activity several millimeters below the infection plane.

In contrast with these observations, acid phosphatases decreased following inoculation with either isolate; activity decreased proportionately from the surface, but the decrease was more pronounced with the pathogenic isolate. Another acid phosphatase of fungal origin was detected in tissue inoculated with the pathogenic isolate; activity was highest in the first layer. It was concluded that the acid phosphatase of the host was inactivated in the first layer, whereas the fungal (pathogenic) acid phosphatase retained activity.

Malic dehydrogenase activity decreased proportionately from the surface following inoculation with nonpathogenic or pathogenic isolates. Similar changes in esterase activity were observed in the susceptible host; in the resistant host, activity was very low and difficult to detect.

The increases and decreases in enzyme activity inferred that the fungus induced changes in the host's protein synthesis or enzyme activities several millimeters away from the site of infection. The similarity in the changes induced by the nonpathogenic isolate to those by the pathogen suggested that inoculation with a nonpathogenic isolate might cause changes in a susceptible host that would induce immunity or resistance.

To examine this possibility, sweet potatoes (susceptible) were cut into sections 2 cm thick and divided into three groups. One group was incubated without treatment, a second was treated with sterile culture medium, and the remainder were inoculated with mycelial fragments of the nonpathogenic isolate of *C. fimbriata.* The following day, sections from the three groups were inoculated with mycelial fragments from the pathogenic isolate. Other sections from each group were also inoculated with the pathogen on additional days (up to 6 days). The results (Fig. 2) indicated that the earlier inoculation with the nonpathogenic isolate did indeed produce an acquired immunity or resistance toward the pathogen; immunity or resistance was limited to a few cell layers from the plane of inoculation with the nonpathogenic isolate. The immunity could be induced on only one side of a section and to some extent on one half of the same side. This suggested that the acquired immunity was a local response related to a stimulation by the nonpathogenic isolate of the host's defense mechanism in those few cell layers immediately adjacent to the surface. When this resistant layer was removed by scraping just before the second inoculation, the tissue became susceptible. Likewise, cutting through this resistant layer allowed the fungus to reach the underlying susceptible tissue and to grow. The sections that were cut only, treated with sterile culture medium, or inoculated with the nonpathogenic isolate, all developed callus on the surface. All subsequent inoculation with the pathogenic isolate on surfaces which had been cut only, or had been treated with sterile culture medium, resulted in infection, whereas surfaces which had been previously inoculated with the nonpathogenic isolate remained resistant or immune. This suggests that callus formation alone was not the cause of resistance. Filtrates or homogenates of cultures of either isolate did not induce the immunity or resistance when applied to the cut surface.

The concept of acquired immunity has been associated with phytoalexin formation (*10*), but acquired immunity without association with a phytoalexin has been reported (*11*). In sweet potato, Uritani has associated resistance with ipomeamarone formation (*2*) which has been classed as a phytoalexin (*12*). Ipomeamarone formation does not seem to account for the induced immunity reported here; ipomeamarone could not be detected with Ehrlich reagent in the tissue inoculated with the nonpathogenic isolate whereas it was easily detected during this same period in the tissue inoculated with the pathogen. Mueller (*10*) treated white potatoes with an avirulent strain of *Phytophthora* and obtained resistance to a virulent strain; resistance was associated with a necrotic reaction. In contrast, the acquired immunity to *C. fimbriata* in sweet potato was not associated with necrotic reaction.

Evidence from this investigation would favor an explanation of the mechanism of resistance in terms of altered protein synthesis or enzyme activity. The stimulus for this change has been shown to be supplied by a nonpathogenic fungus. The rate of response to such a stimulus may be a factor which determines whether a host is resistant or susceptible. Alterations in enzymes and isozymes have been observed in other plant diseases (*13, 14*). Whether changes in isozymes in plants play a role in plant diseases is yet to be established, but the changes observed in this investigation would appear to favor this concept.

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Crustacea: A Primitive Mediterranean Group also Occurs in North America

Abstract. *A new species of the genus* Monodella *(class Crustacea, order Thermosbaenacea) has been found in a cave pool in Texas. Previously the order was believed to be restricted to the Mediterranean area. The new evidence indicates that the order is older than was believed, or that invasion of fresh or brackish water has occurred more than once within the order.*

Until the recent discovery of a new species in Texas, the primitive crustacean order Thermosbaenacea was throught to be restricted to the area of the Mediterranean Sea. These small crustaceans (1 to 3 mm long) lack

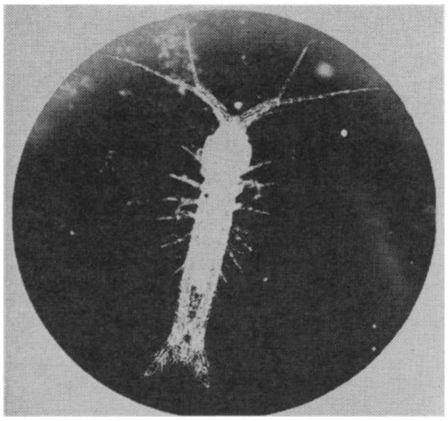

Fig. 1. A female, 3 mm long, of the new species of *Monodella* found in Texas.

pigment and are eyeless. They occur in a salty hot spring in Tunisia (*1*), in waters [slightly brackish (*2*) or fresh (*3*)] of two caves very near the coast of Italy, in coastal, interstitial salt water, and brackish water of a nearby cave, in Yugoslavia (*4*), and in a salty hot spring on the shore of the Dead Sea (*5*). The monospecific genus *Thermosbaena* is found just south of the Mediterranean. Three of the four previously known species of the other genus, *Monodella*, live on the north shore; the fourth is found about 100 km east of the Mediterranean.

Because of this distribution and because four of the five previously known species within the order live in salty or brackish water, many investigators have supposed that the order only recently left the sea and that adaptation of the known species to their present habitats was associated with fluctuation of sea level in the Mediterranean during the Quaternary, perhaps specifically during regression of the sea in the late Pliocene (*6*).

Six specimens of the new species of *Monodella* have been captured in cool freshwater of Ezell's Cave at San Marcos, Hays County, Texas (*7*) (see Fig. 1). The cave is 200 km from the nearest coastal salt water and 180 m above sea level. The discovery suggests two hypotheses. The first is that invasion of freshwater by these crustaceans from the sea occurred independently and recently in at least two widely separated parts of the world, that marine members of the order then became extinct (or have not yet been found), and that the adaptations accompanying the separate invasions of fresh or brackish water have involved little morphological change. The alternative hypothesis is that the Thermosbaenacea arose sufficiently long ago to have dispersed between the land masses of the eastern and western hemispheres, either through fresh or coastal waters of land bridges (perhaps they persisted through the breakup of the Gondwana land mass, if such a land mass existed).

The known present distribution of the order indicates a restriction to highly specialized habitats which contain relatively few species and in which relict representatives of other groups have commonly survived. Hence these animals may be the scattered remnants of a once widespread group. Since knowledge of the distribution of cave- and spring-dwelling animals is very limited, further speculation at present is unlikely to be profitable.

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Epidermal Papillomas with Virus-like Particles in Flathead Sole, Hippoglossoides elassodon

Abstract. *Epidermal papillomas frequently occur on the external surfaces of flathead sole,* Hippoglossoides elassodon, *in the waters of San Juan Islands, Washington. Virus-like particles and associated granular bodies, also of possible viral nature, are commonly found in the neoplastic epithelial cells of these tumors. Similar structures are not observed in normal epidermis.*

Epidermal papillomas occur frequently on flathead sole, *Hippoglossoides elassodon*, collected from the waters of San Juan Islands, Washington (*1, 2*). Similar neoplasms have occasionally been observed in other species of Pleuronectidae (*2, 3*). In this report we describe some gross, microscopic, and ultrastructural features of the epidermal papillomas of the flathead sole.

All fish were collected with an otter trawl in East and West Sounds of Orcas Island during July and August 1963. For light microscopy, tumors and normal skin were fixed in 10 percent formalin or Bouin's fluid, embedded in paraffin, and stained with hematoxylin and eosin. For electron microscopy, the same tissues were fixed in 1.33 percent OsO_4 buffered with *s*-collidine, embedded in Epon 812, and cut into thin sections on an LKB ultrotome equipped with a diamond or glass knife. Sections stained with lead were examined under an RCA EMU 3G electron microscope.

Spreading, cauliflower-like skin tumors were present on 73 fish (7.6 percent) of a total collection of 964 (Fig. 1). The tumors were located anywhere on external surfaces and individual fish possessed one to four tumors. By light microscopy, the tumors were typical epidermal papillomas composed of thick folds of epidermal cells supported on connective tissue stroma. The epidermal cells were ovoid to polygonal, with eosinophilic cytoplasm. Nucleoli were often conspicuous.

By electron microscopy, numerous virus-like particles (*a* in Fig. 2*B*) averaging 44 mμ in diameter were observed in the cytoplasm of most neoplastic cells, located in the space outside the sacs of endoplasmic reticulum. Similar virus-like particles were observed at the edges of osmiophilic, homogeneous cytoplasmic bodies which averaged about 150 mμ in maximum dimension (*b* in Fig. 2, *A* and *B*). These bodies with their associated virus-like particles were interpreted as possible loci of virus formation; similar bodies and particles were never seen in normal epidermis. The virus-like particle appeared to be enclosed by a single membrane 60 to 70 Å thick, and often had an eccentrically placed, internal, osmiophilic granule measuring 60 Å in diameter, which was tentatively interpreted as a nucleoid.

The epidermal cells of the papillomas contained a second type of osmiophilic granular body not observed in normal epidermis (*c* in Fig. 2, *A* and *B*). This body, enclosed in a membranous sac, measured 160 mμ to 200 mμ in diameter. In electron micrographs it appeared to be composed of numerous, small, dense granules. The center was often less dense than the edge, with sometimes a suggestion of radially oriented capsomeres that were