twine with each other. A hyphal sheath, generally one cell layer thick, usually completely invests the tubes (Figs. 1 and 2). The filaments have cross walls (septa) that are often far apart (121 μm in one case), such that the filaments appear to be coenocytic. The septa are complexly perforate (Figs. 3 to 6). An apparently elliptical aperture (Figs. 5 and 6) occurs in a border that encloses a cavity (Figs. 4 to 6; Fig. 4 is a section close to the aperture). The septal pores superficially resemble the circular bordered pits of conifers, without the pit membrane and torus of the latter. Despite the numerous reports of septate hyphae or filaments in fossil algae and fungi (*20*), including such complicated structures as fungal clamp connections (*21*), there are to my knowledge no previous reports of the occurrence of perforate septa in extinct plants.

Various types of pores and pit connections occur in the septa of the extant red algae (*22, 23*) and the higher fungi (*22, 24, 25*), but these structures are sufficiently different from the septal pores of *Prototaxites* to obviate any close relationship between these taxa. The perforate septa of *Prototaxites*, however, do show a closer resemblance to the dolipore septa of many Basidiomycetes (*22, 24*) or the similar septa of certain Mucorales (*25*) than to those of other modern groups. *Prototaxites* lacks both septal plugs, which are characteristic of the red algae and some fungi (*22*), and septal pore caps (parenthesomes), which are distinctive of many Basidiomycetes (*22, 24*).

The septal pores of *Prototaxites* might represent an "evolutionary experiment," perhaps leading toward the type of septal structure that is characteristic of the more specialized algae and fungi. Alternatively, one should not dismiss the possibility of convergent evolution of pitlike systems that are comparable to those of the tracheary elements of vascular plants, especially since other members of the Nematophytales (*Nematothallus* and *Nematoplexus*) (*7, 8*) have spirally or annularly thickened tubes that simulate the water-conducting elements of the higher plants (*26*). The Silurian and Devonian are now known to have been very active periods of evolution for multifarious plant groups and structures (*26, 27*).

The large tubes of *Prototaxites* (Fig. 2) have no counterparts in the plant kingdom. Any resemblance to sieve-filament elements, trumpet hyphae (filaments), mucilage canals, and thick-walled hyphae of the brown algae, particularly the seaweeds Laminariales and Fucales, is strictly superficial (*28*).

Prototaxites remains a taxonomic and structural enigma. Indeed, mycologists who have seen my portfolio of transmission electron micrographs have denied any relation of *Prototaxites* to the fungi, whereas phycologists have similarly excluded the algae. I believe that the concept of Nematophytales (*7*) as a bizarre group of uncertain relationship seems best supported. The complex ultrastructure of not only the walls of the large tubes of *Prototaxites*, but also of the perforate septa of the filaments suggests that in the nonvascular plants, as in the vascular plants (*16*), very elaborate cell wall structure had evolved by Devonian times.

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18. My findings from electron microscopy largely corroborate Arnold's (*10*) light microscopic data. A detailed account is in preparation.
19. These terms are used interchangeably in descriptive accounts of *Prototaxites*. Hypha, filament, septal pore, and similar terms thus bear no implications with regard to taxonomic relationships.
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29. I thank numerous mycologists, phycologists, and paleobotanists, especially T. L. Phillips, V. J. Vreeland, and S. M. Wolniak for valuable discussions, and C. A. Arnold for the loan of his holotype material of *Prototaxites southworthii* for reinvestigation.

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Premature Specification of the Retina in Embryonic *Xenopus* Eyes Treated with Ionophore X537A

Abstract. *Eyes excised from* Xenopus *embryos at stages 24 to 25 were cultured for 4 to 6 hours in a medium containing the ionophore X537A or in a control medium. The eyes were implanted either upside down or normally in host embryos at stages 28 to 30, and their retinotectal projections were mapped after metamorphosis. Treatment with X537A prevented realignment of retinal axes in eyes implanted into hosts that were capable of producing retinal axial alignment in all control eyes.*

During development, the ganglion cells of the retina project their axons into the brain to form an orderly pattern of connections in the midbrain tectum. The spatial order of the ganglion cells in the anteroposterior (AP) and dorsoventral (DV) axes of the retina is duplicated in the order of axon terminals in the AP and DV axes of the tectum. Although the way this happens is largely unknown, the polarity of the projection in the AP and DV retinal axes is determined (specified) in the early em-

bryo, before the axons grow from the retina to the tectum. In embryos of the clawed frog, *Xenopus laevis*, the retinal rudiment undergoes a change of state, called specification (*1*), during a critical period of about 5 hours, at tailbud embryonic stages [stages 28 to 31 of Nieuwkoop and Faber (*2*)]. The retinotectal projection, mapped electrophysiologically after metamorphosis, was found to be normal after 180° rotation of the eye in early tailbud embryos at or before stage 28. The same operation done a few hours later, at stage 30, produced inversion of the retinotectal projection in the AP axis, and at stage 31 produced inversion of both axes (*3*). Retinal axes of eyes excised from stage 22 to 28 embryos and grown in vitro for 6 to 10 days are stable, and such eyes develop normal morphology and undergo specification in vitro (*4*). The specified state is stable and unmodifiable in eyes maintained in tissue culture for up to 10 days (*5*). Although surgical transection of stage 32 eyes may cause them to revert to an unspecified state (*6*), no conditions have yet been found that prematurely specify eyes before they normally undergo the change at stages 28 to 31.

I now report a condition that precipitates eyes at stages 24 to 25 into the specified state. Hastening of specification resulted from treating excised stage 24 to 25 eyes in vitro with the ionophore X537A (Hoffmann-La Roche). The effect of X537A was discovered during screening of several agents that were expected to alter the development of eyes in vitro. Since X537A increases intracellular free ionized calcium and results in cellular upcoupling in another system (*7*), it was chosen. Its use was based on the hypotheses that (i) many cellular interactions may require communication through permeable intercellular junctions, which depends on the level of cytoplasmic free ionized calcium (*8*), and (ii) intercellular communication between the retina and extraocular tissues and communication among retinal cells may be required for establishing and for reversing the embryonic axes of the retina (*9*).

Xenopus laevis embryos were obtained, their developmental stages were ascertained, they were anesthetized for surgery, and they were reared through metamorphosis (*6*). Eyes were excised from embryos in stages 24 to 25 and maintained for 4 to 6 hours in vitro. The culture medium consisted of an inorganic salt solution (Holtfreter's solution) to which was added bovine serum albumin (600 mg/liter) in the control series or ionophore X537A (30 mg/liter) bound to bovine serum albumin (600 mg/liter) in the experimental series. Both culture media were at *p*H 7.4 to 7.6. Eyes were cultured for 4 to 6 hours and were then implanted in place of one eye of a host embryo between stages 28 and 30 (Fig. 1). The eye was implanted either upside down or normally aligned with the axes of the host.

Table 1. Alignment of anteroposterior (AP) and dorsoventral (DV) retinal axes in implanted eyes.

Culture conditions	Orientation of implanted eye	Alignment of retinal axes in retinotectal map (number of frogs)		
		Normal AP and DV	Inverted AP, normal DV	Inverted AP and DV
X537A	Inverted	2*	1*	11
Control	Inverted	8	1*	0
X537A	Normal	8	0	0
Control	Normal	5	0	0

*Anomalous results

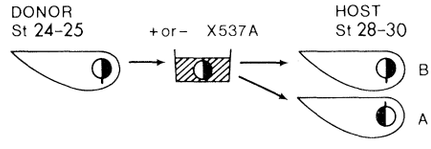

Fig. 1. Unspecified eyes from *Xenopus* embryos in stages 24 to 25 were cultured in the presence or absence of X537A for 4 to 6 hours and then implanted either upside down (*A*) or in a normal orientation (*B*) into host embryos in stages 28 to 30.

After metamorphosis, the retinotectal projections were mapped from the transplanted and the normal eye in each animal by a method described elsewhere (*3, 6*). In the mapping procedure, a platinum-iridium microelectrode was moved to a series of positions 100 μm or 150 μm apart on the tectum; at each electrode position the optimal location of a stimulus in the visual field was determined. The resulting retinotectal map describes the direction of the AP and DV axes of the retina and shows whether the retinal axes are properly aligned with the body axes. In each animal the retinotectal map of the normal eye was an internal control for the axis orientation of the map of the implanted eye. In all cases, the axes of the retinotectal map of the unoperated eye were normally aligned with the axes of the body.

In the control experiments, all eyes that had gone through the in vitro step without ionophore treatment developed normal retinotectal maps (Table 1). This showed that realignment of the retinal axes of the inverted control eyes had occurred and that the conditions in vitro and the surgical manipulations had not prevented realignment of the retinal axes in the control eyes transplanted upside down.

Eyes treated with X537A and reimplanted normally gave rise to normal retinotectal maps in all cases. Therefore, the X537A had not prevented development of the eye, outgrowth of optic nerve fibers, or formation of a normal retinotectal map. However, when eyes were implanted upside down after treatment with X537A, inverted retinotectal maps developed in 11 of 13 cases. Thus the retinal axes in eyes treated with X537A at stages 24 to 25 were not realigned when the eyes were implanted upside down in stage 28 to 30 hosts. The ionophore evidently makes the eye at stages 24 and 25 refractory to the influences of the host, in which one or both retinal axes can be realigned in all control eyes.

Three anomalous results are indicated by asterisks in Table 1. Realignment of both retinal axes occurred in two animals with eyes treated with X537A and of the DV axis occurred in another. Either the ionophore did not affect the eyes or the eyes had recovered from the effect in time to allow realignment of one or both axes. One control animal with eye inversion had inversion of the AP retinal axis only. In normal development the AP retinal axis is specified before the DV axis; in this case the interaction between the implanted eye and the host may have been delayed until after the AP axis but before the DV axis was irreversibly specified.

The results are consistent with the hypothesis that X537A, by causing cellular uncoupling (*7*), prevents cellular interactions that are necessary for reversal of retinal axes in an inverted embryonic eye (*9*). However, the results do not give any direct evidence about cellular mechanisms. Indeed, like other studies of retinal specification (*3-6, 9*), these results have only helped to characterize the transition from the unspecified to the specified state of the retina as a whole without revealing the mechanisms of the change in individual retinal cells. Nevertheless, by showing that X537A can precipitate a change of state in embryonic eyes without producing other developmental abnormalities, these results provide evidence for a specific effect of the ionophore on a developing system.

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Vascular and Brain Dopamine β-Hydroxylase Activity in Young Spontaneously Hypertensive Rats

Abstract. *Dopamine β-hydroxylase activity was higher in mesenteric vessels, adrenal glands, and serum of 3-week-old spontaneously hypertensive rats but lower in the locus coeruleus than it was in the control Wistar-Kyoto rats. The results support the concept that the nervous system is an important regulator of blood pressure.*

It has long been thought that the sympathetic nervous system plays an important part in the regulation of blood pressure in patients with hypertension, but there is no firm evidence of excessive activity of the sympathetic nervous system in development and maintenance of essential hypertension. Dopamine β-hydroxylase (DBH) (E.C. 1.14.2.1) is secreted by the process of exocytosis from sympathetic nerve endings with norepinephrine (*1*). Therefore, DBH in blood was thought to serve as an index of the activity of the sympathetic nerves (*2*). However, results on the plasma level of DBH in patients with essential hypertension were contradictory—several reports indicated an increase in plasma DBH activity (*3*), whereas others showed that this activity does not correlate with blood pressure (*4*). One difficulty in evaluating blood DBH as an index of sympathetic function is the wide variation of DBH activity in man, since this activity is significantly influenced by hereditary factors (*5*). Lovenberg *et al.* (*6*) also reported wide variations in DBH activity in sympathetically innervated organs and brain in various strains of rats.

Studies of blood DBH may be more meaningful in an animal model of hypertension such as spontaneously hypertensive (SH) rats (*7*), since the genetic variations may be small if Wistar-Kyoto rats (from which the SH strain was bred) are used as controls. In fact, we found that both Wistar-Kyoto and SH rats (both strains supplied by Drs. K. Okamoto and Y. Yamori, University of Kyoto) show only slight variations in serum DBH activity, which is fairly constant after the rats are 14 weeks old and is not significantly different between the two strains. However, young SH rats (3 weeks old) were shown to have higher serum DBH activity than Wistar-Kyoto rats of the same age (*8*). One interesting analogy in the blood level of DBH between our study on SH rats and the human study by Stone *et al.* (*3*) is the high activity of DBH before the onset of hypertension and subsequent decrease after development of hypertension.

Since there is considerable controversy over whether DBH levels in serum can be an index of peripheral sympathetic activity, we examined the activity of DBH in peripheral sympathetically innervated tissues and adrenals of 3-week-old SH rats, in order to prove the increase of the DBH level and to investigate the origin of the elevated serum DBH activity in these young rats.

Some central noradrenergic neurons are supposed to have either inhibitory (*9*) or stimulatory (*10*) roles in the regulation of peripheral sympathetic activities; therefore the DBH activity in some noradrenergic regions of the brain was also examined. Tyrosine hydroxylase (TH), which synthesizes dopa from tyrosine as the initial step of biosynthesis of catecholamines and therefore exists in both noradrenergic and dopaminergic brain regions, was also measured in comparison with DBH.

The SH and Wistar-Kyoto rats of the same age were raised under the same conditions. At 3 weeks of age the rats were killed by decapitation; blood samples were obtained by exsanguination and were put into a test tube kept in ice, and the serum was removed. Mesenteric vessels, vas deferens, adrenals, and brain were quickly removed, frozen on Dry Ice, and stored at −80°C. The regions of catecholaminergic neurons (caudate nucleus, substantia nigra, hypothalamus, and locus coeruleus) were dissected out under a microscope from frozen sections of the brain (*11*). The brain tissues were homogenized in 250 to 380 μl of 0.1*M* potassium phosphate buffer, *p*H 7.5, containing 0.1 percent Triton X-100. Mesenteric vessels, vas deferens, and adrenals were homogenized in 2 to 4 ml of the same buffer; the homogenate was centrifuged at 50,000*g* for 30 minutes, and the supernatant was used for the assay of DBH and TH activities. The DBH activity was determined by dual-wavelength spectro-

Table 1. Dopamine β-hydroxylase activity of Wistar-Kyoto (control) and spontaneously hypertensive (SH) rats at 3 weeks of age. Numbers in parentheses are the number of samples. Abbreviation: S.E.M., standard error of the mean.

Source of enzyme	Dopamine β-hydroxylase activity			
	Nanomoles per minute per gram (wet weight ± S.E.M.)		Nanomoles per minute per milligram of protein ± S.E.M.	
	Wistar-Kyoto rats	SH rats	Wistar-Kyoto rats	SH rats
Serum	1.14 ± 0.08 (4)	1.63 ± 0.07* (5)		
Mesenteric vessels	2.2 ± 0.1 (5)	5.8 ± 0.8* (5)	0.30 ± 0.01 (5)	0.76 ± 0.03* (5)
Vas deferens	8.5 ± 0.9 (4)	7.5 ± 0.9 (5)	0.57 ± 0.05 (4)	0.49 ± 0.04 (5)
Adrenals	31 ± 4 (4)	46 ± 3† (5)	0.82 ± 0.07 (4)	1.12 ± 0.08† (5)

*Differs from control (Wistar-Kyoto rats), $P < .01$. †Differs from control (Wistar-Kyoto rats), $P < .05$.

Table 2. Dopamine β-hydroxylase and tyrosine hydroxylase activities in the brain regions of Wistar-Kyoto (control) and spontaneously hypertensive (SH) rats at 3 weeks of age. Values are expressed in picomoles per minute per milligram of protein ± standard error of the mean. Numbers in parentheses are the number of samples.

Region	Dopamine β-hydroxylase*		Tyrosine hydroxylase†	
	Wistar-Kyoto rats	SH rats	Wistar-Kyoto rats	SH rats
Substantia nigra	0‡ (8)	0‡ (8)	51 ± 7 (8)	65 ± 6 (8)
Caudate nucleus	0‡ (8)	0‡ (8)	136 ± 7 (8)	141 ± 7 (8)
Locus coeruleus	249 ± 15 (8)	186 ± 19§ (7)	13 ± 2 (8)	12 ± 2 (8)
Hypothalamus	54 ± 4 (8)	47 ± 4 (8)	15 ± 2 (8)	22 ± 3 (8)

*Substrate, $2 \times 10^{-2}M$ tyramine. †Substrate, $2.5 \times 10^{-3}M$ tyrosine; cofactor, $2 \times 10^{-3}M$ L-*erythro*-tetrahydrobiopterin. ‡Less than the limit of sensitivity of the assay. §Differs from control (Wistar-Kyoto rats), $P < .05$.