

EFFECT OF VERTEBRATE HYPOGLYCEMIC AND β -CELL CYTOTOXIC AGENTS ON INSECTS*

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Abstract—1. The effect of several vertebrate hypoglycemic and β -cell cytotoxic chemicals on insects was examined.

2. The dietary LC₅₀'s for phenformin fed to *Manduca sexta*, *Plodia interpunctella*, and *Tribolium confusum* were 1.4×10^3 , 3.3×10^3 and 13.0×10^3 ppm.

3. Chlorpropamide and tolbutamide were similarly effective against *M. sexta* at 8.5×10^3 and $\sim 17 \times 10^3$ ppm.

4. Chlorpropamide produced a 23% hypotrehalosemic response in *M. sexta* fed 10^4 ppm supplemented diet.

5. The β -cell cytotoxic agents, alloxan and streptozotocin, produced 50% mortality when injected into the hemocoel of fourth-instar *M. sexta* at doses of 150 and 85 $\mu\text{g/g}$ body weight.

INTRODUCTION

The discovery of physiologically active, insulin-like polypeptides in the neuroendocrine system of insects indicates that the invertebrate and vertebrate hypoglycemic factors are structurally related (Dixit & Patel, 1964; Falkmer *et al.*, 1973; Meneses & Ortiz, 1975; Tager *et al.*, 1976; Ishay *et al.*, 1976; Kramer *et al.*, 1977). It also suggests that pharmacological agents which affect insulin action in vertebrates should probably function in insects. As part of a study to determine how the insect neuroendocrine system regulates carbohydrate metabolism, we determined the toxicity of hypoglycemic and β -cell cytotoxic chemicals in several insects: the tobacco hornworm [*Manduca sexta* (L.)], the Indian meal moth (*Plodia interpunctella* Hübner) and the confused flour beetle (*Tribolium confusum* Jacquelin du Val). The five drugs tested (Fig. 1) were alloxan and streptozotocin which were injected into the body cavity and phenformin, tolbutamide and chlorpropamide which were administered orally. We also examined the effect of chlorpropamide on carbohydrate levels in hemolymph and fat body of *M. sexta*.

MATERIALS AND METHODS

Chemicals

Phenformin HCl [*N*-(2-phenylethyl)imidodicarbonimidic diamide monohydrochloride], chlorpropamide [4-chloro-*N*-[(propylamino)carbonyl] benzenesulfonamide] and tolbutamide [*N*-[(butylamino)carbonyl]-4-methylbenzenesulfonamide] were obtained from Ciba-Geigy Corporation, Ardsley, NY, Pfizer Pharmaceuticals, Inc., Barceloneta, P.R. and Upjohn Co., Kalamazoo, MI., respectively. Streptozotocin [2-deoxy-2-[[methylnitrosoamino]carbonyl]-amino]-D-glucose] was supplied by Dr Howard Tager, The

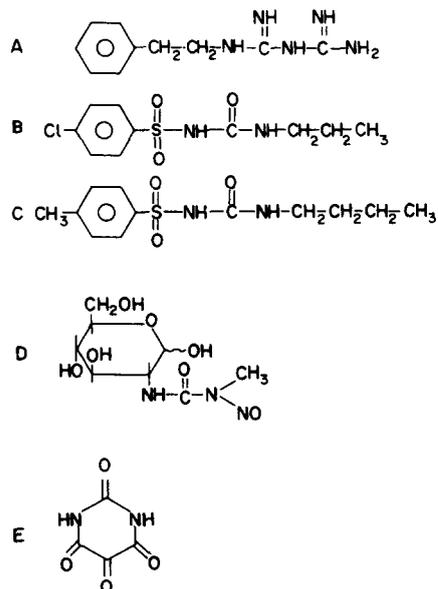


Fig. 1. Hypoglycemic and hyperglycemic chemicals. (A)—Phenformin; (B)—chlorpropamide; (C)—tolbutamide; (D)—streptozotocin; (E)—alloxan.

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University of Chicago. Alloxan [2,4,5,6-(1H,3H)-pyrimidinetetrone] was purchased from Sigma, St Louis, MO.

Insects

Manduca sexta eggs were obtained from Dr J. P. Reinecke (Agricultural Research Service, U.S. Department of Agriculture, Fargo, ND, U.S.A.). Larvae were reared at 28°C and 60% relative humidity with a 16-hr light photoperiod and a standard diet (Bell & Joachim, 1976). After cooling to 60°C, the oral drugs were thoroughly blended into the agar-based diet. Diet (~13 g) was poured into 110 cm³ plastic beakers and after it had hardened, one neonate larva was added. The mean weight of 10–20 larvae fed control or drug-supplemented diet was compared at 1–4 day intervals for about 40 days. Fecal matter was removed at each observation. *P. interpunctella* and *T. confusum* were obtained from cultures at the U.S. Grain Marketing Research Laboratory. Moth medium was prepared according to Kinsinger (1975). Then 50–75 Indian meal moth eggs were placed in 100 g of medium and moths were counted as they emerged. Also 50 beetle eggs were placed in 5 g of enriched flour. After 10 days, the mean weight of 10 insects was recorded at regular intervals for approx 2 months and adults were recorded as they emerged. All experiments were conducted at 27°C and 60% RH. The oral toxicity was evaluated as the ppm per weight of diet necessary to obtain 50% mortality (LC₅₀ with 95% confidence limits given in parentheses). Probit analyses of the data were conducted according to Finney (1952).

When alloxan was to be injected it was dissolved in insect saline solution (10 mg/ml) and injected (100–500 µg/insect) into the hemocoel of fourth-instar *M. sexta* (0.8 ± 0.1 g). The insects were observed on alternate days until adult eclosion or mortality. For the tests with streptozotocin, a 1% solution in 25 mM citric acid, 0.9% NaCl, pH 4 was prepared immediately before injection (25–200 µg/insect). The data are presented as that amount which produced 50% mortality when injected (LD₅₀).

Carbohydrate analysis

Newly molted fifth-instar *M. sexta* (0.9–1.1 g) were transferred to the agar-based diet with and without 10⁴ ppm chlorpropamide. Insects were allowed to feed for approx 3 days until they weighed 2.2 ± 0.3 g. Hemolymph and fat body were collected and subjected to trehalose and glycogen analysis by using the anthrone reagent (Roe, 1955) as described previously (Tager *et al.*, 1976; Kramer *et al.*, 1977). The mean values for 8 control and 8 drug-fed hornworms (± S.E. of those values) are reported.

RESULTS AND DISCUSSION

Phenformin

Despite the long usage of biguanides in human diabetes therapy, the mode of action is not clear. Phenformin is a biguanide drug that apparently enhances the glycolytic utilization of glucose in vertebrates and thus lowers blood sugar (Duncan & Clark, 1965; Danowski, 1968). To find out whether this chemical might adversely affect insects, we determined dose-mortality relationships for 2 moth and 1 beetle species. *M. sexta* larvae did not grow on diet supplemented with 5 × 10³ ppm drug (20 mM). At 2.5 × 10³ ppm (10 mM), larvae attained their maximum weight 4–5 days later than the control group, but they were unable to complete pupation. At lower doses (0.5 × 10³ ppm), some animals did undergo adult eclosion, the number being inversely proportional to the drug concentration. The LC₅₀ for *Manduca* was 1.4 (0.9–1.8) × 10³ ppm.

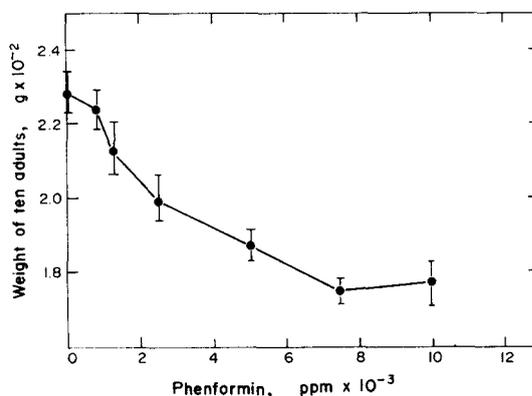


Fig. 2. Effect of phenformin on weight of the confused flour beetle, *Tribolium confusum*.

The LC₅₀ for *Plodia* was 3.3 (1.8–6.1) × 10³ ppm when the drug was homogeneously mixed in the ground wheat moth medium. Phenformin was even less active against the coleopteran, *T. confusum*: the LC₅₀ was 1.3 (1.0–1.9) × 10⁴ ppm in enriched flour. At 2 × 10⁴ ppm phenformin, only 34% of the *Tribolium* could develop completely in approx 60 days, whereas untreated adults emerged in 40 days. A weight reduction occurred with the adult insects in a manner that was dependent on the drug concentration (Fig. 2). For example, beetles grew to only ca. 80% of the normal weight when fed 8–10 × 10³ ppm phenformin.

Chlorpropamide and tolbutamide

Sulfonylurea drugs also are used as antidiabetic agents and these compounds are believed to stimulate the synthesis or release of endogenous insulin in vertebrates (Levine, 1957; Duncan & Clarke, 1965). The LC₅₀ for chlorpropamide was approximately 8.5 × 10³ ppm (34 mM). Tolbutamide was estimated to be about half as active. To determine whether the sulfonylurea produces a hypoglycemic response in insects, as it does in vertebrates, we fed

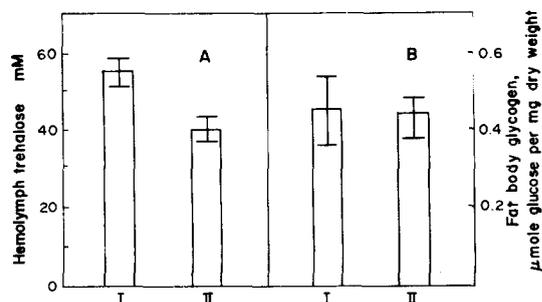


Fig. 3. Effect of chlorpropamide on hemolymph trehalose and fat body glycogen of *M. sexta*. Mean values ± S.E.M. for eight experimental subjects are shown. Fourth-instar larvae (1.0 ± 0.2 g) were placed on normal diet or on diet containing 10⁴ ppm chlorpropamide. Those weighing 2.2 ± 0.3 were killed 4–6 days later. (A)—Hemolymph trehalose content from control hornworms (I) and hornworms fed diet supplemented with 10⁴ ppm chlorpropamide (II). (B)—Fat body glycogen content from control (I) and chlorpropamide-treated (II) hornworms.

last instar *M. sexta* larvae a diet that was treated with chlorpropamide at the LC_{50} value and checked carbohydrate levels in *M. sexta* hemolymph and fat body. Chlorpropamide caused hemolymph trehalose to drop 23% from a control value of 54 ± 4 mM to 42 ± 2 mM (Fig. 3A). The drug did not significantly alter the fat body glycogen level of 450 ± 140 μ mole of glucose per gram of desiccated tissue (Fig. 3B). This hypotrehalosemia is consistent with a mode of action that increases circulating insulin levels.

Alloxan and streptozotocin

Alloxan and streptozotocin are pancreatic β -cell cytotoxic agents that can produce hyperglycemia when injected into vertebrates (Lazarus & Shapiro, 1972; Orci *et al.*, 1972). Both compounds are used experimentally to induce diabetes and the latter is also used therapeutically as an antiinsulinoma drug. The estimated LD_{50} 's for *M. sexta* were about 150 and 85 μ g/g body weight, respectively, values similar to the effective doses for vertebrates [50–200 mg/kg (Rakieten *et al.*, 1963)]. Previously Ishay (1975) found a marked increase in glucose concentration in *Vespa orientalis* that had been fed alloxan. To our knowledge, this is the first report concerning a streptozotocin effect on insect growth.

Concluding remarks

When we examined the effects of vertebrate hypoglycemic and β -cell cytotoxic agents on insects, we found that administering five of these drugs inhibited growth and development. One chemical, chlorpropamide, depressed the blood carbohydrate level, producing hypotrehalosemia. The physiological effects of the other drugs were not determined. We do not know whether the stresses caused by the drugs are hypoglycemic hormone (insulin)-mediated responses or secondary effects. Our results and those of others (see references cited in Introduction, Seecof & Dewhurst, 1974; Mosna & Barigozzi, 1976) support the hypothesis that insects have the potential to suffer from endocrinopathic conditions caused by insulin dysfunction such as diabetes. Interestingly, the hyperglycemia associated with caudery of the medial neurosecretory cells of the *Calliphora* brain also results in manifestations (i.e. polydipsia) similar to those found in vertebrate diabetes (Normann, 1975). As they act in vertebrates, the sulfonylurea compounds may increase insulin output in insects, while alloxan and streptozotocin may degranulate or kill cells responsible for producing insulin in the neuroendocrine system. The biguanide derivative may induce peripheral tissue such as fat body and muscle to accelerate glucose uptake and oxidation. Final proof of the pharmacological actions of these drugs, however, must await more detailed biochemical and morphological analyses of their cellular effects in insects (Nagabhushanam & Hanumante, 1977).

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