were obtained at 80 kV and with a cathode current of 5 μA. The results were fed into a Kontron computer and following their 10-fold optimalization they were registered by a recorder. The diameter of the analyzed spot was 1 μm and the time of analysis was 60 sec. Particular attention was paid to positioning of the Birbeck granule in the center of the electron beam. Subsequent analyses were performed by shifting the beam beyond the analyzed area. Sections of embedding medium, grids and reagents used for preparation of the material served for control analysis.

Results and discussion. The emission spectrum obtained in studies on Birbeck granules is presented in figure 2 (solid line). Two specific maxima are noted, corresponding to the M_a and L_{a1} lines of Au ($M_a = 2.123$ KeV, $L_{a1} = 9.713$ KeV). Identical peaks have been noted from X-ray analysis of colloidal gold granules, obtained according to Horisberger and Rosset[6] and applied onto the grids. Spectral curves obtained upon analysis of sites in the neighborhood of Birbeck granules are exemplified by the curve (broken line) in figure 2. No peaks indicating the content of Au could be noted on this curve or on spectral curves for the nucleus (fig. 2, dotted line), mitochondria, lysosomes, cytoplasmic reticulum or cell membrane.

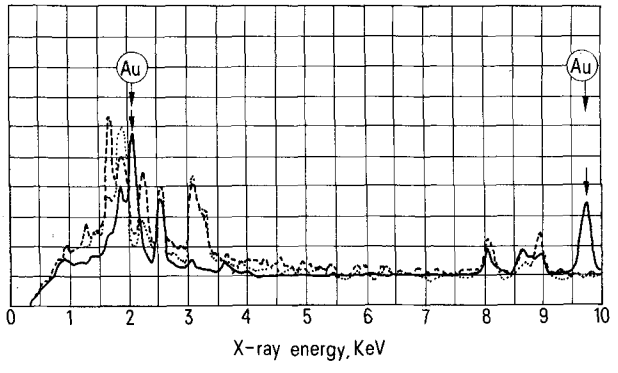

Figure 2. EDX-spectra of Langerhans cell granules. Solid line = evident maxima for lines corresponding to $M_\alpha = 2.123$ KeV, and $L_{\alpha 1} = 9.713$ KeV of Au, broken lines = close neighborhood of the granules, and dotted lines = chromatin of the cell nucleus. The arrows with circle indicate the maximum for gold standard.

The presence of gold was demonstrated in macrophages, kidney, adrenal gland and ovary after treatment of experimental animals with drugs containing gold used in rheumatoid arthritis[7]. Some authors[8,9] have suggested that macrophage phagocytic activity is progressively reduced in patients receiving the above mentioned drugs. However, the information obtained from the literature does not suggest any probable physiological role for gold in the Birbeck granules of Langerhans cells. Probably the presence of gold in them cannot be related to a specific ability of the granules to bind heavy metals, since there was no evidence with EDX-spectra of the presence of Cr, Pb or other metals with which the rats studied presumably have more frequent contact. The granules may, however, be expected to bind Au specifically since trapping of exogenous gold has already been demonstrated in this type of cell by Langerhans[10] 100 years ago. It is conceivable that gold (at this time we cannot determine whether it is metallic or ionic) plays a role in the formation of Birbeck granules which are known to possess a unique structure. It should be mentioned here that gold has been found in human spermatozoa using radionucleic techniques[11], but its role, as in the case of Birbeck granules, is not clear.

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Characterization of free amino acids in the hemolymph of *Achaea janata* L. larvae (Lep. Noctuidae)

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Summary. 19 free amino acids were separated and quantified from the hemolymph of 5th instar larvae of the moth *Achaea janata*. Proline, histidine, threonine/glutamine/asparagine, lysine, valine and serine were the predominant amino acids in the hemolymph. Changes in amino acid concentration are discussed in relation to metabolic and other physiological activities.

Insect hemolymph is characterized by a very high content of free amino acids (FAA) and their derivatives[2,3]. 50-85% of the non-protein nitrogen in insect blood is in the form of free amino acids[4]. Various reviews on FAA of insects indicate that these compounds are of great importance for both growth and differentiation[5-9]. Amino acids are not only the substrate for protein synthesis[3] but enter into diverse metabolic pathways and participate in various physiological activities. At least some of these roles seem to be peculiar to insects[10].

Although changes in FAA during the development of some lepidopterans have been studied[11-14], no systematic quantitative analysis of FAA during a particular instar of an insect has been carried out as yet. Reported herein are the changes in the FAA concentrations in the hemolymph of *Achaea janata* L. (Lep. Noctuidae) during the 5th instar.

Materials and methods. Larvae of *Achaea janata* for experiments were taken from a stock culture maintained in the laboratory according to Ramdev and Rao[15] and under these conditions the 5th instar larval period was found to

be, on average, 5.45 days. Hemolymph samples from 5th instar larvae of *Achaea janata* were taken at 0, 24, 48, 72 and 96 h after moulting. Samples were centrifuged (3500 rpm) to separate hemocytes from plasma. Plasma proteins were precipitated by adding 400 µl 95% alcohol to 100 µl plasma (for each sample). The protein precipitate was removed by centrifugation (3500 rpm) and washed with 95% alcohol thrice. All the supernatants from each sample were pooled. The samples were then analyzed using a Technicon Sequential Multisample Amino Acid Analyzer, with nor-leucine as internal standard. Amino acids were identified by their retention times by comparing them with previously run standards.

Results and discussion. 19 free amino acid peaks, including 1 unidentified peak, were observed (table). The amino acids recorded could be divided into 3 categories, as follows:

a) Amino acids which showed reduction in concentration with increasing larval age, namely, lysine, aspartic acid, serine, glycine and cystine;

b) amino acids which showed an increase in concentration with the development of the larva, namely glutamic acid, isoleucine, leucine and tyrosine, and

c) amino acids which showed an increase up to the middle of the instar and thereafter decreased, namely, histidine, arginine, threonine/glutamine/asparagine group, proline, valine, methionine and phenylalanine.

Nath and Srivastava[16] reported qualitatively 19 amino acids in the same stage of *Achaea janata*. However, they reported that lysine, phenylalanine and valine were absent from the hemolymph; these amino acids were found to be present in detectable amounts throughout the 5th instar in the present studies. The difference could be due to differences in the methods of analysis.

The most predominant amino acids during the 5th instar were proline, histidine, threonine/glutamine/asparagine, lysine, valine and serine; the quantity of proline was the highest. A high level of proline has been recorded in various insects[17,18]. It was shown in *Glossina* that a large quantity of proline is required for energy production[19,20] and this may also be the case in *Achaea*.

The presence of glycine and alanine in considerable concentration probably reflects their importance as intermediates between carbohydrate and amino acid metabolism. Their fluctuations may be related to their metabolism in the insect. The high level of the threonine/glutamine/aparagine group in the hemolymph could be related to glutaminase activity in the fat bodies[21,22]. Although the concentration of the individual amino acids of this group has not been worked out, due to the limitations of the method adopted for analysis, it is known that glutamine has an important role in transamination in insects[6,8,23,24]. Aspartic acid, which is known to participate in transamination reactions, was found to be almost absent. The reasons for its absence are difficult to suggest.

Concentration of tyrosine increased at the time of prepupation, which could be expected as it is required for cuticle tanning and melanogenesis; however, no such increase was observed in phenylalanine, which is also known to be involved in the synthesis of insect cuticle[25]. Lysine concentration decreased gradually with the age of the larva and this was probably due to its utilization in protein synthesis. The importance of the high concentration of cystine at the beginning of the 5th instar is difficult to understand. As emphasized by Sutcliffe[26,27] the participation of inorganic ions in the hemolymph osmotic pressure tends to decrease with the phylogenetic position of the animal. The more evolved the animal is, greater is the importance of small organic molecules in the osmotic pressure of the blood. Thus, in the present case it could be that some amino acids play a role in maintaining the osmotic pressure of the hemolymph.

Free amino acids (µm/ml) of 5th instar larvae of *Achaea janata*

Amino acid	Time after moulting (h)				
	0	24	48	72	96
Lysine	10.17	3.94	5.74	3.27	2.07
Histidine	–	5.94	21.18	10.31	6.62
NH_3	18.60	2.26	11.59	12.99	13.56
Arginine	2.63	3.40	4.31	1.35	2.60
Asparagine	0.32	Traces	Traces	–	–
Threonine/glutamine/ asparagine	7.81	5.46	11.26	4.50	4.27
Serine	8.46	6.45	4.27	2.21	2.95
Glutamine	1.50	1.56	2.70	2.89	2.40
Unidentified	0.77	12.16	7.92	19.13	16.28
Proline	16.45	23.08	20.69	14.23	16.28
Glycine	4.02	3.82	3.47	1.16	1.74
Alanine	3.95	1.57	4.97	1.62	2.17
Cystine	6.18	–	–	–	–
Valine	4.64	1.46	9.01	3.53	1.87
Methionine	0.78	3.26	4.92	2.07	1.53
Isoleucine	1.29	0.81	3.05	2.11	3.38
Leucine	1.86	1.20	5.60	2.92	8.57
Tyrosine	1.15	1.66	3.29	2.86	3.41
Phenylalanine	1.21	1.15	2.28	0.92	1.24

Values presented are the average of 3 replications.

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