Reprint No. 939

Reprinted without change of paging from Contrib. Boyce Thompson Inst. 20 (6) : 363-364, April-June, 1960

THE SULFUR METABOLISM OF INSECTS. V. THE ABILITY OF INSECTS TO USE SULFATE IN THE SYNTHESIS OF METHIONINE[1]

THOMAS H. HAINES, S. MARK HENRY, AND RICHARD J. BLOCK[2]

SUMMARY

$Na_2S^{35}O_4$ was administered to 13 species of insects. The Japanese beetle was able to utilize the sulfur of $Na_2S^{35}O_4$ for the synthesis of methionine and other divalent sulfur-containing compounds.

Of the remaining insects, only the cockroaches, known to contain intracellular symbionts, were able to effect this reduction.

INTRODUCTION

Although various organic sulfur-containing compounds have been found in animals after the administration of $Na_2S^{35}O_4$, S^{35}-methionine has not been reported in the absence of bacteria (1). Unless methionine can be made from sulfate-sulfur by the animal *per se*, administration of sulfate merely has a sparing action on the methionine requirements. It has been previously shown (1) that *Blattella germanica* (L.) is not able to synthesize methionine in the absence of its intracellular symbionts. In this investigation, five additional species of cockroaches and eight other insects were tested for their ability to synthesize methionine from $Na_2S^{35}O_4$.

EXPERIMENTAL

Insects. The following insects were used:

Orthoptera:	Florida cockroach, *Eurycotis floridana* (Walker)
	Oriental cockroach, *Blatta orientalis* L.
	Australian cockroach, *Periplaneta australasiae* (F.)
	Madeira roach, *Leucophaea maderae* (F.)
	Beetle roach, *Diploptera punctata* (Eschscholtz)
Lepidoptera:	Banded woollybear, *Isia isabella* (J. E. Smith)
	Southern armyworm, *Prodenia eridania* (Cram.)
Coleoptera:	Mexican bean beetle, *Epilachna varivestis* Muls.
	Broad-necked root borer, *Prionus laticollis* (Drury)
	Ground beetle, *Carabus* sp.
	Japanese beetle, *Popillia japonica* New.
	Yellow mealworm, *Tenebrio molitor* L.

[1] The data presented here represent a part of a dissertation submitted by S. Mark Henry to the Faculty of the Graduate School of Arts and Science of New York University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Aided by grants E-2608 and RG-4987, National Institutes of Health, U. S. Public Health Service, Bethesda, Maryland.

Hemiptera: Large milkweed bug, *Oncopeltus fasciatus* (Dall.)

The beetles, *Popillia*, *Carabus*, *Tenebrio*, and *Prionus* were in the adult stage. All other insects used in the investigation were nymphs or larvae.

Methods. Five to ten μl. (1 μc./μl.) of carrier-free $Na_2S^{35}O_4$ were injected into the hemoceole of the cockroaches, beetles, and woollybear caterpillar with a micrometer controlled syringe. The southern armyworm and Mexican bean beetle larvae were maintained on filter paper wet with a $Na_2S^{35}O_4$ solution. After 24 hours the insects were homogenized in hot 80 per cent (v/v) ethanol containing 0.001 per cent (w/v) *N*-ethylmaleimide and the insoluble material was removed by filtration (1). The residue was then washed and hydrolyzed with 3*N* HCl.

The radioactive metabolites were identified by means of autoradiograms of two-dimensional chromatograms (1).

RESULTS

Cockroaches. The five species of cockroaches produced methionine, cystine, and the other sulfur-containing compounds when given $Na_2S^{35}O_4$, as was previously reported for *Blattella* and *Periplaneta* (1).

Japanese beetle. This insect, in which intracellular symbionts have not been reported, formed methionine, cystine, and traces of glutathione, sulfite (or thiosulfate), and taurine from $Na_2S^{35}O_4$.

Other insects. None of the other insects tested converted the sulfur of $Na_2S^{35}O_4$ into methionine or even cystine.

DISCUSSION

It appears from the present report and previous studies (1) that the characteristic intracellular symbionts of cockroaches enable this family of insects to incorporate sulfate-sulfur into methionine and other organic compounds. On the other hand, the remaining insects tested were unable to effect this conversion except for the Japanese beetle. Although the authors are not aware of any reports in the literature that Japanese beetles contain intracellular symbionts, this possibility cannot be excluded at the present time. Furthermore, the fact that the $Na_2S^{35}O_4$ was administered parenterally does not exclude absorption via the malpighian tubules into the gut and subsequent reabsorption of methionine synthesized by the gut microbiota. Experiments are in progress to characterize the metabolism of sulfate in the Japanese beetle.

The authors wish to thank Dr. Albert Hartzell for identification of the insects.

LITERATURE CITED

1. HENRY, S. MARK, and RICHARD J. BLOCK. The sulfur metabolism of insects. IV. The conversion of inorganic sulfate to organic sulfur compounds in cockroaches. The role of intracellular symbionts. Contribs. Boyce Thompson Inst. **20**: 317–329. 1960.