Intestinal Uptake of Ouabain and Digitoxin in the Milkweed Bug, *Oncopeltus fasciatus*

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Summary. The absorption of ouabain and digitoxin into the gut of the milkweed bug was studied by a tissue-accumulation technique. Uptake of both compounds obeyed diffusion kinetics, was Na^+ sensitive, and for digitoxin, was concentrative.

The large milkweed bug, *Oncopeltus fasciatus* (Dallas), is one of several insects known to concentrate cardenolides present in food plants[3]. These digitalis-like poisons discourage vertebrate predation[4]. Ouabain and digitoxin are inhibitors of (Na^+–K^+) ATPase and ATPase-linked nutrient transport systems in vertebrate intestines[5]; yet *O. fasciatus* can ingest and concentrate these cardenolides as reported by DUFFEY and SCUDDER[6]. These workers found that unaltered ouabain, which is polar, and two polar metabolites of digitoxin, a nonpolar compound, are concentrated in the dorsolateral complex (glands) of milkweed bugs.

The present study was undertaken to investigate the role of the gut of *O. fasciatus* in the concentration of these two representative cardenolides.

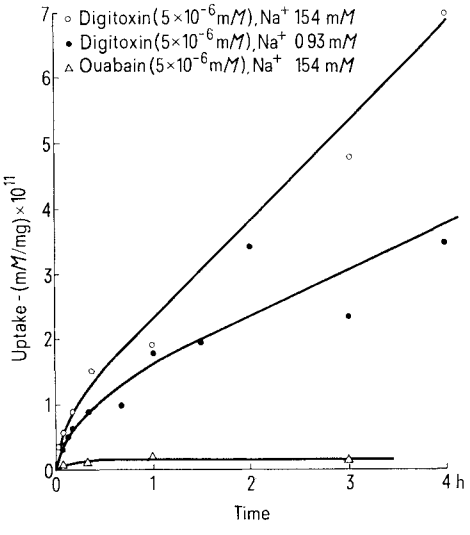

Fig. 1. Uptake of ouabain and digitoxin into the gut of *O. fasciatus* at 21 °C. Each point represents a single determination on tissue pooled from 3 randomly selected bugs.

O. fasciatus were raised in the laboratory on *Asclepias syriaca* L. seeds or husked sunflower seeds and water. ^{3}H-Ouabain and ^{3}H-digitoxin were obtained from New England Nuclear. Non-labeled ouabain and digitoxin were obtained from Sigma Chemical Co. Uptake of ouabain and digitoxin into the cells of the gut of adults of *O. fasciatus* was determined as follows: the gut was removed and held at 0–4 °C in phosphate buffer (pH 7.2; Na^+ concentration 154 m*M*; K^+ concentration 4.5 m*M*)[7] prior to a 30-min preincubation at 21 °C. Labeled substrate was then added at various concentrations. After incubation the gut was rinsed, weighed and added to 10 ml of scintillation solution[8], and the mixture was agitated on a vortex prior to counting by scintillation spectrometry. Uptake was also determined using low Na^+ (0.9 m*M*) buffer, the depleted Na^+ replaced by isosmotic mannitol, and using high K^+ (20 m*M*) buffer. Uptake of digitoxin was determined in the presence of an excess of ouabain (0.05 m*M* ouabain; 5×10^{-6} m*M* digitoxin). The reverse test was not run because of the limited solubility of digitoxin. Unless otherwise noted, all animals tested were fed cardenolide-free[9] sunflower seeds.

[1] The authors express their appreciation to Dr. JAMES A. SLATER and Ms. FLAVIA O'ROURKE, Biological Sciences Group, University of Connecticut, Storrs, Conn. (USA) for supplying milkweed bugs. This work was supported by the University of Maine Honors Program and the University of Maine Agricultural Experiment Station.

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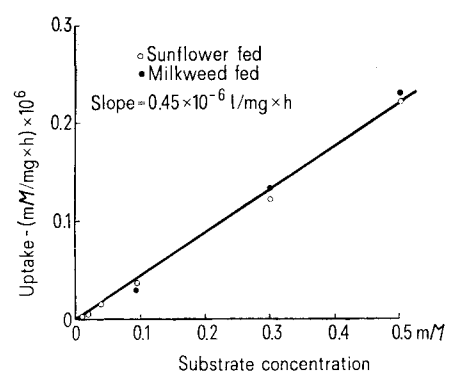

Fig. 2. Uptake of ouabain into the gut of *O. fasciatus* at 1 h. Other conditions are the same as in Figure 1.

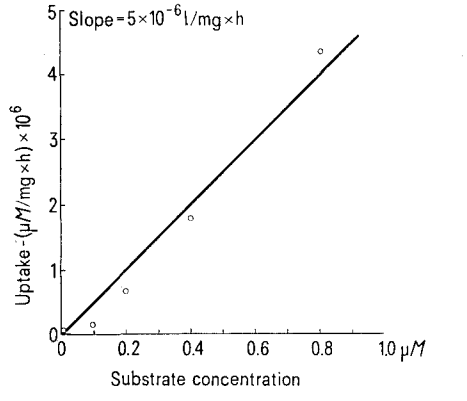

Fig. 3. Uptake of digitoxin into the gut of *O. fasciatus* at 1 h. Other conditions are the same as in Figure 1.

Figure 1 shows the differential uptake of equimolar digitoxin and ouabain into the cells of the gut of *O. fasciatus* over time. The distribution ratio of internal to external substrate concentration (DR) at 4 h is at an equilibrium level of 0.4 for ouabain compared to 17.5 for unequilibrated digitoxin (assuming 80% tissue water content). Low Na^+ buffer effected a decreased uptake of digitoxin; DR at 4 h is 8.7. Similar results were obtained with ouabain; DR at equilibrium is 0.09. A high concentration of K^+ (20 m*M*) had no effect on absorption of either substrate at 2 min, 10 min and 1 h of incubation; nor did an excess of ouabain decrease digitoxin uptake at 1, 2, and 20 min or 2 h. Figures 2 and 3 show that the uptakes of ouabain and digitoxin are nonsaturable and directly proportional to concentration in the ranges shown. *O. fasciatus* fed *A. syriaca* seeds contain cardenolides[6]; these animals showed no significant difference in ouabain uptake from bugs fed sunflower seeds.

Uptake of both ouabain and digitoxin into the gut, as reported in this study, exhibits characteristics of diffusion. The fact that digitoxin, being only slightly water soluble, has a high lipid-water partition coefficient may account for its observed concentration into the gut; ouabain is highly water soluble by comparison. In addition, digitoxin, but not ouabain, is observed to cross intestinal membranes rapidly in vertebrate systems[10]. If metabolism of digitoxin occurs within the cells, this might further account for the inability of this substrate to reach equilibrium even at 4 h.

The preferential absorption of the nonpolar cardenolide digitoxin into the gut of *O. fasciatus* is to be contrasted with the high concentration of polar cardenolides, e.g. ouabain and digitoxin metabolites, found in the dorsolateral glands[6]. Metabolic and selective concentrative processes must bring about this reversal.

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Study of the Characteristics of the Inotropic Effect of Insulin in Rabbit Papillary Muscle[1]

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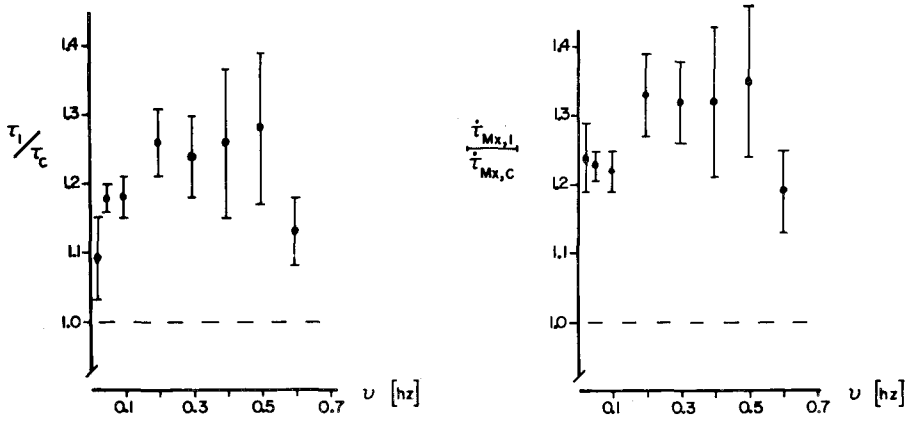
21 June 1976.

Summary. The effect of insulin was examined with emphasis on the alteration in the force-frequency relation. The results show that insulin does not change the time to peak tension nor the time of contraction. The inotropic effect was significant and did not depend upon the frequency of stimulation. However, there was a definite dependence of the magnitude of the inotropic effect on temperature. Previous studies have indicated that the inotropic effect is not a result of increased substrate availability or changes in cAMP phosphodiesterase activity. These results and those reported here are consistant with the hypothesis that insulin's inotropic effect is due to increases in intracellular Ca^{++}.

Although the dominant action of insulin is in facilitating glucose entry into the cell, recent results indicate that it is also an inotropic agent[2]. Since the preferred myocardial substrate is fatty acids and not glucose, it is doubtful that the inotropic effect is a result of alteration in glucose metabolism. Another explanation for insulin's inotropic could be its effect on cAMP phosphodiesterase (PDE)[3]. Preliminary studies comparing the effects of insulin with other PDE effectors (theophylline and imidazole) argue against this. Since very little insulin penetrates into the sarcoplasm[4], the cause of the inotropic effect might be an alteration of ion transport. The studies presented here were conducted to examine more closely the characteristics of the change in performance due to insulin using the changes in the force-frequency relation.

Methods. Cardiectomies were performed on stunned female rabbits weighing 2–3 kg. A papillary muscle from the right ventricle was tied (6–0 silk suture) and then suspended between two silver stimulating electrodes. The mean muscle diameter (1 g load) was 1.1 ± 0.4 mm,



Effect of insulin on mechanical performance of rabbit pappillary muscle. Abscissa = frequency of stimulation [Hz]; Ordinate = ratio of performance in the presence of insulin (10 mU/cc) vs control. τ = maximum twitch tension; $\dot{\tau}_{max} = (d\tau/dt)_{max}$. Maximum time derivative of the tension. Points represent mean ± S. E. M. (N = 11). Butyric acid (10 m*M*) substrate.