Ultrastructure of Photoreceptor Cells in a Vitamin A-Deficient Moth (Manduca sexta)

In vertebrates the presence of vitamin A (retinal) in photoreceptor cell membranes is crucial to the structural and functional integrity of these cells. Retinal is reported to be the chromophore of the insect visual pigment molecule. This molecule is also presumed to be the major functional and structural component of the arthropod rhabdom, and it has been isolated from extracts which those workers believe principally contained rhabdomere fragments.

A recent light microscopic study on night blindness of the invertebrate, Manduca sexta, demonstrated that when vitamin A or its precursors were omitted from the larval diet of this nocturnal Sphingid moth, the photoreceptor cells underwent pathological changes which could be largely reversed with the addition of carotenoids in the larval diet. Recently, ultrastructure studies of retinulae from first generation moths reared on a β-carotene diet showed a partial reversal of the pathology noted in deficient moths.

In the present inquiry, photoreceptor cells from normal and deficient moths have been examined with the electron microscope. The purpose of this preliminary note is twofold: first, to report some unusual subcellular changes observed in the retinular cells of vitamin A-deficient moths and compare these to the fine structure of the photoreceptors in moths provided with a normal carotenoid intake; and secondly, to supply an initial interpretation on the apparent compensatory mechanisms which occur as these highly specialized cells respond to this avitaminosis.

**Method.** Larvae of *M. sexta* were continuously reared on tobacco plants or subsisted for over 20 generations on a vitamin A-deficient diet. The moths were dark phate-buffered glutaraldehyde (pH 7.4) and post-fixed in 1% buffered osmium tetroxide. Thin sections were stained with uranyl acetate in methanol and lead citrate and examined with a Zeiss EM 9 A electron microscope.

**Results.** In a plant-reared moth, the rhabdom of each retinula was clearly outlined and the rhabdometric microvilli were well aligned with conspicuous cell boundaries (Figure A). However, retinulae from vitamin A-deficient moths were found to differ considerably from the normal pattern but some variability in pathology was noted among the deficient specimens. Even at the very distal end of the retinula, there was a misalignment of rhabdomeric microvilli (Figure B). Islands of obliquely or cross-sectioned microvilli (generally oriented parallel to the long axis of the ommatidium) were conspicuous.

Retinular cell boundaries were distinct and large vacuoles existed in many cells. These vacuoles are considered part of the pathological syndrome and are not artifactual, as tissue from 'normal' (plant-reared) moths was not vacuolated using identical fixation procedures. In Fig-

ure C the usual type of microvilli (oriented perpendicularly to the long axis) have also proliferated and only a scant cytoplasm remained which contained numerous membrane-bounded narrow channels (suggestive of a proliferated Golgi complex). Mitochondria filled up the retinular cytoplasm. Multivesicular bodies were also numerous, which may indicate lysosomal activity. Cell boundaries were obscured and the original rosette of cells simulated a syncytial unit. A nipped response to light was evident in the microvilli of the retinula, one to several vacuoles formed and a meshwork of microvillar membranes were seen invading the vacuole (Figure C). This tenuous meshwork became extensive at more proximal levels. At a still more proximal level (Figure D) there was a change in the character of the cytoplasm which assumed a finely granular, homogeneous appearance, being considerably more electron dense. At this level the retinulae had a decreased cross-sectional area and the interommatidial matrix encroached on the retinular space. The microvilli were reduced in diameter and the contents of each microvillus became considerably more electron dense as compared to those at more distal levels. At the Figure D level there was a total lack of microvillar organization. In sections stained for acetylcholinesterase, areas of the retinulae showed elaborate swirls, scrolls and completely concentric bodies (Figure F). No two retinulae showed a similar pattern of disorganization at this level, although at higher magnifications one or several trunks of retinular tissue appeared to give rise to microvilli in most retinulae. Among the interstices of the surrounding tracheoles, the aforementioned granular retinular reticulum was observed with occasional cross sections of axon-like processes which were similar to cross-sectioned, small, unmyelinated axons. Numerous microtubules (170 Å diameter) with a pale halo (about 100 Å thick) and a central light area (75 Å diameter) were observed in interretinular cell cytoplasm (Figure E inset). Microtubules without halos were found in intertracheolar spaces.

At lower levels the microvilli exhibited less tight swirling patterns and the intervening matrix had a similar appearance to that found on the perimeter of the retinula. At levels just above the basal lamina where there were very few rhabdomeric microvilli, the central core of the retinula was observed to consist of an electron dense cytoplasm (Figure F). A less electron dense matrix aggregated into 4 or 5 discrete clumps which, together with the tracheoles, impinged in a medial direction upon the retinula so that the latter assumed a stellate shape in cross-section. The impinging matrix formed from extra-retinular elements when traced distally.

Discussion. Considerable information exists on the effects of deficiency, adequacy or abundance of vitamin A in vertebrate systems. Far less knowledge is available as to the role of vitamin A in invertebrates and particularly insects. It is believed that insects do not synthesize vitamin A, but rather accumulate carotenoids primarily through dietary intake. If vitamin A or carotenoids are not assimilated, vision can be impaired. Some visual dysfunction has been reported in house flies reared from the deficient diet exhibiting receptor potentials of normal amplitude and spectral sensitivity, although behaviorally there was little or no orientation to light. These incongruities await further study.

Hyperplasia of epithelial surfaces is one general physiological response of vertebrate tissue to thisavitaminosis, and this condition usually contributes to early death unless retinoic acid is added to the diet. Unlike vertebrates, this deficient moth exhibits normal growth, reproduction and longevity without the use of any vitamin A analogs. This immunity from the systemic and lethal symptoms of deficiency increases the suitability of this moth as an experimental animal for the study of visual impairment caused by dietary deficiency.

The only reported pathological response to lack of chromophore is that found in the retinular epithelium. By 'lack' we mean the prevention of the conjugation of vitamin A aldehyde with opsin which may come about in at least 3 ways, resulting in a similar retinal pathology. These are: (the aforementioned) dietary deficiency of vitamin A, genetic factors and excessive bleaching of the visual pigment.

Finally, the general membrane phenomena we have witnessed in deficient cases appear to be similar to other findings in which an excess of vitamin A was permitted to fibroblasts in tissue culture. This paradox will remain

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