Presence of Lateral Eye Lens Crystallins in the Median Eye of the American Chameleon

Abstract. *With the use of immunofluorescence techniques, gamma globulin antibody specific for the crystallins of* Anolis carolinensis *lateral eye lens was applied to sections through the median (parapineal) eye of* Anolis carolinensis. *Only the median eye lens exhibited fluorescence specific for the crystallins; other structures were negative. These results indicate that the lens of the reptilean median eye shares tissue-specific antigenic determinants with the lens of the lateral eye. This suggests a possible evolutionary relation between the two structures, based on biochemical, as well as previously reported anatomical, criteria.*

The functional significance of the pineal or epiphyseal complex (derivatives of the brain wall), has been the subject of much conjecture since its characterization by Descartes as the "seat of the soul." In birds and mammals this complex is wholly internal, encased within the skull, whereas in lower vertebrates it may exist as a visible median or third eye (*1*). During embryonic development, the pineal evaginates from the dorsal aspect of the diencephalon roof (*2*). The pineal complex thus shows no developmental correlation with the lateral eye. The median eye has reached the greatest degree of morphological specialization in the lizards, especially in the iguanid lizards. In these organisms it is termed the parapineal or parietal eye, and it possesses a retina, cornea, and lens—structures resembling, in histology, the corresponding elements of the lateral eyes (Fig. 1A). The median eye lens of *Anolis carolinensis*, the American chameleon, has a light microscopic appearance suggestive of lateral eye lens fibers (Fig. 1A), and the ultrastructural similarity between reptilean median eye lens and developing mouse lens has been noted (*3*). Unlike the normally pigment-free vertebrate lateral eye lens, the median eye lens of *A. carolinensis* possesses a central pigmented area (unpublished observation; not shown in Fig. 1A). This lens is derived from neuroectoderm, rather than from epidermal ectoderm, the source of the lens of lateral eyes (*2*). Photosensory, as well as secretory, capabilities have been suggested for the median eye (*1*), although most of these conjectures rely on structural similarities with tissues of comparable function. I report here, using immunofluorescence techniques, the presence of crystallins, the characteristic proteins of the vertebrate lateral eye lens, in the lens of the median eye of the iguanid, *A. carolinensis* (*4*).

Adult *A. carolinensis* were obtained locally and kept in the laboratory at 21° ± 1°C for 1 week before they were killed. The lateral eye lenses were removed and the lens protein was prepared for use as antigen (*5*, *6*). The antibody obtained was sensitive to homologous antigen at concentrations as low as 8.6 μg/ml. The median eye (0.3 mm in diameter) was removed microsurgically and fixed either in Bouin's fixative for light microscopic study, or in cold 95 percent ethanol for immunofluorescence examination (*6*). The antibody gamma globulin to the *A. carolinensis* total lateral eye lens proteins was absorbed three times with tissue powder prepared from decapitated *A. carolinensis*, in order to remove reactivity with common tissue antigens.

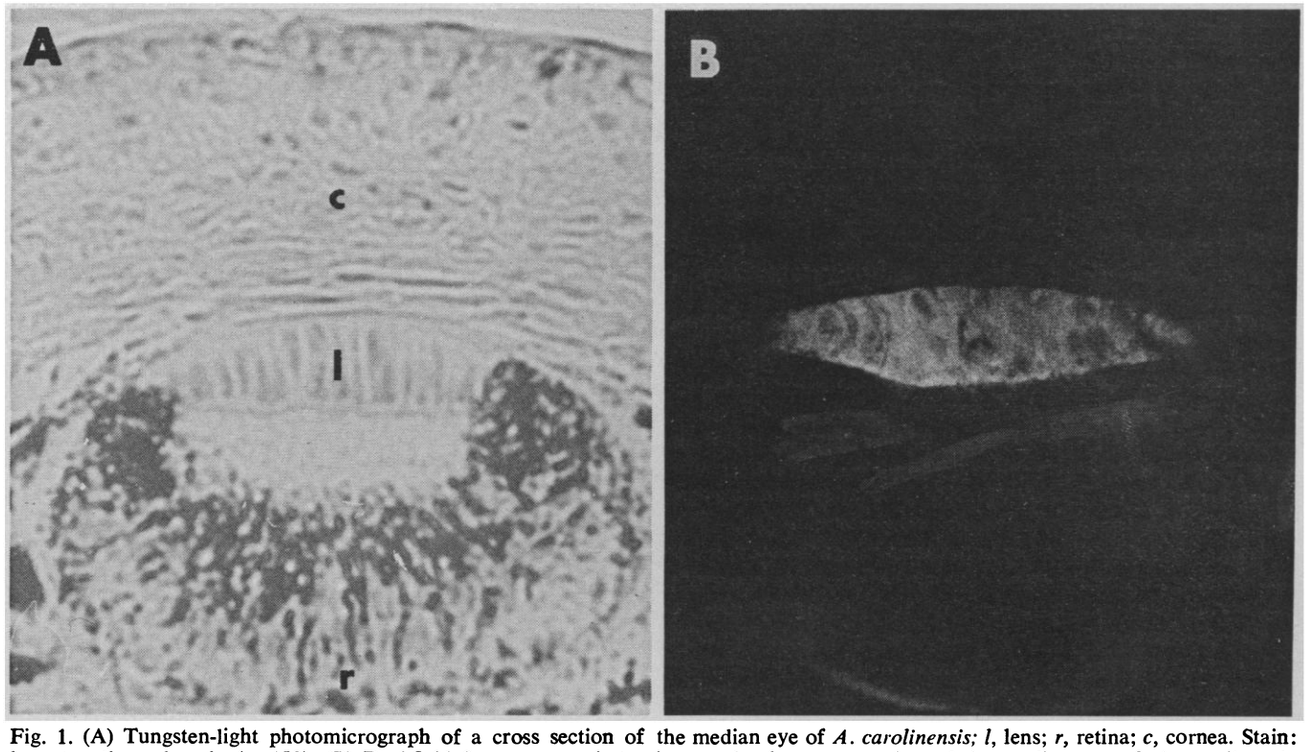

Fig. 1. (A) Tungsten-light photomicrograph of a cross section of the median eye of *A. carolinensis; l*, lens; *r*, retina; *c*, cornea. Stain: hematoxylin and eosin (× 450). (B) Darkfield fluorescence photomicrograph of a cross section of the median eye of *A. carolinensis*. The section, comparable to that of (A), was treated with antibody to total lens protein of the lateral eye in the indirect immunofluorescence technique. Detached lens tissue in the lumen of the median eye appears as two small, strongly fluorescing areas (× 450).

The specificity of this absorbed antibody for *A. carolinensis* crystallins, the lens-specific proteins, was determined by its lack of reactivity (in immunoelectrophoretic and immunodiffusion analyses) with extracts (10 mg/ml) of other *A. carolinensis* tissues such as liver, brain, skin, serum, and eye minus lens. Immunoelectrophoresis of this antibody with its homologous antigen confirmed the presence of three main classes of crystallins—α, β, and δ crystallins—in the lateral eye lens of this organism, as reported for birds and reptiles (*7*). Such an antibody is suitable for use in immunofluorescence detection of corresponding antigens, that is, crystallins, in tissue sections. Alcohol-fixed sections (3 μm) through the median eye of *A. carolinensis* were processed, subjected to the antibody to the crystallins in the indirect or "sandwich" immunofluorescence technique (*8*), and examined as described (*6*). A positive immunofluorescence reaction for *A. carolinensis* lateral eye lens crystallins, with the apple-green fluorescence of fluorescein isothiocyanate as antigen marker, was detected in the median eye. The fluorescence observed is significant not only because of its presence, but also because of its intensity and specific localization; it was restricted to those cells comprising the lens of the median eye (Fig. 1B). Specific immunofluorescence was absent from other median eye tissues, including the retina and the cornea. Controls, consisting of substituting nonimmune rabbit gamma globulin for the antibody to total lens protein, also gave negative results for crystallins.

The lateral eye lens contains proteins shared by widely divergent vertebrate classes, a characteristic which is indicative of its immunologic "organ specificity" (*9*). These results suggest that this evolutionary conservatism of lens proteins (crystallins) may also be shared with other tissues in the same organism, that is, there may exist an exception to their acknowledged "tissue specificity" (*6, 10–12*). The fact that crystallins are found in a vestigial structure (*1*) thought to share functional properties (light-sensitivity), with its homolog (the lateral eye), lends credence to current hypotheses of gene action based upon the concept of variable gene activity. Supporting the latter view are observations (*13*) that (i) differences in DNA sequences in differentiated cells cannot be detected; (ii) only approximately 10 percent of the genome is engaged in DNA or RNA synthesis; and (iii) a distinct spectrum of genes is active in each tissue.

A possible relationship between such gene versatility and crystallin appearance is suggested by the well-known ability of the dorsal margin of the iris to regenerate a lens after lentectomy in the newt (*14*). In this case, a tissue not having or producing crystallins becomes one that does so (*11, 12*). It has also been reported (*15*) that larval *Rana pipiens* lens epithelial cells, which do not normally produce detectable amounts of γ crystallins, can be stimulated to produce this lens-fiber specific protein (*6, 12, 16*) by placing them in hanging-drop cultures, thus changing the normal cellular milieu.

Presumably, the lens tissue of the median eye has a similar gene complement to that of lens tissue of the lateral eye. Expression of these genes, that is, production of crystallins, is demonstrated by both tissues, whose functions are ostensibly the same, but whose anatomical location and embryonic origins differ significantly.

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Triiodothyronine: The 3′-Iodine Is Proximal to the α-Ring in Crystal Structure Conformation

Abstract. *The crystal and molecular structure of the thyroid hormone* L-*triiodothyronine has been determined by x-ray diffraction. The two phenyl rings are almost perpendicular to each other, the acute angle between their normals being 82 degrees. The 3′-iodine is situated* proximal *to the α-ring, rather than* distal *as inferred from chemical studies. Theoretical calculations indicate this* proximal *conformation to be energetically favored over the* distal *one.*

The thyroid hormones L-thyroxine (T_4) and 3,5,3′-triiodo-L-thyronine (T_3) exhibit many important biological effects and are essential for normal growth and development and the control of oxidative metabolism. T_3 appears to be at least three to four times as potent as T_4 in most biological tests, and there is evidence that it is the more important hormone both in mammalian physiology and in most clinical situations (*1, 2*). It has been shown (*3, 4*) that more than one-third of circulating T_3 may arise from extrathyroidal conversion of T_4 to T_3 in normal human subjects, and this has given support to the speculation that T_4 may act primarily as a prohormone, and exert its effect only after transformation to T_3 (*2, 4*).

Although the biological importance of L-triiodothyronine is thus well established, the mechanisms of its actions are poorly understood. A great many thyroid hormone analogs have been prepared, and their hormonal activities have been measured in attempts to establish chemical features essential to thyromimetic action (*5*). It has been suggested that conformational structure, as well as chemical features, is of prime importance to hormonal action. Jorgen-