PHYSICAL AND INSECTICIDAL STRESS EFFECTS ON THE FORESUT OF THE COCKROACHES, LEUCOPHAEA MADERAE (FABR.) AND PERIPLANETA AMERICANA (L.)

by

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TABLE OF CONTENTS

INTRODUCTION

Stress has been defined as the reactions of the animal body to forces of a deleterious nature, infections and various abnormal states that tend to disturb its normal physiological equilibrium (Stedman, 1961).

Cannon (1914) first observed that animals react in integrated metabolic activities when subjected to the emotional stimuli of pain, fear, or rage. Selye (1936) stated the principles which were later developed into his theory of a General Adaptation Syndrome for mammals (Selye, 1950). He defined the syndrome as the sum of all non-specific systemic reactions of the body which ensue upon long-continued exposure to systemic stress. The General Adaptation Syndrome was divided into three phases: 1. The alarm reaction in which the stressor first causes shock phenomena in the animal resulting in a series of physiological events such as depressed nervous function, decreased muscle tone, deranged capillary and cell membrane permeability, general tissue breakdown, and others, which eventually lead to leucocytosis, eosinopenia, and acute gastrointestinal erosion. This is due to the rapidly developing systemic damage caused by the stressor. The animal, if not fatally stressed, then enters part two of the alarm reaction or counter shock. This results in adrenal cortex enlargement and increased hormonal activity to counter the effects of shock. 2. The resistance phase results from prolonged exposure to the stressor. The overall effect is one of increased resistance to the specific stressor with decreased resistance to other stimuli. The metabolic and biochemical changes of the alarm reaction disappear. 3. Exhaustion is the result of a general breakdown of the resistance mechanisms due to prolonged overexposure to the stressor. Initial lesions may reappear and further resistance is not possible.

The syndrome may result from a wide variety of stressful conditions, e.g., cold, burns, excessive muscular exercise, drugs, diet, and others; each of which may induce a specific response but all result in a similar pattern of physiological reaction and hormonal secretion.

The concept of a stress syndrome in insects is hardly as well defined as that for mammals, but evidence is accumulating which shows that physical and chemical stressors can cause the release of neuroactive substances. These substances are very likely found in relatively small amounts as a normal occurrence of physiological activity. But under conditions of chemical or physical stress, they may be released in sufficient quantities to cause widespread unbalance of several physiological systems causing abnormal behavior and eventually paralysis and death. Chemical stress refers to the condition to which an insect is subjected when treated with toxic or biologically active chemicals. Physical stress may be imposed by enforced muscular activity, electrical shock, immobilization which causes a struggling reaction, or by several other stressors including extreme temperatures and radiation. Any of these, if carried far enough, will result in autointoxication or prostration from a physical stressor due to the release of neuroactive substances. Both chemical intoxication and autointoxication are found to have factors in common. Among these are abnormal release of neurosecretory substances, metabolic disturbances such as increased oxygen uptake and water balance disruption, and a similar course of intoxication leading to prostration and death.

Realizing that stress-induced neurosecretion can cause widespread metabolic and physiological changes, the present work was planned to investigate the correlation of internal and external symptoms of intoxication from several

types of both chemical and physical stress. Previous work by Hopkins, et al. (unpublished data) indicated that the crop of the Madeira cockroach, Leucophaea maderae (Fabr.), would become bloated with gas when subjected to prolonged intoxication from parathion. The present study was designed to determine if other groups of organic insecticides and several types of physical stressors would cause similar symptoms. This would provide further evidence for the existence or absence of a non-specific stress syndrome in insects, in which dissimilar stressors could lead to the dysfunction of common physiological systems. Since most of the previous work on insect stress was done with the American cockroach, Periplaneta americana (L.), this species was used in comparison with L. maderae to determine if there was any species variation in the symptom of primary interest. The second phase of experimentation was intended to determine the cause of gas accumulation, the composition of the gas, and the correlation of any additional stress symptoms observed and to bring them into the overall picture of the stress syndrome.

LITERATURE REVIEW

General Symptomatology

The gross external symptomatology of insecticide intoxication in insects is well known. The general sequence of events caused by many organic insecticides can be summarized as follows: (1) A period of normal activity following treatment, (2) hyperactivity in which the insect shows periodic bursts of great agitation, (3) gradual loss of coordination with an extension or stiffening of the legs, (4) prostration in which the insect is unable to right itself, (5) paralysis, and (6) death. Prostration may

last for days while a gradual weakening of the individual occurs. On the other hand, the entire intoxication syndrome may be compressed or obscured by the extremely rapid action of certain compounds or by the unusual species susceptibility of certain insects. Various insecticides may also elicit certain specific symptoms according to their modes of action. These symptoms are accompanied by biochemical and physiological changes which are discussed in greater detail in several reviews. A general coverage of insecticide symptomatology may be found in Metcalf (1955) and Roan and Hopkins (1961). DDT symptomatology is discussed by Wigglesworth (1955), and organophosphorous poisoning by Jochum (1956), O'Brien (1960), and Chadwick (1963). A review covering both autointoxication and insecticidal stress with much detail on neurosecretion and other symptoms was written by Sternburg (1963). Only a brief discussion will be attempted here.

The first discussion of paralysis or autointoxication due to physical stress was by Beament (1958). He observed that the immobilization of the cockroach, P. americana, for three to four days resulted in eventual paralysis. The cockroaches, although appearing unharmed, would gradually become incapable of locomotion either upon release or within a few days after their release. The percentage of affected cockroaches varied with the number of days immobilized, the number of legs free during immobilization, and the position used for immobilization. Parabiotic and injection experiments in which the blood from paralyzed cockroaches was allowed to mix with the blood from normal cockroaches resulted in paralysis of the normal cockroach indicating that a blood factor is involved. Mechanical stress by constant motion in a revolving powder mill also was capable of paralyzing cockroaches with

the appearance of a paralyzing blood toxin as was found in the immobilized cockroaches. Electrical stimulation was not successful in inducing paralysis.

Several symptoms have been found to be common to physical and insecticidal intoxication. According to Heslop and Ray (1959), a common stress syndrome may exist for all types of insecticidal intoxication and the variations observed with different insecticides may be due to the masking or enhancement of certain aspects of this syndrome. This would also appear to be true for physical stress, as neurosecretory factors, increased oxygen uptake, acetylcholine level increases, and disruption of water balance are common to both types of stress.

Acetylcholine Levels and Stress

Tobias et al. (1946) reported a buildup of acetylcholine (ACh) to about 200 per cent above normal in the nerve cord during the late prostrate stage of DDT poisoned American cockroaches and houseflies, Musca domestica. The level of bound ACh was found to be greatly decreased or even absent in poisoned insects with a corresponding increase in nerve cord ACh. Lewis (1953) obtained smaller increases in ACh with the blowflies, Calliphora erythrocephala and Lucilia sericata, attributing the proportionally larger amounts shown by Tobias to synthesis in the extract rather than the ACh content of the insect itself. The organophosphorous compounds TEPP, parathion and malathion, when topically applied, were found to result in sizable increases in ACh in the head of the housefly and TEPP increased ACh in the nerve cord of the American cockroach (Smallman and Fisher, 1958). This was verified in the cockroach by Colhoun (1958, 1959a) who also observed two peaks, one small increase in ACh content corresponding to the period of

hyperexcitability and a second larger increase during prostration. Waller and Lewis (1961) found increases as high as 300 per cent above normal for whole thorax extractions of γ-BHC treated American cockroaches. DDT, aldrin, and pyrethrum evoked smaller increases in ACh.

Lewis et al. (1960) subjected American cockroaches to DDT as well as physical stress by enforced activity on a turntable and by desiccation with anhydrous calcium chloride. Cockroaches treated with DDT showed nearly three times as much ACh in the whole thorax at late prostration as did normal controls. Mechanically induced prostration gave smaller increases of up to 94 per cent for the abdominal nerve cord and 141 per cent for the whole thorax. Desiccation resulted in considerably smaller increases of around 39 per cent in whole thorax extractions.

Neurosecretion and Stress

Beament (1958) has demonstrated the involvement of a blood factor with parabiosis and injection experiments described previously. Hodgson and Geldiay (1959) subjected the cockroach, Blaberus craniifer, to electrical shock with steel electrodes implanted in the head, and to physical stress by inverting the cockroach, causing it to struggle to right itself. Although neither treatment induced prostration, a histological investigation of the corpora cardiaca revealed a marked decrease of neurosecretory materials normally present in those organs. Extracts of corpora cardiaca also showed a loss in potency in depressing spontaneous activity in the cockroach nerve cord in vitro.

Other investigators have found stress-induced blood toxins with both insecticidal treatment and electrical shock. Sternburg and Kearns (1952)

discovered a blood factor which was not DDT in DDT poisoned American cockroaches. This factor increased in amount toward prostration and caused bursts of activity and nerve trains followed by blockage of impulses in isolated nerve cords. Symptoms of poisoning were induced in the flesh fly, Sarcophaga crassipalpis, by the injection of blood from poisoned cockroaches. Further work by Sternburg et al. (1957) and Shankland and Kearns (1959) attempted to characterize, isolate and identify the factor. It was found to be present in the blood of DDT-prostrate silkworms, houseflies, and crayfish, and was not acetylcholine, adrenaline, noradrenaline, or histamine. Sternburg et al. (1959) also failed to identify the toxic factor although they showed it to be released into the blood by electrical stimulation or DDT treatment of the whole American cockroach, or to be released into saline from an isolated nerve cord when the attached cerci were treated with DDT or electrically stimulated. Chromatographic analysis indicated that the blood toxin was common for both stresses. Later, Sternburg (1963) demonstrated a substance which was chromatographically similar to the DDT factor in the blood of American cockroaches physically stressed on a powder mill. Colhoun (1959b, 1959c) further complicated the picture by demonstrating the release into the blood of hormones from the corpus cardiacum and the corpus allatum, and three unknown biologically active compounds, one of which was hypothesized to be 5-hydroxytryptamine, in TEPP and DDT-prostrate American cockroaches. One of these substances was possibly the blood toxin of DDT discovered by Sternburg and Kearns (1952). Both physical and chemical stress may induce the release of the corpus cardiacum hormone which causes abnormal physiological changes in the cockroach, but Milburn et al. (1960) demonstrated that this secretion does not have the same effects on nerve activity as Sternberg's DDT toxin, which is elaborated from the ventral nerve cord.

Oxygen Consumption and Stress

Lord (1949) reported an increase in oxygen uptake when the saw-toothed grain beetle, Oryzaephilus surinamensis, was treated with lethal dosages of DDT and its analogs, γ-BHC, and pyrethrins. Sublethal dosages had no effect and uptake decreased considerably with the onset of death. A second study by Lord (1950) on the red flour beetle, Tribolium castaneum, showed that the oxygen uptake increased with the onset of symptoms when the insects were treated with chlordane, toxaphene, 3,5-dinitro-o-cresol, γ-BHC, pyrethrins, DDT, parathion, and HEPT (Hexaethyltetraphosphate). The peak of O_2 uptake was not correlated with external symptoms but a decrease occurred with the onset of late paralysis and death. Ouye et al. (1961) stated that the increased consumption is associated with paralysis in the housefly poisoned with malathion. Jochum (1956) noticed two peaks of increased O_2 consumption with parathion poisoned Colorado potato beetles, Leptinotarsa decemlineata. The first peak began just before the excitation stage, possibly due to increasing metabolism to maintain water balance. The uptake reached a maximum when paralysis had caused all movements to stop. A second, smaller peak occurred after the stopage of movements, perhaps due to the disintigration of tissue. Heslop and Ray (1959) found a single peak preceding paralysis of the American cockroach treated with DDT. Cockroaches were immobilized and those which became prostrate also showed a similar respiration peak with the onset of prostration. Free moving controls and immobilized cockroaches not becoming prostrate gave no indication of any increased oxygen uptake. This would again indicate a common stress syndrome.

Water Balance and Stress

Water balance is another example in which a similarity between auto-intoxication and poisoning exists. Jochum (1956) reported that during parathion poisoning, the amount of body fluid in Bombyx mori and Dendrolimus pini caterpillars is greatly decreased and within 48 hours, little blood could be removed. The intestine, however, was found to fill with fluid until it became turgid. Beament (1958) also reports that after 10 to 15 days of paralysis, the hemolymph within the American cockroach is reduced, an event which might be expected since a cockroach deprived of water also has reduced blood after a period of time. The hind gut, however, was found to contain abnormally large quantities of water, similar to that of the poisoned caterpillars.

Miscellaneous Symptoms

Two symptoms which have been little discussed by most workers are the evagination of the gut or reproductive structures and the bloating or presence of air in the alimentary system during poisoning. Chadborne and Rainwater (1953) reported that evagination of the hind gut occurred when larvae of the pink bollworm, Heliothis armigera, were treated with dieldrin. It was felt that the peristalic movements of the gut may have been the cause. Jochum (1956) found that parathion poisoning of the adult Colorado potato beetle, Leptinotarsa decemlineata, resulted in the spreading of the abdominal segments and the protrusion of the copulatory organs as if under increased internal pressure. Roan (personal communication to Dr. T. L. Hopkins, 1960) mentioned that a majority of third instar larvae of Costelytra zealandica (Coleoptera)

would show evaginated hindguts 30 minutes after rapid immersion in a 0.3 per cent diazinon emulsion. Three out of forty larvae dipped into a dieldrin solution also showed this symptom.

The subject of gas or flatulance of the gut is reported by only a few workers. Salkeld (1951) observed a bubble of gas in the midgut of six of ten honeybees, Apis mellifera, which had been fed DDT in a sucrose solution. The fore and hind guts were of normal diameter but abnormally dark brown in color. The cause of the gas was felt to be due to DDT effects on the frontal ganglion of the stomatogastric nervous system which would result in abnormal swallowing activity, causing the swallowing of air. However, honeybees fed parathion in the same manner as DDT did not develope a bubble of gas in the midgut. It might be pointed out that parathion acted within ten to twenty minutes to produce symptoms. The bees would turn small, rapid circles and the tongue was almost in continuous motion. Then it would be rubbed between the front legs and regurgitation would occur. After this, the bee would become weaker and death would occur about five minutes after regurgitation. All this appears to take about half an hour while the DDT symptoms took twenty-four or more hours before death.

A second reference made to the accumulation of gas was by Wiesman and Kocher (1952) in their study with the carbamate, pyrolan. They found that with pyrolan poisoning, a latent period was followed by typical hyperactivity symptoms leading to paralysis of the legs and loss of coordination. Insects which had been in contact or fed pyrolan moved backwards for a long time and then suddenly fell on their backs and began to exhibit intervals of tetonic tremors spaced by short periods of quiescence. The mouthparts were in constant motion which led them to believe that air was swallowed resulting in

the distention of the abdomen. This buildup was reported in the American cockroach, the blood sucking Hemipteran, Rhodnius prolixus, and the tick, Argas reflexus. No mention was made of the area of the gut where bloating occurred to cause the abdominal distention.

Jochum (1956) believed the ingestion of gas into the gut of M. domestica and Dendrolimus caterpillars was due to the swallowing of froth formed from the ingestion of small amounts of fluid material during the period when the mouthparts were in constant motion due to poisoning. The fluid may have been the result of small amounts of material regurgitated and then sucked in again. In M. domestica small air bubbles in the intestine appeared to become more numerous as poisoning progressed. He regarded air accumulation as a symptom of weak poisoning action.

Patton (1963) in discussing DDT intoxication briefly states that: One symptom of poisoning, observed both in laboratory study and in the field, is an acute flatulence that stretches the intersegmental membranes of the abdomen and even causes rupture of the digestive tract. No reference is given or any evidence presented to show how the information was obtained, the species involved, or the frequency at which such a symptom presents itself.

Hopkins et al. (unpublished data) have found gas accumulation in the foregut of L. madaerae that occurred approximately at the prostration stage during parathion intoxication.

MATERIALS AND METHODS

Experimental Insects

The principal insects used in this study were adult female Madeira cockroaches, L. maderae. The American cockroach, P. americana, was utilized in

fewer tests for comparative purposes. Both species were reared at approximately 24°C. on Purina Lab Chow pellets and water.

To obtain insects of uniform age and physiological condition, female nymphs of L. maderae were separated from the main colonies and placed in a separate rearing tub. At weekly intervals, the newly emerged adults were removed and placed in gallon jars with food and water and kept for two to three weeks before use. In some tests, it was necessary to use adult female L. maderae directly from the stock colonies, as noted in results.

Adults of P. americana were taken directly from the main colony and the sexes separated for testing. Because of the small size of the colony, it was not possible to select and use only virgin females as with L. maderae. In some cases, males were also utilized, as noted in the results.

Anesthesia

The insects were anesthetized with CO_2 for insecticide treatment and X-ray purposes. In experiments on physical stress (turntable, powder mill), the insects were simply placed in the containers with no anesthesia. Later in the insecticide studies, it was found that anesthesia was unnecessary during X-ray exposure, although it was still used during treatment. One reason for abandoning anesthesia during the X-ray procedure was the discovery that most cockroaches moved little while in the frame. This allowed for easier handling as well as eliminating the potential stressing effects of CO_2. In some cases where the cockroach was hyperactive or unable to remain still, anesthesia was employed to prevent blurring of the radiographs. It was also found that P. americana were more difficult to anesthetize and tended to revive during the one minute X-ray exposure, whereas, L. maderae were

affected by the CO_2 for longer periods of time. When either species revived too quickly from the CO_2, tremors and movement of the abdomen resulted in blurring of the radiograph.

X-ray Techniques

Insects to be X-rayed were placed in a 5 x 7 inch frame with six compartments constructed of 1/4 inch plexiglass (Plate I). Each compartment was 1 x 2 1/4 inches, about the size of a cockroach so as to restrict the insect's movement. The entire frame was covered with three mil polyethylene plastic held in place with double coated Scotch tape.

The X-rays were taken with a General Electric grain inspection X-ray machine (Model CH-1). Kodak type M industrial X-ray film was placed over the frame and exposed for one minute at 5 ma. and 25 kv., developed with G. E. Supermix X-ray developer, and fixed for 5 to 10 minutes in G. E. Supermix X-ray fixer. Development time, depending on temperature, was established by using the maximum time on a Kodak development time indicator for X-ray film.

The X-rays taken were placed on a ground glass X-ray viewer and a value given to the bloated condition on the following basis (Plate II, Fig. 1-5): 1. None - The foregut is normal with no gas other than small bubbles in the crop. 2. Slight bloat - Gas content is somewhat greater than a small bubble and usually less than 1/4 inch in diameter. 3. Moderate bloat - Gas causes the crop to fill much of the thorax and into the abdomen but the X-ray lacks density. 4. Heavy bloat - Gas causes the crop to fill the anterior half of the abdomen with increased density of the X-ray. 5. Extreme bloat - Gas was still contained within the crop but enough was present to force the crop into the abdomen, nearly filling it with a dark, dense area shown by the X-ray. It should be pointed out that density in a radiograph is caused by the greater

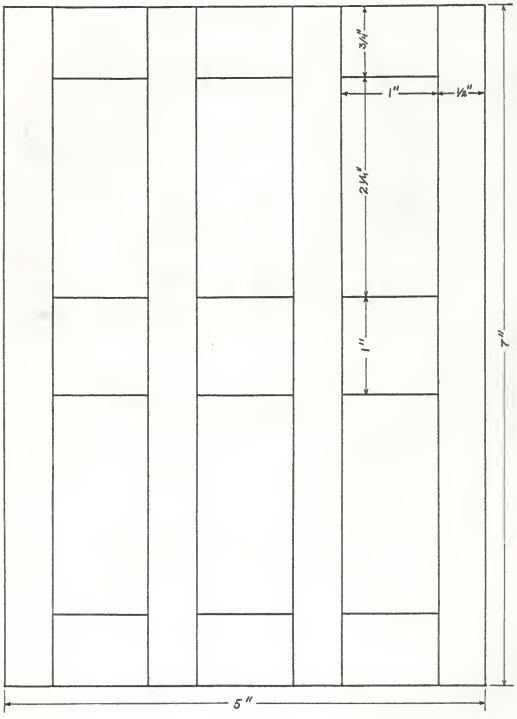
EXPLANATION OF PLATE I

Frame used for holding cockroaches during exposure to X-rays.

PLATE I

X-RAY FRAME

MATERIAL: 1/4" PLEXIGLASS



SCALE 1"=1"

EXPLANATION OF PLATE II

Fig. 1. No bloating. Control *L. maderae*. (22 hours).

Fig. 2. Slight bloating. Ronnel treated *L. maderae*. (28 hours).

Fig. 3. Moderate bloating. Parathion treated *L. maderae*. (24 hours).

Fig. 4. Heavy bloating. Malathion treated *L. maderae*. (46 hours).

Fig. 5. Extreme bloating. Ronnel treated *L. maderae*. (21 hours).

Fig. 6. *P. americana* control. (48 hours).

Fig. 7. *P. americana* female treated with parathion. (20 hours).

Fig. 8. *P. americana* male treated with carbaryl. (20 hours).

Fig. 9. Powder mill control *L. maderae*. (6 days).

Fig. 10. Powder mill exercised *L. maderae*. (6 days). Arrow shows dilated coxal trachea.

Fig. 11. Diatomaceous earth desiccant control *L. maderae*. (24 hours).

Fig. 12. Diatomaceous earth desiccant JC-109 treated *L. maderae*. (24 hours). Arrow shows dilated coxal trachea.

Fig. 13. Extreme bloating of foregut of *L. maderae* treated with parathion and ligated at the neck. (96 hours).

Fig. 14. Extreme bloating of foregut of *L. maderae* treated with electric shock. (3 days). Lines are silver electrodes.

PLATE II

passage of radiation through the gas filled crop and appears darker on the film. In Plate II, positive prints made from the radiographs were used. As a result, greater density becomes a lighter region masking internal detail while lower density appears more grey caused by the blockage of radiation by internal structure.

The intervals at which the X-rays were taken varied with the different treatments. With the insecticide studies, the first radiograph was taken when several cockroaches were entering prostration. Further X-rays were taken at intervals of six to fifteen hours depending on the progression of symptoms in all cockroaches, and were no longer taken when all cockroaches were in late prostration or paralysis. Physically stressed cockroaches were X-rayed once at the end of the treatment and again at later intervals as symptoms were noticed.

Insecticidal Stress Techniques

The compounds selected for use in the chemical stress experiments represent the major groups of synthetic organic insecticides (Table 1). The organophosphorus insecticides parathion, ronnel, malathion and dichlorvos; the carbamate compound carbaryl; and the organochlorine compounds DDT, lindane, and dieldrin were used.

Insecticide solutions were prepared by weight-volume and technical acetone. The dosage of insecticide to be applied was determined by initially testing a range of three concentrations. The dosage of insecticide which would cause prostration of the cockroaches in 18 to 36 hours was selected (Table 2). Dichlorvos was found to act more rapidly with a knock-down time of approximately five hours, while DDT was much slower acting, producing

Table 1. Experimental insecticides, purity, and source of supply.

Insecticide	Chemical name	Source	Purity
Organophosphates			
Dichlorvos (DDVP)	O,O-dimethyl 2,2-dichlorovinyl phosphate	Shell Chemical Co.	Analytical Std. 99%
Ronnel	O,O-dimethyl O-2,4,5-trichlorophenyl phosphorothionate	Dow Chemical Co.	99%+, Recrystalized
Parathion	O,O-diethyl O-p-nitrophenyl phosphorothionate	American Cyanamide Co.	Purified 99.6%
Malathion	O,O-dimethyl S-(1,2-carboethoxyethyl) phosphorodithioate	American Cyanamide Co.	Purified 99.5%
Carbamates			
Carbaryl (Sevin)	1-naphthyl N-methyl carbamate	Union Carbide Chemicals	Analytical Grade 99.85%
Organochlorine			
DDT	1,1,1-trichloro-2,2-bis (p-chlorophenyl)ethane	Nutritional Biochemical Co.	Tech. 77.2% (E.S.A.[1] Ref. Standard)
Lindane	γ-isomer of 1,2,3,4,5,6-hexachlorocyclohexane	Nutritional Biochemical Co.	99+% (E.S.A. Ref. Std.)
Dieldrin	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo, exo-5,8-dimethanonaphthalene	Nutritional Biochemical Co.	Tech. 85% (E.S.A. Ref. Std.)

[1] Entomological Society of America designated reference standard.

Table 2. Dosages of insecticides and solvent volumes applied to two species of cockroaches.

Insecticide	Leucophaea maderae		Periplaneta americana	
	Dosage ug/insect	ul of acetone	Dosage ug/insect	ul of acetone
Dichlorvos	5.0	2.0	-	-
Ronnel	100.0 80.0	5.0 8.0	- -	- -
Parathion	2.5	10.0	2.0	8.0
Malathion	500.0	5.0	-	-
Carbaryl	300.0	30.0	300.0	30.0
DDT	1500.0	5.0	1500.0	20.0
Lindane	25.0	5.0	20.0	2.0
Dieldrin	50.0	10.0	-	-

paralysis after 73 hours with a dose of 1500 µg per cockroach, an application which would leave a slightly waxy layer on the surface of the insect.

The amount of acetone solvent applied varied from 5 to 30 µl depending on the insecticide. Carbaryl was found to be soluble to 10 µg/µl and so required the application of 30 µl of acetone. Application of this amount of acetone to L. maderae controls gave no indication of adverse effects but three P. americana controls receiving 30 µl of acetone showed signs of bloating as early as 20 hours and developed paralysis from 122 to 168 hours. Three P. americana controls which received CO_2 anesthesia only showed bloating as early as eight hours but gave no sign of paralysis throughout the test.

Insecticides were applied to the insects using a microdrop applicator (Roan and Maeda, 1953). A 1/4 cc syringe was fitted with a 26 gauge hypodermic needle which was blunted and bent about 90 degrees. All insecticide applications were topical to the tergal intersegmental membranes of the abdomen.

After application, both the treated and control cockroaches were held in screen covered pint Mason jars without food or water. These were placed in a temperature-humidity control room kept at about 78 $\pm$ 2° F. and 74 $\pm$ 4 per cent relative humidity.

Physical Stress Techniques

Turntable. A turntable was constructed similar to that of Lewis et al., 1960, with a 24 inch disc of 1/4 inch masonite mounted on a bicycle hub and driven through pulleys with a sewing machine belt. A gear reduction was used which would drive the turntable at approximately 3 rpm, chosen to approximate

the walking speed of the cockroach. Rods were mounted from ring stands to hold an inverted petri dish in place about nine inches from the center of the rotating turntable. A short length of wire was crimped over the rim of the petri dish to prevent it from catching the tarsi of the moving cockroach. A petri dish was used as it was shallow enough to prevent the cockroach from climbing the side of the container. In this way, no shocking apparatus or grid was necessary as was used by previous investigators. Cockroaches were forced to walk for three days on the moving surface as a means of causing stress by constant movement. Longer periods of time resulted in high mortality.

Powder Mill. Another device, resembling a ferris wheel, was first used by Beament (1958) and later by Heslop and Ray (1959). The apparatus was constructed with eight 4 oz. plastic jars rigidly attached 5 5/8 inches from the center of a vertically mounted wheel of 1/4 masonite. The wheel was mounted on an inexpensive bicycle hub as the drive bearing. This was attached through pulleys by means of a sewing machine belt to a gear speed reducer and an electric drive motor (Plate III). The wheel turned at 20 rpm as mentioned by Heslop and Ray (1959) and the plastic jars were modified by gluing a glass rod lengthwise to the inside wall of the container. The cloth pads used by Beament to keep the insects in motion were replaced with three one-inch styrofoam balls. Both modifications increased the agitation of the insects and also increased tumbling action.

Electric Shock. Electrical shock was applied with a Phipps-Bird electronic inductorium through electrodes implanted in the head of each cockroach. The electrodes were of four mil silver wire and implanted by piercing the head capsule with a minuten pin and inserting two wires, one on each side of the vertex near the eyes. The wire was then held in place with a drop of

paraffin. The cockroaches were inverted and pinned through the prothoracic flange and across the abdomen to limit movement. The inductorium was then connected and set to produce a moderate twitch in all the cockroach's appendages.

Immobilization. Nine L. maderae cockroaches and twelve P. americana were pinned in the manner described by Beament (1958) in which they are held in place with wire staples across the back to a balsa block. The legs were rendered immobile with pins crossed over them. The insects were removed from the block by cooling them in a deep freeze until all movement ceased. Carbon dioxide was not used because of the struggling it caused. The insects were held in pint Mason jars and observed for signs of paralysis and then placed in the frame and radiographs obtained.

Desiccant Studies. A diatomaceous earth desiccant was tested to determine if bloating of the foregut would occur under the stress of water loss and desiccation. Twenty cockroaches were treated with JG 109, a product supplied by John Galt Company, San Antonio, Texas. A layer of the desiccant 1/4 inch deep was placed in a crystallizing dish. The cockroaches were placed in the dust and allowed to walk about for one minute. This was sufficient for the insects to acquire a coating of the material. They were then removed and placed in pint Mason jars for observation before X-raying.

Gas Analysis of Foregut Contents

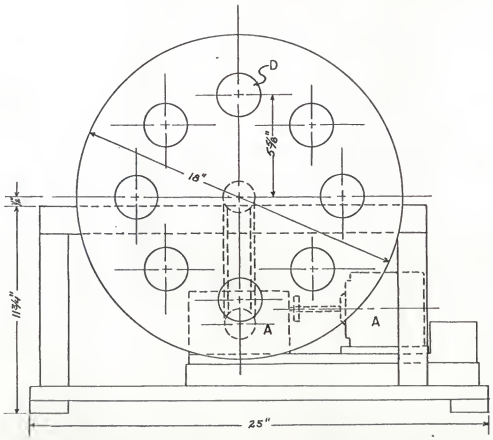
A total of 36 L. maderae females were treated with 2.5 µg of parathion each, held 24 hours until prostrate, and then X-rayed to determine the extent of foregut bloat. Those that were found to contain extensive accumulations of gas were dissected by cutting away the wings and making an incision along

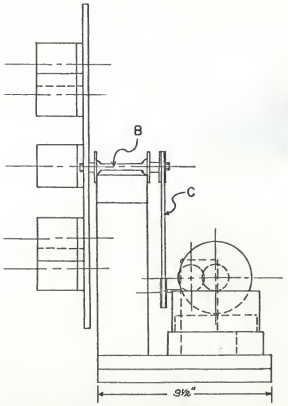
EXPLANATION OF PLATE III

Drawing of powder mill stressing apparatus for inducing constant motion in cockroaches.

POWDER MILL STRESSING APPARATUS

A. ARMY SURPLUS GEAR REDUCER & MOTOR
B. BICYCLE HUB
C. SEWING MACHINE BELT · SEARS ROEBUCK 60903
D. 4 OZ. PLASTIC BOTTLE · CELLUPLASTICS INC. #46





SCALE 3/16" = 1"

the dorsal meson. The tergum was then pinned back, exposing the alimentary canal. This was done gently to prevent puncturing the greatly distended crop. The body cavity was then flooded with cockroach heart and blood saline (Yeager, 1939).

Gas was withdrawn from the crop using a one inch 27 gauge hypodermic needle attached to a Hamilton 1750 gastight syringe. The needle was inserted through the foregut wall, the gas contents withdrawn, and then the volume adjusted to either 0.1 or 0.2 ml depending on the amount available within each crop. This sample was injected into a Fisher-Hamilton Model 29 gas partitioner and analyzed for CO_2, O_2, and N_2 content. The peaks obtained were compared to peaks from a known concentration as a ratio to obtain the per cent composition of the injected gas. Standard samples were run at both volumes before, in the middle, and at the end of the run.

Of the 36 cockroaches treated, 16 were found to contain sufficient gas for analysis. Of these 16, the data from five could not be used due to some unknown difficulty resulting in a total gas content far short of 100 per cent for all three components. Of the remaining 11 cockroaches, in most instances at least three samples could be obtained due to the refilling of the crop almost immediately after the first sample was obtained. Samples were withdrawn at 1/2 hour intervals from certain cockroaches to determine if there were any changes in the composition of gas with time after the initial bloat.

OBSERVATIONS AND RESULTS

Insecticidal Stress and Symptoms

Gas Accumulation in the Foregut of L. maderae. A summary of the data for the insecticide-treated Madeira cockroaches will be found in Table 3. The data taken for individual insecticide studies is found in Appendix Tables 8-14.

Table 3. Madeira cockroaches showing gas accumulation in the foregut, and prostration at time of X-ray when treated with organic insecticides.

Treatment	Number of insects	Number prostrate	Time of X-ray hours	Gas accumulation in foregut				
				None	Slight	Moderate	Heavy	Extreme
Controls (All groups)	17	1	5-92	10	4	2	1[1]	-
Dichlorvos	10	7	9-22	1	-	2	3	4
Ronnel (100 µg)	10	10	21-28	-	-	4	3	3
80 µg)	9	8	44	-	-	2	5	2
Parathion	93	55	24	2	33	26	27	5
Malathion	10	10	29-46	1	1	1	2	5
Carbaryl	10	9	22-35	-	-	2	4	4
DDT	10[2]	9	78-92	-	1	3	-	5
Lindane	10	10	30-45	-	1	-	6	3
Dieldrin	10[2]	8	36-61	-	1	-	1	7

[1] Dichlorvos control, bloat at 2.5 hours, died at 22 hours.

[2] One treated cockroach died before first X-ray.

Dichlorvos. The first symptoms of poisoning occurred at 2.5 hours with ataxia in two and prostration in two of the ten Madeira cockroaches treated. At 22 hours, one was dead after having shown moderate bloat at nine hours, four had extreme, three had heavy, and one had moderate bloat. Three treated cockroaches showed little external effect of poisoning at 22 hours although one was found to contain extreme amounts of gas and one had moderate bloating while the other showed no gas. Females with developing ova were used and several lost brood capsules. One of the two controls developed prostration with extreme bloat at five hours. Presumably, this was due to insecticide contamination, possibly from the fumigant action of dichlorvos (Table 8).

Ronnel. In the first series, ten cockroaches were treated with 100 μg of insecticide per insect. Seven were prostrate at 21 hours and all ten prostrate at 28 hours. Three cockroaches showed extreme and two had heavy bloat at that time. There was a general tendency toward a decrease in gas toward 46 hours after the initial build up. One control had moderate bloat at 28 hours and heavy bloat at 46 hours although no paralysis was noted. (Table 9).

The second series received 80 μg of insecticide per cockroach and only one set of radiographs was taken at 44 hours. Nine cockroaches were treated and all but one appeared normal at 28 hours. At 44 hours, all but one were prostrate and two showed extreme, five heavy, and two moderate bloating of the foregut. Two controls had slight bloating but exhibited no ill effects (Table 9).

Parathion. Parathion was tested on virgin L. maderae females which had been starved 24 hours. Ninety-three were treated in the course of several studies to induce bloating of the foregut for analysis of gas composition. Controls were not run with these as it was found that only a very low portion

of the controls run by Hopkins, et al. (unpublished data) showed even moderate gas accumulations in the crop. Starvation for 24 hours was necessary to clear the foregut of food so that gut material would not clog the syringe when removing samples of gas from the crop for analysis.

Of those treated, five showed extreme bloat, twenty-seven showed heavy bloat, twenty-six showed moderate bloat, thirty-three showed slight bloat, and two showed no bloat at 24 hours. About 60 per cent of the insects were prostrate in 24 hours. The remainder were either unaffected or hyperactive. (Table 3).

Malathion. Ten Madeira cockroaches were treated with insecticide and the two controls received 5 µl of acetone. Five cockroaches were prostrate at 22 hours. Only one showed moderate bloating and only one of the other four had slight gas. The remaining three had no gas accumulation. By 35 hours, all cockroaches were prostrate and three showed extreme bloat, two moderate bloat and two slight bloat, while the remaining two showed no gas. At 29 hours, only one cockroach was active, although it had extreme bloat. The controls remained normal throughout the test. (Table 10).

Carbaryl. Six of the ten treated cockroaches were prostrate at 22 hours and five showed heavy to extreme gas content. The sixth had moderate bloat. With the remaining four, three showed moderate to heavy bloat when they became prostrate at 35 hours, and all contained heavy amounts of gas at 46 hours when the fourth also became prostrate. The controls showed no gas build up. (Table 11)

DDT. At 73 hours, three of the ten cockroaches treated had become prostrate and one had died. Two of these had heavy and moderate bloat and the third had slight bloat. At 94 hours, all nine were prostrate. Six

developed extreme bloat between 85 and 101 hours, one contained moderate bloat and two had slight bloat. One control was normal while the other had slight bloat. Both had received 5 µl of acetone. (Table 12).

Lindane. Ten cockroaches were treated with lindane and two controls were given 5 µl acetone each. One cockroach became prostrate by 10 hours, but the remaining cockroaches did not become prostrate until 22 to 30 hours. Two cockroaches were found to have had extreme bloat, four had heavy bloat, two had moderate and two had slight bloat. One control developed moderate bloat at 22 hours and was dead by 35 hours. This may have been due to accidental insecticidal poisoning, althouth the other control showed no effects. (Table 13).

Dieldrin. Of the ten cockroaches treated, at 36 hours one had died and only two were prostrate, the foreguts of the latter showing extreme bloat which nearly dissipated by 53 hours. All but one of the remaining cockroaches were prostrate at 61 hours and these showed heavy to extreme bloat. The ninth cockroach became prostrate at 72 hours, at which time extreme bloat was present. The control insects did not develop any abnormal symptoms or bloating. (Table 14).

Gas Accumulation in the Foregut of P. americana. A summary of the data for insecticide-treated American cockroaches will be found in Table 4. In the initial study utilizing P. americana, three female cockroaches were treated with 30 µl of acetone and anesthetized with CO_2 for X-raying while the other group of three controls received CO_2 only. Two of the three parathion treated cockroaches were prostrate in eight hours and the third became prostrate by eleven hours. Larger amounts of gas were found to be present at both times in the two cockroaches which became prostrate first. At twenty hours, all three had severe bloat. From that time, the amount decreased in

all three until their death. The carbaryl-treated cockroaches became prostrate by 11 hours. One which was prostrate at eight hours showed extreme bloat at that time and the other two showed heavy bloat at 11 hours. Maximum bloat occurred at 24 hours and then the gas content diminished until death by 168 hours. Tremors in all three DDT-treated cockroaches were reported at 48 hours when the first signs of any volume of gas occurred. By 72 hours, one was prostrate and by 83 hours, another had been affected. It was not until 116 hours that all three cockroaches were prostrate. At this time, one had severe bloating, one had heavy bloating and the third had moderate bloating, but because of the length of time involved in knockdown, lindane was substituted for DDT in subsequent tests with P. americana.

The controls run with this group were found to have slight to moderate bloat at 24 hours, although one had heavy bloat at that time. Two of the controls treated with acetone were unable to right themselves by 168 hours and one was dead by 192 hours. The controls receiving CO_2 anesthesia only, did not show these symptoms in 192 hours.

The second series used five males and four females for treatment and had two female and one male untreated control. In the parathion series, all of the treated insects were prostrate and showed heavy or severe bloat at eight hours even though the amount of movement varied from violent, persistant tremors to only slight and occasional twitches. The male control also was found to have heavy bloating and the females had only slight bloating.

The carbaryl-treated cockroaches were prostrate at seven hours. The males showed the severest bloat with nearly the whole abdomen appearing as a pocket of gas and only a thin body wall showing (Plate II, Fig. 8). The females also showed severe bloat but not as extreme as the male (like Plate

Table 4. American cockroaches showing gas accumulation in the foregut and prostration at the time of X-ray when treated with organic insecticides.

Treatment	Number of insects	Number prostrate	Time of X-rays hours	Gas accumulation in foregut				
				None	Slight	Moderate	Heavy	Extreme
Series 1								
Controls - no treatment	3	0	8-192	-	-	1	1	1
Controls - Acetone treated	3	2	8-192	-	-	2	1	-
Parathion	3	3	8-24	-	-	-	1	2
Carbaryl	3	3	11-24	-	-	-	-	3
DDT	3	3	72-122	-	-	1	1	1
Series 2								
Parathion Treated	9	9	8-20	-	-	-	4	5
Control	3	0	8-20	-	2	-	1	-
Carbaryl Treated	9	9	7-20	-	-	-	5	4
Control	3	0	7-20	-	1	1	1	-
Lindane Treated	9	9	7-20	-	1	1	4	3
Control	3	0	7-20	-	1	2	-	-

II, Fig. 7). At 20 hours, the female controls showed slight and moderate accumulations of gas while the male had heavy bloat.

With lindane treatment, all of the males and two females were prostrate at seven hours. The males showed heavy or extreme bloat while the prostrate and one normal females had heavy bloat. Two treated females were still active at 20 hours but showed slight and moderate bloat. In the controls, the male and one female showed moderate bloat and the other female showed slight bloat.

Miscellaneous symptoms. The Madeira cockroach is considered a viviparous insect in that the ova are deposited in a brood capsule which is held within an internal brood pouch until the first instars hatch. In many instances, when female L. maderae with developing ova were treated with insecticides, they were found to extrude the brood capsule during early prostration when the body is undergoing violent tremors. During the initial test in which the concentration of dieldrin was determined, it was observed that one of the cockroaches had extruded her brood capsule and the brood pouch had remained exposed rather than being withdrawn into the abdomen. It was found to be filled with hemolymph and particles of white matter which appeared to be fat body. The radiograph showed that the foregut contained a heavy amount of gas. To determine the frequency with which the symptom might occur, twenty females were removed from the rearing tub on the assumption that many would contain brood capsules. These were treated with 50 µg of dieldrin per cockroach. In twenty-four hours, only two had evaginated the brood pouch, although several insects had lost brood capsules.

To test the effect of other insecticides, 7.5 µg of parathion was applied to nonvirgin females. Five of the eight cockroaches treated were

EXPLANATION OF PLATE IV

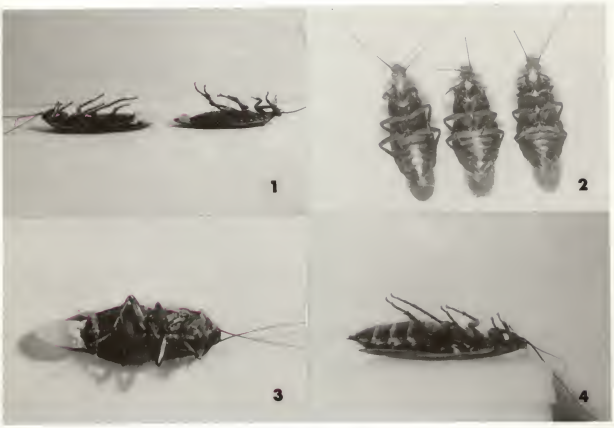
Fig. 1. Paralyzed Madeira cockroaches showing the extruded hemolymph-filled brood pouch after treatment with 7.5 µg of parathion. (24 hours).

Fig. 2. Paralyzed Madeira cockroaches showing stretching of the neck after treatment with 5 µg of Parathion. (24 hours).

Fig. 3. Paralyzed Madeira cockroach showing hemolymph-filled brood pouch extruded after treatment with 7.5 µg of parathion. (24 hours).

Fig. 4. Paralyzed Madeira cockroach showing great distention of abdomen after treatment with electric shock. (3 days). Radiograph of same cockroach in Plate III, Fig. 14.

PLATE IV



found to have the brood pouch extruded (Plate IV, Fig. 1 and 3). In three of the five, the pouch had dried considerably but one had the hemolymph filled pouch extruded about 1/4 inch from the abdomen. The fifth cockroach had its pouch extended approximately 1/2 inch and the white masses in the hemolymph were readily visable. Unfortunately, radiographs were not taken of these cockroaches to determine if gas accumulation may have been a factor in pushing out the pouch.

In one case, a dichlorvos-treated cockroach was observed to have a protruding brood pouch and an X-ray was taken. This showed the insect to have extreme bloat, but because it was an isolated incident, no conclusions on the simultaneous occurrence of the two symptoms can be drawn.

Often during advancing prostration, Madeira cockroaches were observed to have the head extended far forward causing the neck to be stretched (Plate IV, Fig. 2). It was at first thought that this was caused by gas pressure within the crop of the insect, but in comparing the symptom with the gas content of the crop, it appeared to be independent of extreme bloat, since in several cases none was found. Therefore, it was most likely due to the contraction of the neck muscles, similar to muscle contraction observed in the legs of poisoned cockroaches due to the disruption of nervous control.

Ligation Study

To determine if the gas in the foregut originated from swallowing of air by the intoxicated cockroaches ligation experiments were performed. Forty-six cockroaches were ligated at the neck with cotton thread. Twenty-five received 2.5 µg of parathion per insect and the remaining twenty-one were used as nontreated, ligated controls. In twenty-four hours, twenty-

three of the treated cockroaches were prostrate and five showed extreme bloat and three, moderate bloat. Externally, extreme bloating would appear similar to the severely distended abdomen of the electrically stressed cockroach (Plate IV, Fig. 4). At 96 hours, one cockroach not showing bloat previously had a moderate amount of gas. Another which had severe bloat at 24 hours had increased the amount sufficiently so that it appeared to force out the hindgut (Plate II, Fig. 13).

Behavior of the ligated controls was modified due to the complete elimination of nervous connection and neurosecretion from the grain. The insects would jump nervously when probed but would rarely run. When handled, they would cling tenaciously to anything offered them and refuse to move or show any escape behavior. Often it was observed that still insects would rub the tip of their abdomen on the wings in a side-to-side motion.

One ligated control showed slight bloat at 24 hours and extreme bloat in 96 hours. This insect differed from treated cockroaches in that it maintained some mobility even though ligation and the distended abdomen restricted it considerably. The remaining ligated controls failed to develop any sign of gas in the foregut.

A second ligation series used thirty-six cockroaches in groups of nine. One group was ligated and treated with 2.5 µg of parathion, the second group was ligated controls, the third group received parathion treatment only, and the fourth group served as untreated controls. All but one of the ligated and treated cockroaches were prostrate at 28 hours. The active cockroach failed to become prostrate through 100 hours. At 100 hours, none of the ligated-treated or the ligated-untreated cockroaches showed any bloating. The parathion-treated cockroaches showed two with extreme bloat, five with

heavy bloat and two with slight bloat. Three of the untreated controls had slight amounts of gas and two showed moderate bloat.

Physical Stress Symptoms

Gas Accumulation in the Foregut of L. maderae. A summary of the data for physically stressed Madeira cockroaches will be found in Table 5.

Turntable. Sixteen cockroaches were forced into activity on the turntable, while eight insects were placed in pint jars as controls. At the end of three days, the cockroaches were removed from the turntable and X-rayed. Most did not become prostrate in this time, but were weakened to the point that they only attempted escape movements showing some co-ordination when they were probed.

The radiographs showed that one cockroach had heavy bloat, three showed moderate bloat, and remaining four showed pockets of gas (of slight proportions) in the crop.

Other changes were noticed in the turntable exercised cockroaches. The midgut would often show a coil filled with gas or a small pocket would accumulate in the rectum. In some cases, this was in addition to the larger pocket found in the foregut. The tarsi and antennae would usually become brittle after treatment on the turntable, probably due to the abrasive action of the masonite surface. The exhausted insects would lie motionless on the moving table for irregular intervals of time between the enforced walking.

Two of the controls died from unknown causes and one cockroach died during the three days on the turntable.

Powder Mill. Twenty-four 2 to 4 week-old virgin female L. maderae cockroaches were placed in the jars and rotated for five to six days. At the end

of this time, X-rays showed that bloat was a common occurrence. Five of those treated died before being X-rayed. Extreme bloat occurred in four of the 19 remaining physically-stressed cockroaches. These cockroaches were weaker than the others. Nine showed heavy bloating while the remainder showed only slight or moderate bloating. One group of controls also had moderate amounts of gas in the crop but differed from the treated cockroaches in that they did not show dilated trachea and generally appeared denser in the radiographs than those which had undergone activity. The fat body and hemolymph appeared to have been depleted by the enforced activity. When stressed cockroaches were dissected, they were observed to contain less hemolymph than normal cockroaches and the fat body seemed less extensive. Both factors may account for a loss of weight amounting to a 27.1 per cent average for four stressed cockroaches. Two controls held for the same length of time lost only 8.7 per cent of their initial weight. Often the cuticle of those treated was dry and hard at the end of six days, as was observed in the turntable-stressed cockroaches. This was thought to be due to the physical abrasion of the cuticle. Prostration was expressed by a general weakening of the insect. Co-ordinated struggling movements were possible, but the insect was unable to right itself when inverted and would make only feeble, unsuccessful movements to escape when in the normal position.

Electrical Stress. Two groups of Madeira cockroaches were electrically stressed. Both groups consisted of six cockroaches with one normal control, two controls with implanted electrodes and three with implanted electrodes attached to the inductorium. The first series was shocked for two days and then released into pint jars. One treated cockroach died and the other two were prostrate when released. One, which showed no bloat 24 hours after

Table 5. Madeira cockroaches showing gas accumulation in the foregut when subjected to several types of physical stress.

Treatment	Number of insects	Mortality before X-ray	Number prostrate	Time of X-ray days	Gas accumulation in foregut				
					None	Slight	Moderate	Heavy	Extreme
Turntable Controls	8	2	0	3	6	-	-	-	-
Treated	16	1	0	3	-	11	3	1	-
Powder Mill Controls	12	1	0	5-6	5	4	2	-	-
Treated	24	5	7	5-6	-	3	3	9	4
Electrical Shock Control I[1]	4	0	0	3	1	2	1	-	-
Control II[2]	2	0	0	3	2	-	-	-	-
Treated	6	2	1	3-5	-	1	-	2	1
Desiccant[3] Controls	4	0	0	1	4	-	-	-	-
Treated	20	5	12	1	2	9	4	-	-
Treated[4]	4	1	3	2	-	-	3	-	-
Immobilized Controls	2	0	0	7	2	-	-	-	-
Treated	9	1	0	7	3	3	1	1	-

1/Electrodes implanted in head but no electrical shock.
2/No electrodes.
3/Diatomaceous earth, JG-109 supplied by John Galt, Texas, San Antonio.
4/Tracheal dialation study.

release, became fairly active although it could not right itself. The other remained paralyzed and developed a severely distended abdomen and an unusually high gas content (Plate II, Fig. 14, and Plate IV, Fig. 4).

The second series received the shock treatment for four days before release and were X-rayed 24 hours later. Again, one of the treated cockroaches died. The other two were able to walk slowly, but were weakened so they were unable to turn over. One cockroach showed heavy bloat and the other, moderate amounts of gas.

One control of the group of four with implanted electrodes showed moderate amount of bloat and the other three only slight amounts of gas. The untreated controls remained normal.

Immobilization. Of the nine Madeira cockroaches immobilized for eight days, eight survived. One was found to have severe bloat, four moderate bloat, while the remaining four had no gas in the foregut. The trachea were not dilated and none of the surviving cockroaches showed any signs of paralysis, although some were weaker than the controls. The two controls showed no sign of gas accumulation.

Desiccant Study. Of the group of 20 cockroaches treated with diatomaceous earth, fifteen survived for 24 hours. Only four contained a moderate amount of bloat. Three of these were prostrate and the fourth still showed normal activity. The remaining nine prostrate cockroaches showed only slight bloat and the two with normal activity and the controls had no sign of gas. Activity of the prostrate cockroaches consisted of occasional small stiff movements of the legs. These showed co-ordination and no tremors, but were weak and made only with great difficulty.

A second group was treated for tracheal dilation studies and is discussed in that section.

Gas Accumulation in the Foregut of P. americana. A summary of the data for physically stressed American cockroaches is found in Table 6.

Turntable. Four American cockroaches were stressed on the turntable for three days. Three showed heavy bloat and one had extreme gas accumulation at that time. One cockroach was prostrate and the other three showed nearly normal activity and would run for cover when disturbed. The exhausted cockroach was unable to right itself and could make only feeble, but coordinated leg movements. The tegmina and cuticle tended to be hard and dry rather than have the normal soft, waxy texture. One control died for unknown reasons before the third day and the other showed no gas accumulation.

Powder Mill. Fourteen of the sixteen cockroaches treated survived three to four days in the powder mill. All four of the insects showing heavy bloating were prostrate. Of the ten with moderate bloat, six were prostrate and four showed normal activity. The remaining two cockroaches with slight and no gas, were normally active. Prostration and cuticle appearance were similar to those on the turntable. In both stresses, tarsi would often become hard and brittle.

Immobilization. Two series of P. americana were immobilized (Table 6). The first series consisted of four cockroaches which were released after six days. One was dead, and the three that remained were placed in a pint jar and held for 24 hours. At that time, two showed prostration; the third was still active. Radiographs of the three immobilized cockroaches showed they had heavy cases of bloat. A control removed from the main colony had no gas accumulation.

In the second series, twelve cockroaches were pinned down as before. Four controls were placed in pint jars. At four days, four were removed from the balsa block. Upon release, all cockroaches appeared to be fairly

Table 6. American cockroaches showing gas accumulation in the foregut when subjected to several types of physical stress.

Treatment	Number of insects	Mortality before X-ray	Number prostrate	Time of X-ray days	Gas accumulation in foregut				
					None	Slight	Moderate	Heavy	Extreme
Turntable Controls	2	1	0	3	1	-	-	-	-
Treated	4	0	1	3	-	-	-	3	1
Powder Mill Controls	8	0	0	3-4	4[1]	3	-	-	-
Treated	16	0	9	3-4	1	1	10	4	-
Immobilization Controls	4	0	0	9	-	-	-	3	1
Series 1 7 day treated	4	1	2	7	-	-	-	3	-
Series 2 4 day treated	4	0	4	9	-	-	-	1	3
8 day treated	8	5	0	9	-	-	-	1	2

[1] One control undetermined due to blurring of X-ray.

normal in activity. The remaining cockroaches were removed at eight days, placed in pint jars and held 24 hours. At that time, all cockroaches were X-rayed. The cockroaches which had been released at four days were alive but had gradually slipped into a prostrate state by eight days. Five of those released at eight days were dead and the remainder moved somewhat stiffly, but were not in a prostrate state. All four controls were normally active after nine days without food or water.

Bloat was most severe in three of those cockroaches which were released at four days. Their abdomens had shown some distention before X-raying. The fourth insect had less gas but was near death and could only make slight movement of palps. Those cockroaches which were released at eight days showed lesser amounts of gas but there was still heavy bloating. All of the controls also possessed moderate to heavy amounts of gas.

Tracheal Dilation. Examination of the X-rays of physically stressed Madeira cockroaches showed spindle shaped pockets of gas in the region of the meso and metathoracic coxae (Plate II, Fig. 10 and 12). This was observed in the weaker individuals in all of the physically induced stresses except electrical shock and immobilization and was assumed to be a dilation of the trachea in the coxal area of the leg. The same symptom to a lesser extent was found in cockroaches in late prostration from treatment with ronnel, malathion, DDT, and dieldrin. It was most quickly induced in the desiccant studies and could be easily used to determine the external condition of the insect from the X-ray. Bloating of the foregut was variable in desiccant-treated cockroaches, but the dilation of coxal tracheae was always present and increased with the progression of prostration.

A second group of desiccant-treated cockroaches was set up for dissection to determine if the coxal tracheae were indeed inflated with gas. Dissection of normal cockroaches had indicated that there was a large trachea in the coxa, probably for aeriation of the muscles of the coxa, femur, and tibial regions. This is referred to here as the coxal trachea. The treated cockroaches were freed of large accumulations of desiccants before being placed in jars. A radiograph at 36 hours showed one cockroach to have heavy bloating, one moderate bloating, and two with slight accumulations of gas, but only one cockroach showed any stiffening of movements. By 48 hours, one cockroach had died and the other three were prostrate and able to make only stiff movements. The gas content of the crop was moderate but the tracheal region showed great dilation. Dissection of the region showed that the coxal muscles had diminished in size with the coxal trachea inflated and filling the entire space. The muscles were not as moist with hemolymph as they normally were and this may have been a contributing factor to the shrinkage of the muscle.

Gas Analysis

Twenty-four samples were taken from 11 Madeira cockroaches treated with parathion. Eight yielded two or more samples because of rebloating of the crop after dissection. Little movement of the mouthparts was observed during rebloating, but the bubble of gas could be easily seen forming in the foregut. The lowest concentration of CO_2 found in the gut was 0.53 per cent or about 17 times larger than the CO_2 concentration of air and the maximum 7.23 per cent or about 240 times the content of air (Table 7). Both were first samples removed. The average of 11 first samples from the cockroach was 2.32 per cent. The average nitrogen content was about two to three per

cent higher than the normal percentage in air. Oxygen tended to vary inversely with the content of CO_2. Removal of gas at 1/2 hour intervals from rebloating cockroaches resulted in an average CO_2 content of 1.41 per cent with a range from 0.76 to 2.16 per cent.

Eight cockroaches were also placed on the powder mill and rotated for seven days. Six insects survived showing weak, but otherwise normal, activity and the foreguts contained sufficient gas for analysis. Greater care was needed for the dissection, as the cockroaches were generally thinner than the Parathion-poisoned cockroaches and the foregut was closer to the body wall. The foreguts were found to contain a smaller volume of gas than the latter, but the average percentage of CO_2 was 3.62 per cent, about one per cent higher than the chemically-stressed cockroaches. Rebloating did not occur so this data is based on the initial sample taken which was often found to be higher than subsequent samples.

DISCUSSION AND CONCLUSIONS

Abnormal accumulations of gas in the alimentary tract induced by dissimilar stressors provides more evidence for the occurrence of a stress syndrome in insects. Gas accumulation and bloating of the foregut has been the result of exposing two species of cockroach to a wide variety of stressful situations. The organophosphorus and carbamate insecticides as cholinesterase inhibitors, the organochlorine compounds which act in other sites in the nervous system, and physical stressors including enforced activity, immobilization, and electrical shock, produce this symptom in common. The exact cause of the gas accumulation has not been determined, but the composition of the gas has been explored. The extent to which flatulence appears as a symptom has been expanded from its occurrence with

Table 7. Per cent composition of gas found in the bloated foreguts of parathion-intoxicated and physically-stressed Madeira cockroaches as compared with air.

	CO_2	O_2	N_2
Parathion intoxication			
First sample			
Average of 11 samples	2.32	17.62	79.71
Range of 11 samples	0.53 - 7.23	8.76 - 20.46	76.63 - 85.83
Immediate resampling			
Average of 3 samples	0.79	21.14	81.33
Range of 3 samples	0.77 - 0.83	20.78 - 21.75	80.23 - 83.01
30 minute resampling			
Average of 10 samples	1.41	19.59	80.54
Range of 10 samples	0.76 - 2.16	17.64 - 20.78	78.31 - 82.57
Powder mill stress			
Average of 3 samples	3.62	13.01	83.23
Range of 3 samples	3.29 - 3.79	12.11 - 13.77	81.31 - 84.62
Air [1]	0.03	20.99	78.03

[1] Data taken from Handbook of Chemistry and Physics, 39th ed., Chem. Rubber Co., Cleveland. 3213 p., 1957-1958.

parathion intoxication to an association with a larger number of insecticides and for the first time with physical stressors.

In general, insecticidal stress resulted in a high incidence of moderate to extreme bloating of the foregut from all insecticides tested. The parathion data on the Madeira cockroach appeared to show a lower incidence of bloating than the other insecticides. This was because only a single radiograph was taken at 24 hours to determine bloating for gas analysis. A lower percentage were prostrate at this time interval and if the remainder had been allowed to progress to prostration or paralysis, a much greater number of insects would have displayed the symptom as was previously demonstrated by Hopkins et al. (unpublished data).

The American cockroach displayed unusual susceptibility to bloating with all types of stress treatments. Several factors may be important: (1) Generally higher sensitivity to all forms of stress including deprivation of food and water, immobilization, and prolonged insecticide intoxication; (2) Physiological factors including age, sex, and reproductive state were not controlled as closely as with L. maderae; or (3) General behavioral differences between the two species and their relative threshold of sensitivity to stimuli. The Madeira cockroach was more resistant to physical stress than the American cockroach under immobilization and enforced exercise.

There is a remarkable difference in the behavior of the two cockroaches, both normally and under conditions of stress. P. americana tends to be highly nervous and makes rapid escape movements at the slightest stimulation. The Madeira cockroach, on the other hand, is more docile, less easily disturbed and does not move as rapidly. When placed on the turntable, the American cockroach would walk almost continuously until exhaustion, while the former would rarely walk for more than an hour and would then fold its legs, rest

against the side of the petri dish, and slide until disturbed when it would again walk for a short interval. During immobilization, the American cockroach struggles more violently against the restraining pins than the Madeira cockroach, which does not become prostrate by that method of stressing. Beament (1958) found that immobilization of Rhodnius, a slow moving Reduviid, would not result in prostration because it would not struggle when pinned down. The greater struggling and nervous activity of the American cockroach would seem to be an important factor in its susceptibility to stressing. Further evidence that the behavior of the Madeira cockroach may play a significant part in its response to physical stress is the observation that the most frequent prostration occurred in the powder mill, electric shock, and desiccation treatment in which the cockroach had little means of escape from the stressor. Immobilization, dependent on struggling, and the turntable were less successful since the cockroach did not find activity imposed on it and little struggling was necessary.

The sensitivity of the American cockroach to treatment is shown by the high incidence of heavy or extreme bloating when subjected to physical stresses. The Madeira cockroaches tended to show more bloating in the slight to moderate range under physical stressors. Chemical stress also displayed a similar trend with the additional observation that there is a higher frequency of bloating in the controls of the American cockroaches than Madeira controls. This is likely due to sensitivity to starvation and thirst of the former which also must be considered stressors.

One difficulty of enforced exercise as a physical stress, is that it can result in severe tumbling and abrasion of the insect, causing the loss of legs, hardened cuticle, abraded tarsi, and a general disruption of the

exoskeleton. This could affect water balance as the outer layers of the cuticle are important in preventing water loss. The desiccation studies would indicate that bloating may occur even under conditions of high cuticular water loss. Physical abrasion during other types of physical stressing may contribute to the appearance of symptoms, as they do when desiccants are used.

In general, gas accumulation appears to accompany prostration in insecticide-treated Madeira cockroaches. However, it was found that gas may be present without symptoms of poisoning, and slight or even moderate accumulations of gas may occur when no other stress symptoms are manifested in both treated and control cockroaches. Small amounts of gas may be considered normal, but larger accumulations such as heavy or extreme bloating in an active cockroach may indicate that only part of the syndrome is being manifested, or that the other symptoms are being masked in a resistance phase. Prostration may also occur without the presence of gas. This may be due to a rapid progression of symptoms to paralysis by passing the extended prostration phase in which the insect struggles and undergoes tremors, or to regurgitation of the gas during anesthesia or handling. Intermittant bloating has been observed in insecticide-intoxicated cockroaches.

The problem of the source of gas present within the crop has not been solved by this study but two possibilities now exist to explain the symptom. Gas may be swallowed as suggested by Salkeld (1951), Wiesman and Kocher (1952) and Jochum (1956), or enter the crop by gas diffusion through the trachea. The evidence presented by the neck ligation study is insufficient to prove that swallowing is not the cause. The fact that ligated-treated cockroaches showed a much smaller bloating frequency when compared to nonligated-treated cockroaches, indicated that swallowing could be important. This does not

explain why eight of twenty-five ligated-treated cockroaches did show gas accumulation while the ligated-nontreated controls generally did not.

The possibility exists that the gas may enter the crop via the trachea which supply the alimentary tract in large numbers. The gut wall, under the influence of poisoning, could develop a change in permeability to gas so that it would be taken into the crop but not allowed to escape through the trachea again, the normal route of CO_2 excretion. If this was a primary cause, then the ligation experiments should have showed an incidence of bloating similar to that of nonligated-treated insects. Here the function of the brain is unknown and, as the ligations completely eliminated blood through the head as well as nervous connection to the brain, neurosecretion from the brain could have unknown effects. In one series, paraffin was used to block the mouth and allow the nervous system to remain in tact. This was unsuccessful as the insects worked the wax loose before they became prostrate, so that blockage of the alimentary canal was doubtful. If this line of experimentation was followed, and a material used which was less likely to be loosened by the insects, much light could be shed on the cause of bloating as well as the involvement of neurosecretion.

When rebloating occurred in the dissected cockroaches, little, if any, simultaneous movement of the mouthparts was observed. The violent movement reported by Wiesman and Kocher (1952) to be responsible for swallowing the gas does not seem entirely necessary for crop filling. The trachea were not completely pulled away from the crop of the dissected cockroach so that this may still represent a source for the gas. Further testing such as the ligation of rebloating cockroaches or tracheal blockage or removal could be used to elucidate the phenomenon of bloat.

In view of the possible importance of metabolism to the gas composition, it is interesting to note that the CO_2 concentration of the initial sample taken from the crop was higher than that removed immediately after rebloating and at 30 minutes. One would expect that if metabolism was contributing to the gas composition, then CO_2 would increase with a concomitant decrease in O_2 as time elapsed. This was indicated in the present study. It has not been shown how soon cockroaches on the powder mill might develop bloating, but several days of constant motion would likely result in greater concentrations of CO_2 than when the samples were taken 24 hours after treatment. Salkeld (1951) reported histological changes in the secretory cells in the midgut of the honeybee poisoned with DDT but not with parathion. Jochum (1956) also found histological changes indicating breakdown of the epithelium of the midgut, salivary glands and malpighian tubules with parathion treatment of the American cockroach. The parathion treated bees reported by Salkeld (1951) died within 30 minutes, so rapidly that cellular changes may not have had sufficient time to take place. The modification of the cells may indicate an increase in metabolism due to the presence of the poisoning factor. An increase in metabolism of the epithelium would likely increase the output of CO_2 which may be the cause of increased concentration of CO_2 in the bloated foregut.

Brood pouch extrusion was associated only with female Madeira cockroaches with developing ova. It was most frequently observed in the studies used to determine insecticide dosage, as a large percentage of cockroaches from the main colony used in these range finding tests contained egg capsules. The cause of this extrusion was difficult to determine, but in view of its frequency in cockroaches receiving three times the usual dose of parathion, it may be associated with the rapid development of symptoms of

poisoning perhaps caused by increased hydraulic pressure of the blood during tremors. This may evaginate the brood pouch beyond the distance from which it would normally be retracted. The part that foregut bloating might play in the evagination of the pouch is unknown, but it appears in one case that extreme bloating in the foregut of a ligated cockroach was a factor in causing evagination of the hindgut. Displacement by the expansion of the foregut was suggested because the distended brood pouch was always filled with hemolymph.

Dilation of the tracheae in the coxal segments occurred as a result of both physical stress by enforced activity or desiccation and by prolonged intoxication by insecticides. A change in permeability may occur which prevents the air from entering the body cells thus causing a build up of the gas within the tracheal system, the pressure forcing the muscles apart. However, only the coxal trachea become distended. The other possibility can be explained through water loss which results in the drying and shrinking of the muscles. The adherence of the tracheal wall to the muscle would cause a pocket of gas to form. This would be the most likely explanation, since dilation usually occurs in the late stages of insecticide poisoning, physical stress, and desiccation, when water loss would be expected to be pronounced.

SUMMARY

1. This study was designed to determine the correlation of foregut bloating in the Madeira cockroach under insecticidal stress with the major groups of synthetic organic insecticides: organophosphorus compounds, carbamates, and organochlorine compounds; and with physical stress by enforced activity on a turntable and powder mill, immobilization, electrical shock, and desiccation. The American cockroach was used for comparison in some

tests. Other experiments were designed to determine the source and content of the gas present, and observations were made on other symptoms associated with poisoning and autointoxication. This might provide further evidence for a stress syndrome common to physical and insecticidal stressing in insects.

2. Bloating was detected through the use of X-ray techniques. Acetone solutions of the eight insecticides used were applied topically with a microdrop applicator. Physical stress was applied with a turntable, powder mill, an inductorium with electrodes implanted in the insect's head, and a diatomaceous earth desiccant. Gas composition of the foregut contents was analyzed with a Fisher-Hamilton model 29 gas partitioner.

3. Moderate to extreme quantities of gas were found in the foreguts of both L. maderae and P. americana when subjected to all insecticides used. The greatest quantities of gas were usually found after prostration, but a loss of gas was often noticed as the insect progressed toward death.

4. Physical stress resulted in fewer cases of extreme bloating in the Madeira cockroach. Desiccation and enforced exercise in the powder mill caused the highest prostration, while turntable activity and immobilization were not effective in inducing prostration. The heaviest bloating occurred with powder mill and electrical shock treatments. The American cockroaches showed heavy bloating and high incidence of prostration with turntable, powder mill, and immobilization stresses. It was hypothesized that the difference in behavior between the two cockroaches may have been a primary factor in the difference in response to physical stress. The Madeira cockroach is much more docile and unreactive to stimulation than the American cockroach.

5. Gas analysis showed that the crop gas accumulation was composed of CO_2, O_2, and N_2. In insecticidally stressed Madeira cockroaches, CO_2

averaged 2.32 per cent for an initial sample and 1.41 per cent for a sample taken 30 minutes after rebloating. The average CO_2 content of physically-stressed cockroaches was 3.62 per cent. Oxygen content varied inversely with the CO_2 content while nitrogen averaged 81 per cent or slightly above atmospheric nitrogen.

6. Ligation experiments showed that eight out of twenty-five cockroaches ligated at the neck and treated with parathion developed bloating without being able to swallow. This was not as high a frequency as that shown by the treated and nonligated cockroaches but still indicated that swallowing may not be completely responsible for gas accumulation in the foregut. Permeability changes in the gut wall to gases from metabolism and the tracheal system are suggested as another possibility to explain gas accumulation.

7. Other symptoms of insecticidal stress were evagination of the brood pouch filled with hemolymph from female L. maderae with developing ova, and stretching of the neck during prostration. Dilation of the coxal trachea commonly occurred in the Madeira cockroaches subjected to physical stress and occurred less frequently in the later stages of insecticidal stress. This symptom appeared to be related to water loss after long periods of exercised desiccation due to cuticular abrasion.

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APPENDIX

Table 8. The external and internal symptoms of Madeira cockroaches treated with dichlorvos. Number and letter designations indicate gas accumulation and external symptoms respectively 1/

Insect No.	Time of X-ray - Hours			
	2.5	5	9	22
Control 1	4-N	4-Pr	4-Pa	D
Control 2	1-N	1-N	1-N	1-N
Treated 1	1-N	2-N	3-N	4-N
2	1-H	2-N	2-Pr	5-Pa
3	1-Pa	1-Pa	1-Pa	4-Pa
4	1-N	2-Pr	4-Pr	4-Pr
5	1-Pr	1-Pr	1-Pr	5-Pr
6	1-N	1-N	5-Pr	5-Pr
7	1-N	2-N	3-N	3-N
8	1-N	1-N	1-N	1-N
9	2-N	3-Pr	4-Pr	5-Pr
10	3-H	3-Pr	3-Pr	D

1/ Gas Accumulation

1. None
2. Slight
3. Moderate
4. Heavy
5. Extreme

External Symptoms

N. Normal
H. Hyperactive
Pr. Prostrate
Pa. Paralyzed
D. Dead
s. Stretching of the neck
Tr. Dilation of the coxal trachea

Table 9. The external and internal symptoms in Madeira cockroaches treated with ronnel. Number and letter designations indicate gas accumulation and external symptoms respectively[1].

Insect No.	Time of X-ray - Hours			
	Series 1 (100 µg)			Series 2 (80 µg)
	21	28	46	40
Control 1	2-N	2-N	3-N	2-N
Control 2	1-N	1-N	1-N	1-N
Control 3				2-N
Treated 1	5-Pa	5-Pa	5-Pa	4-Pr
2	3-Pa	3-Pa	D	3-Pr
3	4-Pr	1-Pr	1-Pa	4-N
4	2-H	3-Pr	2-Pa	4-Pr
5	4-Pr	5-Pa	D	3-Pr
6	3-Pr	2-Pr	D	5-Pr
7	3-Pr	1-Pr	3-Pa	5-Pr
8	5-N	5-Pr	2-Pa^{Tr}	4-Pr
9	4-H	4-Pr	1-Pa^{Tr}	4-Pr
10	3-Pr	4-Pr	3-Pa^{Tr}	

[1] See footnote 1, Table 8.

Table 10. The external and internal symptoms in Madeira cockroaches treated with Malathion. Number and letter designations indicate gas accumulation and external symptoms respectively 1/

Insect No.	Time of X-ray - Hours			
	22	29	35	46
Control 1	1-N	1-N	1-N	1-N
Control 2	1-N	1-N	1-N	1-N
Treated 1	2-N	1-Pr	4-Pr	3-Pa
2	2-Pr^{S}	4-Pr	5-Pr	D
3	1-Pr^{S}	1-Pr^{S}	1-Pr^{S}	1-Pa
4	2-Pr^{S}	1-Pr^{S}	4-Pr	5-Pa^{Tr}
5	3-N	2-Pr	5-Pr	3-Pa
6	3-Pr	1-Pr	3-Pr	D
7	1-H	5-H^{S}	2-Pr	3-Pa
8	1-H	2-Pr	1-Pr^{S}	2-Pa
9	1-H	2-Pr^{S}	5-Pr^{S}	5-Pa^{Tr}
10	1-Pr^{S}	4-Pr^{S}	2-Pr	1-Pa^{Tr}

1/ See footnote 1, Table 8.

Table 11. The external and internal symptoms in Madeira cockroaches treated with Carbaryl. Number and letter designations indicate gas accumulation and external symptoms respectively [1].

Insect No.	Time of X-ray - Hours			
	22	28	35	46
Control 1	1-N	1-N	1-N	1-N
Control 2	1-N	1-N	1-N	1-N
Treated 1	5-Pr	5-Pr	4-Pa	4-Pa
2	5-Pr	5-Pa	2-Pa	2-Pa
3	5-Pr	5-Pa	2-Pa	5-Pa
4	4-Pr	3-Pa	2-Pa	2-Pa
5	3-Pr	4-Pa	3-Pa	2-Pa
6	5-Pr	4-Pa	4-Pa	4-Pa
7	2-N	2-N	4-Pa	4-Pa
8	3-N	3-N	3-N	4-Pr
9	3-N	3-N	3-Pa	4-Pa
10	1-N	3-N	4-Pa	4-Pa

[1] See footnote 1, Table 8.

Table 12. The external and internal symptoms in Madeira cockroaches treated with DDT. Number and letter designations indicate gas accumulation and external symptoms respectively 1/

Insect No.	Time of X-ray - Hours				
	73	78	85	92	101
Control 1	1-N	2-N	2-N	1-N	1-N
Control 2	1-N	1-N	2-N	2-N	2-N
Treated 1	3-N	2*	2-N	2-Pr	5-Pa^{Tr}
2	4-Pr	4*	5-Pr	4-Pa^{Tr}	2-Pa^{Tr}
3	1-N	3*	2-N	2-Pr^{Tr}	2-Pa^{Tr}
4	1-Pr	1*	2-Pr	5-Pa^{Tr}	2-Pa^{Tr}
5	1-N	2*	3-Pr	2-Pa	3-Pa
6	3-Pr	3*	2-Pr	2-Pa^{Tr}	2-Pa^{Tr}
7	D	D	D	D	D
8	2-N	3*	4-N	5-Pr^{Tr}	5-Pa^{Tr}
9	1-N	1*	1-N	5-Pr	3-Pa^{Tr}
10	3-N	4*	5-N	2-Pr^{Tr}	5-Pa^{Tr}

* No observation.

1/ See footnote 1, Table 8.

Table 13. The external and internal symptoms in Madeira cockroaches treated with Lindane. Number and letter designations indicate gas accumulation and external symptoms respectively [1].

Insect No.	Time of X-ray - Hours					
	10	22	30	35	45	53
Control 1	1-N	1-N	1-N	1-N	1-N	1-N
Control 2	1-N	3-N	3-N	D	D	D
Treated 1	1-N	1-H	4-Pr	3-Pa	3-Pa	2-Pa
2	1-N	2-H	2-Pr	4-Pa	2-Pa	5-Pa^{S}
3	1-N	1-H	3-Pr	4-Pa	3-Pa^{S}	2-Pa
4	1-N	2-H	2-Pa	4-Pa	5-Pa^{S}	4-Pa^{S}
5	2-Pr	3-Pa	2-Pa	2-Pa	2-Pa	2-Pa
6	2-N	4-H	2-Pa	3-Pa	5-Pa^{S}	2-Pa^{S}
7	2-N	2-Pa	4-Pa	3-Pa	2-Pa	3-Pa^{S}
8	2-N	4-Pa	4-Pa	3-Pa	2-Pa^{S}	2-Pa
9	2-N	5-Pa	4-Pa	2-Pa	3-Pa	2-Pa^{Tr}
10	1-N	2-Pa	5-Pa	D	D	D

[1] See footnote 1, Table 8

Table 14. The external and internal symptoms in Madeira cockroaches treated with Dieldrin. Number and letter designations indicate gas accumulation and external symptoms respectively 1/.

Insect No.	Time of X-ray - Hours					
	36	45	53	61	72	78
Control 1	2-N	1-N	1-N	1-N	1-N	1-N
Control 2	2-N	2-N	3-N	2-N	2-N	1-N
Treated 1	D	D	D	D	D	D
2	3-H	5-Pr	4-Pr	3-Pr	4-Pr	4-Pa
3	2-N	4-Pr	5-Pr	5-Pr	2-Pr	2-Pa
4	1-N	4-N	4-Pr	5-Pr	5-Pa	D
5	5-Pr	4-Pr	2-Pr	2-Pr	2-Pr	2-Pa^{Tr}
6	5-Pr	5-Pr	3-Pr	2-Pr	3-Pr	3-Pa
7	1-N	2-N	4-N	4-Pr	3-Pr	3-Pa
8	1-N	2-N	1-N	2-H	5-Pr	4-Pa
9	1-N	2-N	2-H	5-Pr	4-Pr	3-Pa
10	1-N	3-N	5-Pr	5-Pr	3-Pr	3-Pa

1/ See footnote 1, Table 8.

PHYSICAL AND INSECTICIDAL STRESS EFFECTS ON THE FOREGUT OF THE COCKROACHES, LEUCOPHAEA MADERAE (FABR.) AND PERIPLANETA AMERICANA (L.)

by

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The Madeira cockroach, Leucophaea maderae (Fabr.), was subjected to chemical stressors from the major groups of organic insecticides which included dichlorvos, ronnel, parathion, malathion, carbaryl, DDT, lindane, and dieldrin; and physical stress imposed through enforced activity on a powder mill and turntable, struggling by immobilization, electrical shock, and desiccation by cuticular abrasion to determine the correlation of foregut bloating with stress. The American cockroach, Periplaneta americana (L.), was used for comparison in some tests. Other experiments were designed to determine the source and content of the gas present, and observations were made on symptoms associated with poisoning and autointoxication. The presence of gas was detected by X-ray techniques and gas analysis of the foregut contents was made with a Fisher-Hamilton gas partitioner. A scale of none, slight, moderate, heavy, and extreme bloating was established on the basis of X-ray density and gas volume.

Insecticidal treatment of both L. maderae and P. americana resulted in moderate to extreme quantities of gas and a high incidence of prostration with all insecticides tested.

Physical stress with a powder mill and electrical shock were successful in producing the heaviest bloating in L. maderae. Desiccation by inert dusts and the powder mill activity produced the greatest number of prostrate insects. The American cockroach developed heavy bloating and high incidence of prostration with the turntable, powder mill, and immobilization stresses. This may be attributed to the more docile nature and lower sensitivity to stimulation of the Madeira cockroach.

Gas analysis revealed that the crop gas accumulation of insecticidally stressed Madeira cockroaches contained an average of 2.32 per cent CO_2 for

an initial sample and 1.41 per cent for a sample taken 30 minutes after rebloating as compared to 0.03 per cent in air. The average CO_2 content of cockroaches physically stressed on the powder mill was 3.62 per cent. Oxygen content varied inversely with the CO_2 content while nitrogen averaged 81 per cent or slightly higher than atmospheric nitrogen. The high CO_2 tensions in the foregut may indicate an abnormal level of cellular respiration combined with changes in membrane permeability to diffusing gases.

Of twenty-five Madeira cockroaches treated with parathion and ligated at the neck to prevent swallowing, eight developed the bloating symptom. This was not as high a frequency of bloating as that shown by treated - nonligated cockroaches but indicated that swallowing may not be the only source of gas accumulation in the foregut. Permeability changes in the gut wall to gases from metabolism and in the tracheal system may also account for the bloating.

Other symptoms of insecticidal stress were evagination of the brood pouch filled with hemolymph from female Madeira cockroaches with developing ova, and stretching of the neck during prostration. Dilation of the coxal trachea commonly occurred in Madeira cockroaches subjected to physical stress particularily by desiccation and was observed less frequently during late prostration from insecticidal stress.