linity and oxygen-isotope composition. According to the calcium carbonate isotopic temperature scale (*17*) the calcite in both samples would be in equilibrium with standard mean ocean water at 27°C. Extrapolated values for the difference in equilibrium δO^{18} between well-ordered stoichiometric dolomite and calcite are estimated to be 6 to 10 per mil (*18*) and 4 to 7 per mil (*19*). (ii) Alternatively, the δO^{18} of dolomite represents nonequilibrium formation from calcium carbonate in hypersaline brine, whereas the δO^{18} of calcite represents original skeletal material plus recrystallization or isotopic exchange with meteoric waters, brines, or sea water of normal salinity. If the original sedimentary protodolomite-water isotopic fractionation was close to that of calcite-water at the same temperature, then the high O^{18} composition of the dolomite would represent formation in water of high δO^{18} owing to evaporation. Similar nonequilibrium formation of dolomite in standard mean ocean water would necessitate an unreasonably low surface temperature (*17*).

Recent work suggests that the second explanation is more nearly correct. Isotopic analysis of co-existing calcite and dolomite in modern sediments (*18*) has shown that the two actually are of very similar oxygen isotopic composition and do not represent the expected equilibrium ΔO^{18} of about 6 per mil. This similarity was interpreted to mean that dolomite forms by the solid state replacement of Ca in $CaCO_3$ by Mg. However, the occurrence of banded dolomite as concentric crusts in voids (*1*) and as rhombohedra with compositional zoning strongly supports an origin by crystal growth from solution and not by diffusion replacement in the solid state.

The dolomite, once formed, would not be expected to exchange oxygen with meteoric waters or subsurface fluids nearly as readily as calcite (*15*). In this way the original isotopic composition of dolomite would be preserved, whereas that of calcite could approach equilibrium with the surrounding water by exchange or recrystallization (*20*) (see Fig. 1).

Confirmatory evidence for a hypersaline origin of the dolomite beneath the mid-Pacific atolls must await further, more reliable tests for original hypersalinity. Weber (*21*) in a recent paper states that three samples of Funafuti reef dolomite were found to contain an unusually high concentration of chloride ion and this finding can be interpreted as additional possible evidence for hypersalinity.

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24 December 1964

Lens Fiber Differentiation and Gamma Crystallins: Immunofluorescent Study of Wolffian Regeneration

Abstract. *From the adult lens of* Triturus viridescens, *a fraction of proteins was isolated which corresponds to γ-crystallins of higher vertebrates. Tests by immunoelectrophoresis indicate that the antiserum against this fraction reacts with γ-crystallins, but not with α- or β-crystallins. With this antiserum, an immunofluorescent reagent has been prepared for detection of γ-crystallins from newts. In the normal lens of the adult newt, these crystallins are detected in fiber cells and fiber material, but not in the epithelial cells. During transformation of the iris into the lens after lens removal, the staining reaction is negative in the regenerating tissue up to the time the prospective primary fiber cells begin to elongate. Subsequently, without exception those cells in fiber differentiation indicate a γ-crystallin reaction. When the secondary fiber cells are produced at the equatorial zone of the regenerating lens, they also begin to show a γ-crystallin reaction. Thus, γ-crystallins characterize fiber differentiation.*

A number of cellular events are closely connected with the control mechanism of γ-crystallin synthesis. When the lens is removed from the eye of adult newts belonging to the family Salamandridae, a part of the iris is transformed into the lens (*1*). This type of regeneration, called Wolffian lens regeneration, has been the subject of a series of cytochemical and immunochemical investigations (*2*), and the acquisition of tissue specificity by such regenerating tissue is the subject of this report.

In our earlier study (*3*), lens specific antigens became detectable in this system, by immunofluorescence, after the lens vesicle showed the first sign of lens fiber differentiation (lens regeneration stage IV after Sato, *4*).

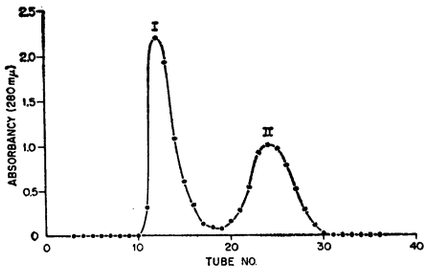


Fig. 1. Gel filtration on Sephadex G-75 of newt lens proteins. Column, 2.2 × 60 cm; load, 40 mg protein; flow rate, 25.2 ml/hr; effluent collected in 4.2-ml fractions.

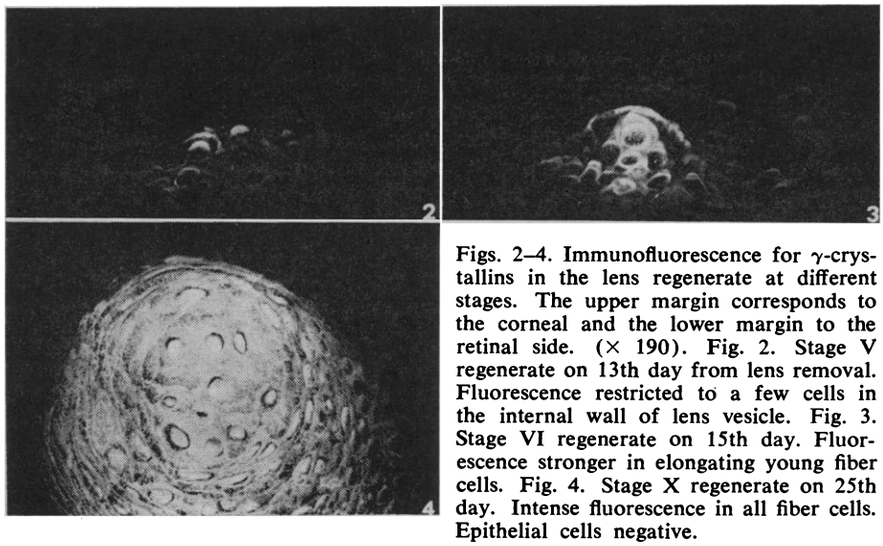
The antigens were localized at this stage in the prospective primary lens fibers. Subsequently they were detected in all secondary as well as primary lens fiber cells in all phases of fiber differentiation. When the lens epithelium was well defined at stage X and later, the lens epithelium also indicated the positive reaction. The newt lens antigens detected in that study are represented by more than eight components which can be classified into three groups. According to their behavior in immunoelectrophoresis, immunodiffusion, and gel filtration, these groups correspond to α-, β-, and γ-crystallins of higher vertebrates. Efforts have been made to separate crystallins of adult *Triturus viridescens*, to obtain antiserums against them, and to test the specificity of the antiserums by immunoelectrophoresis and immunodiffusion. So far, satisfactory results have been obtained only for γ-crystallins.

According to Björk's method of separating γ-crystallins (*5*), a supernatant from the lens homogenate of the adult newt, *Triturus viridescens*, was applied to a Sephadex G-75 (Pharmacia, Uppsala) column. Measurement of absorbancy at 280 mμ indicated two peaks (Fig. 1). Fraction II, which corresponds to γ-crystallins, was collected, concentrated, and dialyzed overnight. Rabbit antiserum was prepared against this fraction in the following way. Specific precipitates formed by reacting fraction II protein with rabbit antiserum to whole lens were incubated in an excess amount of fraction II protein, emulsified in Freund's adjuvant, and injected subcutaneously in two steps separated by an interval of 2 weeks. Three weeks later an additional injection of the specific precipitate from fraction II was administered intraperitoneally. The total amount of protein injected was approximately 25 mg. Two weeks later the rabbits were bled. The serum thus obtained gave positive interfacial tests at a dilution of 1:5000. One antiserum was used for the following study.

A series of tests was made for immunological specificity of the antiserum to fraction II as well as fraction II by immunoelectrophoresis and immunodiffusion. Tested with antiserum to the whole lens of the newt, fraction II showed only the precipitation lines belonging to the γ-crystallin region. The antiserum to fraction II produced those lines alone when tested with a lens extract which contained all of the crystallins. The data were interpreted as indicating that fraction II and its antiserum have the immunological specificities required for the present purpose. When fraction II was tested with antiserum to fraction II (10 mg of antigen per milliliter), three to four precipitation lines were distinguished in the γ-crystallin region. The globulin fraction from the antiserum or from normal serum was labeled with fluorescein isothiocyanate, absorbed with mouse tissue powder (*6*), and used as immunofluorescent reagent for newt γ-crystallins or as its control, respectively. Paraffin sections (3 μ in thickness) of the regenerating cell population at various stages of lens regeneration in the adult newt, *Triturus viridescens*, were treated with this reagent and its control (*3, 7*). For fluorescent microscopy, exciter filters (Schott) BG 12, BG 3, and UG 5, and barrier filters 44 and 47 were employed in conjunction with a dark- or bright-field condenser.

Applied to sections of the normal lens, the immunofluorescent reagent for γ-crystallins intensely stains all fiber cells, but does not stain any of the epithelial cells. On the other hand, the control reagent does not stain the normal lens. Normal iris, pigmented iris after lens removal (stage I), depigmenting iris (stage II), and lens vesicle in the process of formation (stages III–IV) fail to show immunofluorescence for γ-crystallins. The immunofluorescence becomes positive in the prospective primary lens fiber cells in the internal (posterior) wall of the lens vesicle at stage V, when they begin cell elongation after cessation of cell multiplication (Fig. 2). Within the cell, the immunofluorescence is localized in the cytoplasm and nucleus. Subsequently, in all primary lens fiber cells, as they elongate and grow in size, intense immunofluorescence is detectable (Fig. 3).

The nuclear immunofluorescence tends to increase in intensity in the later phase of fiber differentiation (Fig. 4). The sequence of events is repeated by the secondary lens fibers during their cellular growth and differentiation (Fig. 4). Cell-labeling experiments with H^3-thymidine indicate that lens epithelial cells are transformed into the secondary lens fibers step by step at the equatorial zone in the regenerating lens, in conformity with what is known for the normal growing lens. From our data, this transformation is obviously coupled with the appearance of γ-crystallins.



Figs. 2–4. Immunofluorescence for γ-crystallins in the lens regenerate at different stages. The upper margin corresponds to the corneal and the lower margin to the retinal side. (× 190). Fig. 2. Stage V regenerate on 13th day from lens removal. Fluorescence restricted to a few cells in the internal wall of lens vesicle. Fig. 3. Stage VI regenerate on 15th day. Fluorescence stronger in elongating young fiber cells. Fig. 4. Stage X regenerate on 25th day. Intense fluorescence in all fiber cells. Epithelial cells negative.

In contrast to fiber cells, the cells in the lens epithelium fail to show immunofluorescence for γ-crystallins in all stages of regeneration studied (Fig. 4). The same applies to the cells in the external wall of lens vesicle at stages V to IX (Figs. 2 and 3). It should be pointed out that those cells negative in immunofluorescence are dividing in contrast to fiber cells which are nondividing. According to our preliminary data, α- and β-crystallins are present in the lens epithelial cells as well as in the fiber cells. Thus production of γ-crystallins is characteristic for lens fiber differentiation. This conclusion is in good agreement with biochemical data obtained on the calf lens (*8*). If lens fiber differentiation is the essential event of lens differentiation, the synthesis of γ-crystallins should be one of the crucial molecular processes in lens differentiation.

Comparison of our data with that obtained by H^3-thymidine autoradiography shows that the prospective lens fiber cells begin to synthesize γ-crystallins at a definite time interval after the last DNA-synthesizing phase. Electron microscopic data on ribosomes (*9*) and autoradiographic data on intracellular distribution of RNA in the present system imply that synthesis of γ-crystallins starts after the formation of a new ribosomal population is completed.

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Plant Parasitic Nematodes: A New Mechanism for Injury of Hosts

Abstract. *Pathological effects of* Ditylenchus dipsaci *and* Meloidogyne hapla *are related to the disturbance of the auxin balance in the host by the nematode. The parasites produce an auxin inactivator, apparently enzymatic, that enables* Ditylenchus dipsaci *to stunt host stem apices and* Meloidogyne hapla *to reduce its galling potential.*

Evidence that nematodes inactivate auxins provides an explanation for some of the pathological effects of nematode infection. The mechanisms by which parasitic nematodes injure host plants have been largely a matter of speculation based upon plant symptoms of nematode injury. They have usually been described in gross terms—that is, in terms of the mechanical injury resulting from nematodes feeding or moving through the tissues of the host, or of plant tissue reactions to secretions injected by the feeding parasites, or both (*1*). That nematode pathogenicity of host plants has a sound chemical basis was demonstrated by Mountain (*2*), who showed that *Pratylenchus penetrans* in peach roots is able to hydrolyze amygdalin to release benzaldehyde and hydrogen cyanide which diffuses to neighboring cells and brings about necrosis. In research on the relation between growth regulators and pathogenesis (*3*) the role of nematodes has been virtually neglected. Although fundamental disturbances of plant growth regulatory mechanisms in pathogenicity have been suggested, there is no experimental evidence of the biochemical mechanisms involved (*4*).

The possibility that nematodes can disturb plant tissues by secreting auxins was reported recently (*5, 6*). We have now investigated the roles of auxin-inactivating systems originating from the nematode in host parasite relationships. Freshly collected nematodes such as *Ditylenchus dipsaci* and *Meloidogyne hapla* upon incubation in water release an auxin-inactivating system (*7*). A characteristic manifestation of heavy infections of *Ditylenchus dipsaci* on alfalfa is severe stunting; apparently the stem apex is unable to elongate. According to conventional auxin theory, auxin synthesized in the growing point moves downward and as part of its physiological activity plasticizes the walls of the young cells, allowing them to absorb water and enlarge and elongate. Since *D. dipsaci* invades and develops in the stem apex, stunting can be visualized as a result of inactivation of auxin synthesized by the apical meristem.

Differential growth responses, obtained by application of natural and synthetic auxins to the shoot apices of infected and healthy intact plants, were consistent with the notion that indole-3-acetic acid (IAA) was inactivated in infected plants whereas naphthalene acetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) were not inactivated. However, in view of the great variability of response of intact plant stem apexes to auxins, the difficulty of establishing equivalent concentrations of IAA, NAA, and 2,4-D for purposes of comparison, the nonuniformity in the numbers of nematodes infecting shoot apexes, and the fact that the stem apex quickly grows away from the region of influence of the sedentary nematodes, more data were required.

If auxins are inactivated in infected plants, it would be reasonable to expect less auxin in infected shoot tips than in healthy ones. We found this to be the case (Fig. 1). When infected and noninfected shoot tips were lyophilized, extracted for auxin in the conventional manner, and tested by the avena "first internode test" (*8*), less IAA was found,

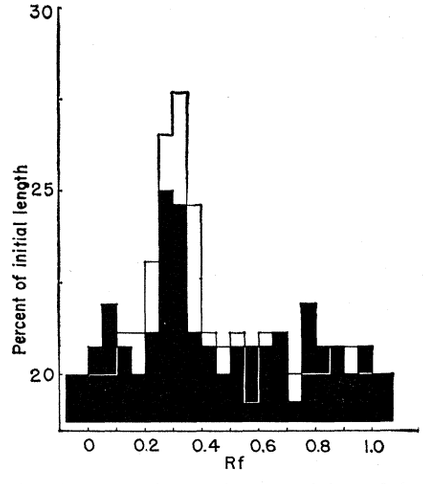

Fig. 1. Growth regulator activity of infected (with *Ditylenchus dipsaci*) and noninfected alfalfa shoot tips. Histograms indicate the distribution of plant growth regulator activity (indicated as percentage increase in growth of the first internode of *Avena*) with increasing R_F values in chromatograms of auxin extracts of infected (shaded) and noninfected (unshaded) alfalfa shoot tips. Tissue extracts were developed with a mixture of water, methanol, and isobutanol.