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Residual Quantities of Streptomycin Sulfate in Haemolymph and Tissue of *Heliothis virescens* Reared on Treated Diet

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SIKOROWSKI, P. P. AND A. C. THOMPSON. *Residual quantities of streptomycin sulfate in haemolymph and tissue of* Heliothis virescens *reared on treated diet.* PHARMACOL BIOCHEM BEHAV 23(4) 525–528, 1985.—The study showed that streptomycin sulfate was present in both tissue and blood of insects fed with the antibiotic-treated diet. Changes from larval to pupal to adult stages were accompanied by decreases in antibiotic concentration. During transformation from pupa to adult most of the antibiotic was discarded with the meconium. Eggs from adults reared from larvae fed on the antibiotic-treated diet were free of streptomycin. The antibiotic did not adversely affect insect development.

Streptomycin sulfate Haemolymph and tissue Heliothis virescens

HELIOTHIS oligidic and meridic diets are highly susceptible to microbial contamination and spoilage. To prevent microbial contamination, antimicrobial agents such as streptomycin often are incorporated into the medium or are spread over its surface.

Streptomycin, an aminoglycoside, is active against a wide range of gram-positive and gram-negative bacteria. In feeding studies with increasing concentrations of streptomycin sulfate, *Heliothis virescens* larval feeding did not change significantly up to 8 g streptomycin sulfate/500 g diet [7]. However, there was a significant decrease in the 8-day-old larval weight at antibiotic concentrations above 2 g/500 g diet. Singh and House [5,6] studied the effects of increasing concentration of streptomycin on *Agria affinis* and found that the rates of growth, development and survival decreased with increasing levels of antibiotic in their diet. Streptomycin mixed with corn cob grit spread over the surface of *Anthonomus grandis* diet had no effect on weevil development, egg production, hatch or pheromone production [4]. Thompson and Sikorowski [7] reported that *H. virescens* could tolerate up to 4 g streptomycin sulfate per 500 g of diet, and the accumulation of lipids, carbohydrates and proteins in the larvae was only slightly affected. In our study, experiments were conducted to determine residual quantities of streptomycin sulfate in the tissue of *H. virescens* larvae, pupae and adults, in the haemolymph of larvae and pupae, and meconium and eggs.

METHOD

Source of Insects

Healthy *H. virescens* used in the tests were taken from laboratory culture. Moths were fed honey-water and held at 27°C and 35% RH. Eggs were surface-disinfected with 0.2% sodium hypochlorite according to the method of Ignoffo [2]. After the eggs hatched, larvae were transferred with a sterile camel's hair brush to separate 20 ml plastic cups containing either medicated or unmedicated semisynthetic diet [1].

Testing for Residual Concentration of Streptomycin in Assay Samples

Tests for residues of streptomycin sulfate were conducted using the disc method as described by Sabath [3]. *Bacillus subtilis* (Ehrenberg) Cohn (The American Type Culture Collection, catalogue 1980, strain 6633) was used to assay for streptomycin sulfate. The standard spore suspension contained about 3×10^{10} colony-forming spores per ml. Two-tenths ml of the spore suspension was added per 100 ml of medium. Ten ml of the agar-spore mixture were dispensed to each Petri dish (100 × 15 mm). The antibiotic inhibition zones were measured with a precision caliper (Manostate, Swiss made) or with a Fisher-Lilly antibiotic zone reader.

Preparation of Samples With Whole Larvae, Pupae, and Adults

Nine-day-old larvae were starved for 24 hr, weighed, frozen at −15°C for 1 to 2 hr and minced (Polytron, Brinkmann Instruments, Westbury, NY 11590) in 6 ml vials with the appropriate volume of buffer of pH 6—KH_2PO_4(8.2 g) + K_2HPO_4 (1.8 g) per liter of distilled water. New pupae were treated the same way as the larvae; new adults were

TABLE 1

RESIDUAL STREPTOMYCIN SULFATE IN HAEMOLYMPH AND TISSUE OF *HELIOTHIS VIRESCENS* LARVAE, PUPAE, ADULT AND MECONIUM

	μg streptomycin sulfate/20 μl					
	Larvae (9-day-old)		Pupae (New)			
% streptomycin in diet (w:w) fed to larvae	Tissue Fluid	Haemolymph	Tissue Fluid	Haemolymph	Wingless Adult (New) Tissue	Meconium
			Standard			
0.00	0.12	0.10	0.12	0.10	0.12	0.30
0.00	0.25	0.20	0.25	0.20	0.25	0.38
0.00	0.50	0.40	0.50	0.40	0.50	0.75
0.00	1.00	0.80		0.80		1.50
			Test			
0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.05	0.00	0.00	0.00	0.00	0.00	0.00
0.10	0.00	0.00	0.00	0.00	0.00	0.03
0.20	Trace	Trace	0.00	0.00	0.00	0.07
0.40	0.18	0.07	Trace	Trace	Trace	0.21
0.80	0.40	0.22	Trace	Trace	Trace	0.26
1.60	1.10	0.57	0.13	Trace	Trace	1.48

TABLE 2

ZONES OF INHIBITION SURROUNDING THE DISCS TREATED WITH TISSUE FLUID, HAEMOLYMPH AND MECONIUM OF *HELIOTHIS VIRESCENS* (ZONE SIZES AGAINST *BACILLUS SUBTILIS*)

	Zone diameters in mm Mean and SD					
	Larvae (9-day-old)		Pupae (New)			
% streptomycin in diet (w:w) fed to larvae	Tissue Fluid[a]	Haemolymph[b]	Tissue Fluid[c]	Haemolymph[d]	Wingless Adult (New) Tissue[e]	Meconium[f]
			Standard (doses in Table 1)			
0.00	8.18 ± 0.76	5.42 ± 2.69	9.51 ± 0.22	8.23 ± 0.50	0.00	7.85 ± 0.30
0.00	12.19 ± 0.77	8.59 ± 0.55	12.83 ± 0.17	9.80 ± 0.66	11.39 ± 1.18	11.98 ± 0.53
0.00	14.61 ± 0.72	12.39 ± 0.76	15.55 ± 0.26	12.25 ± 0.41	14.28 ± 1.00	13.31 ± 0.53
0.00	17.59 ± 0.84	14.51 ± 0.88		15.50 ± 0.51	18.24 ± 1.56	16.20 ± 0.84
			Test			
0.00	0.00	0.00	0.00	0.00	0.00	0.00 ± 0.00
0.05	0.00	0.00	0.00	0.00	0.00	0.00 ± 0.00
0.10	0.00	0.00	0.00	0.00	0.00	1.36 ± 2.78
0.20	3.65 ± 4.44	1.66 ± 3.00	0.00	0.00	0.00	3.05 ± 3.61
0.40	10.18 ± 3.65	3.95 ± 3.83	1.27 ± 2.83	0.89 ± 2.32	0.48 ± 1.76	5.97 ± 2.44
0.80	13.83 ± 2.82	8.99 ± 3.38	3.48 ± 4.51	2.50 ± 4.01	0.35 ± 1.51	8.63 ± 2.01
1.60	18.61 ± 2.86	13.37 ± 1.98	9.78 ± 2.07	3.36 ± 4.07	4.70 ± 1.92	15.99 ± 1.02

Number of zones.
Measured range.
Standard [a] 21–28, [b] 11–14, [c] 35–56, [d] 14–28, [e] 16–26, [f] 16–42.
Test [a] 98–104, [b] 47–59, [c] 105, [d] 25–30, [e] 56–96, [f] 8–34.

TABLE 3

EFFECTS OF STREPTOMYCIN SULFATE ON WEIGHTS OF LARVAE, PUPAE AND ADULTS OF *HELIOTHIS VIRESCENS*

% Streptomycin in diet (w:w) fed to larvae	Weight (mg) Mean ± SD: Larvae (9-day-old) (4 rep; 10 insects/rep)	Pupae (new) (3 rep; 6 insects/rep)	Wingless Adults (new) (3 rep; 6 insects/rep)
0.00			
0.00	178.5 ± 77.7	296.8 ± 3.0	140.3 ± 13.2
0.00			
0.00			
0.00	178.5 ± 77.7	296.8 ± 3.0	140.3 ± 13.2
0.05	200.3 ± 70.9	307.2 ± 18.3	118.9 ± 17.0
0.10	213.1 ± 73.3	307.2 ± 18.3	133.1 ± 5.8
0.20	186.9 ± 74.1	298.1 ± 4.1	125.8 ± 22.4
0.40	157.7 ± 53.2	309.2 ± 5.2	137.0 ± 2.7
0.80	111.7 ± 42.3	306.2 ± 9.2	136.8 ± 19.9
1.60	210.5 ± 64.4	286.3 ± 35.5	135.0 ± 1.4

dewinged before treatment. The ratio of buffer to *H. virescens* tissues—at all stages—was 1 cc buffer to 1 g tissue.

The minced tissue was passed through two layers of cheese cloth. Twenty µl of the tissue fluid was pipetted onto a sensitivity test disc (BBL, Cockeyville, Maryland) placed on the surface of the assay medium and incubated at 37°C for 24 hr. Each test was replicated four times and over 400 insects were used in the study.

Preparation of Haemolymph Samples

Droplets of haemolymph issuing from wounds caused by removal of larval prolegs were collected in 100 µl micropipettes. Each sample of 100 µl of haemolymph represented a pooled sample of ca. 10 larvae. Pupal haemolymph was obtained from wounds caused by puncturing intersegmental membrane with the tip of a scalpel. For haemolymph standards, 5 µl buffer mixed with an appropriate concentration of streptomycin sulfate was added to 500 µl haemolymph. For tests, undiluted haemolymph was used. Residue tests were conducted on 20 µl samples haemolymph per sensitivity disc. The study was replicated four times, with 10 larvae or pupae per replicate.

Meconium Samples

Meconium is a substance excreted by adults soon after their emergence from pupae. As standards, meconium-contaminated empty puparia originating from untreated insects were added to flasks containing the amount of distilled water required to wash off meconium from the puparia. Meconium-contaminated water was decanted from the flasks, filtered through cheese cloth and dispensed into 20 cc plastic cups (1 to 2 cc/cup). The cups were incubated at 50°C until the water was evaporated; afterward the cups containing dry meconium were placed in a desiccator containing phosphorus pentoxide for 24 hr. The dried meconium was mixed with the buffer containing appropriate concentration of the antibiotic (3 µl buffer: 1 µg meconium). For tests, meconia obtained from moths that originated from larvae fed the antibiotic treated diet, were treated as given above except that antibiotic-free buffer was used. Residue tests were conducted on 20 µl samples of meconium per sensitivity disc.

Egg Sampling

Pupae obtained from larvae cultured on medium treated with streptomycin (w:w 0.80 and 1.60% diet) were incubated at 27°C. After adult emergence, ca. 10 pairs were placed per 4 l glass jar. A 14 × 18 cm cotton cloth was suspended from the mouth of each jar for oviposition, and the top was covered with a piece of cotton cloth held in place by a rubber band. Moths were fed honey-water and incubated at 27°C. Eggs were removed from the cloth with sodium hypochlorite aqueous solution, rinsed three times in sterile water and macerated in a glass tissue grinder, some with buffer and some without buffer. Each sample consisted of several thousand eggs. Residue tests were conducted on 20 µl samples of egg homogenate per sensitivity disc.

RESULTS AND DISCUSSION

The residual quantities of streptomycin sulfate in the tissue fluids and haemolymph of *H. virescens* larvae are given in Tables 1 and 2. They showed presence of streptomycin sulfate in tissue fluid and in haemolymph of the larvae. The antibiotic was absorbed through the alimentary canal of the larvae and it contaminated haemolymph which in turn contaminated the insect body. During metamorphosis from larva to pupa, the antibiotic content was greatly reduced both in tissue fluid and in haemolymph (Table 1). We speculate that reduction in the antibiotic content in the pupae may be explained as follows: During larval development, the insect continually fed on antibiotic-treated diet, and any loss of streptomycin due to its metabolic degradation was replenished from the diet. *Heliothis* does not feed in the pupal stage and losses of the streptomycin—during the reconstruction of tissues that takes place at the pupal stage—are not replenished. The antibiotic content was further re-

duced in the tissue fluid of adults (Table 1). During metamorphosis from pupa to adult the antibiotic was eliminated from the insect body in the meconium (Table 1). Any antibiotic in eggs from adults originating from antibiotic-treated larvae, even with 1.60% streptomycin in the diet, was below detectable levels.

Weights of larvae, pupae and adults of treatments and controls were not significantly different (Table 3).

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