impulse propagation cannot be ruled out.

In order to observe any HC-3–induced changes in membrane ionic conductance patterns, we used the sucrose-gap, voltage-clamp technique (*7*) (Table 1). As expected, when HC-3 was applied externally at a 10 m*M* concentration, there was very little effect on either the peak transient (sodium) or late steady-state (potassium) components of membrane conductance. The average value of the peak transient conductance following HC-3 is 99 percent of the control values with the late steady state being 88 percent. However, when applied internally, 10 m*M* HC-3 significantly reduced both the peak transient and late steady-state components. The greater effect was seen on the peak transient component which was reduced to an average value of only 28 percent of control as compared to 66 percent for the late steady-state component. Hemicholinium-3 was able to inhibit both conductance components at a lower internal concentration of 1 m*M*. Again, the effects were partially reversible with washing.

The action of HC-3 on squid axons can best be ascribed to an effect on the ionic conductance mechanisms of the nerve membrane, particularly the peak transient component rather than to a cholinergic mechanism.

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Vitamin A Deficiency: Effect on Mosquito Eye Ultrastructure

Abstract. *Mosquitoes* (Aedes aegypti) *were reared aseptically for one generation on an artificial diet containing neither vitamin A nor its usual precursor in animals, β-carotene. Function (electrical response to light) in the compound eyes of these animals was severely impaired. Ultrastructure of the photoreceptor cells was abnormal in two respects: multivesicular bodies were absent, and masses of smooth membrane lamellae were present near the nucleus. The organization of the photoreceptor organelle, the rhabdomere, was normal. The eyes of control mosquitoes, to whose diet β-carotene was added, were functionally and structurally normal. Multivesicular bodies were normally abundant and the perinuclear membrane masses were not present.*

In vitamin A deficiency, sensitivity of the vertebrate photoreceptor cell to light progressively decreases until blindness occurs and the photoreceptor organelle, the outer segment, degenerates (*1*). The biochemistry of vertebrate and insect vision is similar (*2*), and vitamin A deficiency results in decreased visual sensitivity in flies (*3*). A study with the light microscope revealed some morphological changes in the eye of a moth (*4*).

We have examined the ultrastructure of photoreceptor cells in the compound eyes of yellow fever mosquitoes (*Aedes aegypti* L.) with an induced vitamin A deficiency. Eye morphology (*5*) was compared in adult mosquitoes grown from hatching with or without a source of vitamin A in the larval diet. During the late larval period, the adult eye differentiates. The functional state of the photoreceptors was determined through measurement of electrical response to light (*6*). Three groups of mosquitoes were compared: (i) mosquitoes reared on the nonsterile, undefined diet (dry dog food crumbled in the water of the culture dishes) of the laboratory colony (group 1); (ii) mosquitoes reared aseptically on the minimum standard diet of Akov (*7*) with β-carotene added (0.077 mg/ml) as a vitamin A source (group 2); and (iii) mosquitoes reared aseptically on Akov's diet without a β-carotene supplement (group 3).

Electrical responses from the three groups were similar in wave form but differed in amplitude (Fig. 1). Mosquitoes reared on the defined diet without the β-carotene supplement (group 3) gave responses significantly smaller than those obtained from groups 1 and 2, whose eyes were functionally normal. The decrease of responsiveness in the vitamin A–deficient animals was probably due to a reduction in the amount of visual pigment present, for the wave form of the responses did not differ.

The fine structure of the normal mosquito compound eye is similar to that of other arthropod eyes (*8*). The ultrastructure of the receptor cells was identical in mosquitoes reared on dog food (group 1) and on the artificial diet with added β-carotene (group 2). By contrast, the eyes of those reared on the defined diet without β-carotene (group 3) were abnormal in the following respects. Multivesicular bodies (organelles characteristic of the photoreceptor cells of arthropods) were rarely found (compare Fig. 2, a and b), and masses of endomembrane were found at the proximal ends of the cells (Fig. 2, c and d). These masses, not normally present, were always found

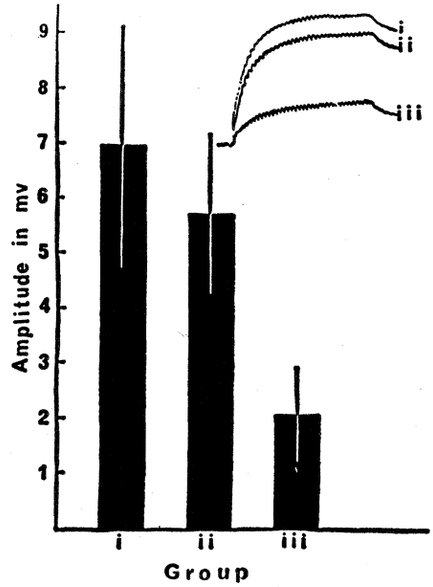

Fig. 1. Magnitude of the negative waves (taken just before cessation of the stimulus) of the electrical responses of mosquito eyes to light. Animals in groups 1 and 2 were reared on diets containing sources of vitamin A, those in group 3 on a diet lacking vitamin A. Each group contained 18 to 23 animals. The vertical lines represent the 95 percent confidence intervals. The inset shows typical electrical responses from each of the three groups.

in the mosquitoes reared on the deficient diet regardless of the conditions of illumination during development, but they were more extensive in adults illuminated continuously for 1 week after emergence (Fig. 2d). The organization of the rhabdomere (the photoreceptor organelle which corresponds to the vertebrate outer segment) does not appear to be affected by the vitamin A deficiency, but it may be reduced in size, especially after prolonged exposure to light.

Our results show that, when mosquitoes are reared with no source of vitamin A in the diet, the function of photoreceptor cells is impaired and their structure is abnormal. In vertebrates, vitamin A deficiency eventually results in complete disappearance of the electrical response and degeneration of the outer segment (*1*). In our experiments with the mosquito, the electrical response did not disappear entirely, nor did the rhabdomere show signs of breakdown. However, it is likely that some vitamin A was still present in the eyes of the mosquitoes reared on the deficient diet, for vitamin A or its precursors may be carried from one generation to the next in the insect egg (*3*). Carlson *et al.* (*4*) found some rhabdomere abnormalities in moths carried for more than one generation without a vitamin A source.

The abnormal membrane masses appear initially to be associated with the nucleus (Fig. 2c), resembling the "nebenkerne" found in various conditions of cell pathology (*9*). They are made up of closed cisternae, rather like arrays of rough endoplasmic reticulum lacking attached ribosomes. In more extreme cases—for example, when the cells have been exposed to light for some time (Fig. 2d)—the masses fill the distal ends of the cells. In the light-adapted eyes (lateral ocelli) of larval mosquitoes given no vitamin A source, the masses may take up one-half of the receptor cell volume (*10*).

The lack of multivesicular bodies in the eyes of deficient animals is particularly interesting because these organelles are also affected by light, being reduced in number during dark adaptation (*11*) or nearly absent in eyes deprived of light (*12*). Thus formation of multivesicular bodies appears to be closely tied to the special function of the cell, and indeed has been used as a measure of cell function (*13*). In other cell types these bodies perform exo- or endophagocytic functions (*14*). It has been suggested that, in the arthropod eye, they serve to remove metabolic by-

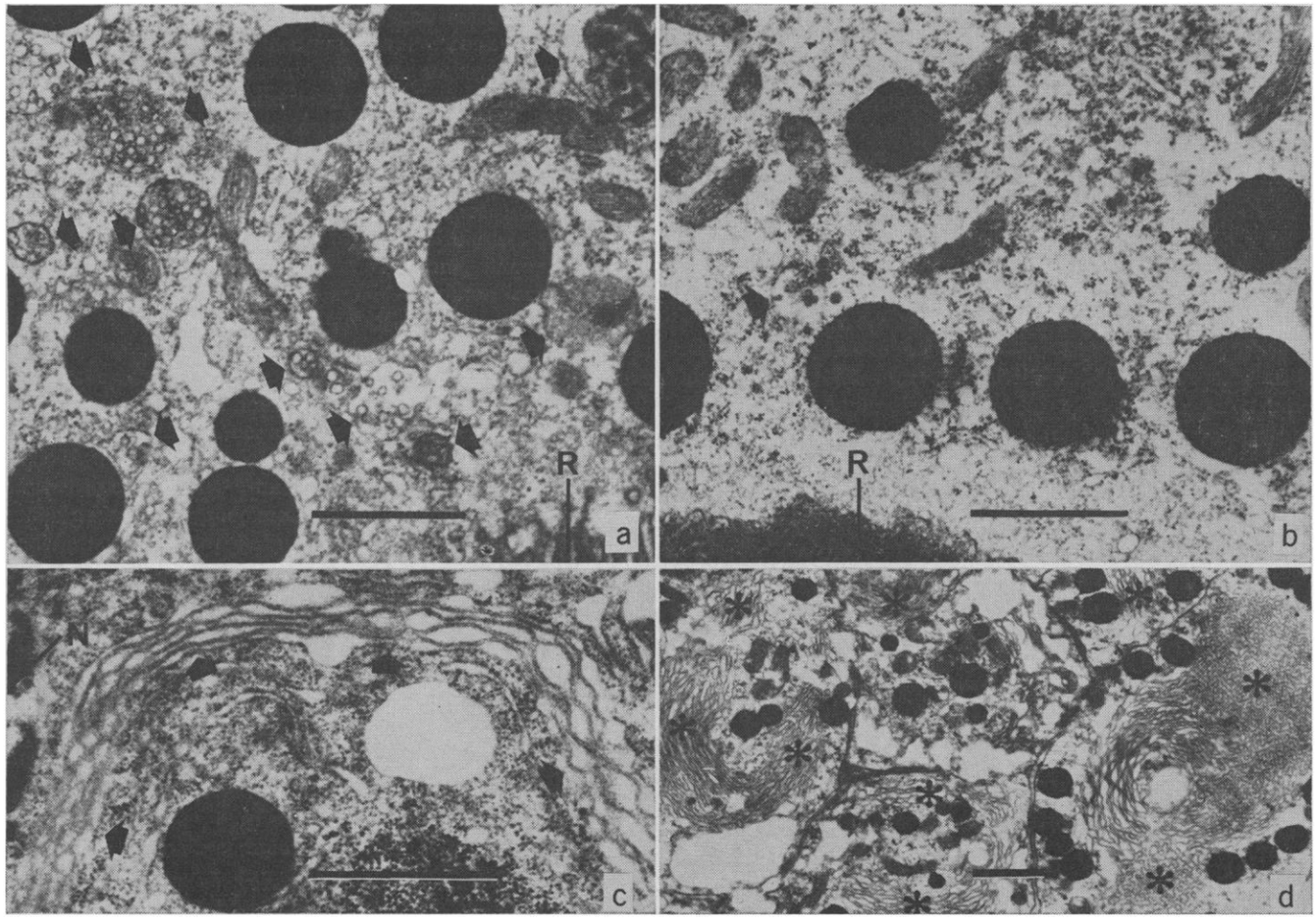

Fig. 2. Electron micrographs of regions in mosquito photoreceptor cells. (a) Region adjacent to the rhabdomere (*R*) from an animal in group 2 given β-carotene as a vitamin A source in the diet. The numerous multivesicular and related lamellar bodies are indicated by arrows (× 13,800). (b) Region corresponding to that shown in (a), but from an animal in group 3 reared without β-carotene in the diet. Multivesicular bodies are rare; only one (arrow) is present in the field (× 13,800). (c) Region near the nucleus (*N*) from an animal (fixed immediately after emergence) in group 3 showing the abnormal membrane lamellae (arrows) found in vitamin A–deficient animals (× 17,000). (d) Masses of membranes (asterisks) at the proximal ends of adjacent receptor cells from a vitamin A–deficient animal illuminated for 1 week after emergence (× 6100). The bar in each figure represents 1 μ.

products of visual function from the illuminated rhabdomere, to take up cast-off rhabdomere membrane, or to sequester hemolymph proteins taken into the receptor cell by pinocytotic vesicles associated with the rhabdomere (*11–13*). Our results show that the receptor cells can continue to function, though at a reduced level, with the system of multivesicular bodies eliminated. The regulation of diet now gives us a second means (in addition to varying illumination) of manipulating receptor cell ultrastructure.

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Oxygen Consumption and Pumping Rates in the Hard Clam Mercenaria mercenaria: A Direct Method

Abstract. *Respiratory and pumping rates in the hard clam* Mercenaria mercenaria *were measured directly in running seawater; the quantity of oxygen used was calculated from the difference in concentrations in incurrent and excurrent water. A linear relationship between pumping and respiratory rates suggests regulation of water transport by oxygen requirement rather than by feeding.*

Reports of respiration in lamellibranch mollusks emphasize the variability in measurements of ventilation rates, and the lack of control of ventilation rate by oxygen requirements because the gill is highly specialized for filter-feeding (*1, 2*). Feeding currents are thought to be greater than those required for respiration and "this flow is kept up for feeding purposes, and the respiratory function is incidental" (*2*).

Indirect measurements of water flow through bivalves, such as clearance rate of particles in suspension, are questionable because the animals may vary unpredictably the percentage of particle retention. Because measurements of oxygen uptake in flowing water systems are calculated on the basis of water flow through the bivalves, many of these are also questionable. Oxygen uptake has been measured in closed standing-water systems of low volume in which metabolites may accumulate (*3, 4*).

Our methods avoid these problems and are especially suited for the siphonate bivalves. The direct method of measurement of water pumpage (*5*) is combined with the polarographic method of measuring oxygen concentration in flowing water (*6*) (Fig. 1). *Mercenaria mercenaria* are kept in aquariums with bottoms covered by sand (15 to 20 cm deep), supplied with running seawater cooled or warmed as needed. Seawater, well aerated and dyed with green food coloring (1 ml/liter), is held in reservoirs at controlled temperature. The dye solution leaving the reservoir passes through a coil set in the aquarium for temperature adjustment, and through a flowmeter to a fine glass tube mounted within a wide tube which carries away the overflow from the aquarium as well as the excurrent stream from the clam, to a weir which maintains a constant level within the aquarium. An adjustable capillary tube is also mounted within the wide tube. The system of tubes is positioned with

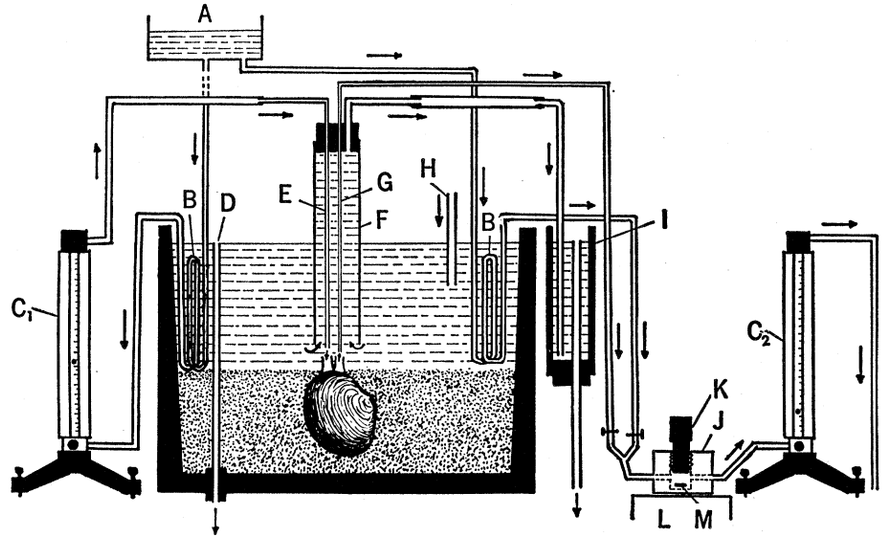

Fig. 1. Apparatus for measurement of pumping and respiration rates in *Mercenaria*. (*A*) Reservoir for dyed seawater, (*B*) coil for temperature adjustment, (C_1) flowmeter in incurrent water, (C_2) flowmeter for outflow from polarographic chamber, (*D*) overflow, (*E*) inflow of dyed seawater, (*F*) aquarium overflow, (*G*) capillary tube for sampling excurrent stream, (*H*) inflow of seawater to aquarium, (*I*) weir to maintain constant level in aquarium, (*J*) polarographic chamber, (*K*) Beckman oxygen electrode, (*L*) magnetic stirrer, and (*M*) stirring bar.