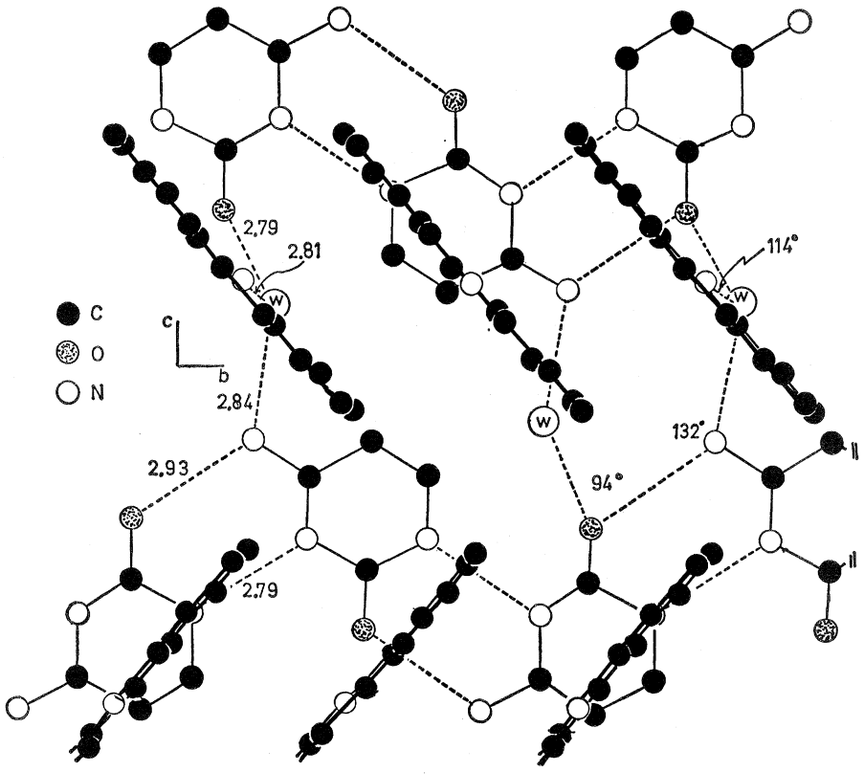


Fig. 1. Projection of molecules down the *a*-axis, showing hydrogen bonds.

respective compounds (*6*, *7*). The acridine molecule appears to be symmetric about the middle ring with none of the differences between the two halves exceeding 1 estimated standard deviation. The acridine and cytosine molecules are essentially planar as the root-mean-square deviations for the respective least-squares planes are 0.003 and 0.005.

From a biological standpoint, the most interesting facet of the structure is the molecular arrangement within the lattice. The packing of the molecules as seen down the *a*-axis is shown in Fig. 1. The cytosine and water molecules form a hydrogen-bonded network perpendicular to *a*. The molecules are held in this sheet-like framework by N1–(H)...N3, N4–(H)...O2, N4–(H)...($H_2$), and O–(H)...O2 hydrogen bonds. The acridine molecules are bound to this layer through a hydrogen bridge between the water and the ring nitrogen. The acridine planes are stacked in a regular fashion perpendicular to the cytosine molecules. These stacked planes are very close to being parallel to one another (there is a 3° distortion), and they have an average interplanar separation of 3.5 Å. The dye-dye stacking thus appears to be governed by normal van der Waals forces, with the specific arrangement of the acridine molecules being influenced by the topological nature of the electron cloud of the ring. The dominant force of this association complex is therefore electrostatic in character, that is hydrogen bonding.

Most interesting is the fact that the predominant features of the packing are consistent with the study of Finkelstein and Weinstein (*7*) on the interaction of proflavin with various synthetic nucleic acids. Their findings with polycytidylate indicated that the interaction is primarily "ionic" and that dye-dye stacking was also present.

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19 February 1968

## Biosynthesis of Pectic Substance in Germinating Pollen: Labeling with Myoinositol-2-$^{14}$C

Abstract. *Growing pollen tubes provide a convenient system for study of the biosynthesis of primary cell wall components of higher plants. Pollen of* Lilium longiflorum *(cv. Ace) which was germinated and grown for periods up to 8 hours in a medium that contained pentaerythritol, a metabolically inert substitute for sucrose, removed myoinositol from the medium and utilized this cyclitol as a precursor of galacturonosyl and pentosyl units for the biosynthesis of pectic substance in pollen tube walls.*

Conversion of myoinositol (MI) to uronosyl and pentosyl units of pectic substance and acidic pentosans has been described (*1*). This process appears to be common among higher plants. Efforts to study the biochemical nature of this conversion in detail have been hampered by lack of a suitable system in which a satisfactory rate of conversion can be obtained during a single working day. Germinating pollen holds promise as such a system (*2*). In this report, uptake and incorporation of label from MI-2-$^{14}$C into pollen tube walls of *Lilium longiflorum* (cv. Ace) are described.

Pollen grains, harvested from greenhouse-grown plants and stored at 2°C for up to 7 weeks, were prewashed and then germinated in Dickinson's pentaerythritol medium (*3*). Dickinson observed that pentaerythritol was not utilized by germinating *L. longiflorum* pollen as a source of metabolizable carbon (*4*). Germination, 60 percent after 2 hours of incubation at 28°C, corresponded to that obtained in medium in which pentaerythritol (0.145*M*) was replaced by sucrose (0.29*M*), but average tube lengths in the latter (1.6 mm in 3 hours) exceeded those in the former (1.2 mm in 3 hours). Average values for percent of pollen germinated and tube length at various times are given in Fig. 1.

In a typical determination, pollen (5 mg) was added to medium (1 ml) containing MI-2-$^{14}$C ($10^{-3}$ $\mu$mole, approximately 30,000 count/min). After incubation, the suspension was filtered in a centrifugal filter holder (Gelman No. 4305) fitted with a type GA-4 membrane. Washed pollen or residue, together with its supporting membrane, was transferred to a liquid scintillation vial that had been prefilled with Cab-O-

Table 1. Distribution of $^{14}C$ between utronic acid and pentose units in fraction II (pectinase hydrolyzate of ethanol-insoluble residue) from *L. longiflorum* pollen germinated and grown in pentaerythritol medium containing myoinositol-2-$^{14}C$. Abbreviation: cpm, counts per minute.

| Incubation (hr) | Carbon-14 incorporated | | Ratio, pentose/uronic acid |
|---|---|---|---|
| | Uronic acid (cpm) | Pentose (cpm) | |
| 4 | 990 | 1820 | 1.8 |
| 6 | 1440 | 3460 | 2.4 |
| 8 | 2120 | 5540 | 2.6 |

Sil (a thixotropic agent) and 15 ml of naphthalene-dioxane scintillation fluid (*5*). The membrane quickly dissolved in this mixture and vial contents were then mixed and counted.

Very little MI was taken up by pollen grains during the first 2 hours of incubation (Fig. 1a). Subsequently, uptake was nearly linear (8 to 10 percent per hour) up to 8 hours, at which time 56 percent of the $^{14}C$ originally present in the medium was recovered in pollen tissue that had been thoroughly washed in unlabeled medium. By contrast, pollen suspended in medium in which pentaerythritol was replaced by 1 percent sodium chloride (*6*) failed to germinate, and removed only a negligible amount of labeled MI from its medium.

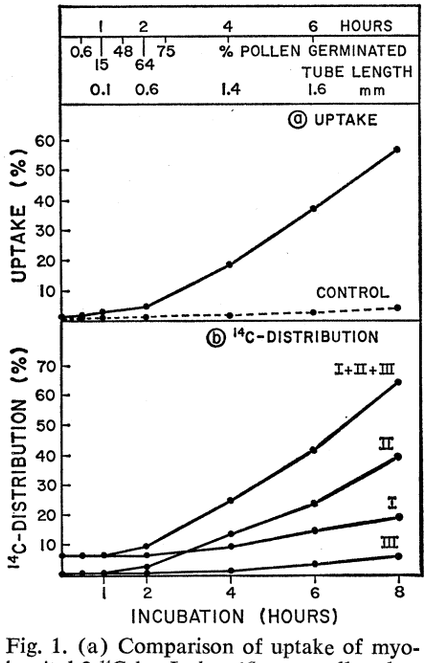


Fig. 1. (a) Comparison of uptake of myoinositol-2-$^{14}C$ by *L. longiflorum* pollen during 8 hours of incubation in pentaerythritol medium and in medium in which pentaerythritol was replaced by sodium chloride. Pollen germination (%) and tube length (mm) at various times are given above the plot. (b) Distribution of myoinositol-2-$^{14}C$ into fractions I (ethanol-soluble), II (pectinase-soluble), and III (pectinase-insoluble) of *L. longiflorum* pollen that had been incubated in pentaerythritol medium. Plots (a) and (b) represent the average of two separate experiments.

To determine the distribution of $^{14}C$ in pollen tissue after germination, samples incubated for periods up to 8 hours were ground in 80 percent ethanol and separated into an ethanol-soluble (I) and an ethanol-insoluble fraction. The latter was further treated with pectinase (*2*) to yield a pectinase-soluble fraction (II) and a final insoluble residue (III). The $^{14}C$ contents of these fractions, plotted as a function of incubation time, are given in Fig. 1b. The small amount of $^{14}C$ recovered in fraction I at 0 hours probably represents MI-2-$^{14}C$ that diffused into pollen grains during the brief period required to transfer the incubation mixture to 80 percent ethanol. As germination and tube growth proceeded, $^{14}C$ in fraction I increased from 6 to 19 percent over 8 hours. Measurable $^{14}C$ appeared in fraction II after 1 hour, increasing to 40 percent after 8 hours. Traces of $^{14}C$, not solubilized by pectinase, were recovered in fraction III. The sum of $^{14}C$ present in all fractions, I+II+III, produced a curve similar to one (Fig. 1a) obtained when germinated pollen was washed with aqueous medium rather than 80 percent ethanol.

Monosaccharide fragments recovered from fraction II after 4, 6, and 8 hours were separated by ion exchange and paper chromatography (*1*). Carbon-14 was present in galacturonic acid, arabinose, and xylose. Glucose and galactose, also present in this hydrolyzate, were unlabeled. Tube growth was accompanied by increasing incorporation of $^{14}C$ into acidic and neutral constituents (Table 1). With time, the ratio of $^{14}C$ in pentose to that in uronic acid increased. Most of the $^{14}C$ present in neutral constituents from fraction II was found in arabinose at 4 hours but longer periods of tube growth resulted in the appearance of labeled xylose as well as two unidentified, labeled constituents that had slower mobilities than arabinose in solvent systems such as ethyl acetate, pyridine, and water (8:2:1 by volume).

If, in a 6-hour incubation, addition of labeled MI to the medium was delayed until 0.5, 1, 2, or 4 hours after the start of incubation, the anticipated decrease in uptake, which was calculated from data in Fig. 1a, was not observed (Fig. 2). Pollen tubes exposed to MI-2-$^{14}C$ during the last 2 hours of growth contained 41 percent of the added $^{14}C$ after filtration and washing as compared with

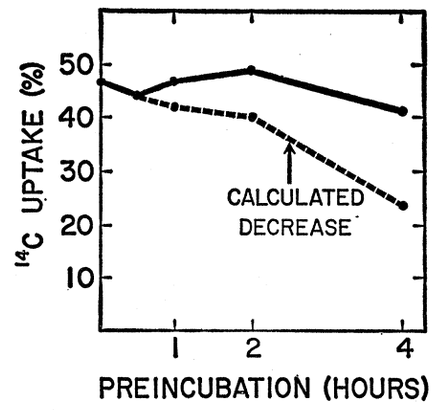


Fig. 2. Uptake of myoinositol-2-$^{14}C$ into *L. longiflorum* pollen which had been preincubated for 0, 0.5, 1, 2, and 4 hours in pentaerythritol medium before addition of label. Each point represents an incubation period of 6 hours. The expected decrease is calculated from data obtained from Fig. 1a.

46 percent for tubes that had been in contact with MI-2-$^{14}C$ throughout the entire incubation. These percentages correspond to an average rate of uptake of 21 percent per hour and 7 percent per hour, respectively.

Present results indicate that MI uptake and incorporation may be described as a three-stage process; first, simple diffusion, as pollen grains take up moisture; second, onset of MI metabolism accompanied by germination; and third, uptake and conversion of MI to pectic substance, as pollen tubes elongate. The MI, supplied exogenously, is utilized in the third stage. A three-phase pattern of respiration with intervals that closely resemble those encountered here has been described by Dickinson for germination and growth of *Lilium* pollen in sucrose and pentaerythritol media (*3, 7*).

Comparative studies with *Petunia* pollen germinated in artificial medium and on pistils reveal fine structural differences related to the biosynthesis of wall substance (*8*). Similar studies with *L. longiflorum* pollen grown on pistils under compatible and incompatible conditions indicate that different biochemical processes may regulate tube wall formation (*9*). To examine pectin biosynthesis by pollen tubes growing through stylar tissue, further experiments are needed in which label from MI is provided to germinating pollen through the intermediate labeling of *L. longiflorum* pistils.

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## Homing Orientation by Olfaction in Newts (Taricha rivularis)


Abstract. *Newts displaced after perfusion of the olfactory epithelium with formaldehyde failed to orient toward home, whereas control animals subjected to oral perfusion with formaldehyde oriented as readily as did untreated controls. Newts with surgically extirpated olfactory nerves failed to home unless their nerves had regenerated. These results strongly suggest a critical role for olfaction in homing behavior.*


The newt *Taricha rivularis* is able to return to its home area after being displaced for distances as great as 8.0 km. This feat is accomplished by direct, oriented migration toward the home area, regardless of its compass direction or the ruggedness of the intervening mountain terrain (*1*). Moreover, newts take correct initial homeward headings upon leaving the release site, even when the displacement distance is as great as 12.8 km (*2*). The maximum distance at which *T. rivularis* can orient its initial migratory movements has yet to be established, though we have found that the farther a newt is displaced, the more likely it is to leave the release site in a random, nonoriented direction. Since blinded animals can home successfully (*3*), it seems unlikely that vision is essential for orientation, although the possible existence of alternate photosensory systems has not been tested.

The experiments we report here provide evidence that in these animals olfaction plays a major role in initial orientation and in the eventual return to the home site.

In the first experiments designed to assess the importance of olfaction in homing, we effected anosmia surgically by extirpating a section of the olfactory nerves. Of 617 such operated newts displaced from Pepperwood Creek to Jim Creek (about 3.2 km displacement distance), only 15 are known to have returned home. These were killed and dissected, and each proved to have regenerated olfactory nerves. The control group for this homing study consisted of 692 normal animals that experienced the same displacement. Of these, 564 were later recaptured at their home sites along Pepperwood Creek. Daily patrols of the stream and frequent collections in the land traps adjacent to Pepperwood Creek preclude the possibility that large numbers of animals could have returned undetected. Although the total return of operated newts was too small to permit any broad conclusions, it is at least clear that not one newt with unregenerated nerves was recaptured at the home location.

In similar experiments designed to test initial orientation rather than complete homing performance, we compared surgically treated anosmic newts with a control group in which an identical opening was made in the skull but the olfactory nerves were left intact. Members of both groups showed random orientation at the release site. Since in the salamander the cerebral hemispheres are predominantly olfactory in function (*4*), the sham operation may have produced anosmia through traumatization of this region. Thus, though the surgical experiments gave unclear results, they did suggest that interventions involving olfactory regions of the central nervous system produced deficits in the ability to take correct initial headings and to complete a homing performance.

We were thus encouraged to attempt more controlled and rigorous lesions in the olfactory system, this time testing initial orientation. We rendered newts anosmic by perfusing the nasal cavities with a 10 percent solution of formaldehyde. A blunted hypodermic syringe was inserted into each external naris, and the solution was injected until it flowed freely from the internal nares into the oral cavity. Excess formaldehyde in the oral cavity was immediately flushed away with fresh water. Histological examination of animals treated in this way showed that the olfactory epithelium had become necrotic.

In another large group of newts only the oral cavity was perfused with 10 percent formaldehyde, which was immediately flushed out with fresh water. A third group was used as a standard with which to compare initial orientation.

All were collected from the same segment of stream and released on land about 1.2 km upstream (Fig. 1). Displacements were made early in the breeding season and each release consisted of approximately equal numbers of anosmic animals and treated controls selected randomly from the total collected each day. The normal control group had experienced the same displacement and recapture routine 1 year earlier. All three groups were marked distinctively by coded toe clipping: 502 anosmic animals, 451 treated controls, and 617 normal controls were released.

Recapture fences were constructed of ¼-inch mesh hardware cloth, arranged so as to funnel migrating newts into escape-proof traps. They were arranged predominantly on the steep, wooded hillside that forms the south bank of Pepperwood Creek in Sonoma County, California. Three fences (30.48 m long) were spaced 41.1, 91.4, and 219.4 m from—and perpendicular to—the stream (Fig. 1). We built a fourth fence on the north side of Pepperwood Creek on flat, open terrain where little migration of newts occurs. Complete four-fence networks of this type were located 228.5 m downstream and 228.5 m upstream from the release point. Captures in two additional fences, one in a densely wooded area farther upstream on the north bank and a comparable one farther downstream augmented the number of recaptures in the main fence networks. In the present tests we made all of our displacements from a downstream collecting site (about 1.2 km) (Fig. 1).

The newt's initial orientation movements upon its departure from the release site were measured by comparing the number of captures in the traps upstream from the release site with the number of captures in the downstream traps. The newts were allowed as much as 3 months to choose their migratory direction when leaving the release site.

The reason for conducting these experiments over long periods and in a