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Rhombic Dodecahedral Secretory Granules in Glucagon Producing Islet Cells*

Rainer H. Lange**

Elektronenmikroskopische Abteilung, Anatomisches Institut der Universität, Gießen

and Catherine Klein***

Laboratoire de Zoologie et d'Embryologie Expérimentale, Université Louis Pasteur, Strasbourg, France

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Summary. By tilting experiments in the electron microscope evidence was found for crystalline islet A_2-granules of *Xiphophorus helleri* H. to be rhombic dodecahedral and thus to belong to the cubic system.

Key words: Pancreatic islets — *Xiphophorus helleri* — A_2-Granules — Crystalline structure — Electron microscopy.

Introduction

A_2-cells of the endocrine pancreas often contain spherical secretory granules (cf. Lange, 1973). It is only by a comparative investigation that crystalline granules are revealed that allow a correlation with isolated hormones, the glucagons. In the teleosts, *Gadus morrhua* L. and *Xiphophorus helleri* Heckel, A_2-cells as identified by their topographical distribution (Thomas, 1970) or by immunohistochemical reaction with antiporcine glucagon serum (Klein and Lange, 1972, 1974) show a preponderance of idiomorphic crystalline secretory granules (Lange, Klein and Thomas, 1973). We report here tilting experiments in the electron microscope performed to determine crystal morphology in *Xiphophorus helleri*.

Materials and Methods

Principal islets in *Xiphophorus helleri* Heckel were dissected and fixed by immersion in glutaraldehyde (2% in cacodylate buffer), osmicated, dehydrated in ethanol and embedded in Araldite. Ultrathin sections, double-stained with uranyl acetate and lead citrate, were examined in a Philips electron microscope EM 201 fitted with goniometer, rotation specimen holder, and anticontamination device. The tilt range was $\pm 60°$. Photographs were taken on Kodak Fine Grain Release Positive Film (35 mm) at magnifications of up to $\times 24500$.

Results

Because of the tiny dimensions of the crystals (diameter in the order of 100 nm), serial sectioning of the granules was not possible as in another case (Lange, Boseck and Syed Ali, 1972). The isodiametric form of granule sections and the

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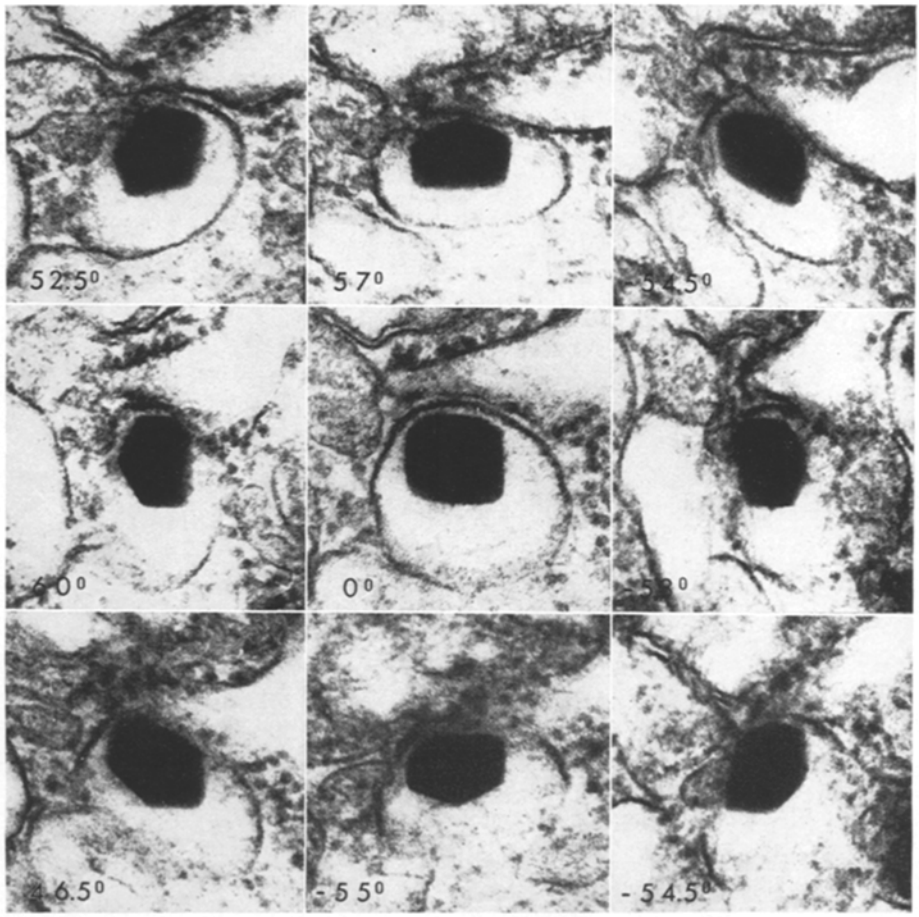

Fig. 1. Full tilting programme with an A_2-granule section in *Xiphophorus helleri*, starting from [100] (central photograph). All angles given are experimental and refer to the 0° position of the central photograph, which represents a distorted octogonal section (near equator). The tilt axis is always normal to the line connecting a peripheral with the central photograph. The incomplete profiles in the tilted positions reflect the fact that a sectioned rather than a whole crystal has been tilted. Electron microscopic magnification ×24500, total ×89000

frequent occurrence of hexagonal, square and octogonal section profiles led us to choose a rhombic dodecahedral model as operational basis for the tilting experiments.

Fig. 1 gives the results of a typical tilting operation, demonstrating the expected 4-fold external symmetry of the crystals when the starting point is a more or less ideal [100] projection. The expected results were also obtained when other typical section profiles were started from, e.g., [211] and [110] projections. The angle between external 3-fold and 4-fold axis was found to be 53.6° (n = 14; theoretical value 54.75°) and that between external 2-fold and 4-fold axis 47.9° (n = 14, theoretical value 45°). Similar results have been obtained in *Gadus*

morrhua. One should realize that the accuracy of such angular measurements is limited by the fact that the point at which a given crystal face runs parallel to the microscope axis can only roughly be determined.

Discussion

The only crystalline form obtained from mammalian and avian glucagons in vitro, to our knowledge, has been the rhombic dodecahedron (cf. Lange, 1973). The impressive predictability of tilting results when starting from a variety of typical section profiles of rhombic dodecahedra (cf. Lange, Boseck and Syed Ali, 1972; Kleber, 1971), the isodiametric form of granule sections and the angular measurements are considered as being good evidence for our morphological interpretation of A_2-granules. Further studies of teleost islet tissues may yield more evidence, it will, however, be extremely difficult to derive absolute proof from thin section electron microscopy. A very interesting question is that of crystal structure in A_2-granules and it has been attacked in a preliminary manner (Lange, Klein, Thomas, 1973). It is clear that structural data allowing comparison with glucagon x-ray data (King, 1959) can be obtained from electron microscopy only with very considerable preparatory and instrumental efforts.

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Prof. Dr. Rainer H. Lange
Zentrum für Anatomie und Cytobiologie
der Universität Gießen
6300 Gießen
Friedrichstraße 24
Federal Republic of Germany