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Changes in the Haemolymph Metabolites, Protein, Carbohydrate, and Free Amino Acid of *Galleria mellonella* L. Parasitized by the Pupal Parasitoid, *Brachymeria lasus* Walker

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***Galleria mellonella* L. was parasitized by the pupal parasitoid, *Brachymerica lasus* Walker in the laboratory. Haemolymph total protein and carbohydrate concentrations from the hosts parasitized by *B. lasus* decreased within 1st day following parasitization. However, haemolymph total free amino acids, glycine, proline, arginine, isoleucine, phenylalanie, leucine, valine, glutamic acid, histidine, serine, and tyrosine increased and free amino acids decreased were threonine, aspartic acid, methionine, cysteine, alanine, and lysine.**

KEY WORDS: Parasitization, Haemolymph, *Brachymeria lasus* Walker, *Galleria mellonella* L. Protein, Carbohydrate, Free amino acids

Hymenopterous endoparasitoids were known to cause many physiological and biochemical changes in the host insect, an action Vinsone and Iwantsch (1980) termed "host regulation." These changes included alternations-in growth, food utilization and development rate of host-that are thought to benefit the developing parasitoid. Changes that have been reported in the host'haemolymph after parasitization include changes in specific gravity, freezing point depression, dry weight, hormone titers, plus differences in amino acid, carbohydrate, lipid, and protein concentrations and in the number of electrophoretically separable proteins (Baldwin and House, 1952; Corbet, 1968; Barras *et al.*, 1969; Dahlman, 1970; Brewer *et al.*, 1973; Thompson and Barlow, 1974; Dahlman, 1975; Vinson and Iwantsch, 1980; Dahlman and Green, jr, 1981; Beckage and Riddiford, 1982; Thompson, 1982).

Brachymeria lasus was best known as a solitary endoparasitoid of fall webworm pupae, *Hyphantria cunea* in korea, and this insect was polyphagous. Currently, there was interest in *B. lasus* as a possible biocontrol agent for gypsy moth, *Lymantria dispar* and fall webworm, *Hyphantria cunea.*

After *B. lasus* parasitizes the host insect, greater wax moth, *Galleria mellonella*, we examined the changes in haemolymph metabolites-protein, carbohydrate, and free amino acid concentrations of host for studies on in vitro culture of the parasitoid.

Materials and Methods

Galleria mellonella pupae were reared at 32°C in darkness, and maintained according to the methods of Dutky *et al.*(1962). Wax moth pupae were removed from their coccons for exposure to the parasitoids.

Wax moth pupae were collected daily for experiments in which pupal age was required, and were used to rear *B. lasus* for colony maintain.

The parasitoids were reared in 9-cm diam petri

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dish at 25 ± 1°C with 16L/8D photoregiom and fed a 10% honey water solution soaked into cotton dental wicks.

Wax moth pupae (O-day-old pupae) were exposed to *B. lauss* in 9 cm diam petri dish at 25 ± 1°C and then the pupae parasitized kept in clear petri dish. The pupae (0-day-old pupae) unparasitized also were kept in the same condition.

Haemolympy samples were obtained by puncturing near the head of pupae with fine needle and being collected into eppendorf tubes in ice with capillary tubes at 24-h intervals after pupae were given control treatment or treatment with the parasitoids. Haemolymph from several pupae was pooled to obtain a minimum volume of 70 μ1, but usually the volume of the sample was 70-100 μ1. To collect the haemolymph of parasitized host pupae, the egg and larva of *B. lasus* in the host pupae was identified by dissecting host pupae in Ringer'solution (128 mM NaCl, 1.8 mM $CaCl_2$, 1.3 mM KCl; pH 7.4) under stereo microscope.

A small amounts of pheylthiourea (PTU) was added to the appropriate amount of haemolymph to prevent melanization and this haemolymph was centrifuged at 10,000 g for 10 min at 4°C to remove haemocytes and tissue debris and supernatant was taken and stored at −20°C for use.

Total protein concentration was determinated by the Folin and Ciocateau's Phenol Reagent with bovine serum albumin as standard (Lowry *et al.*, 1951), and total carbohydrate concentration also was determinated by Anthrone methods with glucose as standard (Joseph H. Roe, 1954).

Free amino acids were estimated by Pico-Tag amino acid analysis system (Waters Assoc) with Pierce H (17 species of free amino acid contained in 2.5 μmol/ml) as standard (Brian *et al.*, 1984). The 50 μl of haemolymph sample and 10 volumes of diluent standard were placed in sample tubes and dried under vacuum. Samples were dried down again after adding 10 μl of ethanol--water-triethylamine (TEA) (2:2:1) to each sample tubes. The derivatization was made fresh daily and consisted of ethanol-TEA-water-phenylisothiocyanate (PITC) (7:1:1:1:). The phenylthiocarbamyl (PTC) derivative of free amino acids was formed by adding 20 μl of ethanol--TEA-water-phenylisothiocyanate to the dried samples and then in the vacuum vials for 20 min at room temperature. Ethanol-TEA-water-phenylisothiocyanate was then removed under using the Workstation. The column used was C18 reverse-phase column with 4 μm packing (150 mm × 3,9 id). Each samples(10 μl) were injected after adding 250 μl of sample diluent to each sample tubes and then dissoving samples. The solvent system consisted of two eluents: (A) an aqueous buffer and (B) 60% acetonitril in water. The typical buffer was 0.14 M sodium acetate containing 0.5 ml/TEA and titrated to pH 6.35 with glacial acetic acid. A gradient which was run for the separation was a linear increase from 0% B to 46% B in 10 min, followed by 46% B to 100% B in the next 4 min.

Results

The concentrations of haemolymph protein and carbohydrate in wax moth pupae parasitized by *B. lasus* has significantly lower levels than that of unparasitized hosts during the entire exprimental periods (Figs. 1 and 2).

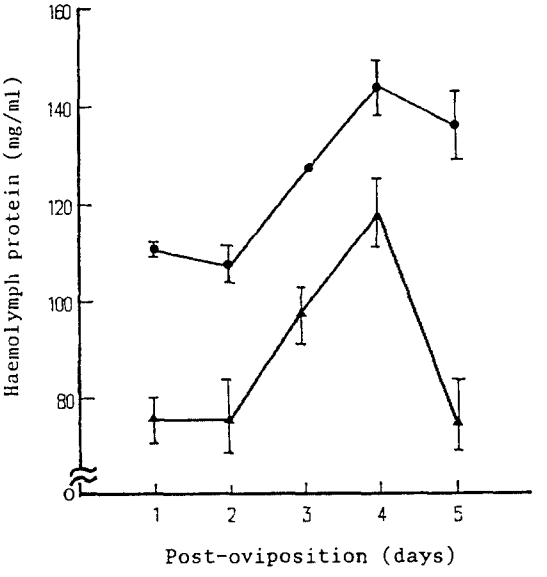

Fig. 1. The haemolymph total protein concentrations of *Galleria mellonella unparasitized* and parasitized by *Brachymeria lasus*, as O-day-old pupae. Each value represents the mean of several individuals. ▲: Parasitized, ●: Unparasitized

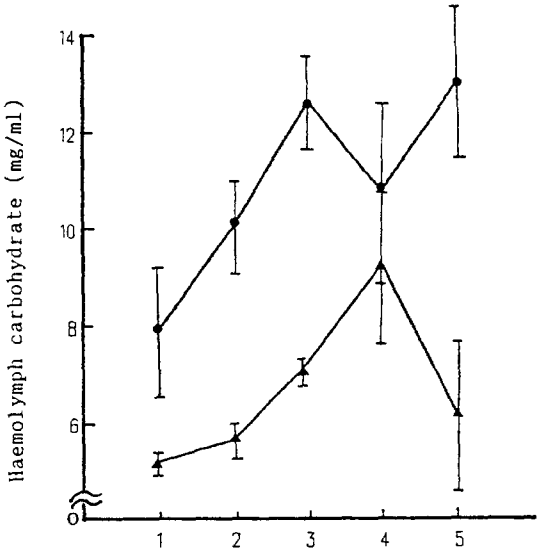

Fig. 2. The haemolymph total carbohydrate concentrations of *Galleria mellonella* unparasited and parasitized by *Brachymeria lasus*, as O-day-old pupae. Each value represents the mean of several individuals. ▲: Parasitized, ●: Unparasitized

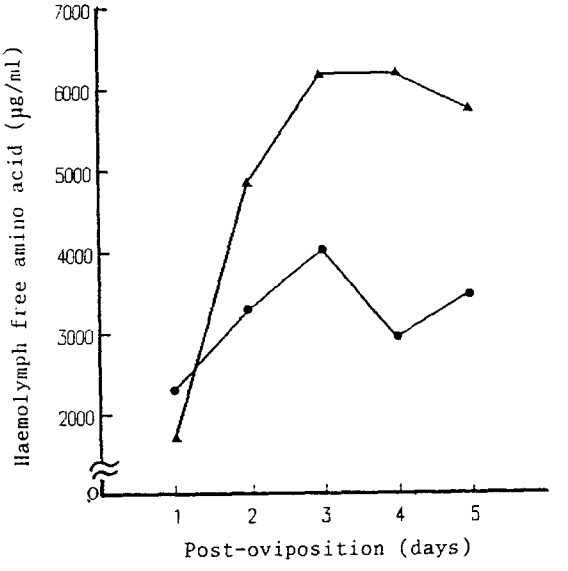

Fig. 3. The haemolymph total free amino acid concentrations of *Galleria mellonella* unparasited and parasitized by *Brachymeria lasus*, O-day-old pupae. Each value represents the haemolymph of several individuals. ▲: Parasitized, ●: Unparasitized

Quantitatively, total free amino acid concentration in wax moth pupae was approximately 3200 μg/ml haemolymph in all the experimental periods, and was dominated by threonine, methionine, lysine, glutamic acid, alanine, proline, tyrosine, and arginine, together with lesser amounts of histidine, valine, leucine, and serine, which varied among experimental periods. Glycine, isoleucine, pheylalanine, aspartic acid, and cysteine were minor constituents in all the experimental periods and were present at variable concentrations all less than 100 μg/ml haemolymph (Tables, 1 and 2).

Parasitization resulted in a increase in total free amino acid concentration except 1st day of the experimental periods (Fig. 3) and a modification in free amino acid compositions. Increased free amino acids were glycine, proline, arginine, isoleucine, phenylalanine, leucine, valine, glutamic acid, histidine, serine, and tyrosine, and decreased free amino acids wer threonine, aspartic acid, methionine, cysteine, alanine, and lysine (Tables. 1 and 2).

Discussion

Various results on the concentration of haemolymph protein and carbohydrate have been obtained from studies with hymenopterous insect parasite. Dahlman (1975) reported that haemolymph trehalose concentrations decreased in *Manduca sexta* parasitized by *Apantales congregatus*. In contrast, the concentartion in *Heliothis virescens* was unaltered after infection by *Cardiochiles nigriceps* (Dalhman and Vinson, 1975). Dalhman and Vinson (1975), however, reported a marked elevation in the haemolymph concentration of trehalose in *H. virescens* parasitized by *Microplitis croceipes*. Similar results were reported for *Trichoplusia ni* parasitized by Hyposoter exiguae (Thompson, 1982).

The effects of the gregarous insect parasite, *Apanteles congregatus* on the total haemolymph protein level in *Manduca sexta* parasitized as first instar larvae was examined by Dalhman (1969). Early fourth and fifth instar host insects had levels similar to uninfected individuals. Smilowitz and Smith (1977) demonstrated alternations in

Table 1. Haemolymph concentrations essential free amino acids in *Galleria mellonella* unparasited and parasitized by *Brachymeria lasus*, as 0-day-old pupae.

Amino acid concentrations (μg/ml)		Post-ovip osition (days) 1 day	Change (%)	2 day	Change (%)	3 day	Change (%)	4 day	Change (%)	5 day	Change (%)	Average	Change (%)	R.C(%)*
Histidine	U**	208.021	−55	128.540	+107	254.661	−32	106.665	+244	234.559	+60	186.489	+37	9.8
	P***	92.795		266.695		173.644		367.050		374.557		254.948		9.9
Arginine	U	408.203	−53	120.912	+321	240.091	+119	170.398	+334	201.497	+305	228.220	+150	12.1
	P	191.577		580.979		525.461		740.104		816.276		570.880		22.3
Threonine	U	34.714	+82	513.902	−71	507.650	−38	231.500	+19	341.153	−33	325.784	−35	17.2
	P	63.172		150.655		313.132		275.927		261.187		212.815		8.3
Valine	U	104.509	−8	184.945	+54	258.963	+90	208.803	+86	221.817	+74	195.824	+68	10.4
	P	95.594		284.345		490.873		388.587		386.438		329.159		12.8
Methionine	U	109.509	+6	396.249	−13	337.551	+7	379.522	−59	219.553	−40	288.477	−23	15.3
	P	116.501		342.844		360.847		153.634		130.848		220.935		8.6
Isoleucine	U	29.826	+84	74.344	+97	127.901	+127	100.728	+128	108.733	+128	88.306	+120	4.7
	P	54.840		146.748		290.011		230.093		248.222		193.844		7.2
Leucine	U	50.840	+71	124.268	+82	268.686	+82	197.858	+91	242.686	+66	176.868	+79	9.4
	P	87.119		226.249		488.786		377.831		403.459		316.689		12.3
Phenylalanine	U	36.312	+44	69.219	+50	131.811	+81	87.324	+94	108.625	+103	86.658	+81	4.6
	P	52.144		103.868		238.214		169.342		220.967		156.907		6.1
Lysine	U	186.022	−25	348.042	+19	393.072	−38	276.467	+32	373.279	+4	315.376	−2	16.7
	P	139.953		413.093		242.452		365.936		387.659		309.819		12.1
Total	U	1168.041	−23	1960.412	+28	2520.385	+24	1759.295	+74	2051.902	+57	1892.003	+36	
	P	893.000		2512.476		3123.420		2068.504		3229.613		2566.003		

*Relative concentration, **Unparasitized, ***Parasitized. Chang (%) $= \frac{\text{(Parasitized-Unparasitized)}}{\text{Unparasitized}} \times 100$

Table 2. Haemolymph concentrations of nonessential free amino acids in *Galleria mellonella* unparasitized and parasitized by *Brachymeria lasus*, as 0-day-old pupae.

Amino acid concentrations (μg/ml)		Post-oviposition (days) 1 day	Change (%)	2 day	Change (%)	3 day	Change (%)	4 day	Change (%)	5 day	Change (%)	Average	Change (%)	R.C(%)*
	U**	3.913		5.546		73.920		41.797		123.912		49.818		3.8
Aspartic acid			−75		−78		+109		−68		−89		−26	
	P***	0.986		1.196		154.269		13.347		13.869		36.733		1.5
	U	128.767		207.792		401.941		217.179		338.461		258.828		19.8
Glutamic acid			−42		−25		+56		+8		+47		+40	
	P	74.587		155.328		628.017		452.357		497.208		361.499		14.5
	U	53.993		159.404		140.422		82.406		112.781		109.801		8.4
Serine			−20		−19		+28		+70		+29		+16	
	P	43.210		129.404		180.244		140.500		145.327		127.796		5.1
	U	209.665		72.439		71.457		46.931		95.208		95.208		
Glycine			−13		+547		+682		+868		+1203		+440	
	P	182.371		487.998		558.817		731.194		611.712		514.418		20.6
	U	200.740		398.355		311.801		244.097		229.058		276.810		21.2
Alanine			−68		−20		−18		+124		+82		−2	
	P	64.359		318.005		254.184		302.090		416.562		271.040		10.8
	U	419.599		269.669		192.739		215.948		227.724		265.142		20.3
Proline			−22		+280		+366		+466		+420		+251	
	P	326.150		1024.767		897.868		1222.754		1185.012		931.310		37.2
	U	99.193		164.869		273.050		281.149		345.139		232.680		17.8
Tyrosine			−3		+20		+25		−15		−2		+4	
	P	96.626		198.325		342.554		238.321		339.860		243.137		9.7
	U	10.138		36.623		23.301		16.435		16.416		20.583		1.6
Cysteine			+28		−54		+56		−36		−41		−16	
	P	13.017		16.817		36.249		10.600		9.740		17.285		0.7
	U	1126.008		1314.697		1488.631		1174.558		1440.421		1308.870		
Total			−29		+77		+105		+165		+54		+91	
	P	801.306		2332.136		3052.202		3111.163		2219.290		2503.218		

*Relative concentration, **Unparasitized, ***Parasitized. Change (%) = $\frac{\text{(Parasitized-Unparasitized)}}{\text{Unparasitized}} \times 100$

haemolymph proteins of *Pieris rapae* parasitized as second instar larvae by *Apanteles glometatus*. Protein concentrations in both uninfected and infected hosts followed a cyclical pattern during host development, but parasitized larvae had somewhat higher concentrations during the late fourth and early fifth instars and significantly lower levels than uninfected insects during the remainder of larval development. The latter decline was coincident with fat body destruction. Thompson (1982) also demonstrated the effects of parasitization by *H. exiguae* on the total haemolymph protein concentrations of fourth instar *T. ni*. Parasitized larvae had significantly elevated haemolymph concentration immediately following parasitization. The higher concentrations were maintained until approx. 4 days post-infection at which time the concentration in unparasitized larvae surpassed that of infected individuals.

Investigations on an alternations in electrophoretic proetin patterns were performed in *Heliothis virescens* parasitized by *Cardiochiles migriceps* (Vinson and Barras, 1970) and in *Heliothis zea* parasitized by *Microplitis croceipes* (Barras *et al.*, 1972). In both studies it was concluded that the developing parasite utilized or destroyed certain haemolymph proteins while causing a shift in free amino acides to the bound state or the secretions of other proteins into the haemolymph.

In investigations such as above, the concentrations of haemolymphy carbohydrate and protein in the host insects parasitized by larval parasitoids show mainly a tendency to increase. However, the concentrations of hemolymph carbohydrate and protein in the wax moth pupae parasitized by *B. lasus* show a tendency to decrease (Figs. 1 and 2). It is suggested that such decrease takes place because the host wax moth pupae parasitized is no feeding.

Amino acids may be of considerable importance in the nutritional and metabolic adaptations of insect parasite. Corbet (1968) reported that *N. canescens* spend a considerable portion of its larval life as a first instar larva and completes most of its larval development during the late last larval stadium of the host. The composition of the host'haemolymph changed dramatically during this period. It was suggested that such changes after feeding behavior of the first instar larva, increasing the rate of nutrient uptake and inducing growth. Among the change occurring in the host'haemolymph was a sharp increase in free amino acid concentration.

In similar investigations, Barras *et al.* (1969) reported that following parasitization of *H. virescens* by *C. nigriceps*, the concentration of certain free amino acid decreased while their levels increased in the peptide and protein fractions. The result, however, was not consistent with all the amono acids. Fisher and Ganesalingam (1970) demonstrated altered amino acid levels in Anagasta Keuhniella one week after parasitization by *Devorgilla canescens*. Phenylalanine and leucine were absent in parasitized hosts while two unknown amino acids, which were absent in uninfected hosts, were reported at high levels in parasitized. Thompson (1986) also reported that following parasitization of *Trichoplusia ni* by *Hyposoter exiguae*, the concentration of total haemolymph glucogenic amino acid decreased.

In vitro studies demonstrated that amino acid alone can fulfill the energy requirements for complete larval development of a number of hymenopterous parasitodis (Thompson, 1076, 1983; Yazgan, 1972). The parasitoid *B. lasus* grew well in vitro on media in which nitrogen was supplied as a mixture of protein and free amino acids. Larvae failed to develop properly, however, on media containing protein alone. Studies on amino acid metabolism of *Exeristes roborator* further indicated the importance of amino acids for growth and development of inset parasitoids. A comparison of the rate of incorporation of carbohydrate precursur into amino acid with the synthesis rate of protein from amino acid indicated that the protein sythesized by amino acid synthesis. Thus, developing larvae require a source of amino acid for maximal growth and development, and a low rate of amino acid synthesis may represent an adaptation of the parasite, which feeds on host haemolymph, a rich source of amino acids (Thompson. 1982).

In this experiment, therefore, the concentration of increased haemolymph free amino acids in the wax moth pupae parasitized by *B. lasus* is thought to benefit to the developing parasitoid because of requiring a lot of free amino acid for maximal growth and development of the parasitoid (Fig. 3,

Tables. 1 and 2). But, it is not known yet whether the concentration of increased hamolymph free amino acids is due to the fat body destruction or haemolymph proteolysis in the host pupae.

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무늬수중다리좀벌(*Brachymeria lasus Walker*)에 의해 기생된 꿀벌부채명나방(*Galleria mellonella L.*)번데기의 혈림프 대사물질에서 단백질, 탄수화물, 유리아미노산의 변화

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꿀벌부채명나방이 무늬수중다리좀벌에 의해 기생 되었을때 이 기주의 혈림프에서 대사물질의 변화 생겼다. 이 기생벌에 의해 기생된 기주와 기생되지않은 기주의 혈림프에서 총단백질과 총탄수화물의 농도를 비교하여보면, 기생된 기주에서 전체적으로 뚜렷이 감소하는 경향을 나타내었다. 그러나, 총유리아미노산은 기생된 후 첫날을 제외하고 기생된 기주에서 전체적으로 뚜렷이 증가하는 경향을 나타내었다. 그리고 유리아미노산 중에서, glycine, proline, arginine, isoleucine, phenylalanine, leucine, valine, glutamic acid, histidine, serine, tyrosine은 뚜렷이 증가하는 경향을 나타내었고, threonine, aspartic acid, methionine, cysteine, alanine, lysine는 약간의 감소하는 경향을 나타내었다.

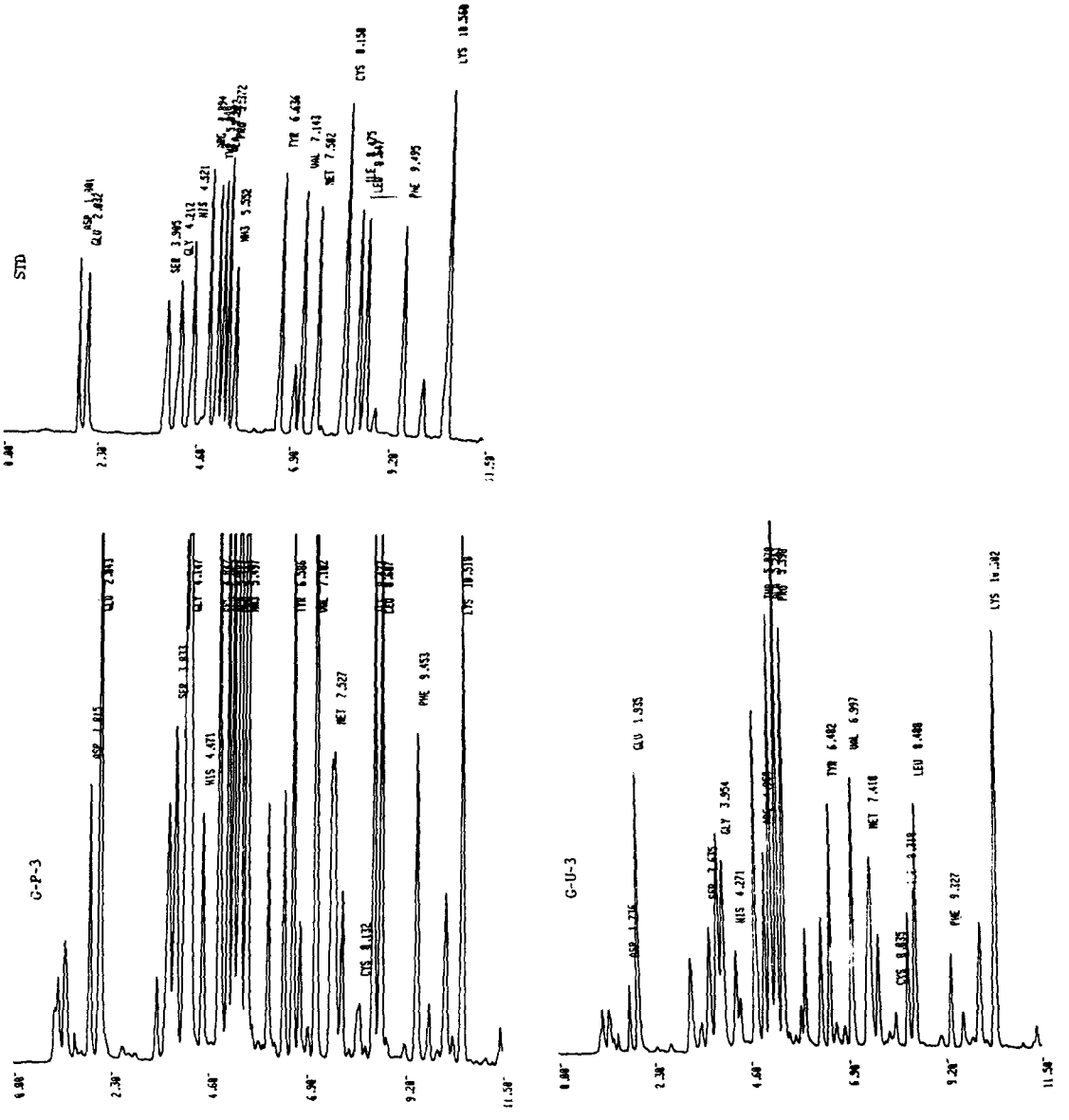

Plate. Chromatograms of free amino acids. STD: Standard, G: *Galleria mellonella*, P: Parasitized, U: Unparasitized, 3: Post-oviposition (days).