A Stable Isotope, Europium-151, as a Tracer for Field Studies of Insects¹

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(Received March 30, 1970)

Larvae of *Hyphantria cunea* and *Spodoptera litura* (formerly *Prodenia litura*) fed with artificial diets containing $^{151}\text{Eu}_2\text{O}_3$ grew and survived at the same rate as larvae fed with normal (control) diet. Dried specimens of larvae and adults were activated by thermal neutron irradiation and examined with a multichannel pulse height analyzer. The larvae reared on Eu-diet showed clear radioactivity peaks of $^{152\text{m}}\text{Eu}$, especially at the point of $\text{SmK}\alpha$ X-ray (40.1 keV). Even adults of *S. litura*, where the last instar larvae were reared on Eu-diet, were also detectable. Under the condition of neutron flux, minimum detectable limit of Eu was about $0.02\text{ }\mu\text{g}$ per medium-sized insect. No traces of Eu was found in larvae and adults reared on control diets. It was concluded that activated Europium-151 is useful activable tracer for insect population studies.

INTRODUCTION

The radioactive isotopes have been used for field studies of insects, since the first attempt by BUGHER and TAYLOR (1949) who reared mosquito larvae in solutions containing ^{32}P or ^{89}Sr . In heavily-populated countries such as Japan, however, the use of radioisotopes is inevitably limited due to its dangerous effects on the human health. If we use a rare element with remarkably large cross section (radioactivity per g of the element) after thermal neutron irradiation as a marker of insects, these dangers can be avoided because the element will be activated after recapture of the marked individuals. The results of preliminary experiments on the suitability of ^{151}Eu , selected because of its rarity on earth, large cross section (3.0×10^{11} c. p. m. at 1 hr irradiation of 4.3×10^{12} neutron/cm²-sec; cf. YULE, 1965) and relatively long half life (9.3 hr), as an activable marker of insects, are reported in the present paper.

MATERIALS AND METHODS

One hundred and sixteen mg of Eu_2O_3 containing 100 mg ^{151}Eu (obtained from ORNL, Oak Ridge, Tennessee) was diluted by 100 ml of 0.02N HCl solution. This stock solution was added to artificial diets to make the Eu-content at 44 ppm for *Hyphantria cunea* and 80 ppm for *Spodoptera litura* (Table 1).

The larvae of *H. cunea* (black-headed strain) and *S. litura*, both of which had

¹ Contribution from JIBP-PT No. 102. A part of this study was supported by a grant for the special project research, 'Studies on the dynamic status of biosphere,' sponsored by the Ministry of Education, Japan.

Table 1. COMPOSITIONS OF THE ARTIFICIAL DIETS

A. *Hyphantria cunea*

Ingredients	Quantity
Water	1000 ml
Agar (powder)	30 g
Cellulose powder	40 g
Sucrose	30 g
Casein	40 g
Dry yeast powder (Ebios)	20 g
WESSON's salt mixture	5 g
Cholesterol	0.5 g
Sodium salt of sorbic acid	2 g
Sodium dehydroacetate	0.5 g
Fresh leaves of <i>Platanus</i>	200 g
Soy bean powder	30 g
Cholin chloride	2 g
Sodium L-ascorbate	2 g

B. *Spodoptera litura*

Water	600 ml
Agar (powder)	20 g
Cellulose powder	40 g
Dry yeast powder (Ebios)	80 g
Sodium salt of sorbic acid	2 g
Sodium dehydroacetate	2 g
Kidney bean (Soaked in water for 24 hr)	400 g
Beet leaves	100 g
Sodium L-ascorbate	7 g
Formalin	4 ml
Hydrochloric acid	5 ml

been reared on mulberry leaves in our laboratory, were fed with these diets after the middle or late larval stage. The control larvae were fed with the same diets without Eu_2O_3 (only the same amounts of HCl was added). The body weight of larvae and adults and the amount of diet consumed were occasionally measured by a Sartorius 2604 automatic balance. (The amount of diet absorbed was estimated by subtracting the weight of feces from the weight of diet eaten.) The larvae used for examination were killed with dry ice-alcohol solution and oven-dried, while the moths were killed in potassium cyanide gas.

Insect specimens individually sealed in polyethylene film and enveloped by parafin paper were incapsulated in 30 ml polyethylene vials and irradiated in JRR-2 reactor with 3×10^{13} neutron/cm² for 1 or 10 min. After the irradiation and cooling for 24 hr, each sample, put in an aluminium cylinder (2 cm in diameter,

7 cm in height and 0.5 mm thickness) was assayed using a 400-channel multi-channel pulse height analyzer (RIDL Model 34-12B) with a NaI (Tl) $1\frac{3}{4}$ in. $\times$ 2 in. ϕ detector. Distance from the crystal to the sample was fixed at 6 cm for cases of 1 min irradiation and 9 cm for those of 10 min irradiation. The linear vertical scale of the multichannel pulse height analyzer was used.

Filter papers, about 0.5 cm² in area, on which fixed amounts (10, 1, 0.1 and 0.02 μ g) of ¹⁵¹Eu were added by means of micro-syringe, were used as reference standards.

RESULTS

Effects of Eu on Growth and Survival

TALBURT and JOHNSON (1967) reported that Eu contained in the medium at the concentration of 0.011 M (about 20 times as high as the Eu-concentration of *Spodoptera*-diet) inhibited the growth of some microorganisms. The effect of Eu on the growth and survival of lepidoptera larvae was, therefore, examined. As shown in Table 2, there is no significant difference between the growth and survival rates of larvae of *H. cunea* and *S. litura* reared on diets with and without Eu. For *S. litura* also, no significant difference was found on the rate of adult emergence and the size of adults for both larvae fed on Eu-diet and control ones.

As the rearing on Eu-diet during 9 days did not show any decrease in the rates of emergence and survival, the harmful effect of ¹⁵¹Eu on lepidopterous larvae would be, if any, negligible in the experiments.

Table 2. GROWTH AND SURVIVAL OF *Hyphantria cunea* AND *Spodoptera litura* LARVAE ON EU-DIETS AND CONTROL ONES

1. <i>H. cunea</i>							
Diet	Initial body weight	Body weight after 3 days	Daily increment % during 3 days	Percent pupation			
Eu-diet for 3 days	111.3 mg	248.7 mg	72.0 %	80 %			
Control diet for 3 days	126.9	248.9	66.0	80			
2. <i>S. litura</i>							
Diet	Initial body weight in mg ^a	Body weight after 5 days in mg ^b	Daily increment % during 5 days	Survival rate of larva %	Rate of adult emergence %	Adult body weight in mg	
						♂	♀
Eu-diet for 9 days	136.5 ± 4.0	616.3 ± 77.7	70.2 ± 9.5	74.3	62.9	139.7 ± 39.3	182.9 ± 22.9
Eu-diet for 1st 3 days and control diet for the last 6 days	—	—	—	88.0	74.0	115.8 ± 22.5	166.4 ± 45.3
Control diet for 9 days	125.7 ± 2.1	442.0 ± 85.6	50.6 ± 20.4	57.5	54.0	139.1 ± 26.8	197.7 ± 33.4

^a The 5th instar larva.

^b The 6th (last) instar larva.

Recovery of Eu-Marked Specimens

The gamma-ray spectra of standard filter papers containing fixed amounts of Eu showed, as BANDO et al. (1969) had reported, two detectable peaks of ^{152m}Eu one of which was at 40.1 keV and the other at 121 keV (Fig. 1). As the peak height at 40.1 keV precisely reflected the amount of Eu contained in the standard filter papers, this peak was used to detect the presence of Eu in insect specimens. All the larvae of *H. cunea* and *S. litura* fed on Eu-diets during 24 hr just before killing showed detectable peaks at 40.1 keV but all the larvae fed with control diets did not show any trace of Eu (Fig. 2). The Eu disappeared, however, from *H. cunea* larvae which were first fed with Eu-diets but thereafter transferred to control diets for one or more days before killing, except for only 1 of the 11 cases studied. In *S. litura*, on the other hand, 6 days rearing on the control diet did not eliminate trace of Eu which was absorbed during the previous 3 days.

Recovery percentages of Eu detected from larvae were about 2 to 4 per cent of Eu absorbed during 24 hr before killing (Table 3). Note that the standard deviations of measured Eu contents were rather small. A question naturally arose: Whether only Eu located in guts of larvae could be detected or not?

The presence of Eu in adults of *S. litura*, where the larvae were reared on Eu-diet affords an answer to this question (Fig. 3). As all the feces were believed

Fig. 1. Gamma-ray spectra of Eu soaked into filter paper and that of control filter paper. The line time (L.T.) corresponds to 6 sec.

Fig. 2. Gamma-ray spectra of *Hyphantria cunea* larvae fed with Eu-diet and the control ones. Note that the radioactivity unit (length of line AB) is not an absolute value but a relative one, because we changed the range of pulse height for each case.

Fig. 3. Gamma-ray spectra of *Spodoptera litura* adults, the larvae of which were reared on Eu-diet and control diet,

Table 3. CONTENTS OF EU-152m IN INSECTS EXAMINED

No. days reared on		No. samples	Eu (R) (Eu μ g recovered)	Live body weight in g	Eu (A) (Eu μ g estimated to be absorbed)	Eu(R) $\times$ 100 Eu(A)
Eu-diet	Control diet					
1. <i>Hyphantria cunea</i> larva						
1	0	12	0.369 $\pm$ 0.154	0.253 $\pm$ 0.024	10.95 $\pm$ 1.49	3.37 $\pm$ 1.42
1	0	3	0.330 $\pm$ 0.255	0.334	6.20	5.33 $\pm$ 4.12
1	0	2	0.208 $\pm$ 0.048	0.205	8.71	2.39 $\pm$ 0.56
2	0	2	0.381 $\pm$ 0.081	0.253	9.90 (0.21)	3.84 $\pm$ 0.81 (182 $\pm$ 39)
4	0	1	0.163	0.172	14.56 (1.09)	1.12 (9.70)
1	→ 1	3	0	0.138	10.43	0
2	→ 2	4	0	0.251	16.50	0
0	1	5	0	0.238	0	0
0	1	5	0	0.246	0	0
0	2	5	0	0.221	0	0
2. <i>Spodoptera litura</i> larva						
9	0	3	1.940 $\pm$ 0.109	—	720	0.27 $\pm$ 0.02
3	0	5	1.791 $\pm$ 0.083	—	242 (80.6)	0.74 $\pm$ 0.04 (2.22 $\pm$ 0.10)
3	→ 6	4	0.017 $\pm$ 0.022	—	242	0.01 $\pm$ 0.001
0	9	8	0	—	0	0
3. <i>Spodoptera litura</i> adult						
9	0	5	0.034 $\pm$ 0.007	0.183	720	0.005 $\pm$ 0.001
0	5	5	0	0.198	0	0

Figures in parentheses mean the amount of Eu absorbed on the last day before killing and the percentage of Eu recovered to Eu absorbed on that day.

to be excreted from the guts of larvae before pupation, a part of Eu in the guts was absorbed into the larval body through the gut membrane. It must, however, be mentioned that only 1.75 per cent of Eu found in mature larvae reared on Eu-diet for 9 days could be recovered in adults (Table 3). In addition, the moulted skin of *H. cunea* reared on Eu-diet for two days showed trace amounts (0.019 μ g for 5 individuals) of Eu.

If Eu can be detected from predators which feed upon the insect reared on Eu-diet, the activation analysis will afford a powerful tool for the study of food-chain relationships. This is the aim for future studies, as well as field trials of the mark-release-recapture experiment of Eu-marked insects.

To sum up, the activated $^{152\text{m}}\text{Eu}$ in larvae and adults of *H. cunea* and *S. litura* reared on diets containing stable ^{151}Eu after the irradiation were detectable. As all the individuals reared on the control diets did not show any trace of Eu, it may be concluded that the source of $^{152\text{m}}\text{Eu}$ detected from insects after the irradiation is due to ^{151}Eu contained in the artificial diets. Thus we can use Eu as a marker for field studies of insects,

ACKNOWLEDGEMENTS

The author expresses his sincere thanks to Dr. C. TOMIZAWA, Division of Agricultural Chemicals of our Institute for his initial stimulation on this work and for reading the manuscript. Thanks are also due to Dr. M. SHIBUYA and Mr. K. YURTA, Chemistry Section of our Institute, for their helpful advice and facilities for the use of the hot laboratory, and to Dr. H. KOBAYASHI, the Radioisotope Laboratory, for his advice.

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