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[1]. Retinal is reported to be the chromophore of the insect visual pigment molecule[2,3]. 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[4] 'believe' principally contained rhabdomere fragments.

A recent light microscopic study[5] 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[6]. 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[7]. The moths were dark adapted and the excised eyes were fixed in 2.5% phosphate-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 rhabdomeric 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[5,8] 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-

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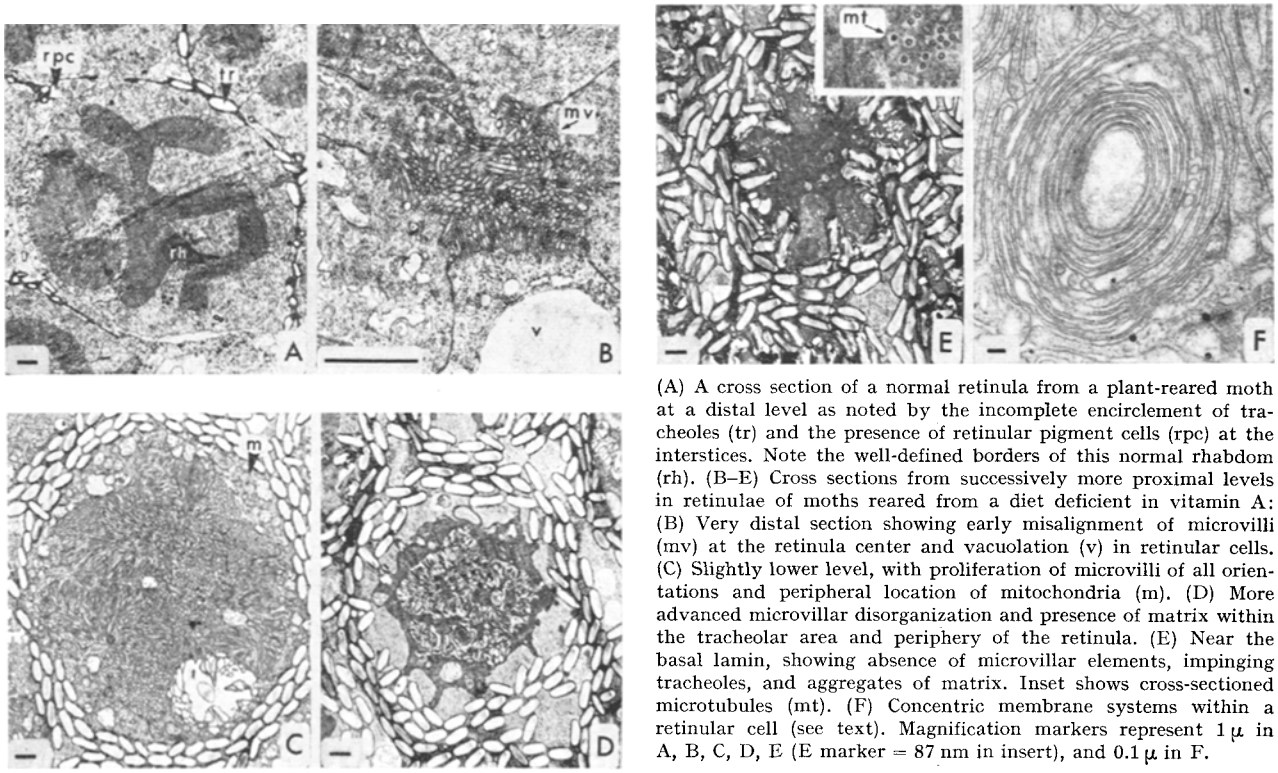

(A) A cross section of a normal retinula from a plant-reared moth at a distal level as noted by the incomplete encirclement of tracheoles (tr) and the presence of retinular pigment cells (rpc) at the interstices. Note the well-defined borders of this normal rhabdom (rh). (B–E) Cross sections from successively more proximal levels in retinulae of moths reared from a diet deficient in vitamin A: (B) Very distal section showing early misalignment of microvilli (mv) at the retinula center and vacuolation (v) in retinular cells. (C) Slightly lower level, with proliferation of microvilli of all orientations and peripheral location of mitochondria (m). (D) More advanced microvillar disorganization and presence of matrix within the tracheolar area and periphery of the retinula. (E) Near the basal lamin, showing absence of microvillar elements, impinging tracheoles, and aggregates of matrix. Inset shows cross-sectioned microtubules (mt). (F) Concentric membrane systems within a retinular cell (see text). Magnification markers represent 1 μ in A, B, C, D, E (E marker = 87 nm in insert), and 0.1 μ in F.

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. On the periphery 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. Patches of partly distended and vesiculated microvilli formed 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 matrix 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 E). 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 extraretinular elements when traced distally.

Discussion. Considerable information exists on the effects of deficiency, adequacy or abundance of vitamin A in vertebrate systems[9]. Far less knowledge is available as to the role of vitamin A in invertebrates and particularly insects[10]. It is believed[10,11] 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[12] in house flies reared on a vitamin A-deficient diet. If a functional difficulty can arise from this deficiency, one might also expect a structural correlate; and in fact a gross retinular pathology was found[5] in the vitamin A-deficient *M. sexta* and, as reported here, in the fine structure of the photoreceptor cells from such deficient moths.

As to our second objective of this study, to interpret these results, certain functional relationships are suggested in the light of recent literature. The extraordinary proliferation of microvilli in deficient moths appears to be produced at the expense of the unspecialized cytoplasm. The first impression is that this increase in microvilli is a compensatory mechanism, but there is no corresponding numerical increase in mitochondria from which extra energy could be derived for this increased surface area. Accentuating this 'inequity' has been the displacement and relocation of existing mitochondria (now found only on the periphery of the retinula) so that the normal continuity has been lost between the mitochondria and the open ends of the microvilli.

The many microtubules within the intertracheolar areas may relate to the increase in microvilli. The added trophic requirements of this situation may evoke a proliferation of microtubules if we assign either a transport or supportive function to these organelles. Such roles have been suggested[13] for these structures in other tissues. Large aggregates of halo-bearing microtubules have been observed after glutaraldehyde fixation of the Schwann cell sheath of shrimp[14].

It has been proposed[12] for flies that the absence of carotenoid lowers the probability that light quanta will be trapped. *M. sexta* moths reared from the deficient diet exhibited receptor potentials of normal amplitude in response to light stimulation[15], although behaviourally there was little or no orientation to light[5]. These incongruities await further study

Hyperplasia of epithelial surfaces is one general physiological response of vertebrate tissue to this avitaminosis[9], and this condition usually contributes to early death unless retinoic acid is added to the diet[16]. Unlike vertebrates, this deficient moth exhibits normal growth, reproduction and longevity without the use of any vitamin A analogs[5]. 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[17] and excessive bleaching of the visual pigment[18].

Finally, the general membrane phenomena we have witnessed in deficient cases appear to be similar to other findings[19] in which an *excess* of vitamin A was permitted to fibroblasts in tissue culture. This paradox will remain

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until a more complete understanding is gained of the instability of membrane constituents when vitamin A is deficient or in excess[20–22].

Zusammenfassung. Die Lepidoptere *Manduca sexta* wurde während mehr als 20 Generationen ohne Vitamin A aufgezogen. Feinstrukturelle Veränderungen traten auf: Die Photorezeptorzellen zeigten starke Zunahme und Desorientierung der Mikrovilli des Rhabdoms. Mitochondrien waren aus der Normallage nahe am Ursprung der Mikrovilli gegen die Peripherie der Retinulazelle verschoben. «Zwiebelkörper» (Sammlungen von konzentrisch angeordneten Mikrovillimembranen) und eine grosse Anzahl von Mikrotubuli wurden in den interretinulären Zellen gefunden. Mit Pflanzendiät aufgezogene Insekten hingegen zeigten keine der beschriebenen Ultrastrukturänderungen.

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Effect of Sodium Chloride on Female Mouse Peritoneal Fluid Cell Content

The detection of changes in cell population and cell morphology of peritoneal fluid has become a useful tool in determining the patho-physiologic status of the abdominal cavity[1–9]. Since abdominal fluid contains relatively large numbers of cells in a small amount of fluid, other workers have used saline to wash out the pelvic cavity in clinical and experimental studies[10–12]. FELIX and DALTON[13] observed an increase in macrophages in mouse peritoneal fluid following a saline i.p. injection. Mast cell counts were unchanged, but histamine release occurs rapidly in the presence of sodium chloride[14]. GARDNER[15] mentioned that the injection of any fluid into guinea-pigs increased peritoneal fluid polynuclears. In the present study, we determined the effect of an i.p. injection which contained 1% sodium chloride on the % distribution of cells in female mouse peritoneal fluid.

Method. We injected adult female CF-1 mice i.p. with 0.1 ml of 1% aqueous sodium chloride solution (Fisher Scientific). 1 h later, serous abdominal fluid was aspirated with a 27 gauge needle from the animal's ventral surface. We spread the aspirated specimen on an albumin-coated slide, stained it by PAPANICOLAOU's procedure[16], and 200 consecutive cells were randomly counted and grouped as mesothelial cells, lymphocytes, polymorphonuclear leucocytes, histiocytes, mast cells, bare nuclei and daisy cells. Bare nuclei are light to dark staining nuclei without cytoplasm. When the nuclei of a cell, most likely a degenerating mesothelial cell, bulged out in a pattern resembling a daisy, we called them daisy cells.

The significance of difference between individual cell counts of control and sodium chloride-treated mice was computed using the formula, S.E. $= \sqrt{\Sigma d^2/N(N-1)}$ and Student's t-test. We calculated the standard error for each mean cell count, the probability value (p), and by dividing the average cell count by 2, the % distribution of each individual mean cell count was obtained.

Results. The % distribution of cells listed in the Table indicates that an i.p. injection of 1% sodium chloride produced in 1 h a marked alteration in the cellular content of female mouse peritoneal fluid. Mesothelial cells, which line the peritoneal cavity and constitute the majority of cells in abdominal fluid, were significantly lowered by the sodium chloride treatment ($p < 0.05$). On the other hand, the % distribution of lymphocytes and polymorphonuclear leucocytes was significantly increased ($p < 0.02$; $p < 0.05$) reflecting a possible irritating effect of the salt solution. We recorded no significant change in the proportion of histiocytes, mast cells and bare nuclei in abdominal fluid, but daisy cells were seen only in cytologic specimens from sodium chloride-treated animals.

Conclusions. 1 h after an i.p. injection of 1% sodium chloride, we found relatively less mesothelial cells, but there were more lymphocytes and polymorphonuclear leucocytes in adult female mouse peritoneal fluid. Histiocyte, mast cell and bare nuclei proportions were relatively

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