# CARBOHYDRATE INVOLVEMENT DURING DDT POISONING OF THE AMERICAN COCKROACH, PERIPLANETA AMERICANA

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This is to certify that the

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ABSTRACT

CARBOHYDRATE INVOLVEMENT DURING DDT POISONING OF THE AMERICAN COCKROACH, PERIPLANETA AMERICANA

By

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In DDT-treated American cockroaches, Periplaneta americana, L. (Orthoptera:Blattidae), glycogen and trehalose concentrations were lowered over a relatively short time span corresponding to the period from late hyperactivity to early prostration. Such a depletion corresponded with a peak in $^{14}CO_2$ evolution from cockroaches receiving glucose-$^{14}C$ injections. The depletion occurred even after supplemental injections of up to 6 mg trehalose per cockroach were made, but was delayed by a rise in temperature. Dieldrin and propoxur poisoning caused a similar carbohydrate depletion. In DDT-poisoned cockroaches carbohydrate depletion is proposed as a link between the effect of DDT on the nervous system and the response by the other tissues of the insect to the insecticide.

Serum from DDT-prostrate cockroaches injected into previously untreated cockroaches caused an increase in the trehalose content. This was not observed with serum from DDE-treated cockroaches. This hyperglycemic activity is present in serum mainly from the prostrate stages of DDT poisoning. The factor is stable to heating and is stable at room temperature for as long as four hours. The active fractions from

molecular sieve chromatography are distinct from the bulk of the serum protein. The physiological significance of this factor is discussed.

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By

Jeffrey Granett

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A one-foot waterfall—

it too makes noises,

and at night is cool.

Issa

to my parents, Sandy and the future.

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# LITERATURE REVIEW

## I. Trehalose in Insects

### A. Trehalose Levels

Wyatt and Kalf (1957) reported the presence of trehalose in representatives of five orders of insects. In four lepidopterous species trehalose comprised 90% of the total hemolymph carbohydrates. Concentrations in the plasma ranged from 306 mg percent (mg per 100 ml) trehalose in the oriental silkworm larva, Bombyx mori, to 1398 mg percent in the silk moth, Telea polyphemus. Evans and Dethier (1957) reported a range of 200 to 3000 mg percent trehalose in the hemolymph of the blowfly, Phormia regina. Steele (1963) found an average of 1070 mg percent trehalose in the American cockroach, Periplaneta americana. Contrary to the high level of trehalose present in most insects, Barlow and House (1960) found that only 1 to 2 percent of the carbohydrates in the hemolymph of the parasitic fly larvae, Agria affinis, was trehalose; glucose accounted for 80% of the carbohydrates. Hansen (1964) found no trehalose in the hemolymph of locusts, but detected high levels of maltose, cellobiose, or other sugars depending on the diet.

### B. Functions of Trehalose

Trehalose, as the predominant carbohydrate in the hemolymph of most insects, is comparable to glucose in mammalian systems and serves as a mobile source of energy (Wyatt and Kalf, 1957; Chefurka, 1965). Utilization of trehalose has been studied during various physiologic

activities. Discussed here will be 1) trehalose utilization during development, 2) utilization in flight, 3) levels at different times of day and 4) the effect of gut absorption on hemolymph trehalose levels.

1. Trehalose Utilization during Insect Development

Between the pupal and adult stages of several silkworm species the trehalose level decreases by one-half (Wyatt and Kalf, 1957). Candy and Kilby (1962) suggest that this drop may be a result of chitin synthesis, although their proposed pathway does not necessarily indicate this.

Diapause hormone injected into silkworm pupae enhances de novo synthesis of trehalase (the enzyme hydrolyzing trehalose to glucose) in pupal ovaries, resulting in higher ovarian glycogen levels (Yamashita and Hasegawa, 1967). Similar effects are shown by pupae injected with ecdysone or cholesterol subsequent to excision of the supraeosophageal ganglia (Kobayashi et al., 1967).

2. Trehalose and Insect Flight

During flight the rate of glycogen decrease in the fat body of Drosophila is directly proportional to the wing-beat frequency (Williams et al., 1943). Clegg and Evans (1961) extended this correlation to the trehalose of the hemolymph with the observation that high trehalose concentrations accompany high wing-beat frequencies. They hypothesized that the source of this trehalose was fat body glycogen and dietary monosaccharides.

3. Circadian Fluctuation in Trehalose Levels

Nowosielski and Patton (1964) reported peak hemolymph trehalose concentrations in the house cricket, Gryllus domesticus, at 3 hours before

dawn (in a 12 hour day).

4. Gut Absorption of Carbohydrates

The absorption of various sugars through the gut and their incorporation into hemolymph trehalose and fat body glycogen was studied in Bombyx mori by Horie (1960). Sugars which increased the trehalose level also increased fat body glycogen. Treherne (1958a and b) observed in the locust, Schistocerca gregaria, that sugars are mainly absorbed by diffusion through the midgut caecae and to a lesser extent through the ventriculus. Once in the hemolymph the sugars are rapidly converted to trehalose, providing a steep glucose concentration gradient between the hemolymph and the gut lumen for the absorption of dietary glucose.

C. Trehalose Metabolism

1. Synthesis and Breakdown of Trehalose

Trehalose in insect hemolymph may be cleaved at the cell membrane into two glucose moieties by trehalase. This enzyme has been isolated and purified from a number of insects (Howden and Kilby, 1956; Friedman, 1960; Derr and Randall, 1966). In trehalose synthesis UDP-glucose and glucose-6-phosphate form trehalose-6-phosphate which is then hydrolyzed by trehalose phosphate phosphatase to yield trehalose (Chefurka, 1965).

2. Regulation of Trehalose Levels

Synthesis of trehalose and glycogen in the insect is competitive. The balance of the two is under feedback and hormonal control.

a. Feedback Control

Excess trehalose inhibits the incorporation of glucose into trehalose and stimulates its incorporation into glycogen in in vitro

fat body incubations (Murphy and Wyatt, 1965). It is believed that trehalose is an allosteric inhibitor of trehalose phosphate synthetase, binding to a site on the enzyme that is separate from the catalytic site. The inhibition can be eliminated by mild protein denaturation.

Trehalose activation of glucose-6-phosphate hydrolysis in fat body extracts of the blowfly, Phormia regina, was demonstrated by Friedman (1967a). Since glucose-6-phosphate is required for trehalose synthesis the net affect is to inhibit trehalose synthesis.

b. Hormonal Control

Steele (1961) extracted a hyperglycemic factor from the corpus cardiacum of the American cockroach, Periplaneta americana. When injected into other cockroaches, this extract increased the hemolymph trehalose 150 percent within 5 hours, increased inorganic phosphate release into the hemolymph and lowered the amount of glycogen in the fat body (Steele, 1963). Steele suggested that this hormone, thought to be peptidyl in nature, affected the phosphorylase activity. Ralph and McCarthy (1964) in similar experiments found that the hyperglycemic factor was present in the corpus cardiacum, brain, corpus allatum, and subeosophageal ganglion (in order of decreasing activity). Bowers and Friedman (1963) noted a hyperglycemic hormone in the cockroach, Blaberus discoidalis. Migliori Natalizi and Frontali (1966) isolated two such factors from the corpus cardiacum of the American cockroach and head homogenates of the honeybee, Apis mellifera. Migliori Natalizi et al. (1970) purified one from a corpus cardiacum extract from American cockroaches using Sephadex column chromatography and demonstrated trypsin sensitivity. Brown (1965) isolated two factors with similar

activity from an American cockroach extract by paper chromatography. These factors were also trypsin sensitive and presumed to be low molecular weight polypeptides.

Friedman (1967b) found that the hyperglycemic hormone was inactive in blow flies fed ad libitum, but was active if they were starved. He suggested that the trehalose synthesis system normally works at full capacity as governed by the feedback inhibition of trehalose synthesis. When the trehalose concentration is low and there are abundant sugars from the gut, these sugars are efficiently converted into trehalose. During starvation, however, glycogen breakdown is the rate-determining factor of trehalose formation; so Friedman hypothesized that the hormone acts on glycogen breakdown. Wiens and Gilbert (1967) studied the effect of the hyperglycemic hormone on phosphorylase activity and the respiration of in vitro fat body preparations. They hypothesized several sites of hormone action, including: a) an increase of phosphofructokinase activity, b) inhibition of glycolysis by pentose phosphate cycle acceleration, c) hexokinase activation and d) trehalose-6-phosphate synthetase activation.

## II. DDT

### A. Introduction

Although DDT was first synthesized in 1894 by Zeidler, its insecticidal properties were not established until 1939 by P. Müller of the Geigy Chemical Corporation. Widespread and continued use came after World War II due to DDT's low cost, extremely wide spectrum of insecticidal activity, stability and low acute mammalian toxicity. This stability and DDT's lipid solubility leads to its accumulation in the

environment and storage in animal adipose tissue. In recent years, fear of chronic poisoning by DDT has caused controversy and as a result it has been banned for many uses in several states and countries.

However, the continued study of DDT, even though its use is declining, is of more than academic interest. First, DDT is indispensible in certain applications and in countries less technically developed than the United States. It will be used in significant quantities for a long time. Secondly, DDT and its metabolite DDE are some of the most widespread environmental contaminants and will be prevalent for many years even if the environmental input should be halted immediately. Thirdly, the action of DDT in organisms appears to affect basic animal processes. An understanding of DDT's action may thus give information on these biological systems.

B. Mode of Action of DDT

1. Symptoms

The acute toxicity of DDT to insects is attributed to the chemical's action on the nervous system reflected in the behavior of the poisoned insects. The abnormal neuronal activity results in tremors, hyperactivity, ataxia and eventual paralysis. Roeder and Weiant (1946) found that the sensory nerves were the most sensitive part of the nervous system to DDT. They also described the high-frequency trains of spike potentials caused by DDT in the axons of cockroach legs.

Increases in respiration rates due to DDT were described by Ludwig (1946), Lord (1949) and Harvey and Brown (1951). Also, weight loss and water loss occurred with DDT poisoning (Ludwig, 1946) and heart beat frequency was affected (Patel and Cutkomp, 1967).

2. Action on Nerves

The mode of action of DDT on nerves has been studied intensively. O'Brien (1967) summarizes some of these studies. Several areas of DDT's action have been further explored since O'Brien's review.

The hypothesis of the charge-transfer complex of DDT with nerve proteins first described by O'Brien and Matsumura (1964) was essentially refuted by Hatanaka et al. (1967). They found that binding of DDT was non-specific and occurred with rat liver, muscle and brain in addition to cockroach nerve cord. In addition, the relatively non-toxic surfactant Triton X-100 mimicked the tissue binding effect. Brunnert and Matsumura (1969) revived the charge-transfer complex hypothesis on the basis of competition studies between DDE and DDT. A small portion (6%) of a 5 nmole DDT solution was bound to a site in the synaptic junction different from the DDE binding site.

Some of the above work was done with rat brain fractions. Similar preparations were used also for studies with ATPase. Matsumura and Patil (1969) reported that DDT inhibited the $Na^+$, $K^+$, $Mg^{2+}$-adenosine triphosphatase found in a fraction of rat brain. The inhibition by DDT was about 1000 times higher than that by DDE. In addition, the inhibition was greater at low temperatures, a phenomenon resembling the negative temperature coefficient of DDT toxicity. However, the validity of this work has been questioned by Akera et al. (1971) who found high inhibition with DDE as well as with DDT. They suggested that the contradictory results were due to the low enzyme activity in the preparations used in the earlier work.

3. Biochemical Changes during DDT Intoxication

Tobias et al. (1946) noted that free acetylcholine in the nervous system of DDT-prostrate flies and cockroaches increased 200 percent while the bound acetylcholine decreased. However, the acetylcholine-forming enzymes were unaffected. Lewis (1953) substantiated the higher acetylcholine levels in DDT-prostrate house flies. In 1960 Lewis et al. found that this rise in acetylcholine was similar to that observed under physically-induced prostration. The magnitude of the acetylcholine increase could be correlated with the degree of neuromuscular activity before prostration. They suggested that the rise was due to release of bound acetylcholine from the axons. The rise could not be attributed to increased activity of the acetylcholine synthesizing enzymes, choline acetylase or acetylkinase (Rothschild and Howden, 1961). Interpreting data of Sternburg and Hewitt (1962), Winteringham (1966) concluded that DDT poisoning increases acetylcholine turnover in the insect ventral nerve.

The high concentrations of amino acids in insect hemolymph lend themselves nicely to monitoring for possible changes resulting from insecticide poisoning. Winteringham (1958) reported accumulation of glutamine in the hemolymph, arising possibly through transamination. Cline and Pearce (1963) found that DDT interfered primarily with proline, formate and glycine metabolism in house flies. A greater percentage of injected formate-$^{14}C$ was converted into uric acid and allantoin than into proline in DDT-poisoned insects. They noted that a carbamate insecticide did not affect formate metabolism. Corrigan and Kearns (1963) found that injected proline-$^{14}C$ was oxidized to carbon dioxide three times faster in DDT-poisoned than in control American

cockroaches. Also it was metabolized to glutamine-$^{14}C$ which Winteringham (1958) had suggested might be an ammonia trap for amino acid oxidation. Corrigan and Kearns also suggested that the demand for oxidizable carbon shifted metabolism to proline. In 1966 Cline and Pearce confirmed the drop in proline by radiotracer studies with house flies. In addition they found a decrease in radiolabelled trehalose after glucose-$^{14}C$ injections in DDT-treated insects as compared to solvent-treated controls. Patel et al. (1968) surveyed all the amino acids and found that their average concentrations decreased 22.7 percent in the ovaries of DDT-poisoned susceptible house flies as opposed to a 5.5 percent increase in poisoned resistant flies.

Several papers have reported a drop in carbohydrates during DDT poisoning in insects. This was reviewed in the introduction to Part I of this thesis. There has also been considerable work on intermediary energy metabolism during DDT poisoning. Winteringham et al. (1960) reported significant breakdown of about 20% of the thoracic ATP in DDT-poisoned flies. The ATP drop and prostrate symptomology was reversed by injection of glucose. Sparing the insect hypermotor activity during DDT poisoning by the use of anesthesia did not sustain the ATP level. With DDT poisoning they also saw a drop in L-$\alpha$-glycerophosphate and phospholipids at prostration.

Agosin et al. (1961 and 1963) studied the influence of DDT on intermediary carbohydrate metabolism in Triatoma infestans. They found that DDT (as well as non-toxic DDE) inhibited anaerobic glycolytic pyruvate production by cell-free preparations. DDT enhanced incorporation of glucose into carbon dioxide, glycogen and fatty acids while DDE did not. Glucose oxidation via the pentose phosphate pathway amounted to

77 percent in DDT-treated insects compared to 22 percent in normal insects. Plapp (1970) confirmed this increase in the pentose pathway in house flies. However, Ela et al. (1970) found no increase in the pentose phosphate pathway over the glycolytic pathway with DDT poisoning in cockroaches.

In Triatoma DDT increased the NADP level, but not the NADP/NADPH ratio, possibly because of increased NAD-kinase. The increased NAD-kinase is thought to be related to detoxification of DDT and resistance (Ilevicky et al., 1964). Increased glutathione turnover (glutathione is necessary for activity of DDT-dehydrochlorinase, a detoxifying enzyme) and increased protein synthesis are also related to DDT poisoning in resistant house flies (Agosin et al., 1966).

4. Neurohormonal Involvement in DDT Poisoning

Sternburg and Kearns (1952) found that the hemolymph of DDT-poisoned cockroaches, when injected into normal insects, produced DDT poisoning symptoms. Since the injected hemolymph did not contain sufficient DDT to cause the symptoms, they concluded that the DDT had induced production of a neurotoxic factor. Using electrophysiological preparations, this toxin was found to cause multiple firing in ganglia and sensory nerve fibers. It was unstable in the hemolymph, but was dialyzable and stable in the dialysate (Shankland and Kearns, 1959). Furthermore, it was found that body stress, such as physical immobilization, forced movement or electrical stimulation produced similar (although not necessarily identical) substances which also caused DDT-like symptoms and paralysis in unpoisoned insects (Beament, 1958; Heslop and Ray, 1959). Cook et al. (1969) proposed that their Factor S derived from nerve cords and heads of normal and stressed

American cockroaches may be a neurohormone, transmitter or modulator substance and may be identical to the neurotoxin of Sternburg et al. (1959). The production and release of this type of substance was observed histochemically in the corpus cardiacum (Hodgson and Geldiay, 1959). With parabiosis experiments, Colhoun (1960) showed that the toxin was not the primary cause of the DDT-induced death.

Isolation and identification of the toxins are difficult because of the small quantities present in the insects. Sternburg (1960) tried to solve this problem by using crayfish which are considerably larger than insects and produce similar toxins. He found that the toxin was not a known neurohumoral agent or a DDT metabolite. Hawkins and Sternburg (1964) partially identified it as an aromatic amine, possibly an ester. Patel and Cutkomp (1968) established that the substance was fluorescent and therefore easily detectable. It was not produced by insects treated with certain organophosphate insecticides and therefore is not a dying tissue response. Davey (1963) found that enforced activity of cockroaches also released a cardiac stimulator in the hemolymph. The literature on such toxins is reviewed by Sternburg (1960 and 1963).

## PART I

### Trehalose and Glycogen Depletion during DDT Poisoning of American Cockroaches, Periplaneta americana

Carbohydrate levels in DDT-poisoned insects were investigated during the initial research on this insecticide. Ludwig (1946) showed a drop in glycogen and glucose in insects during DDT poisoning. Merrill et al. (1946) did further work on the carbohydrate depletion including experiments using anesthesia and glucose injections as possible antidotes to the insecticide's activity. They concluded that the depletion of carbohydrates did not play a primary role in the action of DDT. However, their paper did not include complete data on symptomology of the insects. Winteringham (1956) similarly did not report symptomology in full, although he agreed that the carbohydrate drop was not a primary cause of death. Heslop and Ray (1959) suggested that a biochemical lesion was the cause of the decrease in energy compounds. Cline and Pearce (1966) found that trehalose-$^{14}C$, the major metabolite of injected glucose-$^{14}C$, was greatly reduced in DDT-treated NAIDM flies.

This paper presents detailed data on carbohydrate depletion during DDT poisoning and some evidence for the cause of this depletion. Although it may not be the primary cause of death, the carbohydrate depletion must be considered significant to the understanding of what happens biochemically during the dying process of insects and may directly or indirectly be associated with the other factors characterizing the DDT poisoning syndrome.

I. Materials and Methods

Adult male American cockroaches, Periplaneta americana (L.) maintained under a daily 16 hr photophase were the experimental animals. They were given Purina Dog Chow® and water ad libitum. The cockroaches were treated with the following insecticides and analogs: p,p'DDT (99%, City Chemical Corp., New York, N. Y.); p,p'DDE, 98%, propoxur (97%, o-isopropoxyphenyl methylcarbamate [Baygon®], Pesticide Repository, USPHS, Pesticide Research Laboratory, Perrine, Fla.); and dieldrin (99+%, Shell Chemical Co., New York, N. Y.). Treatments were topical on the ventral portion of the abdomen in 10 or 20 µl acetone as stated in each experiment. After treatment the cockroaches were kept in battery jars with pressboard partitions. No food was available, but humidity was maintained with a damp paper towel.

In experiments with DDT the cockroaches were sampled at times determined by symptoms the DDT-treated groups showed. Terminology used in this paper to describe the stages of poisoning was modified from Heslop and Ray (1959). Initial tremors denotes the first stage of poisoning when the insect is slightly ataxic and shows slight tremors. During the tremors stage, all appendages are in continual nervous motion. In the hyperactivity stage the insect exhibits continual walking and running, with occasional convulsions. The late hyperactive stage indicates a slowing, with the insect having little control over its movements. Tremors are continuous. During the prostrate stage the insect cannot right itself, but has continual tremors. In the late prostrate stage all movements have ceased except for occasional, but slight, twitching. Except for the experiments where cockroaches were sampled hourly, the stages of hyperactivity and late prostration were

found to be convenient for the sampling of groups for carbohydrate determinations. At 24°C with 50-100 µg DDT per cockroach, typical hyperactivity symptoms appeared 3-5 hr after treatment and prostration occurred between 8 and 12 hr after treatment.

Hemolymph for trehalose assays was obtained from the cockroaches by cutting appendages (antennae, tarsi, cerci, styli and phallomeres) and collecting with a capillary pipette the droplets formed. Samples were immediately frozen with dry ice. The hemolymph was lyophilized and then suspended in 2 ml 60% ethanol. The anthrone colorimetric test for trehalose was run according to Wyatt and Kalf (1957) and the results presented as mg trehalose per mg dry hemolymph. These qualitative and quantitative determinations were confirmed by formation of the trimethylsilyl derivatives (Sweeley et al., 1963) and analysis by GLC using a 6 ft x ⅛ in. stainless steel column of 3% OV-1 on 100/120 mesh Gas Chrom Q, isothermally at 220°C with 40 ml/min helium flow and flame ionization detection. Cellobiose was used as an internal standard and the peaks were quantitated by peak height ratios. This column resolved derivatives of cellobiose, maltose and trehalose from each other and from derivatives of monosaccharides for identification purposes. In addition, trehalose was distinguishable from either cellobiose or maltose alone because of its single peak.

Glycogen and trehalose were extracted from homogenates of whole cockroaches. Groups of 3-7 cockroaches were homogenized 15 min in a Lourdes Model MM-1 homogenizer in 15 ml 10% trichloroacetic acid. The homogenates were centrifuged at 10,000 g for 15 min, the pellet resuspended in 5 ml water and the centrifugation repeated. Glycogen was precipitated from a 2 ml aliquot of the combined supernatant

1 ml saturated $Na_2SO_4$ and 13 ml 95% ethanol. The preparation was kept at -20°C overnight, the tubes centrifuged in a clinical desk-top centrifuge and the supernatant containing the mono- and disaccharides removed. The precipitate containing glycogen was resuspended in 10 ml 95% ethanol, kept at -20°C for 2 hr and centrifuged again. The ethanol precipitate from the 2 centrifugations was dried and resuspended in 2 ml water. Glycogen was determined by an anthrone test (Morris, 1948). Standards were prepared containing glycogen and $Na_2SO_4$ equal to that in the sample tubes. Trehalose was determined in the combined ethanol supernatants. A 2 ml aliquot was dried and the trichloroacetic acid removed by rinsing twice with 10 ml anhydrous diethyl ether. The samples were then derivatized and chromatographed as described previously.

In one set of experiments, trehalose was injected into DDT-treated and control cockroaches. A series of one, two or three injections of trehalose (2 mg/20 µl water per injection) was made at 3 hr intervals beginning with the first hyperactive symptoms shown in the DDT-treated groups. Carbohydrate levels were determined at late prostration.

A set of experiments was run in which DDT- and DDE-treated cockroaches were held initially at 18°C. When the DDT-treated group showed late hyperactive symptoms, groups from both treatments were placed at 33°C until late prostration symptoms were shown by the DDT-treated groups. At intervals, samples were taken and carbohydrate levels determined as described previously.

A series of respirometry studies measured $^{14}CO_2$ after the injection of 20 µl of uniformly labelled glucose-$^{14}C$ (0.0255 µCi/0.5 mg glucose per 20 µl water per cockroach). The respired $^{14}CO_2$ was bubbled through 10 ml of monoethanolamine:ethyleneglycol monomethyl ether (1:2, v/v) and

collected hourly. In these experiments, 10 cockroaches were used per treatment of DDT or DDE. The insects were treated with the chemicals 2 hr after the glucose injections.

Two other insecticides, dieldrin and propoxur, were used in some experiments. Acetone was used as the solvent and for the control treatments. Trehalose and glycogen were determined as described previously when the poisoned insects reached a prostrate stage.

## II. Results

At DDT-induced late prostration, the trehalose in the hemolymph had decreased 7-8 fold compared to that of cockroaches treated with DDE or controls sampled simultaneously (Table 1). All treatments showed higher trehalose values at the hyperactive stage than the controls with no acetone treatment.

Table 2 shows comparable results for homogenates of whole cockroaches treated with DDT, DDE and acetone. Both trehalose and glycogen were depleted in the DDT-treated cockroaches at late prostration. In these cases, the levels in DDE-treated insects were essentially the same as those for the acetone-treated controls. Because of this similarity, further tests used only the DDE-treated cockroaches as controls. The hyperactive cockroaches in these experiments showed no difference in the carbohydrates among treatments.

Figure 1 depicts the depletion of the carbohydrates with time and the symptomology of the cockroaches treated with 50 μg DDT or DDE in 10 μl acetone/cockroach at 23°C. Each value represents 3-5 groups of 5 cockroaches each and was calculated by dividing the values for the DDT treatments by the DDE values and plotting as percent versus time.

Table 1. Trehalose levels in the hemolymph of treated American cockroaches.

| Treatment[a] | mg trehalose/mg dry hemolymph ± S.E.[b] | |
|---|---|---|
| | Hyperactive stage[c] | Late prostrate stage[c] |
| DDT | 0.17 ± 0.03 | 0.02 ± 0.01 |
| DDE | .16 ± .02 | .12 ± .02 |
| Acetone | .17 ± .02 | .12 ± .02 |
| Control | .11 ± .01 | .10 ± .01 |

[a]Treatments were 48 μg DDT or DDE/10 μl acetone or 10 μl acetone alone topically per cockroach. The control was without any treatment. Temperature was 25°C.

[b]Each value represents 5-7 groups of 5 cockroaches each.

[c]Stage refers to the time these symptoms were shown by DDT-treated cockroaches.

Table 2. Trehalose and glycogen levels after DDT and DDE treatments of American cockroaches.

| Treatment[a] | Trehalose (mg/g wet weight ± S.E.)[b] | | Glycogen (mg/g wet weight ± S.E.)[b] | |
|---|---|---|---|---|
| | Hyperactive[c] | Late prostrate[c] | Hyperactive[c] | Late prostrate[c] |
| DDT | 3.1 ± 0.27 | 0.15 ± 0.07 | 3.7 ± 0.32 | 0.17 ± 0.02 |
| DDE | 3.1 ± .24 | 2.80 ± .27 | 3.4 ± .32 | 3.40 ± .31 |
| Acetone | 2.8 ± .23 | 2.70 ± .24 | 3.6 ± .45 | 2.90 ± .65 |

[a]Treatments were 50 µg DDT or DDE/10µl acetone or 10 µl acetone alone per cockroach. Temperature was 23°C.

[b]Each value represents 3-7 groups of 5 cockroaches each.

[c]Stage refers to the time these symptoms were shown by DDT-treated cockroaches.

Figure 1. Glycogen and trehalose during DDT treatment as percent of DDE-treated control cockroaches. Vertical lines indicate half standard errors.

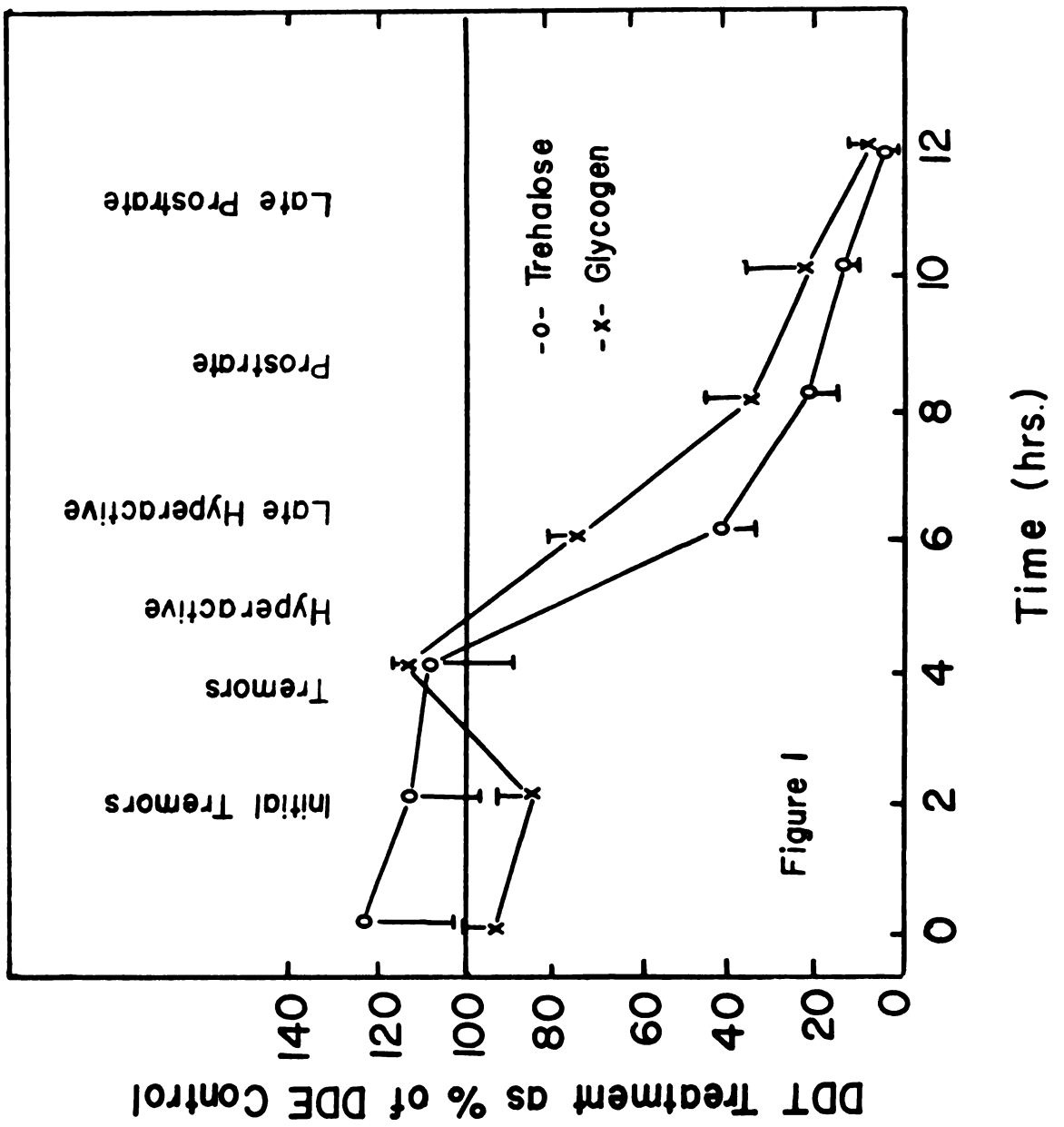
DDT Treatment as % of DDE Control
0
20
40
60
80
100
120
140
0
2
4
6
8
10
12
Time (hrs.)
-o- Trehalose
-x- Glycogen
Initial Tremors
Tremors
Hyperactive
Late Hyperactive
Prostrate
Late Prostrate

Figure 1

The greatest decrease in carbohydrate levels occurred during the late hyperactive stage, although some occurred before and after this stage.

The results for experiments in which trehalose was injected are shown in Table 3. The values are from cockroaches taken when the DDT-treated groups exhibited late prostrate symptoms 22 hr after treatment. Although injections did not appear to reverse the DDT symptoms, the time to death was prolonged. Treated cockroaches showed little adverse response to the repetitive injections other than slowed activity. In all the DDT-treated groups, the carbohydrates were similarly depleted. In the control groups, the injected trehalose tended to accumulate or was metabolized to glycogen.

A series of experiments determined the effect of temperature on carbohydrate depletion. Cockroaches were treated topically with 100 μg DDT or DDE in 20 μl acetone per cockroach. They were moved from an 18 to a 33°C chamber when the DDT-treated groups were in the hyperactive to late hyperactive stages. The DDT symptoms appeared to diminish as evidenced by a decrease in the jerking motions and tremors; however, the late prostrate stage still occurred by 22 hr. Neither trehalose nor glycogen was replenished during the reversal of symptoms (Figure 2). The 8, 10 and 12 hr values in Figure 2 are not significantly different from each other. Each value in this figure represents 3-5 groups of 5 cockroaches each.

Carbon dioxide collection in 3 experiments showed an average of 70.3% (± 2.1% S.E.) of injected glucose-$^{14}C$ recovered by late prostration as $^{14}CO_2$ from DDT-treated cockroaches. This average compared to 34.6% (± 5.1% S.E.) for the DDE-treated insects. Figure 3 shows the hourly

Table 3. Trehalose and glycogen levels in DDT- and DDE-treated American cockroaches after trehalose injections.

| No. injections[a] | Trehalose (mg/g wet weight ± S.E.)[b] | | Glycogen (mg/g wet weight ± S.E.)[b] | |
|---|---|---|---|---|
| | DDE[c] | DDT[c] | DDE[c] | DDT[c] |
| 0 | 2.8 ± 0.34 | 0.14 ± 0.08 | 2.5 ± 0.56 | 0.17 ± 0.04 |
| 1 | 2.9 ± .30 | .25 ± .04 | 3.5 ± .40 | .19 ± .01 |
| 2 | 4.2 ± .21 | .18 ± .06 | 2.9 ± .36 | .17 ± .03 |
| 3 | 4.3 ± .18 | .16 ± .03 | 4.1 ± .29 | .14 ± .01 |

[a]2 mg trehalose/20 µl water were injected at 3 hr intervals beginning at the hyperactive stage (5 hr). Samples were taken at late prostration, 22 hr.

[b]Each value represents 3-6 groups of 5 cockroaches each.

[c]100 µg DDT or DDE/10 µl acetone per cockroach. Temperature was 18°C.

Figure 2. The effect of a temperature rise on trehalose and glycogen levels in DDT-treated American cockroaches. Values are presented as a percent of the DDE-treated controls. Vertical lines represent half standard errors.

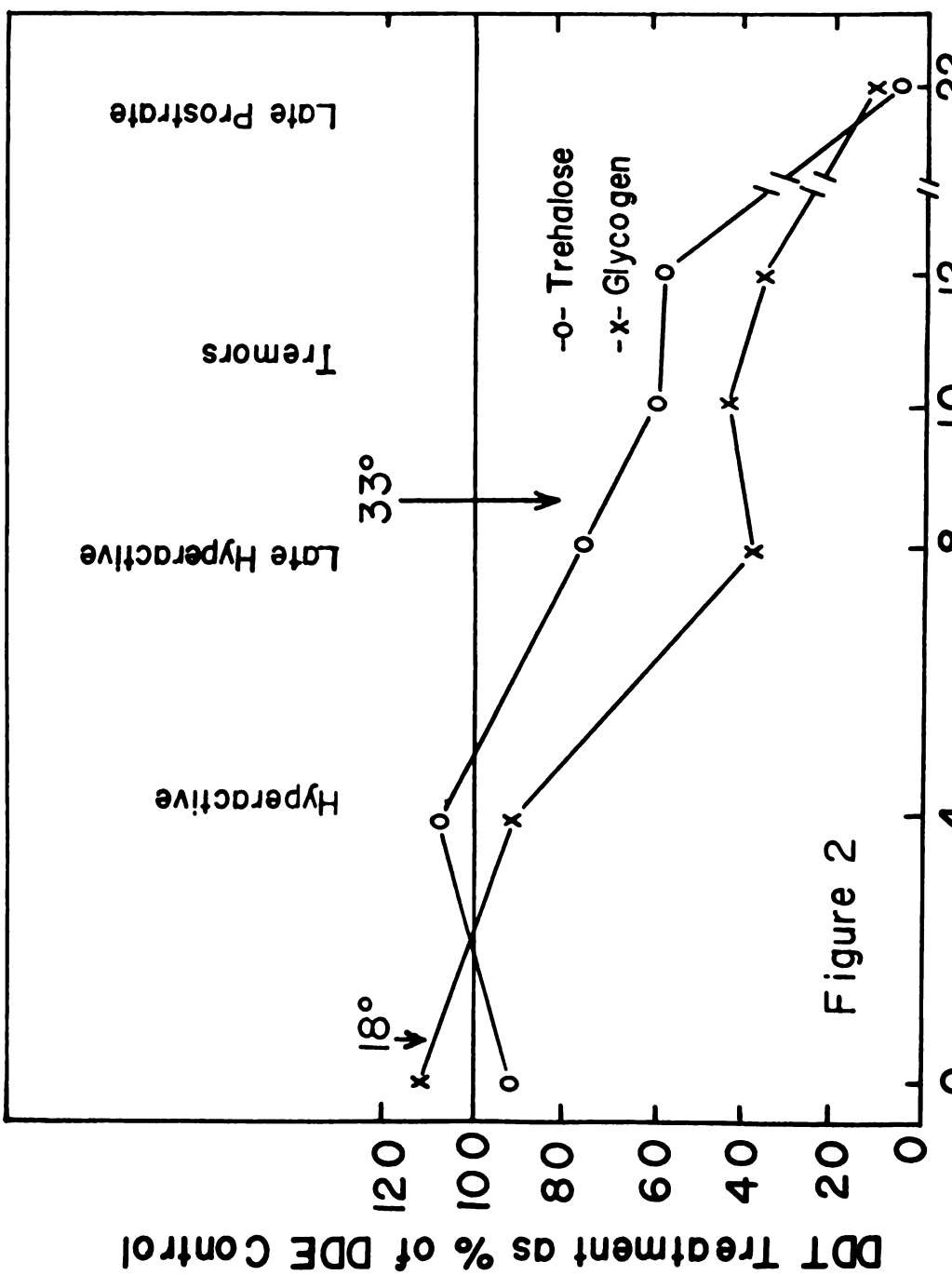
DDT Treatment as % of DDE Control
120
100
80
60
40
20
0
0
4
8
10
12
22
Time (hrs.)
18°
33°
-o- Trehalose
-x- Glycogen
Hyperactive
Late Hyperactive
Tremors
Late Prostrate

Figure 2

Figure 3. Difference between $^{14}CO_2$ respired from DDT- and DDE-treated American cockroaches injected with glucose-$^{14}C$ as a percent of total dpm's injected. Values were calculated by the formula:

$$\frac{\left(\begin{array}{c}\text{DPM } ^{14}CO_2 \text{ from DDT-} \\ \text{treated insects}\end{array}\right) - \left(\begin{array}{c}\text{DPM } ^{14}CO_2 \text{ from DDE-} \\ \text{treated insects}\end{array}\right)}{\text{total DPM injected}} \times 100\%$$

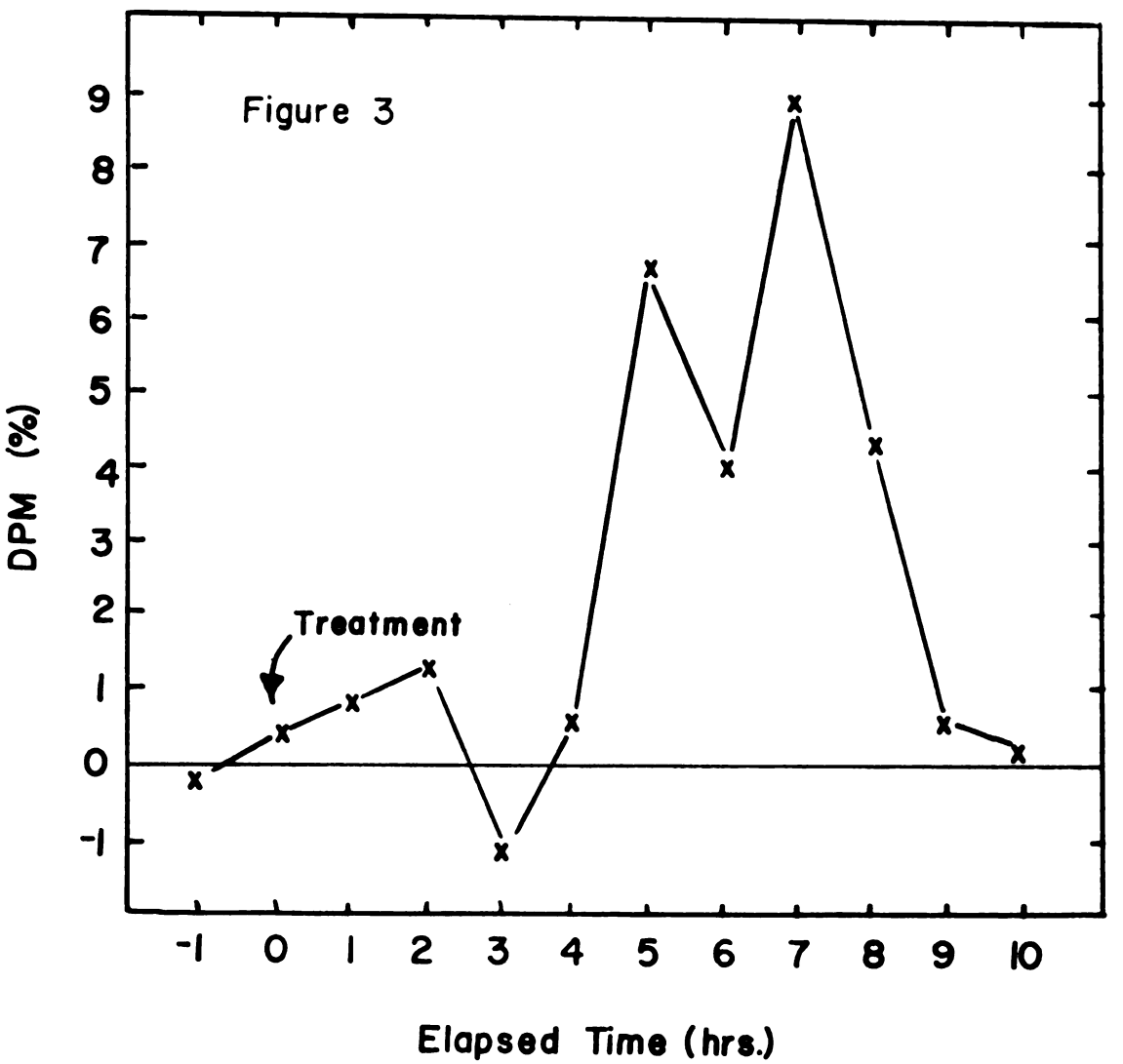
Figure 3
DPM (%)
9
8
7
6
5
4
3
2
1
0
-1
Treatment
-1
0
1
2
3
4
5
6
7
8
9
10
Elapsed Time (hrs.)

recovery of $^{14}CO_2$ from one such experiment in which groups of 10 cockroaches were treated with 100 µg DDT or DDE in 20 µl acetone per cockroach. Similar curves with 2 maxima were obtained with replicate runs for groups of cockroaches as well as with individual cockroaches per respirometer flask. One peak occurred during the hyperactive to late hyperactive stages; the second occurred while the insects were prostrate.

Dieldrin and propoxur were used to determine whether the depletion of carbohydrates was specific to DDT or also could be attributed to insecticides with different modes of action. Table 4 shows the results of experiments with these insecticides. Dieldrin treatment caused depleted carbohydrate levels very similar to the results with DDT. With propoxur, the trehalose level was reduced by a factor of only 2 but the glycogen level was down 20 fold. Because glycogen and trehalose are presumably interchangeable, the trehalose level probably would have been lower had more time elapsed before its determination.

## III. Discussion

The depletion of glycogen and trehalose occurred almost simultaneously and over a relatively short time period during and just after the peak in activity of the DDT-poisoned insects. The depletion was complete by the late prostrate stage, the time when the insect's tremors stopped. The largest amount of $^{14}CO_2$ respired from the glucose-$^{14}C$ injections also came at, and just after the peak in activity and corresponds fairly well with the carbohydrate depletion. The correlations of symptomology, $^{14}CO_2$ respiration and the carbohydrate depletion could suggest that the carbohydrate depletion is primarily a result of the higher amounts of energy used by the insect's muscles during hyper-

Table 4. Trehalose and glycogen levels in dieldrin- and propoxur-treated American cockroaches.

| | Trehalose (mg/g wet weight ± S.E.)[a] | | Glycogen (mg/g wet weight ± S.E.)[a] | |
|---|---|---|---|---|
| | Insecticide | Acetone | Insecticide | Acetone |
| Dieldrin[b] | 0.25 ± 0.07 | 2.0 ± 0.13 | 0.14 ± 0.01 | 3.9 ± 0.29 |
| Propoxur[c] | 1.20 ± .09 | 2.3 ± .10 | .21 ± .03 | 4.1 ± .45 |

[a]Each value represents 4-6 groups of 5 cockroaches each.

[b]Treatment was with 80 µg dieldrin/20 µl acetone per cockroach, topically. Acetone treatment was with 20 µl acetone. Samples were taken at 24 hr while cockroaches were prostrate, but exhibiting strong tremors.

[c]Treatment was with 5 µg propoxur/5 µl acetone. Samples were taken at 5½ hr while cockroaches were prostrate, but exhibiting strong tremors.

activity. However, the possibility of some additional unspecified means of carbohydrate utilization, as Winteringham (1956) suggested, was not ruled out.

The experiment with temperature variations supports the hypothesis that carbohydrate depletion may result from increased muscular activity. When the hyperactive symptoms were lessened by a higher temperature, depletion was delayed possibly because a decreased level of muscular activity results in a slower utilization of the carbohydrates. Merrill et al. (1946) in their experiments with anesthesia used time after poisoning rather than symptomology as a criterion for making analyses. This accounts for their results showing high carbohydrate levels with the poisoned, anesthetized insects, which were probably in early stages of DDT symptomology, instead of the depleted levels shown in our work.

The trehalose injection experiments were performed to determine if the insects could be maintained and DDT symptoms reversed by replacing the carbohydrates lost during poisoning. Although symptoms were reversed somewhat, the insects could not be maintained alive at the trehalose levels used. This experiment also sought to determine whether it was possible to reach the late prostrate stage without a complete depletion of carbohydrates. If it were possible to separate late prostration from carbohydrate depletion, the hypothesis that carbohydrate depletion was necessary for DDT prostration would be disproved. The carbohydrate levels did drop indicating that the depletion may be necessary for the insects to reach the late prostrate stage. Up to 6 mg trehalose were administered and the entire amount used by the time of late prostration. An important distinction here

is between the prostrate and late prostrate designations. During the prostrate stage the insect may be highly active though too uncoordinated to run; however, in the late prostrate stage most activity has ceased. Carbohydrate levels seem to reflect these stages. During the prostrate stage there still may be some carbohydrates present; in the late prostrate stage the depletion is essentially complete.

From these observations and information in the literature, a hypothetical route of DDT poisoning can be drawn. DDT binds with nerve tissue and causes trains of impulses (Roeder and Weiant, 1946; Brunnert and Matsumura, 1969). These trains cause the insect to become hyperactive and the insect becomes increasingly uncoordinated. The hyperactivity depletes the carbohydrate reserves. As the carbohydrate level passes below some threshold concentration the insect ceases to move as a result of the lack of an oxidizable carbon source. Such a sequence would be supported by the reported carbohydrate levels after prostration with dieldrin and propoxur. These insecticides also cause hyperactivity even though their mode of action is different than that of DDT.

This sequence would not be in conflict with data on the influence of DDT poisoning on amino acid levels (Winteringham, 1958; Corrigan and Kearns, 1958; Patel and Cutkomp, 1968). Here stress on the carbohydrate reserves might cause the insect to mobilize the amino acids for use as carbon sources (Corrigan and Kearns, 1963).

In conclusion, this sequence would suggest that although carbohydrate depletion is not the cause of DDT's effect on the nervous system, it is a link between the effect of DDT on the nervous system and the death of the other tissues in the poisoned insect.

## PART II

### A Hyperglycemic Factor in the Serum of DDT-prostrate American Cockroaches, Periplaneta americana

Although the primary action of DDT in insects involves disruption of nervous tissue, there has been considerable speculation as to other possible lesions (See O'Brien, 1967). Sternburg and Kearns (1952) described a neurotoxin other than the poisoning agent, which was produced by cockroaches after DDT-induced prostration. The structure, mode of action and function of this neurotoxin has not been elucidated (Sternburg, 1963). Cook et al. (1969) suggested that their "Factor S," which has properties similar to the neurotoxin and is released during stress, may be a neurohormone, a transmitter or a modulator substance. A hormone has been found in cockroaches which causes nerve trains similar to those produced by the neurotoxin and the Factor S and was described by Migliori Natalizi et al. (1970).

The possibility that the factors described above may influence carbohydrate levels suggested the research described in this paper. Steele (1961, 1963) and Migliori Natalizi and Frontali (1966) have described hormones from cockroaches that increase trehalose levels in the hemolymph. This paper presents data showing the presence of a hyperglycemic factor in the hemolymph of DDT-prostrate cockroaches. Some characteristics of this factor are described and its possible significance discussed.

I. Materials and Methods

Adult male American cockroaches, Periplaneta americana, L. maintained under a daily 16 hr photophase were used as experimental animals. They were given Purina Dog Chow® and water ad libitum. Treatments of p,p'DDT (99%, City Chemical Corp., New York, N. Y.) and p,p'DDE (98%, Pesticide Repository, USPHS, Pesticide Research Laboratory, Perrine, Fla.) were topically administered to the ventral portion of the abdomen in 10 µl acetone at 25°C. Doses were 100 µg DDT or DDE per insect. After treatment the cockroaches were kept in covered battery jars with pressboard partitions. No food was available but humidity was maintained by a damp paper towel.

Procedures for collection of cockroach hemolymph for bioassay were as previously described in Part I. The terminology used for the various stages of DDT poisoning were also as before. Prostration occurred about 20 hr after poisoning under the test conditions. Samples of hemolymph were immediately frozen with dry ice, stored at -20°C and used within 2 days.

After defrosting, the hemolymph serum was separated from cellular debris with a microsyringe. The serum was either diluted with a Ringer's saline[a] (Maddrell, 1968) for use directly in the bioassay or lyophilized for fractionation by molecular sieve chromatography. The lyophilized serum for this chromatography was resuspended in a minimal volume of 0.1 M acetic acid and placed on a 0.9 x 22 cm Sephadex G-25 column with Dextran 2000 dye to determine the void volume. The serum components

---

[a]The Ringer's saline used consisted of 9.82 g NaCl, 0.77 g KCl, 0.5 g $CaCl_2$, 0.18 g $NaHCO_3$, 0.10 g $NaH_2PO_4$ in 1 liter distilled water.

were eluted with 0.1 M acetic acid and collected in 2 ml fractions. These fractions were lyophilized and resuspended in Ringer's saline for bioassay.

The bioassay procedure consisted of the injection of 10 µl of the diluted serum or reconstituted Sephadex eluant into carbon dioxide anesthetized cockroaches. Injections were made through the integument between the second and third abdominal sternites on the left side of the midline. Two hours after injection the insects were again immobilized with carbon dioxide, frozen on dry ice and stored at -20°C until trehalose assays were performed within 4 days.

Trehalose in homogenates of whole cockroaches was assayed by GLC as described previously, except that 11% QF-1 + OV-17 (Applied Science Laboratories, College Park, Penn.) as the stationary phase on 80/100 mesh Gas Chrom Q was used in some assays yielding results similar to those with the OV-1 column.

Heat stability experiments were conducted with the diluted serum. Treatments of the serum ranging from 3 minutes at 60°C to 5 minutes at 100°C were used. Bioassays for hyperglycemic activity in the samples were conducted as described above.

The content of DDT in the serum that was bioassayed was determined by GLC. A 0.5 ml sample of serum was extracted twice with 5 ml of hexane and the combined extracts evaporated to 0.5 ml. DDT was quantitated isothermally at 230°C using a 6 ft x 1/8 in. stainless steel column of 3% QF-1 + OV-17 on 60/80 mesh Gas Chrom Q. Helium flow was maintained at 30 ml/minute and an electron capture detector was used. The peaks were quantitated by comparing peak heights with those of standards.

Protein determinations were made on Sephadex eluants using the Lowry method (Lowry et al., 1951).

## II. Results

The serum of hemolymph from DDT-treated cockroaches at late prostration, when injected into previously untreated cockroaches, was found to increase trehalose levels. Serum from DDE-treated cockroaches had no such hyperglycemic activity. The peak of the trehalose increase was observed 1-3 hr after injection with some activity as long as 7 hr after injection (Figure 4). Each trehalose value represents determinations on 6-8 replicates of 4-5 cockroaches each. The control trehalose values fluctuated around 2.2 mg trehalose per g wet weight. The differences between the absolute values of the controls in each experiment is due to variations in the cockroach colony. Insects judged healthy and from a single cage were used in each experiment.

From two determinations the amount of DDT in the serum injected for the assays averaged 0.025 μg per insect (about 0.03 ppm). No metabolites other than DDE were present. When this amount of DDT was added to serum from DDE-treated insects and this mixture subsequently injected into previously untreated cockroaches, no rise in trehalose was detected. The DDT plus serum injections resulted in a trehalose level of 1.64 mg trehalose/g cockroach wet weight (± 0.12 S.E.) 2 hr after injection. The serum injected controls had a level of 1.75 mg trehalose/g wet weight (± 0.12 S.E.) at 2 hr. These values represent the average of two experiments with 4 groups of 5 cockroaches in each.

Serum from cockroaches at early prostrate and late prostrate stages of DDT poisoning displayed hyperglycemic activity, whereas serum

Figure 4. The level of trehalose in cockroaches treated with serum from DDT-prostrate cockroaches. Values are expressed as a percent of controls injected with serum from DDE-treated cockroaches. Vertical lines indicate standard errors.

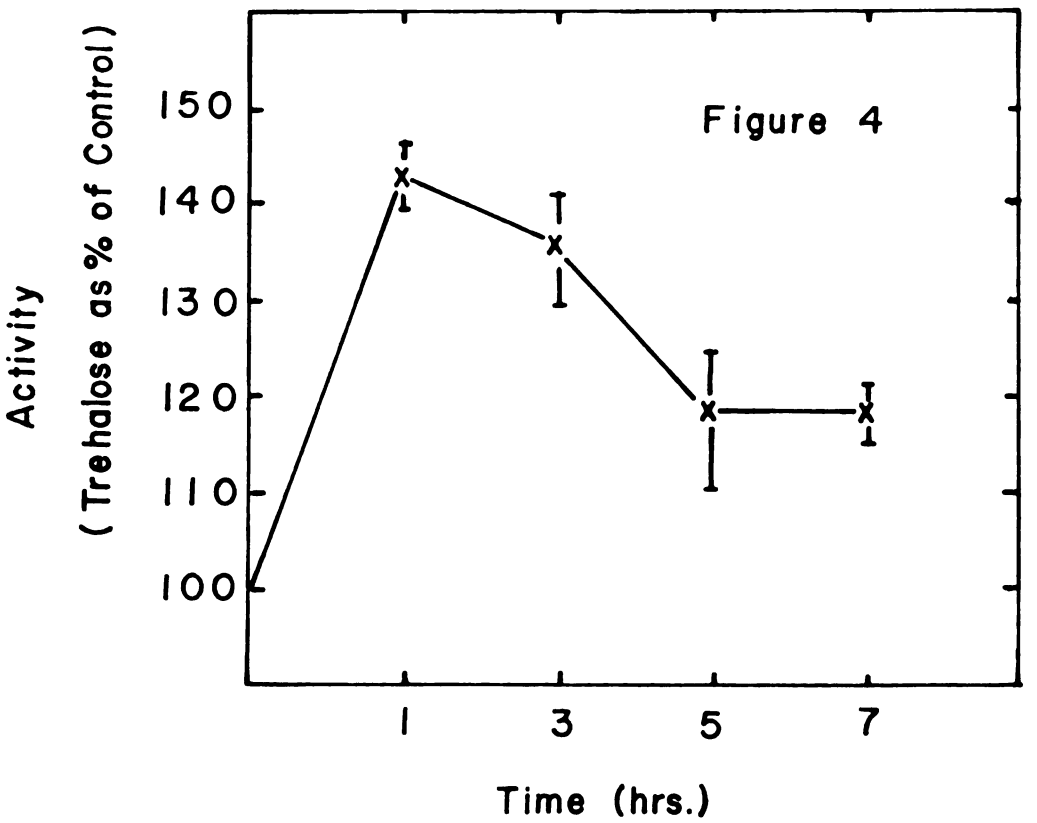
Figure 4
Activity
(Trehalose as % of Control)
150
140
130
120
110
100
1
3
5
7
Time (hrs.)

from cockroaches at the tremors or hyperactivity stages showed little significant activity (Figure 5). Controls were injected with serum from DDE-treated insects. Each value represents averages of two experiments with 4 groups of 5 cockroaches each.

The stability of the hyperglycemic factor to temperature was assayed. The activity of serum left at room temperature averaged 96% that of normal serum for up to 4 hr, but had been completely lost by 24 hr. The activity withstood severe heat treatment. After heating at 60°C for 3 minutes an average activity of 93% remained. Heating to 75°C for 5 minutes similarly left 99% of the activity. However, heating at 100°C for 5 minutes left only 69%. In these experiments Ringer's saline injections were used as controls. Values represent averages from 2-3 experiments with 3-4 groups of 5 cockroaches each.

Increasing dilutions of the active serum produced a graded decline in the trehalose increase in injected cockroaches. It appears that the active hemolymph diluted 1:16 with Ringer's saline produced no hyperglycemic response in the injected cockroaches. Ringer's saline injections were used as controls (Table 5).

The active factor was fractionated from the serum using a Sephadex G-15 column. The most active portions of the eluant, as seen in Figure 6, are the fractions between 13 and 17 ml with a possible later peak at the fraction at 25 ml. Protein determinations of individual fractions showed that the bulk of the protein is eluted before the fractions containing the hyperglycemic activity. This experiment was done twice. In this representative run the bioassay values represent 3 groups of 4-5 cockroaches each.

Figure 5. Hyperglycemic activity from serum of cockroaches in different DDT poisoning stages. Trehalose levels in cockroaches injected with serum from DDT-treated (-x-) and DDE-treated (-o-) cockroaches. Poisoning stages refer to symptoms shown by DDT-treated groups. Vertical lines indicate standard errors.

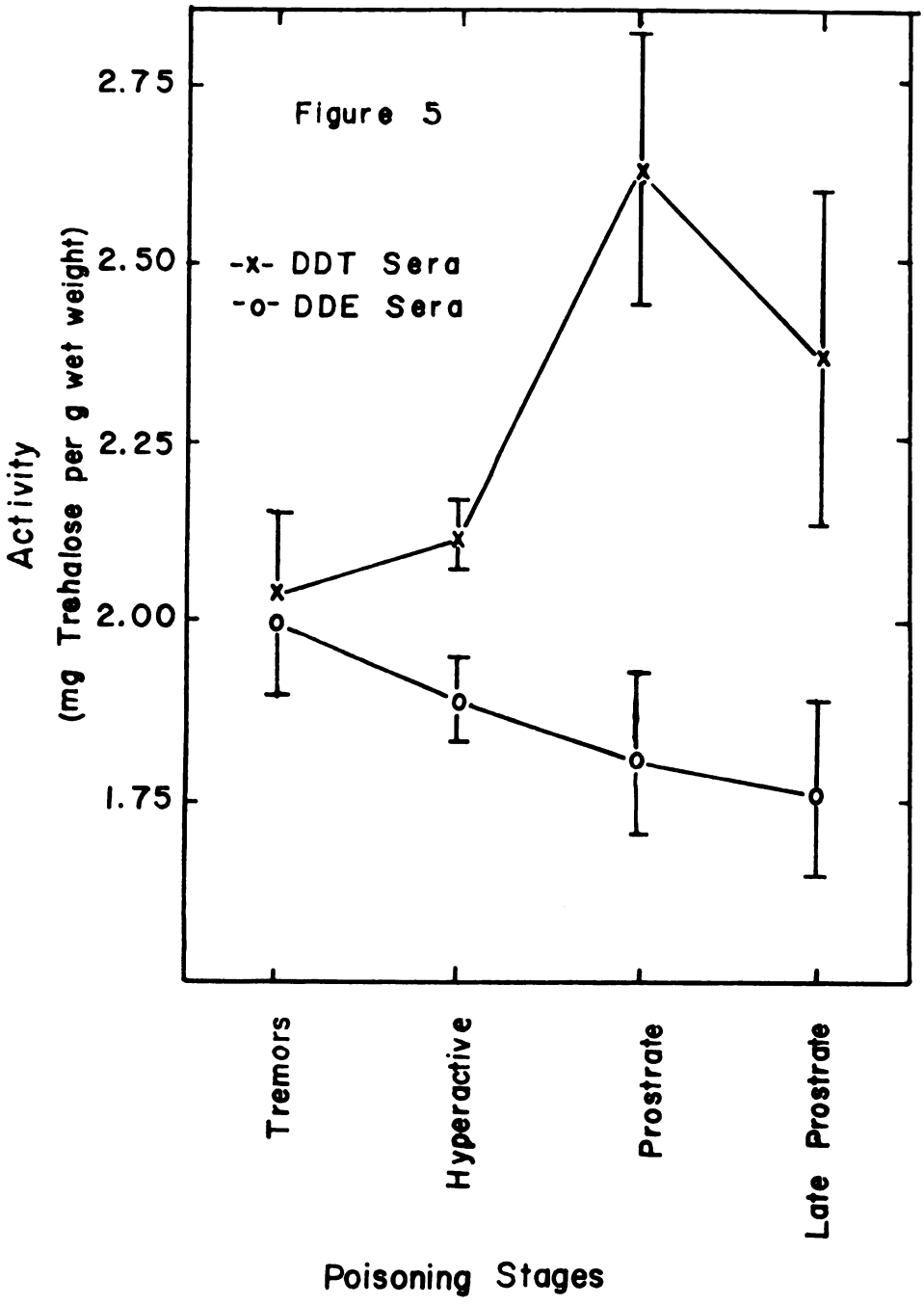
Figure 5
-x- DDT Sera
-o- DDE Sera
Activity
(mg Trehalose per g wet weight)
2.75
2.50
2.25
2.00
1.75
Tremors
Hyperactive
Prostrate
Late Prostrate
Poisoning Stages

Table 5. Hyperglycemic activity of serum from DDT-prostrate cockroaches diluted with Ringer's saline.

| Dilution parts serum:saline | Trehalose (% of control)[a] ± S.E. |
|---|---|
| 1:2 | 149 ± 16 |
| 1:4 | 140 ± 20 |
| 1:8 | 123 ± 10 |
| 1:16 | 102 ± 10 |

[a]Values are the increase in trehalose as a percentage of saline injected controls. Values represent 3 experiments each with 3-4 groups of 4-5 insects.

Figure 6. Sephadex chromatography of the hyperglycemic factor. A 0.9 x 22 cm Sephadex G-15 column was used and eluted in 2 ml fractions with 0.1 M acetic acid. The void volume was 5 ml. Activity of the eluant (-x-) was detected with a cockroach bioassay. The dashed line is the trehalose level of the saline injected controls. Protein levels (-o-) were determined by the Lowry method.

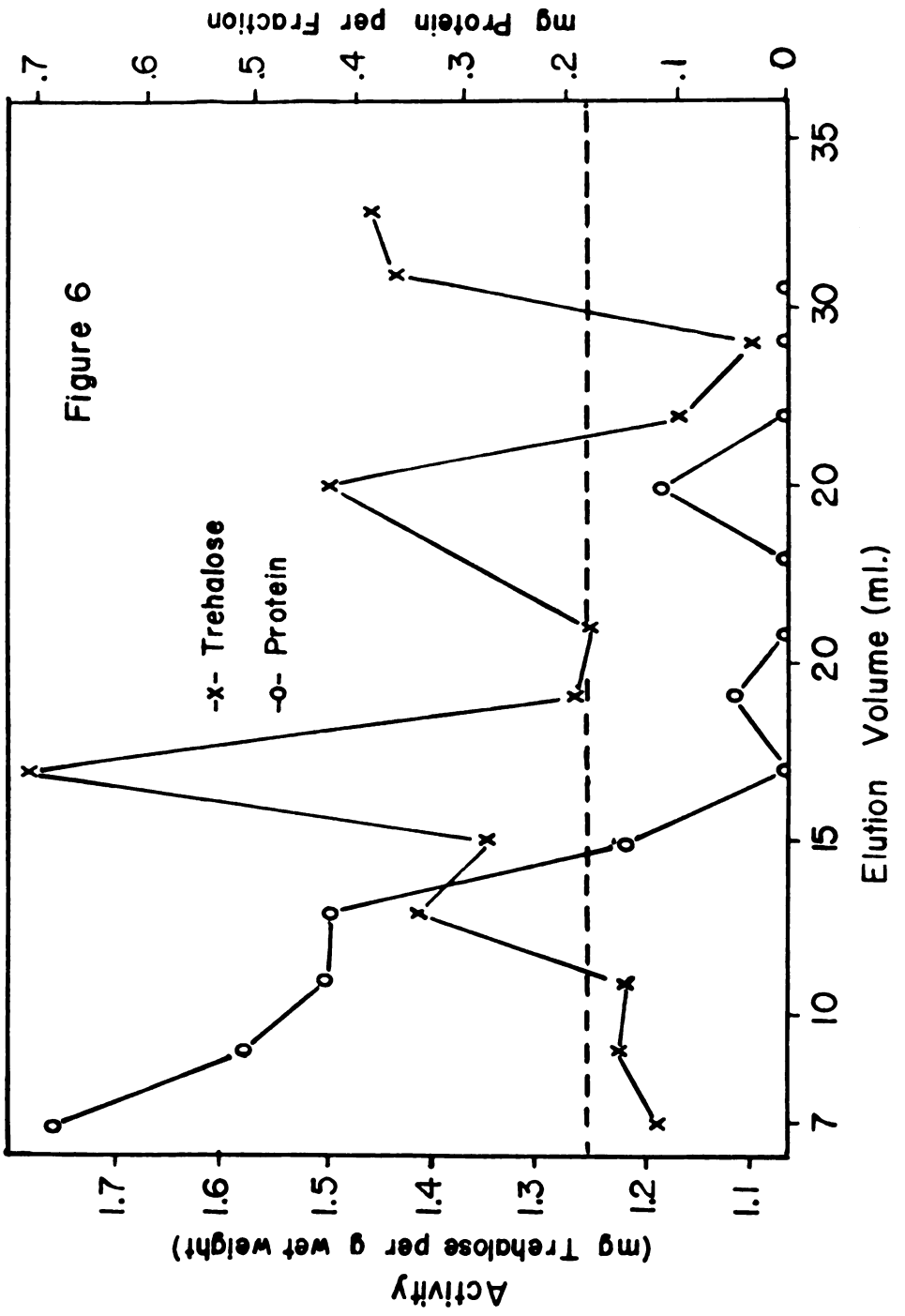
Figure 6
-x- Trehalose
-o- Protein
Activity
(mg Trehalose per g wet weight)
mg Protein per Fraction
Elution Volume (ml.)
1.1
1.2
1.3
1.4
1.5
1.6
1.7
0
.1
.2
.3
.4
.5
.6
.7
7
10
15
20
20
30
35

III. Discussion

The hyperglycemic activity of serum from DDT-prostrate cockroaches is due to a substance other than DDT or some metabolite of DDT. This factor appears analogous to the neurotoxin of Sternburg et al. (1959) in that it is found mainly in prostrate stages of poisoning and is heat stable. The heat stability of the factor and the elution from Sephadex indicate that the factor is probably not a protein. The hyperglycemic hormone described by Migliori Natalizi et al. (1970) had a similar elution pattern from Sephadex G-15. Their hormone was thought to be a peptide due to its trypsin sensitivity.

The physiologic nature of this hyperglycemic factor is open to speculation. First, the factor may be a product of tissue breakdown occurring in the late stages of DDT poisoning. The fat body tissues in cockroaches during the late stages of poisoning appear emptied and disrupted. Release of some pharmacologically active substance likely could occur.

A second possibility is that the factor may be a hyperglycemic hormone released into the hemolymph during DDT prostration. The release may be stimulated by the low levels of trehalose in the hemolymph or, alternatively, release may be stimulated directly by the action of DDT on the glands producing the hormone. These glands are probably associated with the nervous system and might be stimulated by the action of DDT on the nervous system. If the release were indeed effected by DDT, a variety of other hormones might also be released into the hemolymph.

A third possibility is that the factor does not affect carbohydrate metabolism directly, but causes the release of a hyperglycemic

hormone *in vivo*. For example, the factor could exert its action by affecting nerve tissue such as the neurohormonal glands. Such an action would be similar to that of the neurotoxin of Sternburg and Kearns (1952).

# AN OVERVIEW

## I. Interpretations of the Research

### A. Experiments Concerning Carbohydrate Levels

This research has demonstrated that in an insect treated with DDT, control over carbohydrate metabolism is upset during the dying process. This was reported in the first paper (Part I) as the increased utilization of trehalose and glycogen and the depletion of these carbohydrates by the time of prostration. Although circumstantial evidence (intense physical activity and rapid $^{14}CO_2$ respiration from glucose-$^{14}C$) suggests that these carbohydrates were primarily oxidized to carbon dioxide, more evidence would be needed for proof that this is indeed the main source of carbon dioxide and that the higher oxidation is of significance in DDT poisoning.

Tracer studies in which other metabolites are checked would lend significance. For example, what part does amino acid and lipid oxidation play in the increased respiration? Until the extent to which these other possible carbon sources contribute to the increased carbon dioxide production is determined, the significance of the carbohydrate depletion cannot be fully appreciated.

The second problem presented, though not completely solved in the first paper, is the existence or non-existence of a cause-effect relationship in the role of the carbohydrate depletion in DDT poisoning. Experiments other than the ones done could be attempted to substantiate

the interpretation of this paper that there is a cause-effect relationship, but in a complex system such as a whole organism such a relationship is almost impossible to prove. For example, if large doses of DDT were used and produced, hypothetically, a high carbohydrate level in the prostrate cockroach, this could be interpreted to mean that DDT affected the insect differently than the lower doses used in the real experiments. It could be argued that instead of the absence of the cause-effect relationship, the high carbohydrate levels really indicate a qualitative change in the mode of action of DDT at the higher dose, rather than the desired quantitative change.

Merrill et al. (1946) stated that the changes in the carbohydrate levels are not significant in the poisoning process. Arguments that question the procedures used can cast doubt upon this conclusion. They found that if the cockroaches were anesthetized the drop in carbohydrates did not occur. As discussed earlier, no mention was made of the symptoms the poisoned animals were exhibiting when they came out of anesthesia. However, if we assume that the insects did show prostrate symptomology, it could be argued that the experiment is not significant because the role of the anesthetic itself in producing the prostration is not known; there could be a synergistic or antagonistic effect between the anesthetic and the insecticide. Gatfield et al. (1966) demonstrated that phenobarbital anesthesia in mice is accompanied by a reduced utilization of glycogen and glucose in the brain. Such an antagonistic affect might occur with insects.

In essence, then, the chicken-egg (which-came-first) relationship is very difficult to prove or disprove. The experimental manipulations used to demonstrate it may themselves change the relationship being shown.

B. Experiments Concerning the Hyperglycemic Factor

The presence of a hyperglycemic factor is demonstrated fairly conclusively in the experiments. The rise in trehalose is not due to the absence of a substance or a dilution of the trehalose by injection of the agent, since saline injections produce no rise in the trehalose. The factor is probably organic (not an inorganic ion such as $PO_4^{2-}$ or $Ca^{2+}$) since it is deactivated by 24 hr at room temperature and to some extent by boiling for 5 minutes. Of course, direct experimentation into the chemical nature of the factor would give conclusive answers.

The possibility that the factor is a hormone is not discounted by the evidence. The factor is eluted from the Sephadex column in about the same fraction, proportionately, as the hormone studied by Migliori Natalizi et al. (1970). The fact that the factor is not present in large quantities during the tremors or hyperactive stages of DDT poisoning is also amenable to the hormone hypothesis. Maddrell (1964) in studying the diuretic hormone of cockroaches found that it was degraded while it exerted its activity on the malpighian tubules. In DDT poisoning the hyperglycemic hormone can only exert its activity in the early stages since in the prostrate stages no glycogen is available for conversion to trehalose. If degradation of the hormone is similar to that of the diuretic hormone an accumulation in the hemolymph might be expected only at the prostrate stages.

An understanding of the role of this hyperglycemic factor in DDT poisoning is not possible without more information. The discussion above treats this factor as a side effect of DDT poisoning. There is at present no evidence supporting this view or contradicting it. More

about the site of activity of this factor must be known before placing it in the sequence of events in DDT poisoning.

## II. Experiments

Several areas of uncertainty were mentioned in the previous section. Research in this area could profitably go in these and other directions.

### A. Lipid and Protein Levels

In order to understand the significance of the changes in carbohydrates during DDT poisoning, the levels of lipids and proteins in the insect hemolymph at various stages of DDT poisoning are important. Total lipids would be important since they too could be used as a source of energy and might reflect the presence of other hormone-like factors in the hemolymph. Levels of phospholipid precursors would also give an indication of changes in lipid metabolism.

Differing protein levels would either indicate the activity of a hormone-like factor or be an indication of tissue breakdown.

### B. Additional Effects of the Factor

A spectrum of the effects this factor has on the insect would be of significance in understanding its role. First, does it increase phosphate levels and carbon dioxide production in vivo? The hyperglycemic hormone of Steele (1963) increases phosphate. Whether the factor does would give an indication as to its equivalence to the hormone. Second, how does the factor affect the utilization of carbohydrates? Does it increase the metabolism of glucose-$^{14}C$ and what are the ratios of C-6 versus the C-1 incorporation into carbon dioxide? These types of studies would necessitate purification of the factor and an attempt to separate the various activities.

C. In Vitro Fat Body Bioassays

Does the factor directly affect the fat body or work on other systems in vivo? The factor could be incubated with fat body preparations. Increased release of trehalose by the fat bodies would indicate a direct effect of the factor on the fat body and reduce the possibility that some additional factor must act as an intermediate in vivo or that the new trehalose is not produced in the fat body, but released from muscles or hemolymph glycoproteins.

D. Chemical Characterization of the Factor

Additional experiments could characterize the factor chemically. For example, ion exchange chromatography, trypsin sensitivity tests and various tests for active groups could be done. As an adjunct to these tests, utilization of whole cockroach homogenates rather than the hemolymph as a source of the factor should be attempted, since this would allow for the preparation of larger quantities of the factor.

E. Biological Characterization of the Factor

Is the factor released during physical stress or poisoning with other insecticides? This fact is important for comparison to the neurotoxins as discussed previously.

Similarly, is the factor active on nerve preparations? Since the neurotoxin is also present in the hemolymph of DDT-prostrate cockroaches this type of experimentation would also entail an attempt to separate the two types of activity. A tentative identification of the factor as being the same as the neurotoxin and/or the hyperglycemic hormone could be made by their elution patterns from Sephadex columns or other types of chromatography.

III. Significance of these Studies

The significance of this work possibly lies in two areas. First it will produce a better understanding of how insects, and possibly animals in general, respond to stresses. From the work on the neurotoxins, DDT appears to be a nonspecific stressing agent on the insects. Possibly study of this factor and others will indicate that they too are released as a result of many types of stress. An understanding of how an animal reacts to a stress is basic to understanding the methods by which an organism controls its basic functions.

The second area of significance is an outgrowth of the possibility that nonspecific stress is an important part of DDT poisoning. In the past, screening programs for potential insecticidal chemicals have used mortality as the main criterion for selecting candidate insecticides. Since insects in general do not differ grossly from other forms of life biochemically, the resulting insecticides were also biocides. If indeed this factor is part of a hormone system and it is significant in the chain of events leading to mortality, the possibility exists that these studies will be useful in devising insect-specific screening programs for insecticides.

# LIST OF REFERENCES

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