Table 2. Complementation tests between *su* mutants at 37°C.

Mutants crossed	Burst size
IIIa × IIIb	
*su*41 × *su*66	6.7
*su*43 × *su*44	8.7
*su*43 × *su*66	8.0
I × IIIa	
*su*52 × *su*43	88
I × IIIb	
*su*52 × *su*44	163
IV × IIIa	
*su*100 × *su*43	79
IV × IIIb	
*su*100 × *su*66	97

and in particular, that they do not seem to reduce the activity of IIIb. Polarity might actually exist between genes other than the IIIa-IIIb pair and yet not be observed in complementation tests if the products of these other genes are adequate for phage growth, even if present only in small quantities. However, it is clear from our results that polar mutations can noticeably affect IIIb, perhaps indicating that the viral coat protein which IIIb makes (*4*) is a major phage component required in large quantities. Therefore, the fact that *su* mutants in genes other than IIIa do not reduce the activity of IIIb in complementation tests suggests that none of the known genes precedes IIIa in a coordinate translation with IIIa, for otherwise the polarity model would suggest that such mutants should be defective in IIIb. The other genes then must either follow IIIa and IIIb in coordinate translation or else be translated independently of IIIa and IIIb. By the same arguments, we would expect to find that IIIa and IIIb are coordinately transcribed into the same species of mRNA, but it remains to be determined whether any or all other phage genes are included.

Because the mature phage DNA is single-stranded (*10*), it too has a polarity, which can also be determined in reference to the genetic map. It is known for other systems (*11*) that the direction of translation of mRNA corresponds to the direction from the 5′- to the 3′-end of a nucleotide. Since the mature phage DNA is homologous (*8*) to the mRNA and has the same polarity (*12*), the direction of translation must also correspond to the 5′→3′ direction of the DNA (Fig. 1).

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Vitamin A Deficiency: Effect on Retinal Structure of the Moth Manduca sexta

Abstract. *Sphingid moths* (Manduca sexta) *were reared for several generations on an artificial diet deficient in vitamin A and its precursors. Retinal tissue from depleted moths was removed for histological examination. There was extensive histolysis in the retinal epithelium and underlying nervous and connective tissues. This pathology correlated with severe visual impairment, even though normal growth, metamorphosis, and reproduction occurred. In the adult this pathology could be reversed when the larvae were reared only on tobacco (its usual host) or on the artificial diet supplemented with β-carotene or vitamin A palmitate.*

Inadequate intake of the fat-soluble factor termed vitamin A produces a purulent eye condition in rats (*1*), which is usually accompanied by temporary night blindness. A similar type of nyctalopia in humans can often be relieved by dietary intake of large amounts of vitamin A (*2*), the biochemical rationale for this therapy being that the prosthetic portion of rod visual pigment is vitamin A aldehyde (*3*).

The existence of vitamin A aldehyde (retinal) in insects was first reported in the heads of honeybees (*4*) and later in the Lepidoptera (*5*). The role of retinal as the chromophore of the visual pigment of various insect species and the absorption spectrum of this substance have been reviewed (*6*). Others (cited in *7*) have demonstrated that vitamin A is a factor in insect reproduction and metabolism of integumentary pigment. As in other animals, there are no substantiated claims of vitamin A biosynthesis, and this factor must be supplied by the diet. We have found a relationship between vitamin A deficiency and a subsequent pathological condition (or its reversal upon carotenoid intake) of the photoreceptor elements of the nocturnal tobacco hornworm moth *Manduca sexta*. For the first time in an invertebrate, optic pathology has been correlated with vitamin A deficiency.

Earlier electrophysiological studies (*8*) on the compound eye of *M. sexta* reared in the wild revealed a spectral sensitivity peak at 5000 Å; from this observation a rhodopsin system was postulated. Further behavioral evidence indicated that a vitamin A deficiency might effect the development of the retinal structure of the adult moth.

In an attempt to establish the role of vitamin A in the development of the retina and its associated elements in *M. sexta*, we reared larvae for several generations on an artificial diet (*9*) deficient in vitamin A. Other larvae were reared on tobacco leaves (*10*) or on an artificial diet containing 15 mg of β-carotene or 13.75 mg of vitamin A palmitate per 100 g (wet weight) of artificial diet. After this induction period, and 24 hours after adult emergence, the retinulae, postretinal axons, and adhering lamina ganglionaris were excised from the remainder of the optic lobe. These tissues were fixed immediately in a 10 percent solution of acrolein (buffered to *p*H 6.9) and were left in this fixative at 4°C for 2 hours. After fixation, the tissues were transferred to distilled water at 4°C for a period of 12 hours, and were then dehydrated, cleared, and embedded in 2-hydroxyethyl methacrylate

(*11*). Sections were cut at 2 μ, mounted, and stained with acid fuchsin and methylene blue.

Moths reared from larvae fed for many generations on the diet deficient in vitamin A were permitted to reproduce. Their progeny were reared on the basic artificial diet supplemented with either 25,000 U.S.P. units of β-carotene or vitamin A palmitate per 100 g (wet weight) of diet. The retinal histologies of the two groups were not appreciably different. The distal photoreceptor elements of those moths given β-carotene (Fig. 1A) show a generally complete enclosure of the seven or eight retinular cells forming an entire retinula. A large nucleus can be seen in each retinular cell, with an occasional nucleolus also present. The cross section of the ommatidium (Fig. 1B) demonstrates a somewhat cruciform rhabdom, but in slightly more proximal sections this becomes more serpentine and similar to that of the moths fed tobacco (Fig. 1C). Scattered throughout the cytoplasm of each retinular cell are small granular inclusions which we believe to be mitochondria. These are often not aligned on the rhabdom (microtubules) as they are in the moths fed tobacco (Fig. 1E). We noted no appreciable differences between basement membranes in moths reared on tobacco or on β-carotene.

In moths reared on tobacco a cluster of seven or eight retinula cells makes up the basal portion of the ommatidium, with a darker staining eccentrically situated retinular cell evident at the distal end of the aggregate. Although this cell resembles the eccentric cell of *Limulus*, we have, at present, no information on whether this particular cell is a functional bipolar neuron as it is in *Limulus* (*12*). Since this cell has a rhabdomere, it may be akin to the basal retinula cell observed in the commercial silkworm moth (*13*). Occasionally two of these cell types are seen in one retinula. The rhabdom pattern when observed in cross section (Fig. 1E) is somewhat more labyrinthine than that found for *Erebus* (*14*). In *M. sexta* two or more ranks of tracheoles surround each ommatidium. In Fig. 1F, the fenestrate basement membrane is taut, and the emerging postretinal axons project directly to the lamina.

As seen with the light microscope, the pathology of retinal elements from moths reared on a diet deficient in vitamin A is strikingly reminiscent of that described for similarly deprived rats (*15*). In rats, the rod disks appeared broken into vesicles and tubules; many rod outer segments apparently were

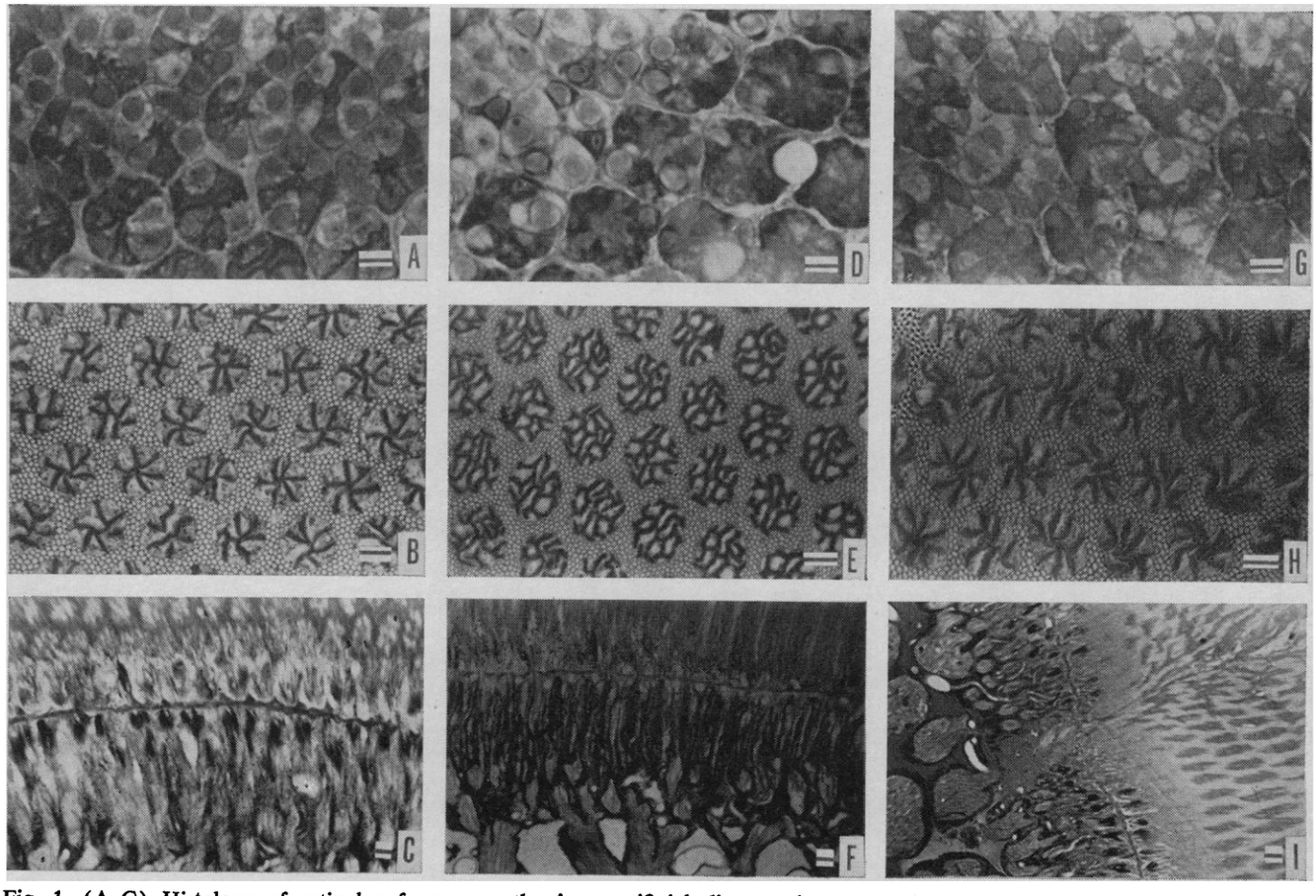

Fig. 1. (A–C) Histology of retinulae from a moth given artificial diet supplemented with β-carotene: (A) Peripheral portion of retinulae. (B) More distal cross section of retinulae than (E). (C) Basement membrane with proximal retinulae above and postretinal axons aggregating into fascicles below. (D–F) Retinular cells from *Manduca sexta* reared on tobacco: (D) Cross section at distal end of retinulae. Note darkly stained eccentrically situated retinular cell in eight-celled retinula. Large vacuole on right side is an artifact caused by the embedding medium. (E) Transection at midpoint of retinula shaft. (F) Longitudinal section showing straight-coursing postretinal axons and complete basement membrane. (G–I) Retinulae of a moth deficient in carotenoids: (G) Cross section at retinula tips showing incomplete retinular-cell membranes and apparent vacuolated retinular cells. (H) Cross section at approximate midpoint of retinulae depicting hypertrophy of rhabdom. (I) Incomplete basement membrane with herniation of retinular and tracheolar tissue into the subretinular space, where convoluted axonal bundles are also seen. Magnification marker represents 10 μ.

missing and others had a "gnawed" (*15*) appearance. The external limiting membrane had nearly disappeared, and only traces remained. Recent electron-microscopic studies (*16*) have summarized the ultrastructural pathology of the rods in rats deficient in vitamin A.

In moths deficient in vitamin A, segments of the outer retinula border were missing (Fig. 1G), presenting a "gnawed" appearance. Certain retinular cells had a retrograde change with a loss or resorption of acidophilic material. The cell contours may have been maintained, but many nuclei appeared suspended by trabeculae. Remaining cytoplasm was granular, and the total appearance seemed similar to the parenchymatous degeneration of vertebrate tissue (*17*). The rhabdom in cross section (Fig. 1H) was diffuse, swollen and more lightly stained. Its overall semblance was more flexuous than that of rhabdomes from rats given two treatments. The basement membrane had disappeared in certain areas, allowing both tracheolar and retinular tissue to herniate into the subretinular area (Fig. 1I). The presence of convoluted and flaccid postretinal axonal fasciculae suggested that degeneration of the nervous tissue had also occurred. This observation tends to be substantiated on a functional basis by records of meager postsynaptic response and by the absence of a retinal action potential in the electroretinograms of these moths (*8*).

A deficiency of vitamin A does not appear to inhibit growth or limit the vitality of these moths. Such deficient moths do, however, show little if any orientation to light and usually do not become successfully adapted to the dark. The ommatidial disks in deficient moths are milky-white in color, while those of animals reared on tobacco are golden. This tuft of retinulae in the moths given diets supplemented with β-carotene or vitamin A is white or very pale yellow. When these latter moths become adapted to the dark a white glow is noted when the cornea is reilluminated, while an orange glow is noted in moths fed tobacco. Dark-adaptation curves reveal that moths given either carotenoid have a higher sensitivity to light than moths reared on tobacco do (*8*).

Apart from our brief remarks on the abnormality of electroretinograms of deficient moths, other studies (*18*) indicate that houseflies can be reared without dietary intake of vitamin A and that they suffer a partial loss of photosensitivity (over a 4-log-unit increase in visual threshold). It has been suggested that a lack in dietary carotenoid can be offset to a degree by storage of the provitamin in the egg or by the presence of microorganisms that produce vitamin A, or by both (*18*). Our results suggest, however, that *Manduca sexta* does not have these sources and that carotenoid intake during larval life is crucial for normal vision ("normal" in this sense referring to night vision) in the adult moth.

Because metaplasias that cause squamatization and keratinization of epithelium (*17*) arise in vertebrates with severe vitamin A deficiency, such animals serve as relatively poor experimental subjects for studies of nutritional night blindness. This problem in rats has recently been overcome (*19*) through use of vitamin A acid (in this case the only metaplasia was that associated with the retinal epithelium). In the moth, however, no such analogs are required, and thus this species might serve well as a laboratory animal in the study of nutritional night blindness as well as other dystrophic diseases of the eye.

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Reagentless Substrate Analysis with Immobilized Enzymes

Abstract. *By coupling an immobilized enzyme system with an electrochemical sensor, the reagent requirement for this glucose method is eliminated. Miniaturization and a further simplification of the instrumentation for the continuous analysis of glucose is achieved.*

Techniques employing immobilized enzyme material for chemical analysis have been reported (*1*, *2*). These techniques eliminate the need for soluble enzyme reagents in analyses based on enzyme-catalyzed reactions. Furthermore, when a soluble enzyme is the only reagent required, a reagentless system of analysis can be achieved. Such a system is demonstrated for the continuous determination of glucose with glucose oxidase.

$$O_2 + \text{glucose} \xrightleftharpoons{\text{G.O.}} H_2O_2 + \text{gluconic acid}$$

In the above reaction, glucose oxidase (G.O.) catalyzes the oxidation of glucose to gluconic acid and hydrogen peroxide. In this method of glucose determination oxygen uptake is taken as a measure of the reaction. Since the oxygen is always contained in the samples, and glucose is the sought-for constituent, the glucose oxidase is the only reagent which must be added to the system for analysis. By immobilizing this enzyme requirement and using an electrochemical method for measuring oxygen tension (*3*), a "reagentless" determination of glucose is achieved.

The preparation and characterization of the immobilized enzyme material used in these studies has been reported (*2*). In the enzyme immobilization procedure, the enzyme is entrapped in a gel matrix by photocatalytic polymerization of acrylamide and *N,N*-methylenebisacrylamide. The copolymeriza-