The Response of Dietary Stressed *Periplaneta americana* to Chronic Intake of Pure Aflatoxin B_1

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Aflatoxin B_1, a common fungal metabolite of *Aspergillus flavus* has been found in a variety of stored agricultural products (GOLUMBIC and KULIK 1969). This metabolite has been reported to be a highly toxic hepatocarcinogen; more toxic than the other three commonly found aflatoxins, B_2, G_1 and G_2 and butter yellow (BUTLER 1965). Most organisms have been found to be susceptible to aflatoxins with young birds and fish being the most sensitive (CARNAGHAN et al. 1963; VERRETT et al. 1964; BAUER and LEISTNER 1969).

Biological assay systems reported for aflatoxin B_1 have included studies on chick embryos (VERRETT et al. 1964; HORWITZ et al. 1970), ducklings (CARNAGHAN et al. 1963), amebae (BAUER et al. 1969), trout (BAUER et al. 1969), zebra fish larvae (ABEDI and McKINLEY 1968), bacteria (BURMEISTER and HESSELTINE 1966), various cell cultures (LEGATOR 1966; NAIR et al. 1970; TERAO and MIYAKI 1970), planaria (LLEWELLYN 1973), cress seedlings (SCHOENTAL and WHITE 1956), mollusk eggs (TOWNSLEY and LEE 1967), lily pollen (LLEWELLYN and DASHEK 1973), rhesus monkeys (TERRIER et al. 1971), guinea-pigs (BUTLER 1966), cows (ALLCROFT and CARAGHAN 1963), yellow-fever mosquito, house fly and fruit fly (MATSUMURA and KNIGHT 1967), weevils (MOORE 1974), and brine shrimp larvae (BROWN et al. 1968; BROWN 1969).

The potential for using insects in studies relating to the mode of action and toxicity of aflatoxins has not been explored in detail. Toxicological studies of mycotoxins in insect species are limited, although there have been some insecticidal properties attributed to the aflatoxins. For example, aflatoxins have been suspected as a cause of honey bee mortality (FOOTE 1966). Also, aflatoxins were found to cause mortality and to reduce both the number of eggs produced and the percentage of eggs which hatched in three species of dipterous insects, *Aedes aegypti, Musca domestica* and *Drosophila melanogaster* (MATSUMURA and KNIGHT 1967). Other groups of insects such as the Isoptera and Lepidoptera (*Heliothis*) have been killed by exposure to aflatoxins (BECKER et al. 1969; GUDAUSKAS et al. 1967).

Since these studies indicate that aflatoxins possess some insecticidal activity, this work reports on the susceptibility of the American cockroach, *Periplaneta americana* (L.) in a dietary stressed environment to purified aflatoxin B_1.

Experimental Procedure

Adult males, *Periplaneta americana* (L.), were chosen randomly from our stock colony and placed individually into feeding chambers. This apparatus was chosen after various unsuccessful attempts were made to maintain and feed the cockroaches in many types of wire cages.

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The apparatus allowed the cockroach to be isolated from most environmental variables as well as providing for data collection. A plastic, transparent drinking cup was inverted and placed inside a plastic, disposable Petri dish. Circular filter paper was cut to fit the bottom of the Petri dish and served for feces deposition. A wire ladder was constructed from window screen to aid the cockroach in reaching the curved drinking tube inserted in to the top of the cup. The capillary tube was calibrated with a syringe and placed in the chamber so the animals could feed. Air holes were provided in the cup.

All animals were weighed weekly and were provided with 0.15 M sterile sucrose, *ad libitum*. Daily consumption of the solution was recorded and corrected for evaporation. Aflatoxin B_1, grade B, dried *in situ*, was obtained from Calbiochem, La Jolla, California. The toxin was transferred by acetone into the sugar water prior to autoclaving. Evaluation of the autoclaved medium by the Virginia Division of Consolidated Laboratories, Mycotoxin Laboratory, (Richmond) indicated the presence of only aflatoxin B_1 at 12 µg/ml. The sensitivity of the above evaluation procedures exceeds 2 ppb. Control medium tested negative for the presence of the toxin (HORWITZ et al. 1970).

Experimental animals received sucrose contaminated with 12 µg/ml pure aflatoxin B_1 and control animals received an identically prepared diet lacking the toxin. Liquid consumption was recorded for all animals and three control chambers without animals were used to determine average evaporation rates. Animals were maintained at 25 ± 2°C in a photoperiod of 12 hours of light.

Fifteen experimental animals were analyzed for aflatoxin B_1 retention in the body. The animals were collected at death or sacrificed and individual bodies were pulverized and extracted with chloroform (HORWITZ et al. 1970).

Six animals designated as overall control were maintained on a diet of Purina Dog Chow. Both food and water were provided *ad libitum* with comsumption levels determined only once a week.

Results and Discussion

The first series of experiments were designed to determine the ingestion rate of the cockroaches when fed sucrose or sucrose plus aflatoxin. The sucrose level fed was considered inadequate for long term studies but was used to study the effects of the toxin on animals on a non-sustaining diet. The sucrose animals (denoted as the controls) consumed approximately 27% more diet than the experimentals (fed aflatoxin plus sucrose). The consumption was measured over a 56-day period, and both groups drank approximately the same quantity over the initial 3 weeks. However, after this interval, the taste of the aflatoxin apparently inhibited the feeding response, and these animals began to drink less. These results are summarized in Figure 1. Overall control animals maintained on adequate solid dog food diet show a significantly lower intake of water.

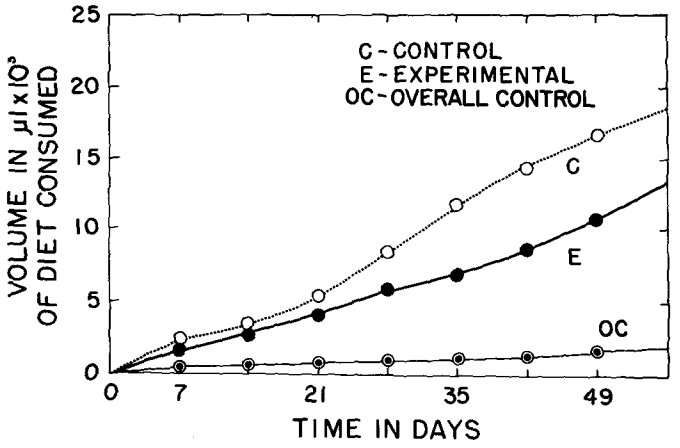

Figure 1. Liquid Diet Consumption for Experimental and Control Animals.

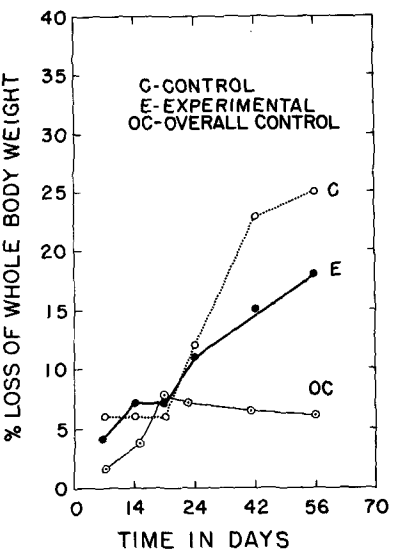

Figure 2. Effect of Aflatoxin B_1 on Animal Weight

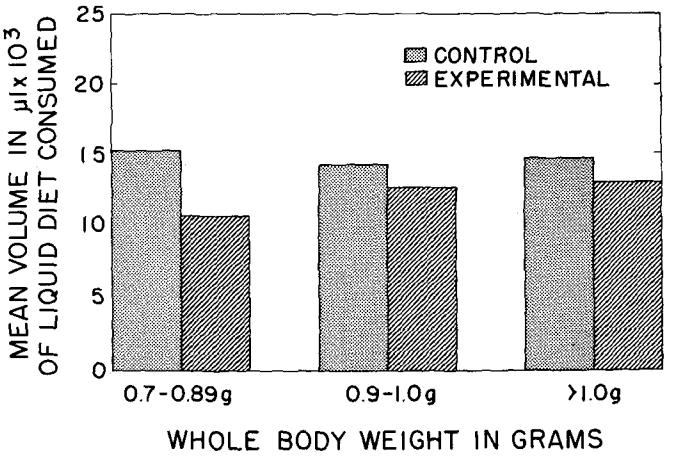

Figure 3. Liquid Diet Consumption as Related to Animal Weight Groups.

A second group of experiments were designed to measure weight loss during the period of food ingestion. Since a protein-free solution of 0.15 M sucrose was fed, it was surmised that this diet would be insufficient to maintain their initial weight. This proved to be correct and both groups lost weight. However, contrary to expectations, the control group which consumed more sucrose also lost more weight. The control group lost nearly 25% of their total body weight while the experimental group lost only 18%. The reason for this phenomenon is as of yet unexplained, but it is postulated that the imbibing of greater quantities of water potentiated the production of urine. It is known that dilute solutions in the gut cause decreased reabsorption by the rectum (SAUER et al. 1970). The animals designated as overall controls and maintained on a normal solid diet consumed considerably less dietary water. These animals also lost significantly less body weight than controls and experimental animals (Figure 2). Aflatoxin animals showed a gradual increase in accumulative values for toxin consumption.

It is possible that the initial weight of the animal may have some effect on the total consumption of liquid and the percent weight loss. However, as shown below, this parameter appears to have little or no effect. The bar graph, Figure 3, represents total µl of consumption by various weight groupings of the control and experimental animals. No statistical differences as related to consumption were apparent due to the original weight of the animals. Also, there was no real difference in the percentage of weight loss of the various weight groups due to variance of original animal weights. This is illustrated in Figure 4, which shows that the controls of the weight groupings lost the greater percentage of body weight.

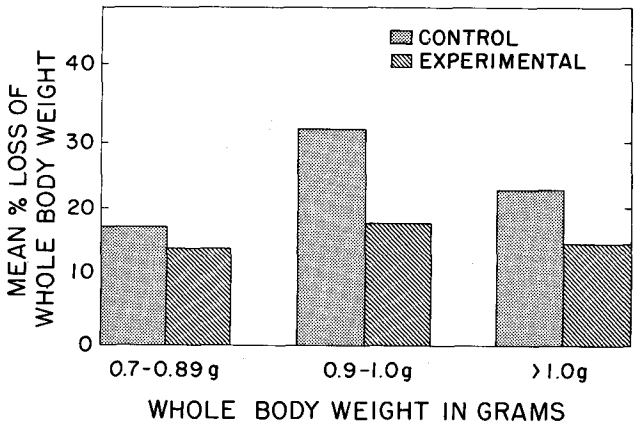

Figure 4. Percent of Animal Weight Loss as Related to Animal Weight Groups.

The LD_{50} for the experimental animals was approximately 24 mg/kg. This is a mean value and does not include the two animals having the highest and lowest toxin consumption. The LT_{50} for the experimental animals receiving this diet and the toxin was 56 days. No control animals died during these studies. The LD_{50} value is 2-3 times higher than the 8-10 mg/kg levels of toxin found to cause 50% mortality in hamsters (HERROLD 1969; LLEWELLYN et al. 1974).

The animals were definitely imbibing the aflatoxin and mortality occurred. This raised a question regarding the possibility of storage and/or detoxification of the aflatoxin B_1 by the animals. Carcasses were analyzed for the presence of mycotoxins. Of the 15 cockroaches that were evaluated for whole body burdens of aflatoxin B_1, 40% showed detectable levels of the toxin (>2 ppb). No metabolites were detected in any of the extracts made from the tested cockroaches, including those animals showing the highest body burden of aflatoxin B_1. Metabolites may have been present but at a level beyond the sensitivity of the detection system. Those experimental animals testing positive had the following body burdens of aflatoxin B_1: 0.075 µg, 0.03 µg, 0.06 µg and 0.06 µg. Those animals living at the LT_{50} showed a slightly higher body burden as compared to those that had succumbed. One animal that was sacrificed for this test had a body burden of 0.24 µg. Although others (NEVINS and GRANT 1971) have reported a 10-fold bioconcentration of the toxin in housefly maggots when fed 0.02 ppm, the adult cockroaches studied herein appeared not to be concentrating the toxin.

Summary

In general these studies seem to indicate that adult male *P. americana* are not particularly sensitive, toxicologically, to aflatoxin B_1, even when maintained on a marginally inadequate diet containing a low level of sucrose and no protein. Also they may be capable of detecting low levels of aflatoxin B_1 in their diet (12 µg/ml) and seem not to concentrate aflatoxin B_1 in their bodies. Even in dietary stressed conditions adult male American cockroaches showed a very limited potential as a bioassay organism for this toxin. Actually it appears that they may be quite resistant to the toxin. Currently there is no definite answer as to the advantages or disadvantages of insufficient dietary proteins or even carbohydrates providing protection against this toxin.

The results show that the toxin would not be an effective cockroach-killing agent and thus could not serve as a bioassay system. However, this insect could serve as a model system in further investigating the mode of action and possible detoxification of aflatoxin B_1.

Acknowledgments

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