may be a change of electrical charge on the cell membrane during cathodal and anodal stimulation. The cause of this electrotonically induced relaxation awaits further study, but this experiment presents some indication of the effect of electrochemical processes in muscle relaxation (*7*, *8*).

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Environmental Significance of Palynomorphs from Lower Eocene Sediments of Arkansas

Abstract. Spores and pollen present in sediments of the lower Eocene Wilcox group in south-central Arkansas are mixed temperate and tropical genera. The source area is postulated to have been temperate highlands adjacent to a tropical coastal plain. A similar interpretation based on plant megafossils has been made. Hystrichosphaerids and dinoflagellates found in the sediments suggest a depositional environment of brackish water.

One of the most extensively studied fossil floras is that present in the sediments of the Wilcox group, lower Eocene in age, in the Gulf Coastal Plain. This flora has been studied by paleobotanists including Lesquereux (*1*), Berry (*2*, *3*), Ball (*4*), and Brown (*5*). Berry conducted the most extensive study, describing 543 species from 180 genera and 82 families. After considering the corrections and additions to Berry's work, Sharp (*6*) listed 137 genera from the Wilcox flora ". . . whose taxonomic position is known with more or less exactness."

There has been considerable speculation about the environmental significance of this lower Eocene flora. Berry (*2*) considered the flora to be tropical and noted that there were no strictly temperate genera. He speculated, however, that ". . . the Wilcox flora probably seemed to be more tropical in character than it really was." Berry also noted the absence of an accompanying brackish water fauna that he believed should have developed in the upper part of the Mississippi Embayment during the lower Eocene.

Brown (*5*) and Sharp (*6*) interpreted the Wilcox flora as being more temperate in nature than did Berry. They pointed out the presence of a number of temperate genera in the flora, including *Betula, Comptonia, Fagus, Sassafras,* and *Staphylea.*

Sharp (*6*) compared the fossil flora of the Wilcox group with the modern floras of a number of regions. He found that some 60 percent of the genera described from the Wilcox sediments still persist in the southeastern United States, the area in which Wilcox sediments were deposited. Thirty of these genera are, however, restricted to Florida. Sharp stated that approximately 53 percent of the Wilcox genera are present in the present flora of central and eastern China. He found the greatest degree of similarity between the Eocene Wilcox flora and that now present in eastern Mexico, an area of high mesas and neighboring coastal plains. Some 68 percent of the Wilcox genera are present in this area.

Two genera, *Quercus* and *Pinus*, which are important elements in the Mexican flora, had not been reported from the Wilcox sediments when Sharp conducted his study. Despite the absence of these two genera in the Wilcox flora, Sharp concluded that the environmental conditions in the Gulf Coastal Plain during the lower Eocene were essentially like those of eastern Mexico at present.

A recently completed palynological study of sediments from the Wilcox group in central Arkansas (*7*) disclosed the presence of 62 spore and pollen types, including both *Quercus* and *Pinus*. The study is based on 60 samples collected from outcrops of the Wilcox group in Pulaski and Saline counties. From this area Berry had described only 12 genera of megafossils. The pollen of *Pinus* is a common constituent of the microflora, present in amounts ranging up to 10 percent of the total forms in some samples. *Quercus* pollen is less common but is present in most of the samples that were analyzed.

The 62 spore and pollen types found in the Wilcox sediments are a mixture of temperate, subtropical, and tropical genera. These include genera such as *Carya, Engelhardtia, Myrica, Manilkara, Symplocos,* and *Anacolosa.* The environmental interpretation of a warm, humid coastal plain with adjacent highlands, such as Sharp described for eastern Mexico, is thus supported by both the megaflora and the microflora. Hystrichosphaerids and dinoflagellates are also present in small numbers in the samples. These fossil groups, considered significant of marine or brackish-water environments, support Berry's postulation that the plant remains and the enclosing sediments were deposited in a brackish-water environment.

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12 June 1961

Hole Drilling by Octopus

Abstract. Octopus bimaculoides and *O. bimaculatus* can drill holes in the shells of their molluscan prey, through which they appear to inject a paralyzing venom.

Octopuses are found among the marine littoral fauna throughout most of the world. Because of their appearance, edibility, and behavior, they are well known to maritime peoples. It has long been known that a large part of the food of the octopus consists of shelled mollusks (*1*). The suggestion has been made that the octopus obtains this food by exerting greater force than the prey can withstand. Bartsch (*2*), writing of *Octopus vulgaris* Lam., says, "Presumably he opens a mussel by attaching some of his suckers to the two valves of the shells and then applying pressure until the valves give way." Phillips (*3*), discussing California species of octopus, states that "abalone divers tell of occasionally finding an octopus patiently exerting pressure on an abalone. The abalone eventually tires, even as an oyster gives in to a starfish. The octopus can also open mussels in this manner."

The investigation reported here was prompted by the observation that the empty shells of small abalones appear-

ing in an aquarium tank, which contained abalones and an octopus, had in them small holes of an unusual nature. This octopus, probably *Octopus bimaculoides* Pickford and McConnaughey (*4*), and three others—one certainly *O. bimaculatus* Verrill, one certainly *O. bimaculoides*, and a juvenile which might have been either of the two species—were isolated in large jars supplied with running sea water. Various shelled mollusks were introduced, and after the octopus had eaten the shellfish, the empty shells were removed and examined. The octopus ate individuals of the following species: *Haliotis fulgens* Philippi, *H. cracherodii* Leach, *Tegula funebralis* Adams, *Chione fluctifraga* Sby., *C. undatella* Sby., *Mytilus edulis* L., *M. californianus* Conrad., *Nassarius fossatus* Gould, and *Ischnochiton conspicuus* Carpenter. In most cases there was a hole of characteristic, somewhat oval, shape in the shell (Fig. 1). The hole drilled through a shell 1.4 mm thick by a 48-g octopus was 0.8 mm long and 0.6 mm wide at the top, narrowing at the bottom to an opening which was 0.3 mm long and 0.2 mm wide. The size of the hole is somewhat dependent upon the thickness of the shell and on the size of the octopus, but even a very large octopus produces a hole which is generally smaller than those typically drilled by carnivorous snails, and clearly distinguishable in shape. A boring snail consumes its prey by inserting its proboscis through the hole and rasping the flesh of the prey. The minute size of the octopus hole makes it impossible for the prey to be eaten in this manner. We believe that the octopus injects venom through the hole, causing an abalone or chiton to release its hold on the substrate, a bivalve to relax the adductor muscles, or a gastropod to weaken the closure of its operculum.

Microscopic examination of the hole, beak, and radula indicated that the beak was not of the necessary shape or sharpness to drill the hole. It appeared that this drilling was done by the radula. The following quotation is relevant: ". . . the line of centrals [teeth of the radula] seen through the open beaks [of *Octopus vulgaris*] resemble the teeth of a circular saw, and doubtless its purpose is that of finishing the work begun by the beaks of carving crustaceans. . . . When we examine the extracted radula we find signs of very hard wear in the front portion, greatest in the central teeth, which are often worn flat, but also severe on the laterals" (*5*).

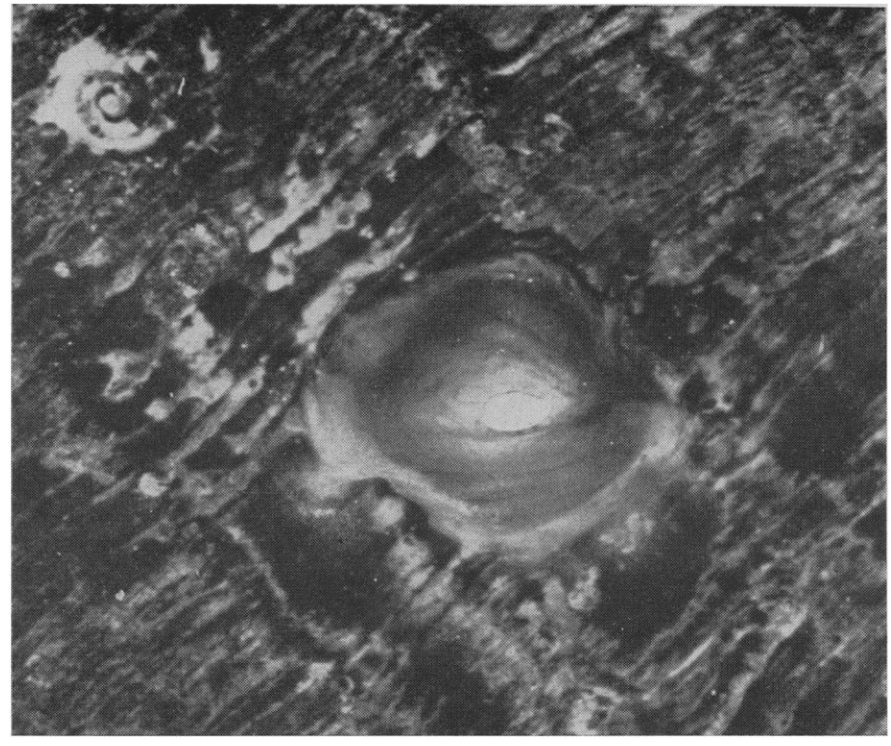

Fig. 1. Hole drilled in an abalone shell (*Haliotis fulgens*) by a large *Octopus bimaculatus* (arm spread, 1.5 m) (about × 18). [A. O. Flechsig]

Once we were fortunate enough to see an octopus in the act of drilling a hole in an abalone (*Haliotis fulgens*). The octopus weighed 48 g, the abalone 19 g. The entire process, from initial attack by the octopus to detachment of the abalone from the aquarium wall, took just 3 hours. The abalone was immediately taken from the octopus. There was a typical hole in the shell. The abalone was placed in another aquarium, where it readily attached itself to the wall and crawled away actively but was unable to hold on strongly. It is almost impossible to detach normal abalone of this size with the fingers, but this individual could be readily detached. Three days later it could still be detached easily, but 2 weeks later it was normal.

It has long been known that the octopus has a venom elaborated chiefly by the posterior salivary glands (*6*), the duct from which opens just beneath the radula (*7*). The use of this venom for paralyzing crabs is also well known (*8*).

An extract was prepared (*6*) of the combined posterior salivary glands (0.8 g, fresh weight) which had been excised from two freshly killed *Octopus bimaculoides;* frozen and stored at − 18°C. In a room at 6°C the glands were ground with sand in a mortar, sea water (9 parts) being added in increments to the homogenate. The suspension was centrifuged, and the opalescent supernatant, kept in ice water, was injected into crabs and abalones. In the crab *Pachygrapsus crassipes* Randall the injections produced death and paralytic symptoms similar to those described elsewhere for octopus venom (*8*); such symptoms were not produced by sea water, nor were they produced by a similarly prepared extract of octopus muscle tissue. Three abalones, *Haliotis fulgens* (19, 20, and 33 g, fresh weight, respectively) each received an injection into the foot muscle of 0.025 ml of extract per gram of abalone. Three other abalones (22, 25, and 31 g, respectively) were similarly injected with sea water. The latter three animals retained their ability to adhere tightly to the aquarium wall and to right themselves when placed on their backs. The abalones injected with salivary extract were easily removed from the aquarium wall and did not right themselves when turned over. They died within 2 days.

Sometimes the octopus does not drill a hole. In such cases it appears to open the bivalve or to pull an abalone off the substrate by force. We are not certain what factors are involved in the choice of the method of feeding, but it seems reasonable to assume that the octopus may try force first and, failing in this, may then drill a hole.

These observations suggest that *Octopus bimaculoides* and *O. bimaculatus* may prey naturally on shelled mollusks by drilling a hole and injecting venom. We have found in nature numerous empty shells of the species *Tegula funebralis* Adams, *Chione undatella* Sby., *Protothaca staminea* Conrad, and *Haliotis* spp. with holes identical in shape to those made by *Octopus* in the aquarium (*9*).

Note added in proof: Dr. S. Stillman Berry has brought a paper by Fujita (*10*) to our attention. Fujita discovered that *O. vulgaris* on the coast of Japan bored holes into the shells of the pearl oyster, and he suggested that venom was injected to weaken the adductor muscle.

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Translocation of Streptomycin from Coleus Leaves and Its Effect on Rhizosphere Bacteria

Abstract. Autoradiographs showed that when streptomycin is applied to leaves of coleus plants (1000 to 3600 micrograms per plant) the antibiotic or some by-product is translocated laterally and downward. The translocated material does not alter rhizosphere microorganisms quantitatively. Gram-negative rhizosphere bacteria, however, are suppressed through the 8th day after treatment.

In studies of the ecology of rhizosphere microorganisms, it became of interest to produce selective quantitative and qualitative alterations in the populations of these organisms in their natural habitat. Two general approaches are available. The first involves treating the entire soil since the location of the rhizosphere cannot be determined beforehand. The second involves application of an active material to the plant (or seed) in a manner that will permit downward translocation of the active material to the roots. The second approach has been used previously (*1*). When Bordeaux mixture, a nonselective agent, was applied to leaves of beans, it increased the levels of copper and reduced the numbers of bacteria in the plant rhizosphere.

The possibility of utilizing selective materials of complex molecular structure was tested by applying streptomycin to leaves of coleus plants (*Coleus blumei* Benth) whose rhizosphere microflora was then periodically sampled. Streptomycin and coleus were selected because (i) this antibiotic apparently moves downward in coleus stems (*2*), (ii) it persists in some plants for as long as 8 weeks (*3*), and (iii) it is degraded relatively slowly in soil (*4*). Also we found that streptomycin is not phytotoxic to coleus at levels of application in excess of 4000 μg per plant.

The movement of antibiotics in plants apparently varies with the plant and the antibiotic employed (*5*). In cherry trees (*6*) and peach trees (*7*) streptomycin moves very little or not at all from treated leaves. In hops (*8*) and beans (*9*) it moves upward with ease, in detectable amounts when applied at low dosages, but not downward. In apples and pears it moves both upward and downward when applied at relatively high dosages (*10*).

All the plants we used were grown in unsterilized soil from split-node cuttings from a single parent plant. The plants were of uniform age and size and generally had four to six fully developed leaves.

Streptomycin sulfate at 10,000 μg/ml of 1-percent glycerol was applied, at rates which varied in the several experiments from 0.03 to 0.10 ml per application, in a band across the widest part of the leaf or leaves. The mixture of antibiotic and glycerol was then spread with a thin glass rod over the upper leaf surface except near the petiole. This method prevented any accidental external movement of the streptomycin down the petiole to the stem. Material in excess of that contained in 0.10 ml of solution was applied to the leaves in equal portions as described above. The leaves were allowed to dry between applications to prevent dripping from the leaf tip.

Table 1. Effect of streptomycin applied to leaves of coleus on the percentage change in numbers of Gram-negative rhizosphere bacteria at different times after treatment. In test 1, 1000 μg of streptomycin sulfate were applied to each plant in one application; in test 2, 3600 μg of streptomycin sulfate were applied to each plant in three applications.

Time after treatment (day)	Change in Gram-negative rhizosphere bacteria (%)		
	Test 1	Test 2	Mean
0	0	0	0
4	−37	−20	−28
8	−22	−22	−22
12	−9	+9	0

The results of a series of experiments are reported here. In the first experiment 1000 μg of streptomycin sulfate were applied to each plant in one application. In the second, 3600 μg were applied to each plant in three applications of 1200 μg each. In both cases the treatment was equally divided between the two leaves arising at a single node. Control plants were treated identically with 1-percent glycerol solution without streptomycin. Four replications were used throughout.

The plants were carefully lifted from the soil 4, 8, and 12 days after treatment, and the superfluous soil was removed from the roots by tapping the plant sharply several times. The roots and adhering soil were immediately transferred to sterile water blanks. After rotary agitation for 30 minutes, dilutions were prepared and dilution plates were made by adding 10 ml of agar medium to 1 ml of the final suspension in a petri plate. Bacteria and streptomycetes were enumerated on egg-albumin agar, and fungi were enumerated on a vegetable juice–dextrose–yeast extract agar containing antimicrobial agents (*11*). The total numbers of the three groups of microorganisms did not

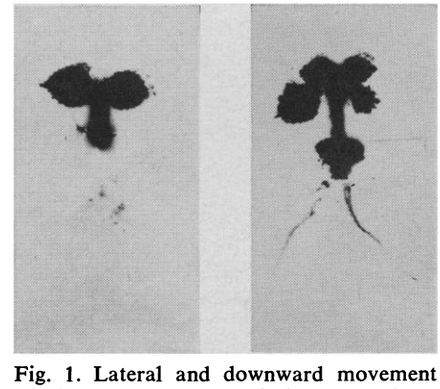

Fig. 1. Lateral and downward movement of C^{14}-labeled streptomycin in coleus plants 6 hours (left) and 24 hours (right) after application of 3000 μg of labeled streptomycin to the upper left-hand leaf of each plant (about × 1/5).