Table II. Influence of carboxypeptidase B on the amount of lysine and arginine, incorporated into the fraction containing aggregation factor

Labelled amino acid	Aggregation factor		Aggregation factor incubated with carboxypeptidase B		Acid soluble radioactivity released by carboxypeptidase B	
	Total (cpm)	Acid insoluble (cpm)	Total (cpm)	Acid insoluble (cpm)	Absolute (cpm)	(%)
L-lysine	2,115	1,885	2,020	710	1,080	59
L-arginine	4,380	4,010	4,495	840	3,285	81

Sponge material (7 g) cut into cubes of 2 mm³ was incubated in 40 ml filtered sea water with 50 µCi ^{14}C-L-arginine (The Radiochemical Centre: spec. activity 318 mCi/mmol) and 50 µCi ^{14}C-L-lysine (The Radiochemical Centre: spec. activity 318 mCi/mmol) in an incubator[5]. After a period of 24 h at 18 °C aggregation factor has been isolated up to step IV (Table I) as described above. The fractions containing the aggregation factor were incubated with 200 µg carboxypeptidase B/ml. Acid insoluble fraction[13] and radioactivity[5] were determined as described.

Zusammenfassung. Chemisch dissoziierte Zellen des Kieselschwammes *Geodia cydonium* reaggregieren aufgrund zweier verschiedener Reaggregationsprinzipien. Der Aggregationsfaktor, auf den die Primäraggregation zurückgeht, ist membrangebunden und wird durch Proteasen nicht inaktiviert. Der sekundäre Aggregationsfaktor wurde 500fach angereichert. Das Molekulargewicht dieses Aggregationsfaktors beträgt etwa 20000 Daltons; er ist mit einem ringförmigen Makromolekül (2×10^9 Daltons) assoziiert.

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Free Amino Acid Composition of the Hemolymph of the Larval Blackfly *Simulium venustum* (Diptera: Simuliidae)

Recent attention has focused upon the possibility of using mermithid nematodes as biocontrol agents of blackflies[1]. However, field introductions of such mermithids cannot be made with probable success until procedures are devised for mass cultivating their infective stage(s). The lack of information concerning physiology (esp. hemolymph composition) of the simuliid hosts is a considerable hinderance to in vitro culture of these nematodes, because they derive nutriment from the host's hemolymph during parasitic development within the insect's hemocoel. The mermithid *Mermis nigrescens* Dujardin synthesizes proteins from amino acids available within the host hemolymph, but not from a dietary supply of dipeptides or proteins[2]. Therefore, this study was done to investigate the free amino acid composition of the hemolymph of the larvae of *Simulium venustum*, a blackfly species susceptible to mermithid parasitism[3].

Field-collected larval blackflies were held in an incubator at 10 °C until their hemolymph could be extracted. Insect larvae sampled for blood were primarily of 'maturing' and 'mature' developmental stages[4]. Using a stereomicroscope, hemolymph was obtained from surface-dried larvae by gently puncturing the insects in their proleg region with a fine insect pin. The fluid which exuded was drawn to fill a 10 µl capillary tube, expelled into a test tube containing the pooled blood sample and stored frozen at −20 °C. The pooled sample comprised blood taken from over 3,000 insects, because only a very small volume of hemolymph (0.3–0.5 µl) could be obtained from each blackfly larva. Therefore, the pooled sample was stored frozen throughout this protracted blood extraction process.

The pooled hemolymph sample was deproteinized by adding 30 mg sulphosalicylic acid, then centrifuged (6,500 g, 4 °C, 20 min). The volume of the supernatant was adjusted to 2.0 ml using a 0.2 *N* sodium citrate buffer (pH 2.2), then analyzed by the Beckman physiological fluids procedure[5] using a Beckman Model 121 amino acid analyzer. To determine total hemolymph levels of amino nitrogen, eight 5 µl aliquots of blood were collected from *S. venustum* larvae. Each aliquot was deproteinized by blowing it into 2 ml of 5% trichloroacetic acid. After centrifugation, 0.5 ml samples of the supernatant fluid were assayed colorimetrically for total amino nitrogen[6]. The mean free amino acid level of larval *S. venustum* hemolymph was found to be 39.3 ± 1.3 mg amino N per 100 ml hemolymph.

Consistent with findings for several other insect species[7–9], the larval *S. venustum* has high concentrations of

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Relative concentrations of free amino acids and ninhydrin positive substances in the hemolymph of *S. venustum*

Free amino acid or ninhydrin positive substance	Hemolymph concentration (μmoles/ml)	Free amino acid or ninhydrin positive substance	Hemolymph concentration (μmoles/ml)
Cysteic acid	0.030	Methionine	0.252
Phosphoserine	0.028	Isoleucine	0.507
Glycerophosphoethanolamine	0.257	Leucine	0.897
Phosphoethanolamine	0.009	Glucosamine	Nil
Taurine	0.267	Tyrosine	0.100
Urea	3.050	Phenylalanine	1.120
Methionine Sulfoxide	0.332	Galactosamine	Nil
Hydroxyproline	Nil	β Alanine	0.136
Aspartic Acid	0.231	β Aminoisobutyric acid	Nil
Threonine	0.894	Hydroxylysine	Trace
Serine	1.590	γ-Aminobutyric Acid	0.014
Glutamine + asparagine	6.400	Ornithine	0.012
Sarcosine	Nil	Ethanolamine	2.170
Proline	2.490	Ammonia	5.050
Glutamic Acid	2.230	Lysine	1.670
Citrulline	0.074	1-Methylhistidine	Nil
Glycine	2.610	Histidine	2.500
Alanine	3.350	3-Methylhistidine	Nil
α Aminoadipic Acid	Nil	Anserine	Nil
α Amino-*n*-butyric Acid	Nil	Tryptophan	0.245
Half Cystine	0.034	Creatinine	Nil
Homocitrulline	*	Carnosine	Nil
Cystathionine	0.065	Arginine	1.790
Valine	0.930	Dihydroxyphenylalanine	2.350

* Two small peaks eluted in the same areas as homocitrulline and norleucine respectively, but positive identification was not possible.

glutamic acid, aspartic acid and amide derivatives, glutamine and asparagine within its hemolymph. Such amino acids have a central role in insect metabolism as reservoirs for transferable amino groups[10] consequently, extremely high levels of alanine (a product of glutamic acid transamination) were also recorded within the hemolymph. The high blood concentrations of phenylalanine and dihydroxyphenylalanine (dopa) may be indicative of cuticular tanning/melanization rather than melanization of the blood sample because the blood did not darken appreciably during storage. The presence of ornithine cycle intermediates (arginine, ornithine, citrulline) and large quantities of ammonia and urea within the hemolymph suggests that the larvae of *S. venustum* may use both ammoniotelic and ureotelic modes of nitrogen excretion, presumably as an adaptation to their aquatic habitat. Taurine, a common blood metabolite (of unknown function) of insects, was present at moderate levels within the simuliid hemolymph. The high level of proline within the hemolymph of *S. venustum* larvae agrees with findings for several unrelated insect species[7,8,11] and may indicate an interrelationship between this amino acid and glutamic acid. Ethanolamine, serine (both phosphatide components), glycine (involved in many metabolic processes) and histidine were also present at high concentration. However, the hemolymph contained very low levels of tyrosine, half cystine (i.e. total of cysteine plus cystine) and cystathionine.

The hemolymph of the larval *S. venustum* constitutes a nutritionally rich microenvironment for the development of parasitic mermithids. These nematodes probably feed by absorbing essentially low molecular weight metabolites from the host hemolymph[12]. Thus, continuing nutritional and physico-chemical studies of the host hemolymph should provide useful information for devising media suitable for culturing these potential biocontrol agents[13].

Zusammenfassung. Nachweis, dass die Aminosäure-Zusammensetzung der Hämolymphe von *Simulium-venustum*-Larven total 39.3 ± 1.3 mg Amino N pro 100 ml enthält, wobei als hauptsächliche Ninhydrinpositive Bestandteile der Hämolymphe Alanin, Asparagin, Asparaginsäure, Dioxyphenylalanin, Äthanolanin, Glutaminsäure, Glutamin, Glycin, Histidin, Phenylalanin und Serin gefunden wurden, während nur kleine Mengen von Tyrosin, Halb-Cystin und Cystathion festgestellt wurden.

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