tion, or whether vitamin D in addition to participating in such an induction process can also function as an obligatory catalyst in the induced system. However, the promotion of calcium absorption by vitamin D probably requires an unimpaired RNA-synthesizing system.

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Red-Light Thresholds in Heterozygote Carriers of Protanopia: Genetic Implications

Abstract. *Absolute thresholds in response to red light were compared in nine normal subjects, six female carriers of protanopia (heterozygotes), and six male subjects with protanopia. The fovea and four peripheral retinal areas were tested, and all data were obtained before the occurrence of the rod–cone break. Elevated thresholds were found in all retinal areas tested in protanopic males, at the fovea in all carriers, and in some peripheral retinal areas in two carriers. The thresholds for carriers were far below those for the protanopic males, and no greater variability of threshold was found in the carriers when they were compared with the normal control group. The findings do not substantiate the occurrence of inactivation at the locus on the X chromosome for protanopia.*

Protanopia and protanomaly are defects of color vision characterized by the partial or complete absence of the ability to discriminate hues in the red-yellow-green region of the spectrum and by a decrease in sensitivity to brightness at the red end of the spectrum. The protanomalous subject can match all colors of the spectrum with mixtures of three hues but requires more red in each mixture than the normal subject. The protanope, on the other hand, can match all colors of the spectrum with only two hues. The genes for these defects are carried at the same locus on the X chromosome. Males, therefore, always show a color-vision defect, whereas females with only one defective gene (heterozygotes) are considered to be carriers.

In a previous study in which we used five males with protanopia and protanomaly and eight female carriers of these defects, we determined absolute thresholds in response to red light at numerous retinal areas in order to test the X-chromosomal inactivation hypothesis of sex-linked inheritance (*1*). Elevated threshold responses to red light of the same magnitude were noted in four of the five color-defective males and in seven of the eight carriers, for a one-degree target located five degrees from the fovea. There was no evidence of greater variability of thresholds in female carriers than in a control group with normal vision.

Since fully dark-adapted eyes were tested in the previous study, the rods probably functioned as red-light detectors at retinal areas tested outside of the macula (*2*). In the study described here, the eyes were adapted to such a level of cone activity that the participation of rods in the detection of red light in peripheral retinal areas was eliminated. Absolute thresholds to red light were compared in normal subjects, female carriers of protanopia defects (heterozygotes), and males with protanopia.

Six protanopes, previously identified with the Nagel anomaloscope, and six female carriers of the defect were tested. All carriers were either mothers or daughters of males known to have protanopia. Carriers Ia and Ib were related to protanope I, carriers IIa and IIb to protanope II, and carrier III to protanope III. Carrier IV was related to a subject with protanopia who was tested in a previous study (*1*). All subjects were normal on routine ocular examination. A control group of nine subjects with normal vision was used.

The method of testing eliminated or minimized the participation of rods in the detection of red light. At the fovea, threshold determinations reflect mainly cone activity, and a monophasic curve (*3*) is obtained if a target of sufficiently small size is used (0.25 degree in this study). However, outside of the fovea, threshold measurements reflect both cone and rod activity and a biphasic curve is obtained. The first portion of the curve reflects cone activity and the second portion rod activity. At the onset of dark adaptation a rapid drop in threshold occurs, and in a few minutes a constant value called the cone plateau is reached. A further drop in threshold occurs after 5 minutes or more (depending on how much light the subject is exposed to before the test begins) and this point is called the rod–cone break. The change in the threshold after the rod–cone break reflects rod activity. We attempted to obtain all our data before the occurrence of the rod–cone break.

Studies of dark adaptation were performed after a period of careful in-

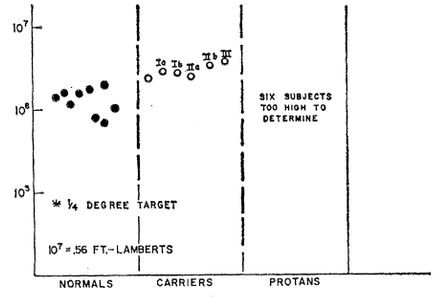

Fig. 1. Foveal thresholds of the three groups, a one-fourth degree target being used. Thresholds could not be obtained in any of the six protanopes. The six carriers have thresholds above the highest threshold in the control group. The five carriers with tested male relatives are identified.

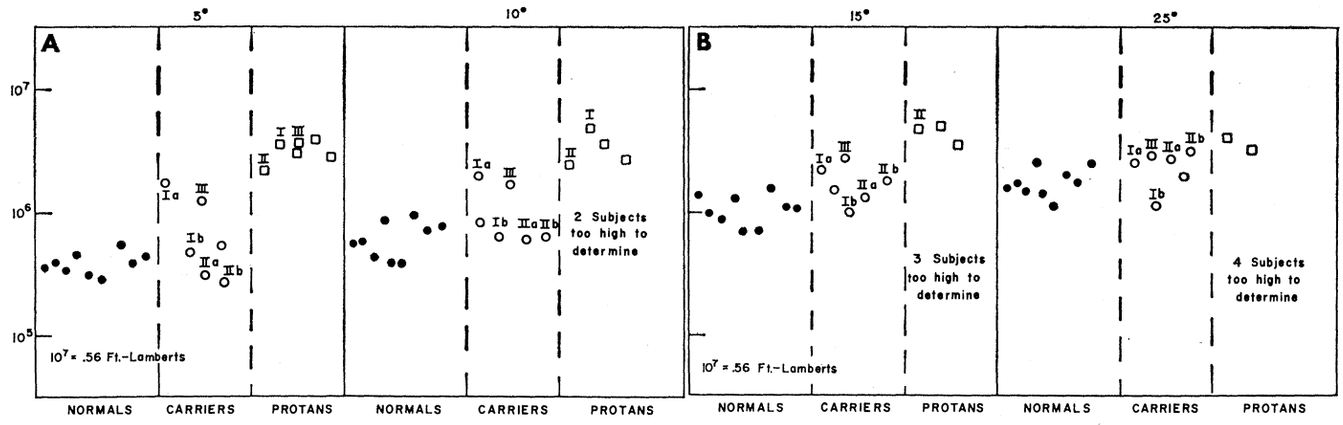

Fig. 2. Peripheral thresholds of all three groups at 5 and 10 degrees (*A*) and 15 and 25 degrees (*B*) with a 1-degree target. The plotted threshold for each area is the mean of the three thresholds obtained in the superior vertical and two horizontal quadrants. Two carriers (*Ia* and *IIIa*) are higher than the control group at 5, 10, and 15 degrees but are not as high as their male relatives (*I* and *III*) at any area. The thresholds from protanopes are elevated at all peripheral areas and are frequently unobtainable. All carriers and protanopes with relatives used in this study are identified.

struction and an initial trial. The tested eye was dilated to 7 to 8 mm with 1.0 percent tropicamide. Each subject was initially adapted to a diffuse white light of 2400-lux illumination for 7 minutes. The method used to obtain an average absolute threshold was described previously (*1*). The fovea was first tested with a target of white light, 0.5 degree in size, until a constant absolute threshold was obtained. The threshold was then measured with a red target 0.25 degree in size. Seven minutes of light-adaptation, as before, was repeated. The threshold response was then determined to a white-light stimulus of 1 degree, at a retinal area 15 degrees directly superior to the fovea. After a cone plateau was reached, threshold responses to 1-degree red-light stimuli were determined at 5, 10, 15, and 25 degrees directly superior to the fovea in the vertical meridian. The red filter was a Kodak-Wratten No. 92 with a dominant wavelength of 646 mμ. The threshold responses to white light at the initial extra-foveal area tested (15 degrees superior to the fovea) was then retested. Usually, no change in the threshold response to white light was noted (the rod–cone break had not yet occurred); however, if a lower threshold was obtained, the initial adaptation to diffuse white light was repeated and thresholds to red light were redetermined in the same areas. These four peripheral retinal areas were then tested in the horizontal meridian both nasally (the direction from the fovea towards the nose) and temporally (the direction from the fovea away from the nose). Again, 7 minutes of initial light-adaptation preceded threshold determinations in each quadrant.

No threshold responses to red light were obtained at the fovea in the six protanopes (Fig. 1). The maximum light intensity with the apparatus used was insufficient to elicit a response. All six carriers were able to see the test light at the fovea, but their thresholds were all above the greatest value obtained in the control group.

In five normal controls, two carriers, and two protanopes, thresholds were not obtained at 15 degrees nasally because that area was probably in the "blind spot" (optic papilla) of the eye in those individuals. All peripheral thresholds in the six subjects with protanopia were higher than the values obtained with normal subjects (Fig. 2). Thresholds were unobtainable in four subjects with protanopia at 25 degrees, in three at 15 degrees, and in one at 10 degrees. Thresholds were definitely elevated in two carriers in at least three peripheral areas. The thresholds for both of these carriers were much below those for their male relatives (which, in fact, were too high to measure at most peripheral areas). A statistical evaluation was made of the variability of threshold in the three retinal quadrants, the carriers being compared with the normal group at 5, 10, 15, and 25 degrees. The variance was greater in carriers only at 10 degrees, a variance ratio of 1.59 being found. When a table of *F* (variance-ratio values) distribution (*4*) was used, this value was not significant even at the .10 level of probability.

In the light of recent findings it is probable that green-sensitive cones function as red-light detectors in protanopes (*5*). If the protanopia locus of the X chromosome underwent inactivation, then both red- and green-sensitive cones should function as red-light detectors in heterozygotes. Dominance of either cone as a red-light detector in the carrier should vary from area to area (if mosaicism exists) and one would anticipate: (i) some areas with normal thresholds (areas with a normal concentration of red-sensitive cones); (ii) areas with thresholds as high as those found in protanopes (areas in which only green-sensitive cones remain); and (iii) areas with an intermediate abnormality of varying degree (areas where the light target used overlapped adjacent areas with normal number of red-sensitive cones and areas without red-sensitive cones). Most important of all, a great variation in threshold should be noted in the heterozygote when testing different areas, since different retinal patches are presumably sampled.

The findings of this study do not lend support to the idea that the locus for protanopia is inactive. Not only were all the thresholds for carriers far below those of the protanopes but also no greater variability of threshold was found in the carriers when they were compared to a control group.

These findings may be explained in two ways. First of all, it is possible that the locus for protanopia is not involved in the inactivation process. If this were the case, both the normal

and the abnormal genes would be active in each cell. Some type of intermediate threshold might be anticipated, and this is what was found. Although inactivation of the locus controlling glucose-6-phosphate dehydrogenase (G-6-PD) formation undoubtedly occurs (*6*), it is not at all certain that the entire human X chromosome undergoes a functional inactivation. It has been suggested that in the mouse it does not (*7*).

On the other hand, it is also possible that inactivation of the protanopic locus occurs, but that the patches of inactivation are so small that a 1-degree target covers such a large area that it gives rise to intermediate threshold values. It was originally suggested (*8*), on the basis of the very early appearance of the chromatin body (*9*), that the number of cells present at the time of inactivation was quite small. However, recent evidence based on electrophoretic variants of G-6-PD indicates that the stage of development at which inactivation takes place may be a much later one (*10*).

Thus, the present studies exclude inactivation of the protan locus of the X chromosome if the inactivated patches are relatively large. However, only techniques which could detect the activity of this gene in small areas containing only a few cone receptors (perhaps even one) would be sufficient to exclude inactivation of the protan locus if the patches are minute.

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Ribosomal-RNA Synthesis in the Absence of Ribosome Synthesis in Germinating Cotton Seeds

Abstract. *Cotton embryos after 72 hours of germination synthesize ribosomal RNA that does not become incorporated into ribosomes and that is very stable. This RNA has many characteristics similar to those of newly synthesized ribosomal RNA of bacteria.*

During the initial phase of their germination cotton seeds synthesize ribosomes as demonstrated by their ability to incorporate ^{32}P-labeled phosphate into ribosomal RNA (rRNA) and ribosomes (*1*). The rRNA synthesized at this period does not contribute significantly to the rRNA present in the mature seed and protein synthesis during this initial phase of germination is apparently catalyzed by ribosomes and stable messenger RNA present in the mature seed (*1*).

We now report that during cotton-seed germination the synthesis of ribosomes ceases at later stages of germination, but that a low level synthesis of rRNA persists. This rRNA is extremely stable but does not become incorporated into ribosomes possibly because of the absence of ribosomal protein synthesis at this point in germination.

In order to follow RNA synthesis during germination, cotton seeds were germinated for varying lengths of time at 30°C in sterile soil and harvested; and the cotyledons were separated from the hypocotyl. The cotyledons were then infiltrated in a vacuum desiccator for 30 minutes with a solution of carrier-free $K_2H^{32}PO_4$ (100 μc/ml); they were then washed thoroughly and placed on moist filter paper for further incubation. All of these operations were carried out in the dark except as otherwise stated.

The preparation of ribosomes and polyribosomes, and of ribosomal RNA, has been described, as have the methods for obtaining the sucrose density-gradient profiles of ultraviolet absorbancy and radioactivity (*1*). In addition, rRNA from the pellet resulting from centrifuging the crude homogenate at 25,000*g* for 20 minutes was purified and characterized with regard to ultraviolet absorbancy and radioactivity on a sucrose density gradient (5 to 20 percent). This pellet contains mitochondria, proplastids, and cellular debris in addition to nuclei, but the extracted RNA is referred to as nuclear RNA. In order to extract rRNA instead of DNA and DNA-like RNA, this pellet was resuspended in 0.1*M* acetate buffer, *p*H 6.0, 0.1*M* NaCl, and bentonite (1 mg/ml); to this suspension was added 1 volume of redistilled phenol saturated with the above buffer solution. After being shaken in the cold the phases were separated by centrifugation, and the aqueous layer was diluted with two volumes of ethanol; the precipitated RNA was collected and suspended in 0.01*M* tris-succinate buffer, *p*H 7.5, 0.001*M* EDTA (ethylenediaminetetraacetate), and bentonite, and reprecipitated by modifying the solution to 1 percent cetyltrimethylammonium bromide (*2*). This RNA was reconverted to the soluble sodium salt and sedimented on a 5 to 20 percent sucrose density gradient containing 0.01*M* tris-succinate, *p*H 7.5, 0.001*M* NaCl, and 0.001*M* EDTA.

Cotton embryos during the first 16 hours of germination incorporate a considerable amount of isotope into ribosomes and polyribosomes (Fig. 1*a*). When these ribosomes and polyribosomes are treated with sodium dodecyl sulfate (SDS) to dissociate rRNA from ribosomal protein components (*3*) and fractionated on a sucrose density gradient, the radioactivity peaks coincide with the absorbancy peaks (Fig. 1*b*); this indicates that the radioactivity incorporated into ribosomes is incorporated into both heavy and light rRNA. When embryos which have germinated in the dark for 72 hours are infiltrated with isotope and then incubated for 3 hours more, the density-gradient profile of isotope incorporation into the ribosomes is markedly different. Although the absorbancy profile is similar to that obtained from ribosomes from 16-hour germinated embryos, virtually no incorporation of isotope into ribosomes has taken place during the 3-hour incubation after isotope infiltration (Fig. 2*a*). Rather, the only substantial incorporation is into material sedimenting more slowly than the 80*S* monomeric ribosome and is not associated with any pronounced absorbancy peak.