ON A CRYSTAL-CONTAINING BODY IN CELLS OF THE OAT COLEOPTILE

ROBERT M. THORNTON and KENNETH V. THIMANN. From the Department of Biology, Harvard University, Cambridge

In the course of an experimental study of phototropism in seedlings (1, 2) attention has been directed to the plastids as possible participants in the light-detection process. This led to a microscopic study of the cells of the oat coleoptile and thence to the discovery of the body which is the subject of this note.

The materials were chosen for their phototropic sensitivity. *Avena sativa* (oats) var. *segerhavre* (Victory) coleoptiles, grown in the dark after being given 5 hours' red light from the initial soaking, were used when 25 to 35 mm long. *Hordeum vulgare* (barley) and *Zea mays* (corn) coleoptiles, also grown in the dark, were used at the same length; the corn was given 3 hours' exposure to red light at the start. *Phycomyces blakesleeanus* was grown as described by Gruen (3) and harvested in stage 4.

Subepidermal cells of the *Avena* coleoptile, especially at and near the apex (0.1 to 0.5 mm from the tip), contain a number of small bodies, a little less than 1 μ in diameter. Each body consists of a single (probably 3-layered) membrane (Figs. 2 to 5) enclosing a matrix denser than the ground cytoplasm of the cell. In this matrix is embedded a single, usually rhomboidal, crystal; the outline of the crystal is sometimes irregularly polyhedral, occasionally rectangular or even hexagonal. Since the lattice lines sometimes appear to cross at right angles, it is not excluded that the crystal packing may be cubic. Usually the crystal is free from the membrane, but in a few cases it appears attached or adherent at one or more sides, and little or none of the matrix may be present at those sides (Fig. 2). In only one instance has any other defined structure appeared in the matrix, and in this case the included material was not lamellar. Thus, a clear distinction exists

between the crystal-containing bodies and any proplastids or mitochondria as yet described.

The lattice spacing of the crystal ranges between 125 and 160 A (Figs. 1, 4, and 5). Thus, in sections about 500 A thick there should be about four layers; varying degrees of overlap between these layers, according to the orientation of the section to the planes of symmetry, doubtless accounts for the varying degrees of contrast observed in the lattice. The possibility that the crystals might be leaflets thinner than 500 A is negated by the fact that the cross-sections which would then be expected, appearing as long narrow strips, are not found. Thus it is more probable that the crystals seen are sections through more or less isodiametric structures. Irregularities in the spacing, suggesting fractures and overlaps or twinning, sometimes appear (Figs. 3, 5). The crystal structure has been seen only in osmium tetroxide-fixed material; permanganate apparently attacks the crystalline substance. So far, not a single crystal-containing body has been seen in any permanganate preparation. Also, what are apparently the crystal-containing bodies can be made out *in vivo* at optical magnification under phase contrast, which should facilitate studies of their distribution.

The crystal bodies have not been found in the epidermis of the coleoptile, though a single anomalous crystal was observed in a cell of the inner epidermis; *i.e.*, the layer lining the cavity. In the cortical cells they are distributed throughout the cytoplasm, and it is not infrequent for one to be closely associated with a proplastid (Fig. 6). A single cortical cell can be calculated to contain from 20 to 200 crystal bodies (*cf.* Fig. 1).

In view of the possibility that these bodies may be connected with phototropic sensitivity, several other positively phototropic organs were studied briefly. No identical crystal-containing bodies could be observed in barley (*Hordeum vulgare*) or corn (*Zea mays*) coleoptiles. Barley apparently lacks the first positive phototropic response, which might be significant in this connection. The corn cells contained some perhaps comparable structures showing fine granulation and variable outline, but composed of densely packed osmiophilic granules. The nature of the membrane is obscure. Whether these structures are analogous to the crystal-containing bodies of *Avena* is not yet clear.

The sporangiophores of *Phycomyces blakesleeanus*, however, do contain bodies comparable to those of *Avena*. The plants were harvested when in their stage of maximal phototropic sensitivity, and sectioned through the growing region; *i.e.*, 0.5 to 1 mm below the sporangium. Relatively large crystal-containing bodies, up to 1.5 μ in longest dimension, are present there. The membrane appears very thin and folded (Fig. 7). In the one photograph in which the lattice spacing could be precisely measured, the spacing was 131 A, exactly the same as in *Avena*.

DISCUSSION

No mention of crystal-bearing bodies of the type described here has been found in the botanical literature. The nearest approach is probably the area of crystalline structure which has been observed several times in the center of plastids or proplastids. Commonly the planes of these crystal lattices are spaced about 375 A apart (for literature, see reference 4). These crystal lattices are notable in the plastids of cells in and around the glands of *Passiflora* (5) and in the leaves of *Chloro-*

Figs. 1 through 4, × 42,000; Fig. 5, × 60,000; Figs. 6 and 7, × 46,000.

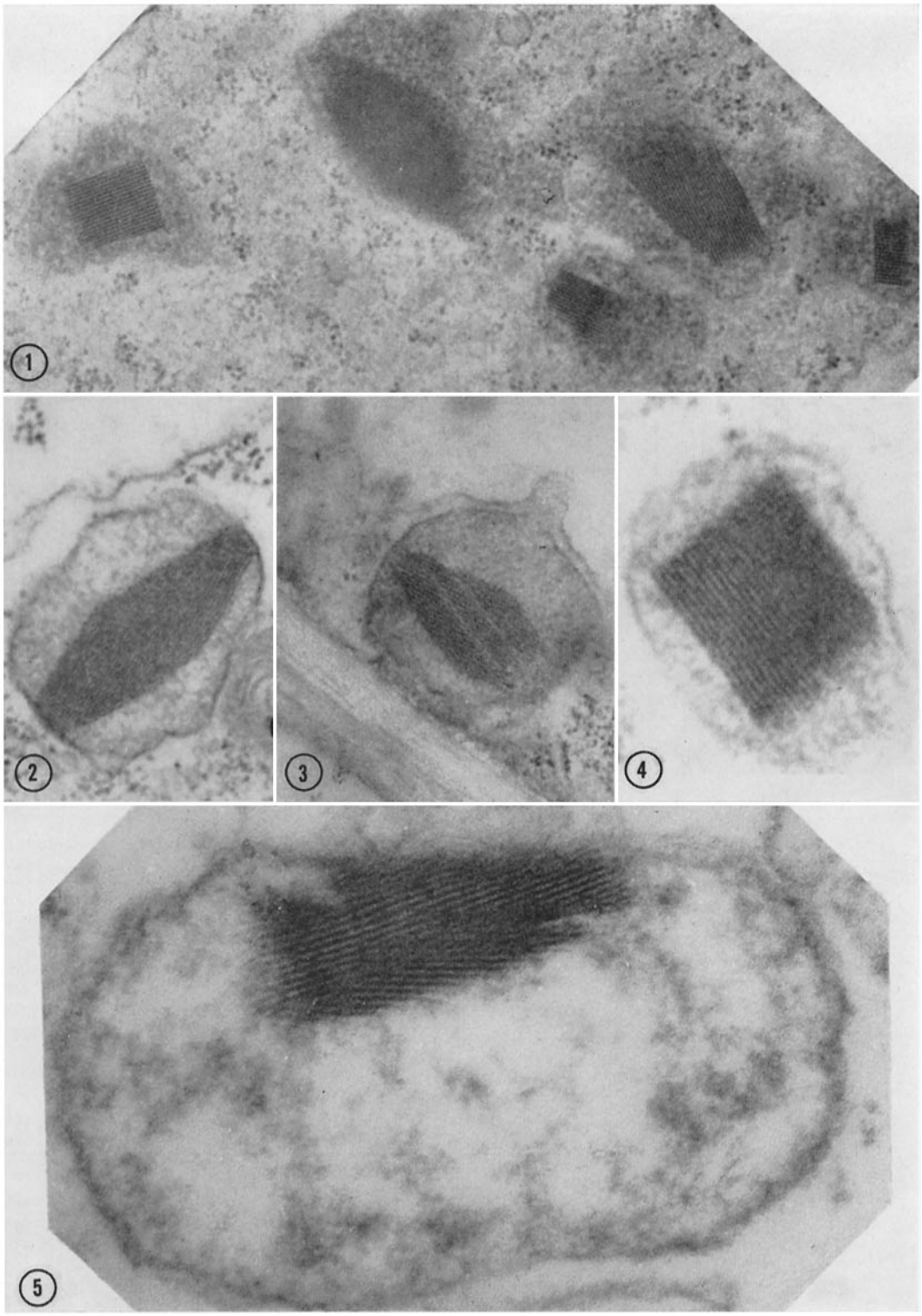
FIGURE 1 Cytoplasm of *Avena* coleoptile subepidermal cell with 5 crystal-containing bodies close together, each in its own matrix. The size of the largest is comparable with that of a mitochondrion.

FIGURE 2 Crystal apparently adherent to membrane at two edges.

FIGURE 3 Crystal shows two lighter bands; note tonoplast membrane outside, and separate from, crystal-body membrane. Note folds of plasmalemma at top left.

FIGURE 4 Crystal-containing body showing 160 A spacing.

FIGURE 5 Apparently twinned crystal within unusually large outer membrane.


1
2
3
4
5

phytum (Liliaceae) (6). In the proplastids of the pea root, some comparable crystalline structures with spacing of only about 100 A are present (4). In all the cases described, the outline of the structures is irregular or broken, quite unlike the regular rhomboids or cubes reported here, so that the structures appear rather as small zones of apparent crystallinity within the plastid. The crystal-containing bodies of *Avena* contain no lamellae of any kind, nor indeed any other structure besides the organic matrix, which makes it difficult to regard them as modified plastids. Their membranes do not appear to be double. The presence of comparable crystal bodies in the fungus *Phycomyces*, of course, makes any identification with plastids *sensu stricto* still more improbable.

The possibility that the crystal bodies might be photoreceptors lends interest to a comparison with the "eyespots" of flagellates, which are rich in carotenoids. However, in *Euglena* the eyespot consists of large granules 100 to 300 mμ in diameter, tightly enough packed to produce the appearance of a hexagonal pattern but not truly crystalline (7). In *Chlamydomonas reinhardi* the granules are 100 to 140 mμ in diameter; each granule contains a dense core and bears no membrane, and they lie in the chloroplast between the lamellae (8). They show no crystalline structure, though in surface view a hexagonal packing is evident, as in *Euglena*. Thus these structures do not resemble the crystal bodies described here.

Chromoplasts rich in carotene, such as those in carrots, are apparently crystalline in shape, often indeed rhomboid, and of varying size up to 20 μ long. Recently, Straus (9) has shown that they contain 19 to 56 per cent of carotene in a protein-lipid stroma. They show no evidence of a bounding membrane.

Three other cases may be mentioned. (*a*) Relatively large cubical crystals (*ca* 6 × 6 μ or more) occur in *Acetabularia* (10). They are believed to consist of a polymerized 3-substituted derivative of indole. No membrane is apparent and their relatively coarse spacing differentiates them completely from the bodies described here. (*b*) Small "polyhedral bodies" with somewhat rectilinear outlines, up to 0.5 μ in diameter, occur in some blue-green algae (11). They show neither membrane nor lattice structure. (*c*) Insect polyhedral viruses sometimes appear crystalline, but these show little resemblance to the structures here described.

It may be mentioned too that "microbodies," believed to be precursors of mitochondria, are similar in size to our crystal-bearing bodies, and have a single membrane, but the dense osmiophilic granule, which often fills most of the space, shows neither a crystalline structure nor a sharp geometrical outline (12).

This work was supported in part by a grant from the National Science Foundation, No. G9084, to Professor K. V. Thimann.

The authors wish to express their warmest thanks to Dr. I. R. Gibbons of the Biological Laboratories for his patient assistance, and to Dr. K. R. Porter for his valued advice and comments.

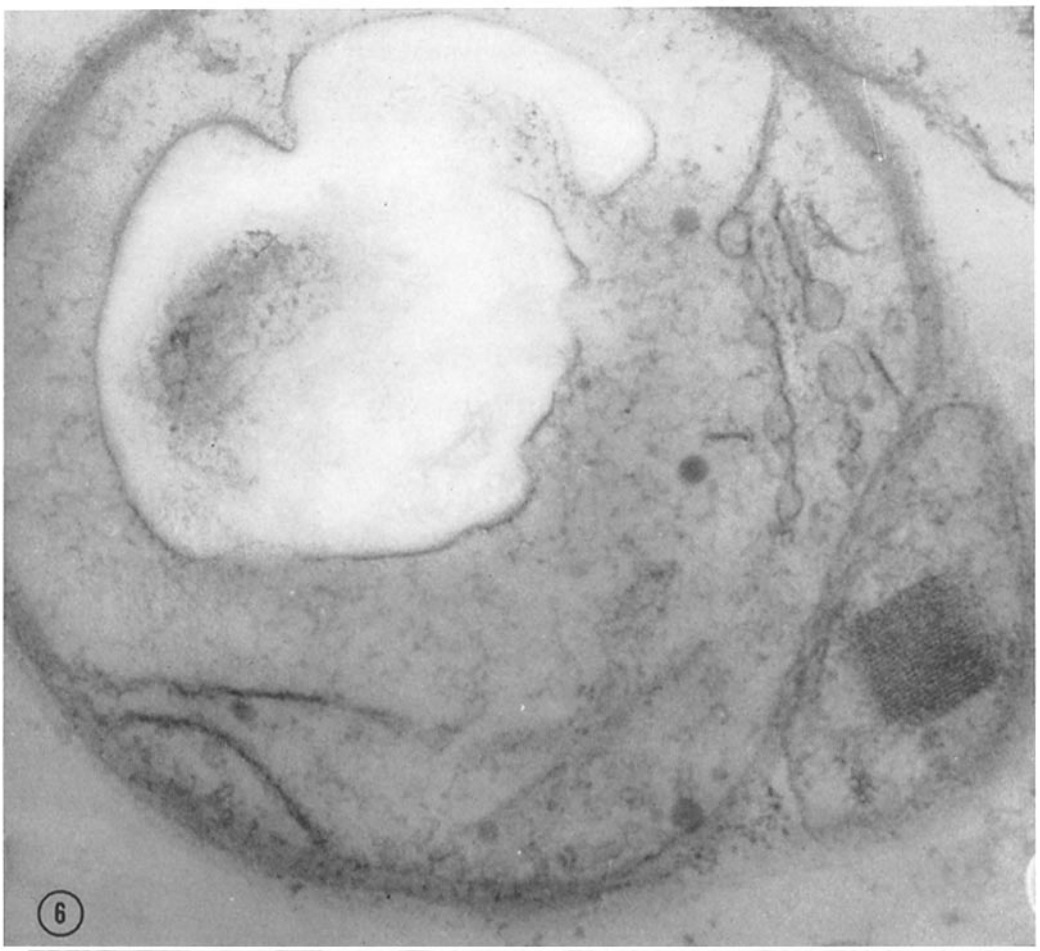
Received for publication, June 5, 1963.

REFERENCES

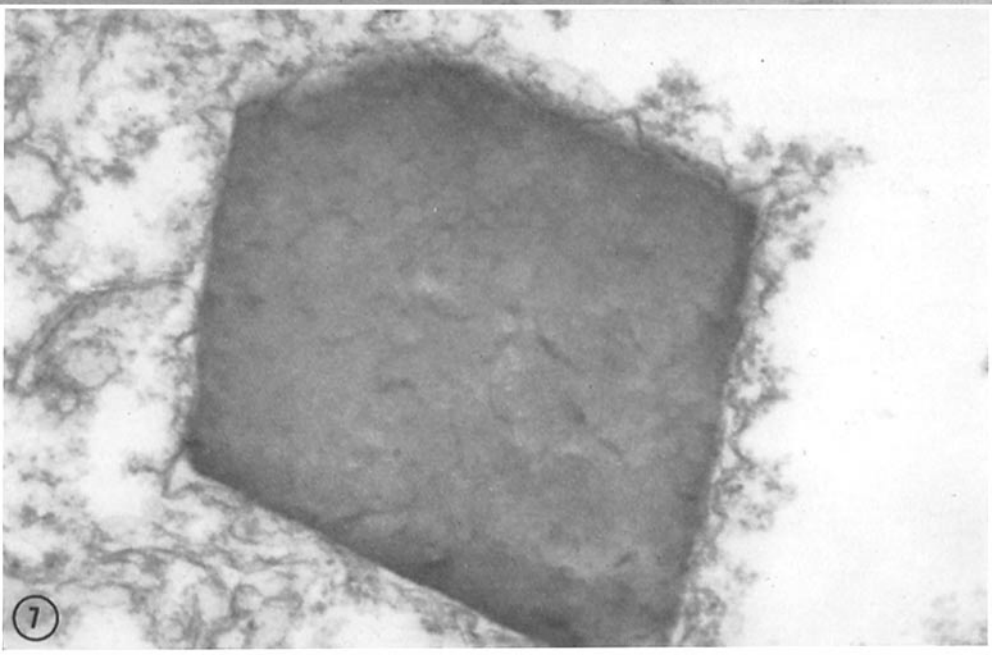
1. THIMANN, K. V., and CURRY, G. M., Phototropism, *in* Light and Life, (W. D. McElroy and B. Glass, editors), The Johns Hopkins Press, 1961, 646.
2. CURRY, G. M., and THIMANN, K. V., Phototropism; the nature of the photoreceptor in higher and lower plants, Progress in Photobiology, Proceedings of the 3rd International Congress on Photobiology, Amsterdam, Elsevier Publishing Company, 1961, 127.
3. GRUEN, H. E., Growth and development of isolated *Phycomyces* sporangiophores, *Plant Physiol.*, 1959, **34**, 158.
4. SITTE, P., Zum Bau der Plastidzentren in Wurzelproplastiden, *Protoplasma*, 1961, **53**, 438.
5. SCHNEPF, E., Plastidenstrukturen bei Passiflora, *Protoplasma*, 1961, **54**, 310.

FIGURE 6 Crystal body apparently adherent to etiolated plastid bearing large starch grain and typically vesiculated prolamellae. Note the outer matrix common to crystal body and plastid.

FIGURE 7 Crystal-containing body in upper part of sporangiophore of *Phycomyces* (½ to 1 mm below sporangium) showing folded membrane close to the crystal.



6



7

6. PERNER, E. S., Die ontogenetische Entwicklung der Chloroplasten von *Chlorophytum comosum*. I. Die Persistenz des Primargranums und seine fragliche Kristalgitter-Struktur im Proplastid. *Z. Naturforsch.* 1956, **11b**, 560.
7. WOLKEN, J. J., A molecular morphology of *Euglena gracilis* var. *bacillaris*, *J. Protozool.*, 1956, **3**, 211.
8. SAGER, R., and PALADE, G. E., Structure and development of the chloroplast in Chlamydomonas. I. The normal green cell. *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 463.
9. STRAUS, W., Studies on the chromoplasts of carrots, *Protoplasma*, 1961, **53**, 405 (and literature there cited).
10. TANDLER, C. J., Bound indoles in Acetabularia, *Planta*, 1962, **59**, 91.
11. PANKRATZ, H. S., and BOWEN, C. C., Cytology of blue green algae. I. The cells of Symploca muscorum, *Am. J. Bot.*, 1963, **50**, 387.
12. ROUILLER, C., and BERNHARD, W., "Microbodies" and the problem of mitochondrial regeneration in living cells, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4 supp., 355.