Table I. Calcium content of ROS disc membranes (nanomoles per milligram of protein)

<table>
<thead>
<tr>
<th>Medium</th>
<th>Frog</th>
<th>Cattle</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Dark</td>
<td>Light</td>
</tr>
<tr>
<td>0.5 mM Ca++</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>2 μM Ca++</td>
<td>15 ± 1</td>
<td>26 ± 1</td>
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<tr>
<td>1 mM EGTA (&lt; 10⁻⁹ M Ca++)</td>
<td>4 ± 0.3</td>
<td>2.6 ± 0.4</td>
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*Single measurement.

The high concentration implied by these considerations suggests that ROS Ca++ must be membrane bound just as in sarcoplasmic reticulum. It is of interest that in the absence of EGTA, ROS Ca++ is always higher in the illuminated than in the dark-adapted preparations. Although at first surprising, this result is not inconsistent with the Ca++ coupling hypothesis. As in muscle sarcoplasmic reticulum, once Ca++ release has been triggered, a Ca++ uptake mechanism is necessary to restore dark adaptation in ROS or relaxation in muscle. Because of the long time delay between light exposure, and separation of the ROS membranes from the medium, it was not possible to demonstrate light-induced Ca++ release in the presence of this uptake mechanism even in the 2 μM Ca++ medium. One millimolar of EGTA was, therefore, added both to complex the 2 MM Ca++ in the medium and to trap any additional Ca++ released to the medium upon illumination. (EGTA is a quadrivalent anion assumed not to penetrate membranes.) EGTA appears to have reduced both dark and light Ca++ content, suggesting Ca++ leakage or exchange from the ROS membrane vesicles. More importantly, illumination is now seen to cause a measurable loss of Ca++ from the membranes. The light-dark Ca++ difference for release in EGTA is only 0.2 Ca++/rhodopsin but the true value may be at least 4- to 5-fold higher (e.g., 1 Ca++/rhodopsin), i.e., the factor by which the Ca++ content of the pellet in 2 μM Ca++ medium exceeds that in EGTA medium.

It is interesting to note that Ca++ determination on supernates in these experiments did not behave linearly until four-fold dilution in SrCl₂. Calcium in the form of microcrystalline calcium phosphate is known to produce such an effect. Since our media contained no phosphate, however, such a complex could only have come from the rod discs themselves.

The preliminary results presented above are sufficiently consistent with the Ca++ coupling hypothesis of visual transduction to merit more thorough investigation of a Ca++ pumping mechanism (ATP dependence, ATPase activity, and ion specificity), proportionality of Ca++ release with fraction of visual pigment bleached, Ca++ leakage rate, and dependence of release mechanism on other ions. Such studies are now in progress.

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Key words: transduction, calcium, membrane, discs, rods.

REFERENCES
Fig. 1. Asteroid bodies present in the gross specimen (×10).

Fig. 2. Scanning electron micrograph of an asteroid body (×2,000).
Brilliant, reflective particles floating in an apparently normal vitreous were described at least one and a half centuries ago. However, this early report describes the particles as "gold dust which settles to the bottom of the eye," a description which more nearly corresponds to the condition we term synchysis scintillans. In 1894, Benson described a different sort of vitreal particles, "The whole vitreous was studded with small, smooth, fixed spheres of a light cream colour. They varied in size about as much as the stars on a frosty night appear to vary in the clear sky." From this resemblance to stars, he originated the name "asteroid hyalitis," which is a misnomer since, in most cases, there are no signs of inflammation as implied by the suffix "-itis." A host of other designations have, therefore, arisen, but probably the term "vitreal asteroid bodies" is the clearest and simplest. The condition is usually monocular, causes no impairment in vision, and has not been associated consistently with any systemic or ocular abnormality.

In 1921, Verhoeff showed that these bodies contain calcium, as well as a material which stains positively with certain "lipid" stains. The "lipid" is insoluble in the standard lipid solvents, and, thus, Verhoeff suggested that this "bound" material might be a soap. However, negative staining with copper acetate, a stain designed for soaps, contradicts the soap-theory. In 1961, Rodman, Johnson, and Zimmerman used polarized light to demonstrate that each asteroid body contains many small crystals embedded in an amorphous matrix. The purpose of the present investigation is to extend the investigation of asteroid bodies using the newer techniques of electron radiation.

**Methods.** The eye of a 79-year-old man was enucleated because of a malignant melanoma and placed in 15 per cent formaldehyde. When the eye was opened, the vitreous was filled with asteroid bodies, which appeared typical with polarized light, oil red O, and alcian blue stains.

**Scanning electron microscopy.** Vitreous was placed on a pure silica plate, lyophilized, and coated with 200 A-thick aluminum. The specimen was viewed in the Cambridge 2A scanning electron microscope.

**Transmission electron microscopy.** Vitreous was placed in chilled 1 per cent osmium tetroxide and dehydrated through a series of solutions of ethanol of increasing concentration. The specimen was placed in propylene oxide and araldite and centrifuged at 1,000 g for two hours. Araldite-embedded sections were cut with a glass knife and viewed in the Siemens 1 electron microscope.
**Fig. 4. Transmission electron micrograph (×50,000).**

**X-ray spectroscopy.** One asteroid body on the scanning specimen (Fig. 2) was then localized under the beam of the Cambridge 5 electron probe x-ray microanalyzer. The subsequent x-rays were dispersed through a mica crystal. The intensity of x-radiation was automatically plotted as the crystal rotated.

**Results.** Fig. 1 shows the asteroid bodies in the vitreous.

Fig. 2 shows an asteroid body under the scanning electron microscope. The "furry" appearance is produced by a network of collagen fibrils adhering to the body. A number of smaller satellite asteroids can be seen on, and partially submerged in, the surface. Fig. 3 shows the satellite asteroids in more detail.

Fig. 4 shows a thin-section of a satellite asteroid under the electron microscope. The body appears to be composed of a number of round particles of equal size and in symmetrical arrangement, growing more dense toward the center of the body. The particles seem to be embedded in a matrix which merges with the surrounding vitreous and appears to be of the same substance.

Fig. 5 is part of the x-ray spectrum for the asteroid body shown in Fig. 2. Since the intensity edges are unique for electron energy levels in the outer shell of an element, they are unique for the element. Well-defined edges are noted corresponding to the energy levels of the electrons in the K-shell of calcium, sulfur, and phosphorus.

**Discussion.** The transmission electron microscope (Fig. 4) reveals round electron-opaque particles of diameter 1,000 Å in a matrix which stains poorly with osmium tetroxide, a "lipid" stain. Fixation in unbuffered formaldehyde apparently was satisfactory in this case, probably because of the stability of asteroids in almost all solvents.

Our data agree with the conclusion drawn one-half century ago that asteroid bodies contain calcium. X-ray spectroscopy (Fig. 5) demonstrates that sulfur and phosphorus are also present.

We hypothesize that asteroid bodies are simply a physicochemical phenomenon or shift of part of the normal vitreous from the liquid to solid phase. Once a "seed" formed in a supersaturated vitreous, crystallization would occur rapidly. Satellite asteroids may form initially and conglomerate in the solid phase to form the bodies seen on bi-
microscopy. The basic process which might lead to this hypothetical supersaturated solution remains unknown.

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Key words: asteroid, vitreous, vitreal opacity, electron probe, scanning electron microscope, ultrastructure, calcium, sulfate, collagen fibrils, mucopolysaccharide.

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REFERENCES