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HEMOLYMPHATIC COMPONENTS IN VECTORS OF Trypanosoma cruzi: STUDY IN SEVERAL SPECIES OF THE SUBFAMILY TRIATOMINAE (HEMIPTERA: REDUVIIDAE).

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SUMMARY

The members of the subfamily Triatominae (Hemiptera : Reduviidae) comprise a great number of species of medical importance in the transmission of the **T. cruzi** (American trypanosomiasis).

The aim of this study was to contribute to the knowledge about the chemical composition in proteins, lipids, lipoproteins, and carbohydrates of vectors of Chagas' disease corresponding to twelve members of the subfamily Triatominae. This study was carried out in ninphs of the fifth instar and adult males of the species: **T. delpontei, T. dimidiata, T. guasayana, T. infestans, T. mazzotti, T. pallidipennis, T. patagonica, T. platensis, T. rubrovaria, T. sordida** of the Triatoma genus, and **D. maximus** and **P. megistus** of the Dipatalogaster and Panstrongylus genera respectively.

The results show on one hand, qualitative differences in the protein composition, and on the other hand, similarity in the lipoprotein profiles. Lipids, proteins, and carbohydrates did not show significant differences between species or/and stages.

KEY WORDS: Chagas' disease; Vectors; Proteins; Lipids; Lipoproteins; Carbohydrates.

INTRODUCTION

The study of the hemolymphatic compounds provides valuable information for the understanding of relationships of one insect with another of the same or different species [17,34].

LOUGTHON & WEST (1965) studied the characteristics of development and distribution of the hemolymphatic proteins in Lepidoptera [22]. The electrophoretic profile has been established in various insects of medical importance, particularly in different members of the subfamily Triatominae (Hemiptera : Reduviidae) [1,23,25,31].

The major sugar found in the hemolymph of insects is a-trehalose, a non reducing glucose-glucose disacharide, responsible for providing energy of rapid consumption for essential activities [33].

The lipids, another indispensable metabolic compound, are involved in many processes such as locomotion, reproduction, embryogenesis and metamorphosis. They also function as hormones in metabolic regulation and as structural components[8]. Lipophorin (HDLp), the major lipoprotein in insects, has been reported as one structure unparalleled in nature by their capacity for lipid binding. The understanding of these biochemical compounds is of great importance in insects which greatly influence upon society through destruction of crops and transmission of diseases [26].

At present, most investigations are concerned principally with two species, that is, **Manduca sexta** (holometabolous) and **Locusta migratoria** (hemimetabolous)[26], but the information about triatomine bugs is scarce, showing a high variation, probably as consequence of the different physiological situations, nutrition conditions, sex or infection by **Blastocrithidia triatomae**[7]. This parasite in **Triatoma infestans** can produce changes in the hemolymphatic profile protein [4], delay and high mortality in their development[27]. Also, modifica-

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tions in the protein pattern have been reported by parasitic actions upon **Heliothis zea**[2] and **Spodoptera frugiperda**[11] as well as metabolic modifications characterized by increase of lipids and diminution of proteins in locust[15]. **HAYAKAWA** (1987) described one inhibitor factor of the lipid transport in parasitized insects[14].

Nowadays, the knowledge of the lipid and lipoprotein composition of Chagas' disease vectors, in standard conditions is related to **T. infestans**[30] and **Rhodnius prolixus**[13].

The study of the metabolic compounds carried out in our laboratory in members of the subfamily Triatominae demanded one previous analysis in standard conditions of the proteic, lipidic, lipoprotein, and carbohydrate compounds. The present study comprises twelve species corresponding to three genera with epidemiologic importance and of different geographic distribution: ten species corresponding to Triatoma genus and two species belonging to Dipetalogaster and Panstrogylus genera. This study was carried out in fifth instar and adult insects.

MATERIALS AND METHODS

Insects:
Genus Triatoma (Laporte, 1832)
T. delpontei (Romaña & Abalos, 1947).
T. dimidiata (Latrelle, 1811).
T. guasayana (Wygodzinsky & Abalos, 1949).
T. infestans (Klug, 1834).
T. mazzotti (Usinger, 1941).
T. pallidipennis (Stal, 1872).
T. patagonica (Del Ponte, 1929).
T. platensis (Neiva, 1913).
T. rubrovaria (Blanchard, 1843).
T. sordida (Stal, 1859).
Genus Panstrongylus (Berg, 1879)
P. megistus (Burmester, 1835).
Genus Dipetalogaster (Usinger, 1939).
D. maximus (Uhler, 1894).

The experiments were carried out using groups with ten insects, fifth instar (NV) and adult males respectively, reared and maintained in the insectary at 28± 1°C, 60-70 % humidity, 16/8 h day/night cycle, free of infection by **T. cruzy** or **B. triatomae** and fed fortnightly on hens. Each group was synchronically moulted, fed and then allowed to fast for 5 days prior to hemolymph collection.

Hemolymph collection: to collect hemolymph, the insects were anesthetized by cooling on ice. The legs were cut off and the insects were placed in a precooled centrifuge tube, and centrifuged at 90 g at 4°C for 1 minute. The hemolymph was then pooled into a precooled tube containing 10 mM ethylenediaminetetracetic acid (disodium salt) (Na_2EDTA), 10 mM dithiothreitol and phenylmethylsulfonyl fluoride (PMSF), N-a-p-tosyl-1-lysine chloromethyl ketone (TLCK) and aprotinin 1 mM in order to avoid proteolysis[10]. Then it was centrifuged at 10.000 g at 4°C for 10 minutes to remove hemocytes. For the carbohydrate determination, the hemolymph was collected only with Na_2EDTA and immediately used. The volume of hemolymph collected in each group ranges between 120 µl and 250 µl for NV and from 150 µl to 420 µl for adult males.

Protein, lipid and carbohydrate determinations: protein concentration was quantified by the Bradford method [3], the total lipid content was estimated according to FRINGS at al.[12] and that of carbohydrates, according to SCOTT et al.[28].

Electrophoresis: in all the cases, 120 µg of proteins were submitted to polyacrylamide gel electrophoresis on 7% acrylamide gels, according to Davis procedure[9]. Scans were performed on a Helena-Scan densitometer. The pre-stained with Sudan Black lipoproteins were fractionated by electrophoresis on polyacrylamide as described previously [6].

Statistical analysis: Student's "t" tests for observations were utilized in all instances [29]. Results are reported as mean ± SEM.

RESULTS

Overall protein values for adult males and NV of the ten species of Triatoma genus were not significantly different (2.8 ± 0.5 g/dl and 2.5 ± 0.4 g/dl respectively). This also applies to **D.maximus** and **P. megistus** (Table 1).

The estimation of total lipids and carbohydrates in the standard conditions did not reveal significant differences. The highest value for the lipids was found in the Triatoma genus for both instars (Table 1).

On the other hand, the fractionation of the hemolymphatic lipoprotein pre-stained with Sudan

Table 1
Protein, lipid and carbohydrate composition in hemolymph of fifth instar (NV) and adult (male) from species of three genera of the subfamily Triatominae (Hemiptera: Reduviidae).

Genus	Protein (g/dl)		Lipid (mg/dl)		Carbohydrate (mg/dl)	
	NV	male	NV	male	NV	male
Triatoma[1]	2.5±0.4(*)	2.8±0.5	108±24	103±31	50.4±8.2	51.2±6.8
Dipetalogaster[2]	1.9±0.4	2.2±0.3	95±23	83±18	55.5±7.0	57.2±6.3
Panstrongylus[3]	2.6±0.5	2.8±0.4	79±25	87±31	54.5±8.2	53.0±3.8

(*): mean ± SEM, from four determinations carried out in duplicate in groups of ten insects.
(1): **T.delpontei; T.dimidiata; T.guasayana; T.infestans; T.mazzotti; T.pallidipennis; T.patagonica; T.platensis;T.rubrovaria; T.sordida.** (2): **D.maximus** (3): **P.megistus.**

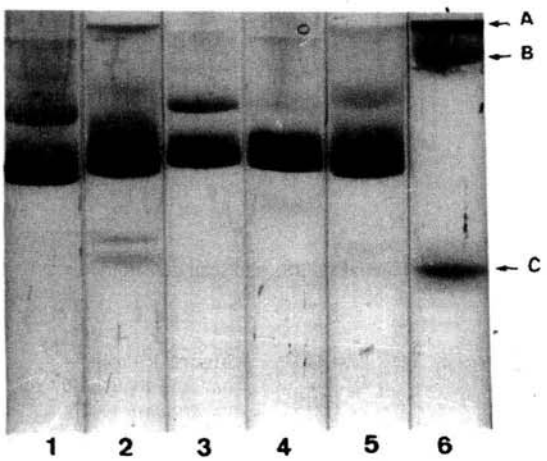

Figure 1: Hemolymphatic lipoproteins separated by polyacrylamide gel electrophoresis.
1-D.maximus (♂); **2-D.maximus** (NV); **3-P.megistus** (♂); **4-T.infestans** (♂); **5-T.platensis** (NV); 6-Human serum (A:VLDL, B:LDL, C:HDL).

Black B showed four fractions for NV and two for adults, in the three genera. These fractions compared with a human normal serum, revealed one migration between HDL (High Density Lipoprotein) and LDL (Low Density Lipoprotein) (Fig.1)

The electrophoretic profiles of the proteic components that characterized the different species showed qualitative differences (Fig.2 A,B and Fig.3). For the Triatoma genus, the number of the components ranges between 13 and 18 for NV and from 14 to 20 for adults; **T. mazzotti** and **T. guasayana** being the ones with the lowest number of bands presented in this instar, while **T. pallidipennis, T. dimidiata,** and **T. patagonica** have the greater number of bands, that is, they have 18. In adults, the species showing the lowest number of bands was **T. guasayana** with 14 and **T. infestans** showed the greatest number with 20. **D. maximus** and **P. megistus** in the fifth instar show 17 and 18 proteic bands respectively whereas adults of **D. maximus** showed 18 and **P. megistus** showed 21.

DISCUSSION

Biochemistry and molecular biology of insects have nowadays become important fields of research where proteic, hormonal, and lipoproteic systems show characteristics similar to mammals so that these systems are considered as biochemical models [19].

Most information found in the literature deals with very few species of insects, particularly **M. sexta, L. migratoria,** and **Bombyx mory** [8]. Comparison of the data obtained is very difficult due to the differences in the physiological state, feed conditions and/or fast, sex or methodology employed [18,32]. Another important factor is the probable infection by parasites which has been proved to produce modifications in the hemolymphatic components [2,4,11] as well as to influence their development and life cycle [27].

We have studied insects from two stages in three genera of vectors of Chagas' disease where some variables were established in order to be able to make comparisons amongst the different stages and genera. This type of study contributes to a better knowledge of the aspects related with the generation of energy, specially the necessary energy for flying. Although the members of the subfamily Reduviidae are not considered "good flyers", recent reports by **LEHANE & SCHOFIELD** (1982)

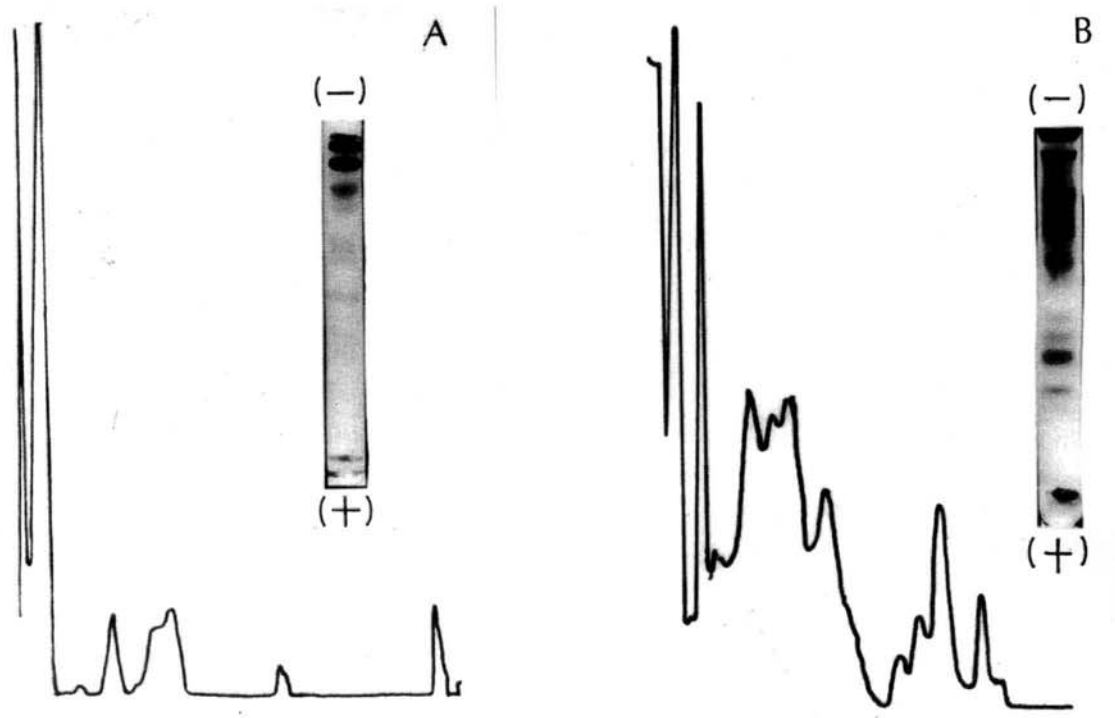

Figure 2: Protein patterns obtained by gel electrophoresis of hemolymph from **T.cruzi** vectors and scan densitometer. A: . **Panstrongylus megistus** (NV); B: **Dipetalogaster maximus.**(♂)

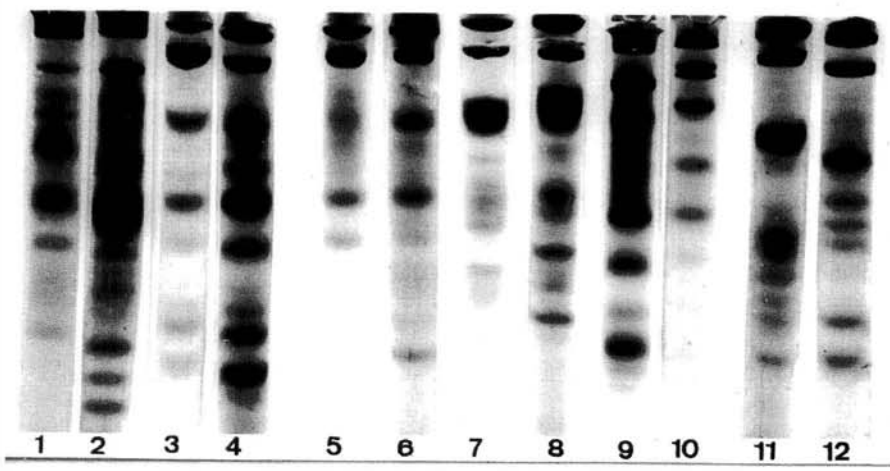

Figure 3: Proteins patterns obtained by gel electrophoresis of hemolymph from Triatoma genus. **T. infestans:** 1-(NV), 2-(♂); **T. rubrovaria:** 3-(NV),4-(♂); **T. guasayana**:5-(NV),6-(♂); **T. mazzotti:**7-(NV),8(♂); **T. patagonica:**9-(NV),10-(♂), **T. pallidipennis:**11-(NV),12-(♂).

point to the possibility that they can fly several kilometers. (Results obtained from experiments performed in the field [21]). In agreement with these reports the presence of structural components of the nervous system - photoreceptors-responsible for the stabilization of flight in **T.infestans** has also been reported [16].

The analysis of the total proteins did not give significant differences among the genera (Table 1) and the results corresponding to the **T. infestans** species agree with those informed for NV[24], whereas the differences found with that reported by **PERASSI** (1972) could be attributed to the longer time elapsed from feeding till the hemolymph was obtained [25].

In the present report, the densitometric evaluation of the obtained proteic profiles allowed to establish qualitative differences among the species

and the stages from Dipetalogaster and Panstrongylus genera (Fig. 2 A,B) and for the members of the Triatoma genus (Fig.3).

The values of total lipids and carbohydrates were not significantly different for the three genera in their two stages (Table 1). The highest value of total lipids corresponded to **T. infestans** in agreement with that reported for the adults stage of this species [30], whereas the values of carbohydrates were in general lower than those reported for phitophague insects [33].

The hemolymphatic lipoproteins have been informed in a variable number between four and seven depending on the insect studied [32].

For the members of the subfamily Reduviidae here reported were shown two to four bands in polyacrylamide; the NV being the stage which showed a higher number. One of these bands was broad which may be indicating a high molecular heterogeneity. All fractions showed mobility between HDL and LDL human lipoproteins (Fig.1). This broad band, present in both stages and in three genera, corresponds to the HDLp fraction obtained by ultracentrifugation whose fractionation on Sepharose 6B showed a bimodal distribution [5]. The additional components shown in the NV stages may correspond to the VHDLp (Very High Density Lipophorin) specific of each stage [19].

The general absence of marked differences in the data from the different analyses studied is not surprising, since the insects studied belong to the same subfamily where several parameters were standardized.

It should be noted that suitable knowledge about the biochemical potentialities of the generation of the energy for flying and demonstration of this ability in several species of triatomines [20], could be relevant in order to design new control programmes and to analyse the aspects related to the transmission of the Chagas' disease.

RESUMO

Componentes hemolinfáticos em vetores do Trypanosoma cruzi: Estudo em doze espécies da subfamília Triatominae (Hemiptera : Reduviidae).

A subfamília Triatominae (Hemíptera : Reduviidae) abrange um grande número de espécies de importância médica na transmissão de parasitoses provocadas pelo **T. cruzi** (Tripanosomiase americana).

Com a finalidade de contribuir ao conhe-cimento da composição química em proteínas, lípides, lipoproteínas e carboidratos da hemolinfa de vetores da doença de Chagas, se apresentam os achados correspondentes a 12 espécies de 3 gêneros, membros da subfamília Triatominae. O estudo foi efetuado com ninfas do 5° estádio e adultos machos das espécies: **T. delpontei, T. dimidiata, T. guasayana, T. infestans, T. mazzotti, T. pallidipennis, T. patagonica, T. platensis, T. rubrovaria** e **T. sordida**, o gênero Triatoma, e **D. maximus** e **P. megistus**, dos gêneros Dipetalogaster e Panstrongylus, respectivamente.

Os achados mostraram diferenças qualitativas na composição proteica, sendo semelhantes os perfis lipoproteicos. Os lípides, proteínas e carboidratos totais não apresentam diferenças significativas entre espécies e/ou gêneros, e/ou estádios.

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