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THE EVALUATION OF HOST EFFICIENCY AND VECTOR POTENTIAL OF LABORATORY JUVENILIZED VECTORS OF CHAGAS' DISEASE. I — EFFECTS OF DEVELOPMENTAL CHANGES INDUCED BY JUVENILE HORMONE ANALOGUES IN *PANSTRONGYLUS MEGISTUS* (HEMIPTERA-REDUVIIDAE) ON THE SUSCEPTIBILITY OF THE INSECTS TO GUT INFECTION WITH *TRYPANOSOMA CRUZI*

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SUMMARY

Recent interest in juvenile hormone analogues and the possibility of their practical application to counteract insecticides in the vector control of Chagas' disease, prompted us to determine the factors that may enhance the efficiency of the juvenilized bug as a *T. cruzi* carrier. The results obtained have shown that hormone-treated bugs increase body weight 2.5 times, as compared with normal bugs of the same age. Due to their stimulated voracity, blood uptake is approximately 2.5 times larger than in normal bugs. In spite of the increased voracity and the larger blood intake, the juvenilized insects were found to be about seven times less sensitive to natural infections with *Trypanosoma cruzi* than normal bugs: The gut infection with *T. cruzi* upon feeding on chagasic patients was 44.9 per cent in the normal bugs against 6.5 per cent in the hormone-treated insects. Similar results, though less striking, were obtained with both groups of bugs when fed on experimentally infected animals. It is suggested that JH analogues which are well known to disrupt the normal development of insects and were found to suppress insect sensitivity to natural infections with *T. cruzi*, should be considered seriously as supplementary means in the control of triatomine bugs.

INTRODUCTION

Juvenilizing effects induced by the application of chemicals mimicking juvenile hormones have been observed in many insect species, including the vectors of Chagas' disease, by WIGGLESWORTH [7] and by BENSON et al. [1]. The main emphasis in these studies was on the screening of chemicals by topical application to the 5th nymphal stage of the insects, or by bringing the bugs into contact with hormone-treated surfaces, and analyzing the morphogenetic effects caused by the chemicals.

Several of the juvenile hormone analogues have been well explored against *Panstrongylus megistus*, and may be soon used in the control against this important vector of Chagas' disease in Brazil. However, no information is available on the effects of induced juvenility with regard to the susceptibility of bugs to gut infection with *Trypanosoma*

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cruzi. Also lacking is information on the behaviour of juvenilized insects as vectors for *T. cruzi*. The evaluation of the juvenile host's efficiency and its potential as vector under natural conditions are considered of importance in the use of JH analogues, because they act by inducing developmental changes and not as killing agents.

The evidence obtained in the laboratory on the effects of JH indicate arrested and uncoordinated development, and inhibition in the reproduction of the 7th instar adults, which developed from supernumerary nymphs. This break in the life-cycle is considered favourable for the practical use of JH in the biological control of triatomine bugs. However, inhibition of reproduction in a long-living insect is not alone a sufficient indicator of the compound practical application in the field for several reasons. In laboratory-bred bugs the juvenilization neither reduces the vigor nor the life span of the insects, and the abnormally large size of the bugs is no doubt due to large uptake of blood caused by their stimulation to great voracity. Consequently, it can be expected that the hormone treated bugs, by feeding on a host infected with *T. cruzi*, will ingest a considerably greater number of the parasites than a normal size insect. It was considered necessary, and of importance, to investigate the host efficiency and vector potential of the laboratory-juvenilized bugs before seriously considering the use of JH analogues as alternatives, or as supplementary means in the control of the vector of Chagas' disease, which is expected to be a good candidate for the integrated methods of vector control, as stated by PERLOWAGORA-SZUMLEWICZ [2].

This paper reports on the susceptibility of juvenilized *P. megistus* to gut infection with *T. cruzi*.

MATERIALS AND METHODS

The *Panstrongylus megistus* used in these studies derived from field collections, maintained in this laboratory since 1969. The insects were from the same stock as used by BENSON et al. [1] in the screening of chemicals which mimic juvenile hormone activity in disrupting normal growth and development in the bugs. From the chemicals tested by the authors, a geranyloxy-methylenedioxy benzene derivate [1- (6,7-epoxy-3-ethyl-7-methylnon-2-enyloxy) - 3,4 - methylenedioxy benzene] was found to be the most active in *P. megistus*, and was therefore used in the present studies.

The procedure for juvenilizing the insects involves exposure of insects to aluminium plates previously coated with "Underseal" over which was sprayed the JH analogue in hexane in a quantity of 10 $\mu g/cm^2$. Plates prepared in 1972 and used during 1973 did not show any measurable loss of activity.

The bugs, one to six days after transition from 4th to 5th instar, were placed in battery jars in which the treated aluminium plates were inserted, allowing the insects to climb and to rest on the treated surface.

The bugs on continuous contact with the treated plates transform into completely juvenilized 6th stage nymphs (grade 1) and into somewhat less juvenilized insects (grade 1.5-2.0) within a period from 14 to 40 days after exposure.

During the process of juvenilization the bugs were fed at weekly intervals by placing the jars beneath chickens for 30 to 40 minutes. At times insect faeces accumulated on the plates and had to be removed by washing with water. The washing did not affect the activity of the treated plates. Juvenilized insects were transferred to clean jars and were kept without feeding until used in xenodiagnosis test.

A total of 612 *P. magistus* were exposed to JH analogues in 1973, but only those insects which showed a complete retention of the nymphal characteristics in the 6th stage (which is adult stage in normal bugs) were used in the experiment. The control insects (normal bugs) were fed at weekly intervals on chickens until transition to 5th instar, and then starved until feeding on naturally and laboratory *T. cruzi* infected hosts.

The following infected hosts were used for the feed of the juvenile and normal bugs: Three dogs, one of which was found positive for *T. cruzi* on a previous occasion, and be-

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longed to a household in which four children were diagnosed serologically and parasitologically for Chagas' disease, one guinea pig and three mice, which were infected experimentally with *T. cruzi* Y-strain [6], a group of 11 mice infected with *T. cruzi* strain from BELIZE [5] and seven human patients diagnosed serologically, parasitologically and clinically positive for Chagas' disease.

All the infected animals exhibited high parasitaemia when feeding the experimental bugs. The bug feeding on some of the patients and on the naturally infected dog was done on a visit to their homes.

Groups of 10 juvenilized and 10 normal bugs were allowed to engorge with blood on the large mammals and on the human patients. Similarly, groups of three treated and three untreated bugs were fed on mice. After feeding, the juvenile and the normal insects were kept in separate jars at room temperature, without feeding until faecal examination, 30 to 45 days later.

The bug faeces for microscopical examination was obtained following the routine procedures. Information on the amount of blood uptake, by juvenilized and by the normal bugs, was obtained by the method described by PERLOWAGORA-SZUMLEWICZ [3].

RESULTS AND COMMENTS

The size difference between the hormone-treated and untreated nymphs of *P. megistus* is shown in Fig. 1 and has been also reported previously [4]. Body weight, determined within seven days after transition to 5th instar in normal insects and to 6th instar in juvenilized bugs, was 107 ± 4.0 mg and 250.9 ± 15.1 mg respectively. Blood uptake, determined by subtracting weight prior to feeding from weight determined immediately after feeding on first meal offered, was 300.1 ± 58.5 in normal insects and 721.8 ± 75.6 in juvenilized ones.

Fig. 1 — A) Supernumerary nymph resulted from JH treated 5th instar nymph of *Panstrongylus megistus* (life size about 25 mm). B) Normal 5th instar nymph of *Panstrongylus megistus* (life size about 15 mm).

The apparent stimulation of the juvenile bugs to ample blood uptake could be expected to increase their efficiency as a host for the flagellates. However, the data summarized in Table I show to the contrary. The gut infection with *T. cruzi* was 44.9 per cent in the normal bugs, against 6.5 per cent in the hormone-treated insects. Similar results, though less striking, were obtained with both groups of bugs which fed on the experimentally infected animals as shown in Tables II and III. In two of the 25 cases tested, the infection in the juvenile bugs was greater than in the control insects (Table III). This may have been due to the extremely high blood parasitaemia in the mice infected with the Belize strain at the time of the feed (in some mice more than 30 trypanosomes per field in wet blood film), or to the refusal of some of the normal insects to engorge on the mice.

TABLE I

Comparison of *T. cruzi* infections in JH analogue treated and untreated *P. megistus* fed on patients with Chagas' disease

Insects \ Patient	Percentage of insects found infected after feeding on patients								
	A.S.B.	M.L.S.S.	O.P.	A.C.S.	O.F.S.	J.M.M.S.	A.M.J.S.	DOG	Total
Juvenilized	0 (0/8)	30 (3/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	20 (2/10)	0 (0/9)	6.5 (5/77)
Normal	30 (3/10)	50 (5/10)	40 (4/10)	40 (4/10)	20 (2/10)	80 (8/10)	78 (7/9)	22 (2/9)	44.9 (35/78)

Figures in brackets indicate the number of insects from which flagellates were recovered over the number examined. Numbers lower than 10 indicate that some insects died prior to examination.

TABLE II

Comparison of *T. cruzi* infections in JH analogue treated and untreated *P. megistus* fed on experimentally infected animals with Y strain of *T. cruzi*

Insects	Percentage of insects found infected after feeding on infected animals						
	Mouse nr.			Dog nr.		Guinea Pig	Total
	1	2	3	1	2		
Juvenilized	0 (0/3)	0 (0/3)	67 (2/3)	100 (6/6)	70 (7/10)	14 (1/7)	50 (16/32)
Normal	100 (3/3)	67 (2/3)	67 (2/3)	100 (10/10)	100 (10/10)	100 (6/6)	94.3 (33/35)

Figures in brackets indicate the number of insects from which flagellates were recovered over the number examined. Numbers lower than 10 indicate that some insects died prior to examination.

TABLE III

Comparison of *T. cruzi* infection in JH analogue treated and untreated *P. megistus* fed on experimentally infected mice with Belize strain of *T. cruzi*

Insects \ Mouse no.	Percentage of insects found infected after feeding on infected mice											
	1	2	3	4	5	6	7	8	9	10	11	Total
Juvenilized	0 (0/3)	33 (1/3)	33 (1/3)	33 (1/3)	33 (1/3)	0 (0/3)	33 (1/3)	0 (0/3)	67 (2/3)	67 (2/3)	0 (0/2) (*)	28.1 (9/32)
Normal	33 (1/3)	67 (2/3)	33 (1/3)	33 (1/3)	67 (2/3)	0 (0/3)	0 (0/3)	33 (1/3)	33 (1/3)	67 (2/3)	67 (2/3)	39.4 (13/33)

Numbers in brackets indicate the number of insects from which flagellates were recovered over the number examined.

(*) One insect died prior to examination

The results obtained are encouraging and suggest the possible use of JH analogues in the control of vectors of Chagas' disease. The overall poor susceptibility of the juvenile bugs to natural gut infection with *T. cruzi* excludes them as more efficient hosts for the parasite.

The beneficial effects, such as a reduction of the naturally infected insect population in human dwellings, leading to a possible decrease in new cases of Chagas' disease would certainly counterbalance the physical discomfort caused by the ferocious juvenilized bugs.

Whether the JH analogues which affect the target host affect the endoparasite, thus modifying the vectorial potential of the bug, will be discussed in a forth-comming paper.

RESUMO

Avaliação da eficiência como hospedeiros e do poder vectorial dos transmissores da doença de Chagas, juvenilizados experimentalmente. I — Efeitos das transformações induzidas por análogos dos hormônios juvenis no P. megistus, *sobre a suscetibilidade à infecção intestinal com o* T. cruzi

O interesse recentemente despertado por análogos dos hormônios juvenis e a possibilidade de integrá-los no combate aos vectores da Doença de Chagas, estimularam-nos a investigar a eficiência do *P. megistus* experimentalmente juvenilizado, como portador do *T. cruzi*. Os resultados obtidos mostram que esses insetos, quando tratados com os mencionados análogos, tiveram sua voracidade exaltada, o que resultou em repasto sangüíneo e peso corporal 2,5 vezes maior que nos espécimes não tratados. A despeito da voracidade aumentada e do maior volume de sangue ingerido, esses insetos, quando alimentados em pacientes chagásicos, revelaram infecção pelo *T. cruzi* de 6,5% contra 44,9% registrados nos espécimes normais. Resultados semelhantes, ainda que menos dramáticos, foram obtidos em ambos os grupos de insetos que se alimentaram em animais com infecção experimental pelo *T. cruzi*. Esses resultados são encorajadores e sugerem a possibilidade de utilizarem-se análogos dos hormônios juvenis no controle dos vectores da Doença de Chagas, dada a reduzida suscetibilidade à infecção natural com *T. cruzi* dos barbeiros juvenilizados, afastando o perigo de serem eles hospedeiros mais eficientes do flagelado que os espécimes normais.

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