95 percent oxygen and 5 percent carbon dioxide in an apparatus similar to that developed by Gibson and McIlwain (*5*). During the recording of electrical activities, the surface of the medium was lowered to the level of the nylon mesh. A pair of stimulating electrodes, consisting of ball-tipped silver wires, was placed on the slice, and stimulation was carried out with 5- to 15-volt square pulses, 0.4 to 0.7 msec in duration. A recording electrode, also of ball-tipped silver wire, rested on the surface of the slice; a reference electrode was put in the medium. The normal medium consisted of (final concentrations): 124 m*M* NaCl, 5 m*M* KCl, 1.24 m*M* KH_2PO_4, 1.3 m*M* $MgSO_4$, 2.6 m*M* $CaCl_2$, 26 m*M* $NaHCO_3$, and 10 m*M* glucose. To replace all or part of chloride ion, propionate or acetate ions were used.

Record 1 of Fig. 1A depicts a potential evoked in normal medium. A single shock generated a negative wave of about 20-msec duration with a short negative deflection superposed on it. When chloride ion was completely replaced by propionate ion (record 3), the same stimulus provoked a long train of seizure discharges with a latency of 40 msec. Individual spikes which composed the seizure train were 3 to 10 msec in duration and varied in size between 0.2 and 6.5 mv. One or two inflections were found on the ascending phase of some of these spikes. In the medium that contained a 13-m*M* concentration of chloride, amplitude of the seizure discharge was much smaller and its total duration was much shorter than these were in a complete absence of chloride ion (record 2). When chloride-free medium was replaced with normal medium, the seizure discharge disappeared immediately (record 4). Besides the seizure discharge described above, several biphasic sharp deflections were generated with a short latency (arrows in records 2 and 3). These deflections also developed concomitantly with reduction of the concentration of chloride ion and were observed consistently in the experiment cited in Fig. 1, but they could be elicited only occasionally throughout the present series of experiments. The stimulus artifacts were larger in records 2 and 3 than in records 1 and 4, though stimulus strength was kept constant. This seems to be due to the change in conductance of the medium. In several experiments, we tried to provoke the seizure discharge in normal medium by increasing stimulus strength up to five times that used to evoke the seizure discharge in chloride-free medium. However, even with such strong stimulation, the seizure discharge was not provoked in normal medium.

A similar seizure discharge was observed when chloride ion was replaced by acetate ion. The seizure discharge was augmented by a 1-m*M* concentration of sodium phenobarbitone but suppressed at a concentration of 3 m*M*. The discharge train had a long refractory period and propagated slowly through the slice at a speed of approximately 1 cm/sec.

As discussed in the introductory paragraph, generation of the seizure discharge in chloride-free medium may be ascribed to the lack of inhibitory processes in the neuronal network. There remains the possibility, however, that removal of chloride ion decreases conductance of the medium, thus reducing the short circuit of stimulus current by the medium. This might increase the efficiency of stimulation so that a single shock produces a train of seizure discharges. This possibility may be excluded by the finding that even with a strong stimulus the seizure discharges could not be provoked in normal medium.

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References and Notes

1. P. Andersen, J. C. Eccles, Y. Løyning, *J. Neurophysiol.* **27**, 592 (1964); J. C. Eccles, *The Physiology of Synapses* (Springer-Verlag, Berlin, 1964), pp. 173–183; M. Ito and M. Yoshida, *Experientia* **20**, 515 (1964).
2. J. Boistel and P. Fatt, *J. Physiol.* **144**, 176 (1958); G. A. Kerkut and R. C. Thomas, *Comp. Biochem. Physiol.* **8**, 39 (1963); A. Takeuchi and N. Takeuchi, *J. Physiol.* **183**, 433 (1966).
3. C. Yamamoto and H. McIlwain, *Nature* **210**, 1055 (1966).
4. H. McIlwain and R. Rodnight, *Practical Neurochemistry* (Churchill, London, 1962), pp. 109–120.
5. I. M. Gibson and H. McIlwain, *J. Physiol.* **176**, 261 (1965).
6. We thank Dr. T. Hirao for his discussion and encouragement, and we thank Drs. K. Iwama and M. Kurokama for reading this manuscript.

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Axonal Delivery of Neuroplasmic Components to Muscle Cells

Abstract. *Substances labeled with phosphate-32 and carbon-14 and applied to hypoglossal nuclei in rabbits traveled down the hypoglossal nerves and after several days began entering only the muscle cells of the tongue. Prevention of axonal delivery on one side caused unilateral labeling of the tongue. Labeled substances delivered by extracellular fluids labeled all cells indiscriminately. The axonal conveyance of neuroplasmic components to peripheral cells may provide a basis for trophic influences of neurons on other cells.*

Maintenance of the axons in peripheral nerves depends on the continual delivery of fresh cytoplasm elaborated in the cell bodies (*1*). The cytoplasm, apparently propelled in a peristaltic manner by the axon (*2*), is continually moved out of the cell body and along the entire length of the axon and all of its branches, supplying them with components that are used in axonal maintenance and activity and that are not (or are insufficiently) supplied by other sources, such as blood or other extracellular fluids and Schwann cells, or by synthesis within the axon. The total volume of neuroplasm may be replaced several times each day (*1*).

Interruption of axoplasmic continuity results in Wallerian degeneration of fibers separated from their cell bodies. After an interval that varies with the length of the distal stump (*3*), degeneration or other trophic changes begin in the muscle or other cells innervated by the interrupted fibers; these changes are clearly distinguished from those induced by interruption of impulses (*3, 4*). We have tested the hypothesis that the trophic dependence of a cell on its innervation is, as in the case of the axon, also based on the continual delivery, by the axon, of substances that originate in the nerve cell. We found that substances (labeled with isotopes) in selected nerve-cell bodies are conveyed down their axons, across the junctions, and into the cells that they innervate (*5*).

We labeled hypoglossal and vagal neurons with P^{32}-inorganic phosphate or C^{14}-amino acids by directly applying solutions of these substances to the posterior tip of the floor of the fourth ventricle in rabbits (1.4 to 2.5 kg) ac-

cording to the method of Miani (*6*). The solutions were deposited in 1-μl portions (each containing 3.5 to 32 μc of radioactivity) at 10- to 20-minute intervals. We assured ourselves that good absorption occurred and that contamination of the cerebrospinal fluid (CSF) had been avoided. A total of 50 to 200 μc were applied to the nerve nuclei in this manner. This report is based on studies on 15 rabbits.

We demonstrated the specificity of our technique in animals killed and examined at various times after the labeling. Scans of the intact animals showed that diffuse labeling rarely occurred and that, when it did, it was slight and transitory. Three-dimensional scanning (*7*) of various nerves excised from the neck showed that, of these, only the vagus and hypoglossal were radioactive. When there had been hemorrhage around the surgical site or flooding of the ventricle with CSF during application of the isotope, radioactivity of the animal was diffuse and specificity was lost.

Radioactivity, as an irregularly shaped wavefront on the three-dimensional scans, advanced along the vagal and hypoglossal trunks at about 5 to 5.5 mm/day, a value comparable to rates of 1 to 11 mm/day reported for other mammalian nerves (*1*) and corresponding well to the rate (5.2 mm/day) of regeneration of the vagus nerve (*8*). Scans of whole organs and autoradiographs of sections (1 to 2 mm) of tissue showed that radioactivity reached the tongue after about 5 days and the atria of the heart after 9 or 10 days. The proximal musculature of the tongue became radioactive earlier and more intensely than the tip. Crushing of one hypoglossal nerve arrested radioactive material at the site of the crushing, where the substance accumulated, and resulted in unilaterally radioactive tongues.

Having shown that radioactive material had been transported to the peripheral tissues only by the nerves, we then used microautoradiographic studies to determine the distribution in the tongue of radioactive substances conveyed by the hypoglossal nerve. Our methods were those described by Kopriwa and Leblond (*9*), in which slides bearing thin sections of tissue fixed, embedded, sectioned, and stained (hematoxylin and eosin) in the usual manner were covered by a thin layer of Eastman liquid emulsion NTB-3. These slides were stored in the dark at 5°C until periodic sampling showed them ready to be developed and prepared for microscopic study.

We dealt with extremely low concentrations of radioactive material in the tongue, in contrast to the amounts that can be introduced by parenteral injection. We therefore cut tissue sections thicker (6 μ) than is desirable for good resolution and still found extended exposure times (up to 5½ months) necessary. However, as we belatedly discovered, we had recovered only a small portion of the radioactivity we had been able to introduce into the nerve cells. Despite the relatively thick sections we could, with careful focusing of the microscope, trace the tracks of silver grains to particular cellular structures, although these relationships are not always distinct in the photographs taken at a single focal level. In order to make the sparse silver grains in the tongue more conspicuous, we have photographed the preparations through phase-contrast optics, which cause the grains to appear as somewhat enlarged bright spots.

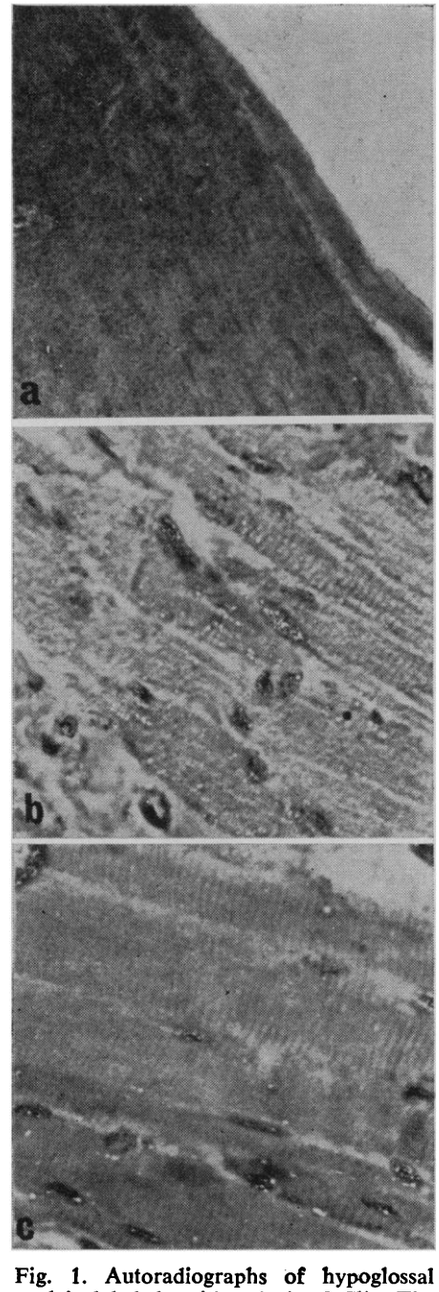

Fig. 1. Autoradiographs of hypoglossal nuclei labeled with glycine-2-C^{14}. The rabbit was killed 9 days after the labeled substance was applied. (a) Longitudinal section of right hypoglossal nerve photographed in bright light (unlike the others, all of which were taken in phase-contrast). (b) Muscle from proximal portion of tongue, right side. Labeling appears as bright spots. Note other cells in same section. (c) Muscle from distal portion of tongue, right side. Note nonmuscle cells. All magnified 512 times.

Figure 1a shows that the radioactive particles were densely packed in the trunk of a hypoglossal nerve whose cell bodies had been labeled 9 days earlier with glycine-2-C^{14}; radioactivity in the nerve sheath was virtually absent. This figure illustrates one of our experimental errors; more time (between labeling and killing the animal) should have been allowed for the nerve to "empty" itself into the tongue.

Figure 1, b and c, are sections from the tongue of the same animal as in Fig. 1a. Labeled molecules were distributed throughout the proximal muscle cells (those in the vicinity of the entry of the nerve), in the fibers, nuclei, and sarcoplasm. Radioactivity on the tip at the same time was still sparse and was apparently concentrated mainly in the nuclei. This difference between base and tip, apparently related to the length of the neural pathway and, possibly, to the density of innervation and to the greater attrition of radioactive substances in the longer passage, was a consistent feature, whether the label was P^{32}-phosphate or C^{14}-amino acids. Labeling of cells other than muscle cells (and, in some sections, branches or axons) was, as in Fig. 1, b and c, negligible or absent.

When neural delivery of labeled substances was limited to one side, there was unilateral labeling of tongue muscle cells (Fig. 2). Sections shown in Fig. 2, a and b, were taken from the tongue of a rabbit in which the left hypoglossal nerve had been cut immediately before the hypoglossal nuclei were labeled with glycine-1-C^{14}. The left side (Fig. 2a) showed early signs

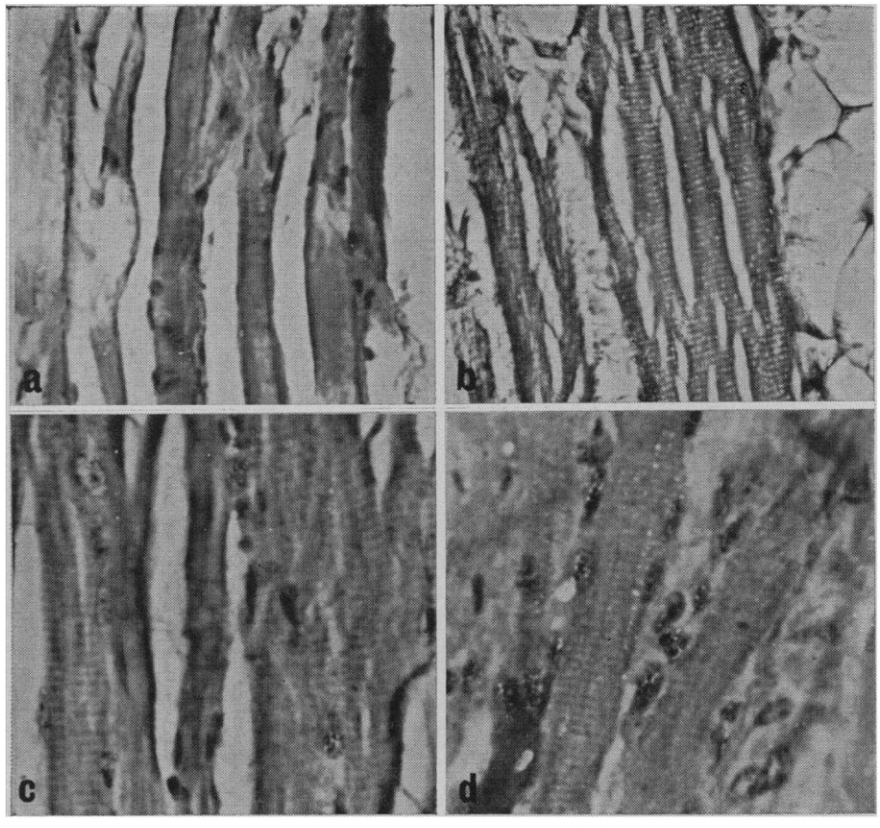

Fig. 2. Unilateral radioactivity of tongue when axonal delivery was prevented on one side. (a and b) Muscle of tongue. Left hypoglossal nerve cut; hypoglossal nuclei labeled with glycine-1-C^{14}. The animal was killed on 8th day. (a) Left side; (b) right side. (c and d) Muscle of tongue. Right hypoglossal nucleus labeled with P^{32}-phosphate. Left hypoglossal nucleus unlabeled. The animal was killed on the 8th day. (c) Left; (d) right.

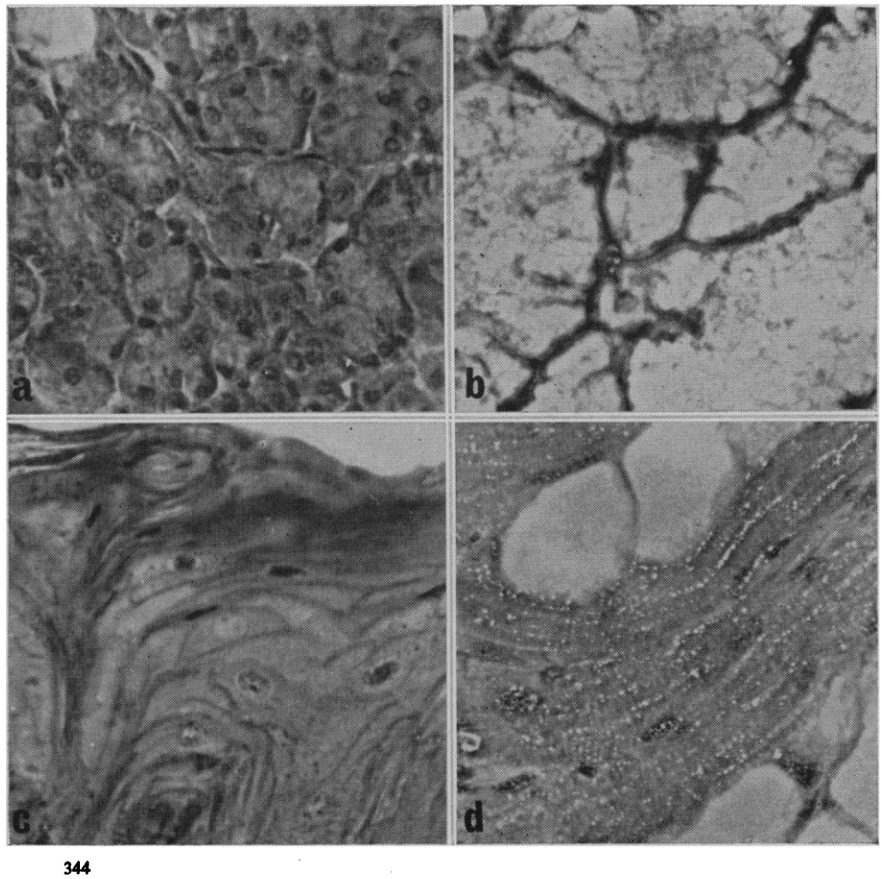

Fig. 3 (a–c). (a) Serous gland, (b) mucous gland, and (c) vallate papilla from tongue shown in Fig. 2d. Note there is no labeling of these tissues. (d) Labeling by way of the extracellular fluid. The cerebrospinal fluid of the fourth ventricle was contaminated with P^{32}-phosphate and the animal killed on the 9th day. Tissues other than muscle are labeled and the pattern of radioactivity in muscle is altered from that shown in Fig. 1–3.

of degeneration and no radioactivity. In the normal, innervated section (Fig. 2b) the muscle cells were strongly labeled. (Figure 2b illustrates a frequent finding in some of our preparations, the transverse alignment of radioactive substances in the muscle fibers. Precise localization awaits autoradiographs of higher resolution.) The results were the same in other experiments in which a small segment of nerve was crushed and axoplasmic continuity thereby interrupted while the gross continuity of the trunk and, presumably, of endoneural fluid spaces was preserved. No labeling of cells other than muscle was found on either side. In two experiments we were able to label the hypoglossal (and vagus) nerve only on one side, while leaving intact the nerves to both sides, thereby eliminating any question of altered physiological state on the denervated side. In one animal (Fig. 2, c and d) only the right hypoglossal nucleus had been labeled (confirmed by microautoradiographs of the hypoglossal nerves) and, correspondingly, only the right side of the tongue was labeled.

Although the tongue consists of a large variety of tissues and cells (muscle, various sensory elements, connective and adipose tissues, epithelium, glands, blood vessels, and so forth) and is, correspondingly, innervated by various nerves (hypoglossal, facial, trigeminal, glossopharyngeal, and sympathetic), only the muscles of the tongue are supplied by the hypoglossal, and we have consistently found that only the muscle cells are labeled in our preparations, as shown by Figs. 1 and 2.

No labeling is evident in other tissues of tongues in which muscle cells were strongly labeled (Fig. 3, a–c). In contrast, when the CSF of one rabbit had become contaminated with the radioactive material, the entire body surface of the animal was radioactive, and all the tissues of the tongue were indiscriminately labeled (Fig. 3d). Another significant feature of this prep-

aration is that, unlike those shown in Figs. 1 and 2, there was considerable radioactivity in interstitial and apparently intravascular spaces.

Our observations indicate that, when radioactive substances were selectively introduced into hypoglossal neurons, these substances, or their derivatives, were conveyed down the axons only to the muscle cells of the tongue and that they reached the muscle cells only via these axons—or very nearly so. The labeled molecules apparently crossed the neuromuscular junction into intracellular components of the muscle. We suggest that the proximo-distal conveyance and intercellular transfer of substances from the nerve cell may underlie the so-called trophic and other long-term influence not based on impulses, of peripheral neurons on the metabolism, function, development, differentiation, growth, and regeneration of the structures that they innervate (*10*).

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References and Notes

1. P. Weiss and H. B. Hiscoe, *J. Exp. Zool.* **107**, 315 (1948); A. J. Samuels, L. L. Boyarsky, R. W. Gerard, B. Libet, B. Brust, *Amer. J. Physiol.* **164**, 1 (1951); H. Koenig, *Trans. Amer. Neurol. Ass.* **83**, 162 (1958); H. Waelsch and A. Lajtha, *Physiol. Rev.* **41**, 709 (1961); P. Weiss, in *Regional Neurochemistry*, S. S. Kety and J. Elkes, Eds. (Pergamon, New York, 1961), p. 220; S. Ochs, D. Dalrymple, G. Richards, *Exp. Neurol.* **5**, 349 (1962); B. Droz and C. P. Leblond, *Science* **137**, 1047 (196→ A. C. Taylor and P. Weiss, *Proc. Nat. Acad. Sci. U.S.* **54**, 1521 (1965).
2. P. Weiss, A. C. Taylor, P. A. Pillai, *Science* **136**, 330 (1962).
3. G. H. Parker, *Amer. Natur.* **66**, 147 (1932); E. Gutmann, Z. Vodička, J. Zelená, *Physiol. Bohemoslov.* **4**, 200 (1955); J. V. Luco and C. Eyzaguirre, *J. Neurophysiol.* **18**, 65 (1955).
4. D. Denny-Brown and C. Brenner, *Arch. Neurol. Psychiat.* **51**, 1 (1944); N. C. Jefferson, T. Ogawa, H. Necheles, *Amer. J. Physiol.* **193**, 563 (1958); E. Gutmann, *Rev. Can. Biol.* **21**, 353 (1962).
5. This work was summarized in preliminary form in I. M. Korr, P. N. Wilkenson, F. W. Chornock, *Fed. Proc.* **25**, 570 (1966).
6. P. Miani, *J. Neurochem.* **10**, 859 (1963).
7. Picker Nuclear Model 610-205.
8. L. Guth and S. Jacobson, *Exp. Neurol.* **14**, 439 (1966).
9. B. M. Kopriwa and C. P. Leblond, *J. Histochem. Cytochem.* **10**, 269 (1962).
10. E. Gutmann, Ed., *The Denervated Muscle* (Czechoslovak Academy of Sciences, Prague, 1962); E. Gutmann and P. Hník, Eds., *The Effect of Use and Disuse on Neuromuscular Functions* (Czechoslovak Academy of Sciences, Prague, 1963); M. Singer and J. P. Schadé, Eds., *Mechanisms of Neural Regeneration, Progress in Brain Research* (Elsevier, Amsterdam, 1964), vol. 13.
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Fungal Morphogenesis: Ring Formation and Closure by Arthrobotrys dactyloides

Abstract. *The formation and the closure of constricting rings by a nematode-trapping fungus were recorded by means of time-lapse cinephotomicrography. Analysis of the film revealed that hyphal rings resulted from a sequence of morphological events not previously described. Cell inflation and ring constriction were induced by touch, increased temperature, and electrical stimulation. The inflation process was not particularly sensitive to metabolic inhibitors and appears to operate without an expenditure of energy on the part of the cell.*

Fungi frequently display morphological change that can be analyzed biochemically (*1*), and nematode-trapping fungi are typical examples. These microorganisms produce specialized structures that function as traps and enable prey to be captured, killed, and consumed (*2*). The most intricate and remarkable type of trap is the so-called "constricting ring." Constricting rings consist of three curved cells on a short two-celled stalk. They are produced at intervals along a fungal filament and usually grow at right angles to it. When a nematode enters, the cells that comprise the ring inflate rapidly and obliterate the opening. The nematode is trapped by occlusion and is later penetrated by hyphae that originate from the ring cells and spread throughout the carcass, absorbing its content.

We employed time-lapse cinephotomicrography to obtain a record of growth, morphogenesis, and predation by various species of nematode-trapping fungi (*3*). The fungi were grown at 28°C on cornmeal extract agar, in microchambers on slides (*4*). They were photographed at the rate of four frames per minute, using a Reichert Zetopan microscope and a 16-mm Bolex camera that were synchronized and automated for time-lapse work (*5*). Analysis of films depicting morphogenesis in *Arthrobotrys dactyloides* (*6*) revealed that the fungal form is under strict control and that constricting rings are produced by the sequence of events illustrated in Fig. 1.

Hyphal branches destined to differentiate into organelles of capture were highly refractile, robust, and readily identified. As they extended, these specialized branches arched and appeared hook-like (Fig. 1, A and B). Growth and curling continued (Fig. 1C), but in no case was a ring formed by the anticipated, simple union of the tip with its supporting branch. Instead, a bud was consistently formed by the more distal of the two stalk cells (Fig. 1D). It formed in opposition to the advancing tip and the two converged and fused to produce a closed ring (Fig. 1, E–G). After anastomosis, the three cells that comprised the ring increased in size and refractility. They attained an individual width of 5 to 8 μ, a length of 20 to 28 μ, and formed rings 20 to 32 μ in diameter. The biochemical basis for curling of fungal filaments has not been ascertained. It may involve localized differences in the rate of synthesis of cell wall material or increased tension within the structural polymer of the wall because of modification of individual components or linkages. The former condition is responsible for the growth toward light of green plants and some

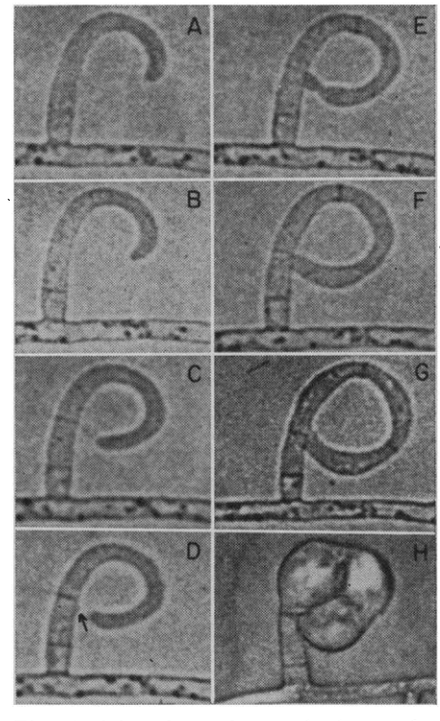

Fig. 1. Ring formation and closure by *Arthrobotrys dactyloides* recorded using time-lapse cinephotomicrography. Rings are initiated as branches that extend to form curved hooks (A–C). The more distal of the supporting cells gives rise to a bud (D) in opposition to the advancing hyphal tip, and the two converge and fuse to produce a closed ring (E–G). Ring cells inflate to triple their normal volume in approximately 0.1 second (H) if induced to do so by touch, increased temperature, or electrical stimulation. An interval of 4 hours was required for this morphological transformation (× 625).