

after operation (fig. 3). The cell diameters in the denervated dogs became large as compared to those in the sham-operated group, even though the pressure gradient was small.

The results presented in this paper, together with the previous study³, suggest that cardiac cell hypertrophy is accelerated by denervation of the heart independently of the degree of pressure-overload or genetic factor. This may imply a neural mechanism which, when present, would inhibit cardiac cell hypertrophy. The mechanism by which cardiac cell hypertrophy is increased after denervation is not clear. However, a report says that denervation leads to a supersensitivity to chemical and physical stimuli⁵, and also results in an increase in the number of adrenergic receptors⁶. Left stellate ganglectomy may lead to a reduction in free lysosomal enzyme activity, implying a possible reduction in protein degradation in the cell⁷. Furthermore, inhibition of glycolysis has been observed in the denervated dog heart⁸. In conclusion, our data supply evidence that cardiac cell hypertrophy in dogs with aortic stenosis may be accelerated by denervation of the heart.

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Granules of Langerhans cells in the thymus contain gold

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Summary. The granules of Langerhans cells in the thymus of the rat were subjected to X-ray microanalysis. It was established that gold is present in these morphological structures.

Langerhans cells contain characteristic granules (fig. 1), the ultrastructure of which was described for the first time by Birbeck and Breathnach¹. Despite numerous studies, their role is still not clear²⁻⁵, so any new information about the granules is of potential significance. With the above in mind we attempted to examine the chemical content of Langerhans cell granules using X-ray analysis.

Material and methods. Studies were performed on male Wistar rats aged 60 days and fed standard laboratory animal chow LSM (Laboratory Diet Factory, Motycz,

Poland). The animals were decapitated and small thymus fragments were fixed for 1 h in 2.5% glutaraldehyde in 50 mM phosphate buffer, pH 7.4. The material was post-fixed in 1% OsO₄ solution in 0.1 M phosphate buffer, pH 7.4, dehydrated in a series of alcohols, passed through acetone and embedded in a low viscosity medium (Spurr, 1969). 100-nm thick sections were collected on nylon grids and subjected to X-ray microanalysis. The analysis was performed using a Link EDX 290 analyzer coupled to a Zeiss EM 10 electron microscope. The emission spectra

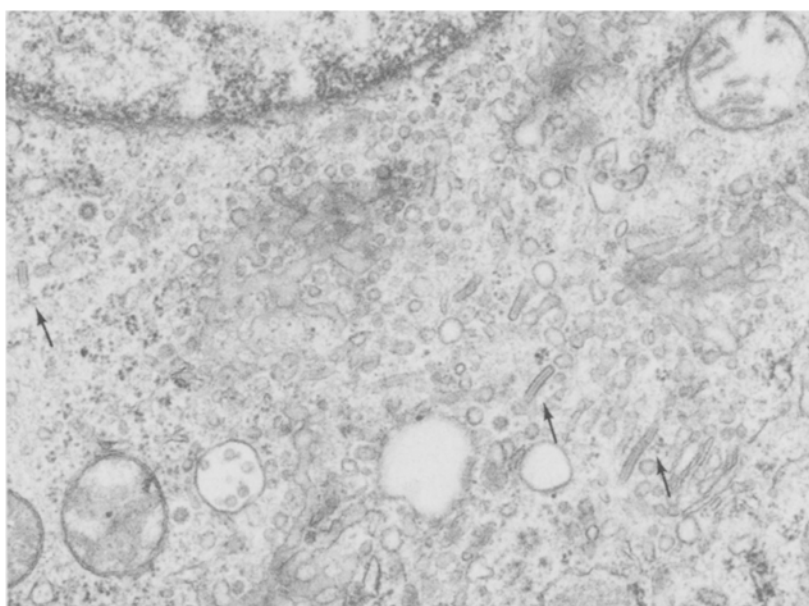


Figure 1. Langerhans cell of rat thymus. Arrows indicate some of the Langerhans cell granules. $\times 26,000$.

were obtained at 80 kV and with a cathode current of 5 μ A. The results were fed into a Kontron computer and following their 10-fold optimization they were registered by a recorder. The diameter of the analyzed spot was 1 μ m 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 Rosset⁶ 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.

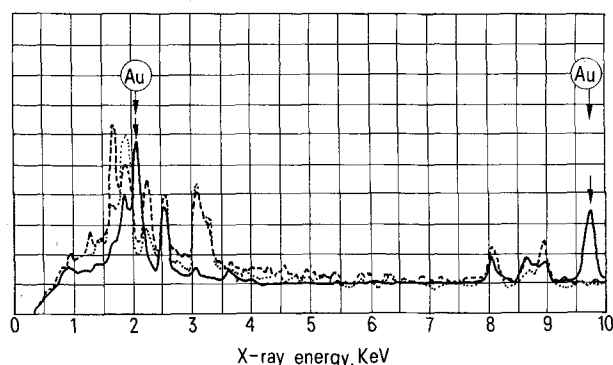


Figure 2. EDX-spectra of Langerhans cell granules. Solid line = evident maxima for lines corresponding to $M_a=2.123$ KeV, and $L_{a1}=9.713$ KeV of Au, broken lines = close neighborhood of the granules, and dotted lines = chromatin of the cell nucleus. The arrows with circle indicate the maximum for 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 arthritis⁷. 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 Langerhans¹⁰ 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 techniques¹¹, 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 acids⁴. 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 synthesis³ but enter into diverse metabolic pathways and participate in various physiological activities. At least some of these roles seem to be peculiar to insects¹⁰.

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 Rao¹⁵ and under these conditions the 5th instar larval period was found to