable measurements with *Tenebrio molitor*.

### DISCUSSION

The results of the foregoing experiments demonstrate that the Malpighian tubes of all three species of insects used in this study

<sup>4</sup> Equal quantities of 1 percent alpha naphthol (in 95 percent alcohol) and 1 percent paraphenylenediamine (in water).

were able to filter the body fluid of the insect and remove soluble salts by a process comparable to the action of vertebrate glomeruli. They also demonstrate that the presence of arsenious ion in a concentration which approximates the lethal concentration has no significant effect upon the ability of the tubes to function in the process of filtration. A lethal dose of arsenic administered buccally to a series of mealworms which were subsequently dissected and the rates of absorption of normal saline determined caused no significant change of rate.

From these data it is apparent that the Malpighian tubes play a definite role in the removal of arsenic from the blood stream of insects poisoned by the ingestion of arsenic-bearing insecticides. There is no significant relation between the susceptibility of the insect and the effects of arsenic upon the tubes.

#### SUMMARY

The rates of absorption of arsenious ion by the Malpighian tubes of three species of insects—*Galleria mellonella*, *Tenebrio molitor*, and *Rhodophora florida*—were determined and compared. *G. mellonella*, the species most susceptible to arsenic poisoning, showed a reduction in the rate of absorption in the presence of arsenic but the reduction was of indeterminate significance. The other two species showed no significant difference. None of the enzyme systems estimated were affected.

From the measurements made, it is apparent that the Malpighian system of insects plays a definite role in the elimination of arsenic from the blood; however, the data give no indication that the function of these organs is the important factor in the relative susceptibility of insects to arsenic poisoning.

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