show the same high degree of specificity previously seen in the rat brain (*3*).

The presence of androgen-concentrating cells in the midbrain of the chaffinch is particularly interesting in view of the suggestion that the midbrain is involved in the control of vocal behavior in a number of avian species (*19–28*). Popa and Popa (*19*) and Brown (*20*) reported that vocalizations could be electrically stimulated in an area near the optic ventricle. More detailed mapping studies of this region by Newman (*21*) and Potash (*22*) have demonstrated two separate functional areas—the ICo (*23*) from which vocalizations can be elicited with a low threshold and the MLd from which evoked responses can be recorded after auditory stimulation. A variety of vocalizations, including alarm calls, have been stimulated from ICo. Many of these elicited vocalizations have been triggered from several other sites in the brain (for example, hypothalamus and septal area), although normal song has not yet been elicited by stimulation anywhere in the brain. The finding of auditory evoked responses in MLd supports previous neuroanatomical and neurophysiological studies showing auditory sensory input to this nucleus (*29*).

Our autoradiographic results suggest a biochemical division within the midbrain of the chaffinch which correlates well with the two functional areas described by Newman and Potash. Cells in ICo have the ability to retain certain androgenic hormones, while cells in MLd do not have this capacity. In view of the stimulation studies referred to above and the androgen dependency of many avian vocalizations, the concentration of labeled cells in ICo after an injection of [^{3}H]testosterone suggests that this area may be a site of androgen action in the regulation of vocal behavior.

RICHARD E. ZIGMOND*
FERNANDO NOTTEBOHM
DONALD W. PFAFF
Rockefeller University,
New York 10021

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10. Two of the four birds had been kept outdoors following the natural photoperiod and were injected with [^{3}H]testosterone in August. The other two birds were kept indoors under an artificial photoperiod which, when the birds were injected, corresponded to early spring.
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12. Control sections indicated that the concentration of silver grains over certain cell bodies was due to the presence of radioactive steroid rather than to chemography and that the absence of grains in other areas was not the result of fading of the latent image.
13. Concentrations of labeled cells have been found elsewhere in the chaffinch brain, particularly in the medial preoptic area, medial hypothalamus, and lateral septum. The preoptic area and the hypothalamus have been shown to be sites of androgen action in the control of courtship behavior and the regulation of gonadotrophin secretion in a number of avian species [F. Gogan, *Gen. Comp. Endocrinol.* **11**, 316 (1968); R. Barfield, *Horm. Behav.* **1**, 37 (1969); J. B. Hutchison, *J. Reprod. Fert.* **11** (Suppl.), 15 (1970); F. E. Wilson, in *Aspects of Neuroendocrinology*, W. Bargmann and B. Scharrer, Eds. (Springer-Verlag, Berlin, 1970), p. 274]. A detailed description of the topography of the androgen-concentrating cells in these areas is in preparation.
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16. The details of dose of [^{3}H]testosterone, route of injection, and the time the animals were killed were the same as in the autoradiographic experiments. The brain nuclei were extracted with dichloromethane. After the solvent was evaporated, the extracted material was run on silica gel, thin-layer plates in a solvent system containing chloroform and diethyl ether (20 : 1). Testosterone, 5α-dihydrotestosterone, and androstenedione were run as standards. Results are expressed as the percentage of the total radioactivity recovered from the plate. The mean percentages of testosterone and dihydrotestosterone in three experiments were 47 percent and 46 percent, respectively. The possibility that the extent of dihydrotestosterone formation varies in different regions is suggested by a single experiment in which we isolated nuclei from different areas of the brain. The percentages of the radioactivity that migrated with testosterone and dihydrotestosterone, respectively, in the three samples were as follows: optic lobes, 62 and 35 percent; striatum, 47 and 42 percent; and diencephalon, 40 and 53 percent.
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* Present address: Medical Research Council, Neurochemical Pharmacology Unit, Medical School, Hills Road, Cambridge CB2 2QD, England.

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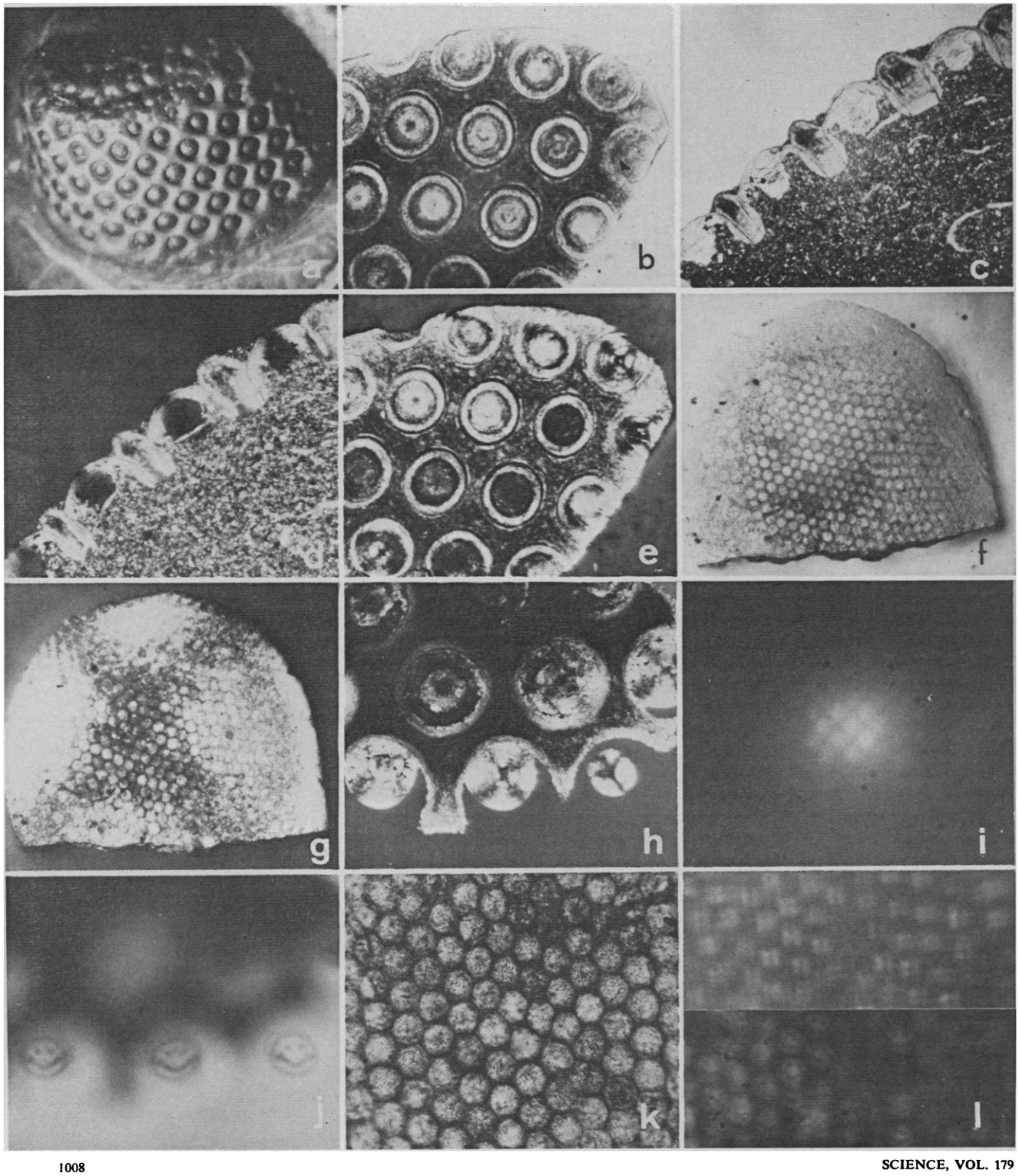
Trilobite Eyes: Calcified Lenses in vivo

Abstract. *The corneal lenses preserved in the eyes of some of the Paleozoic trilobites (Arthropoda) are constructed of calcite that is crystallographically oriented to behave like glass. The calcareous lenses are capable of forming inverted images over a large depth of field and must have been present in the living trilobites.*

The corneal lenses of the eyes in many of the extinct Paleozoic trilobites are commonly preserved as fossil remains along with other parts of the calcitic-chitinous exoskeleton. Although a number of excellent and detailed papers have dealt exclusively with various aspects of trilobite visual organs (*1, 2*) the question of the original composition of the lenses has only briefly been considered. Most workers have assumed that inasmuch as the corneal lenses of modern-day arthropods are modified extensions of the chitinous cuticle (*3*) the same was true for the trilobites. However, the exoskeletons of most trilobites are preserved as fossils not because they were constructed of chitin per se but because this chitin was substantially impregnated with calcium carbonate. Thus, if the lenses of trilobite eyes were unmineralized in life, as is the case with the majority of living organisms, then a problem emerges regarding their fossil preservation. Why should one uncalcified portion of the trilobite cuticle commonly be preserved when other portions,

such as their undersurfaces, are rarely found? Two alternative solutions to this problem seem possible: (i) the corneal lenses of the trilobite eyes were preferentially replaced or impregnated in a postmortem fashion by secondarily deposited calcite, or (ii) the lenses were mineralized with calcite while the animals were alive. The former view appears to have been shared by most earlier workers. The evidence presented below is offered in support of the second point of view.

As is the case with living arthropods, the trilobites as a group have two basic types of eyes—simple and compound. The simple eyes, each having its own lens and socket, are grouped together to form what is called an aggregate or schizochroal eye (Fig. 1a). The compound eye, on the other hand, consists of closely packed prismatic facets or lenses that taken together give the impression of being one large curved corneal lens. As such it is referred to as a holochroal eye. Well-preserved examples of each type were chosen for study. Specimens of *Phacops rana* from the Devonian Moscow Formation (New York) represent the schizochroal type, and *Isotelus gigas* from the Ordovician Trenton Limestone (New York) represents the holochroal type. Polished



thin sections were prepared to provide both tangential and vertical views of the eyes.

Figure 1b is a tangential section through a portion of the schizochroal eye of *Phacops*. As reported in earlier studies, there is a hexagonal packing of the circular lenses, which are separated by a thickened cuticular sclera. A vertical view (Fig. 1c) shows that each lens is biconvex with a corneal covering that is a cuticular extension of the interlensar sclera. Note the sediment infilling beneath the eye. Figure 1d shows this same section in cross-polarized light, and it is apparent that each lens is in complete extinction in this orientation but the corneal covering is only partially so (*4*). The corneal covering consists of radially oriented polycrystalline calcite, while the subcorneal lens is crystallographically uniform. Figure 1, e and h, shows tangential views in cross-polarized light and the two-part lens crystallography is confirmed. The pseudo-optic axis figures, showing a black cross, illustrate the radial orientation of the corneal calcite. Lenses sectioned below their respective corneas (Fig. 1e) are uniformly extinct, which illustrates the apparent single crystal aspect of the subcorneal region. In the cornea the crystallographic *c*-axes of the radial crystals are perpendicular to the external curvature of the lens, while the *c*-axis of the subcorneal lens is always oriented outward through the center of each lens.

A portion of the compound or holochroal eye of *Isotelus gigas* is shown in horizontal tangential section in Fig. 1f and the close-packed hexagonal "facets" can be seen. The same section viewed in cross-polarized light (Fig. 1g) displays one large, black cross over the entire section. Here the whole eye of *Isotelus* behaves as does each of the lenses in the schizochroal eye of *Phacops* and the crystallography of the calcite is similar. Many of the hexagonal prisms behave as one crystal and the black cross is the result of the eye curvature.

As a highly birefringent mineral, calcite has a double refraction so pronounced that it is often used to illustrate the phenomenon. However, light passing in the direction of the *c*-axis (optic axis) is not doubly refracted and the mineral behaves isotropically with an index of refraction of 1.486. It is only in this special orientation that a lens made of calcite would be able to produce an image free of a doubling effect. Thus, the individual lenses of the schizochroal eye of *Phacops* and the facets of *Isotelus* are constructed of calcite so precisely oriented crystallographically that they behave optically as if they were made of glass. This unique crystallographic orientation, which is reproducible from specimen to specimen, cannot be considered an accidental postmortem or secondary replacement by calcium carbonate. Such a consistent and selective preferred orientation can only be due to a process of biomineralization. The calcite lenses must have been present during the life of the animals.

In order to test the "vision" of the trilobite lenses an experiment was performed in which the eyes were embedded in a clear epoxy, mounted face down on glass microscope slides, and sectioned by carefully grinding from the rear only. The sections (Fig. 1h) were placed face down on the stage of a microscope without a condenser and brought into focus. From this point the objective lens of the microscope was racked upward from 1 to 2 mm (*5*) until objects placed beneath the eyes at various points appeared in focus (Fig. 1i). Experimentation revealed that all objects are inverted and appear to remain in focus from a distance of just a few millimeters up to infinity with no change in the position of the microscope objective required. Images appeared with varying degrees of clarity in all lenses sufficiently free of sediment infilling to transmit light (Fig. 1j). In the schizochroal eye the image appeared in each of the individual lenses, while in the holochroal eye the image was repeated in each of the hexagonal facets (Fig. 1, k and l). Eyes sectioned from both sides (Fig. 1b) failed to produce an image, the biconvexity of the lenses having been altered.

These data provide compelling evidence that the eyes of at least some of the trilobites in life had lenses that were constructed of a modified cuticle containing crystallographically oriented, clear calcite. Although it is not known whether the trilobites were capable of form perception, their biconvex lenses were able to form inverted images over a wide depth of field. In most living arthropods the eyes consist of unmineralized cuticle, but the corneal lenses of a few living crustaceans have been found to contain calcite. Wolsky (*6*) and Dudich (*7*) have reported its occurrence in some members of the isopods and amphipods, most of them terrestrial. But the altogether surprising thing about these living examples of calcified eyes is the poor crystallographic orientation of the calcite impregnating the corneal lenses and the fact that it is a mosaic rarely coincident with the facets or ommatidia. The *c*-axes are seldom perpendicular to the surface which, as Wolsky and Dudich point out, means that the lenses would be doubly refracting. Thus, if an evaluation is made solely on the basis of crystallography, some of the extinct trilobites appear to have evolved a significantly better optical system than that of the few living arthropods known to have calcified corneal lenses (*8*).

KENNETH M. TOWE
Department of Paleobiology,
Smithsonian Institution,
Washington, D.C. 20560

Fig. 1. (a) Schizochroal eye of *Phacops* (× 7). (b) Tangential section with hexagonally arranged lenses (× 25). (c) Vertical section with biconvex lenses separated by interlensar sclera (× 40). (d) Same as c, in cross-polarized light. Note the two-part construction of each lens (× 25). (e) Same as b, in cross-polarized light. The outer part of the lenses shows a black cross; the inner part is uniform where the outer part is removed (× 25). (f) Portion of a holochroal corneal lens from *Isotelus* with hexagonal facets (× 35). (g) Same as f in cross-polarized light. A large black cross covers the entire section. (h) Schizochroal eye tangentially sectioned from behind, showing black crosses in cross-polarized light (× 40). (i) Image through one lens of a screen with mesh openings of 0.7 mm, placed 10 cm from the eye (× 180). (j) Repeated image seen in several lenses (× 40). (k) Hexagonal facets of the holochroal eye (× 90). (l) Mesh screen and happy faces imaged in the facets of k (× 90).

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