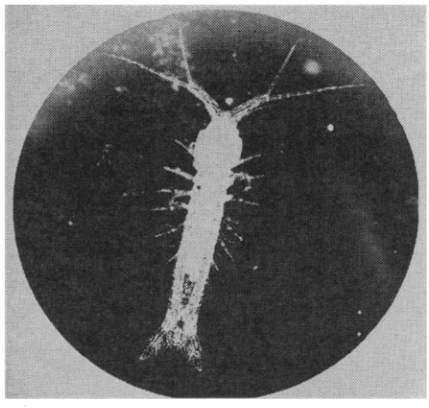

Fig. 1. A female, 3 mm long, of the new species of *Monodella* found in Texas.

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

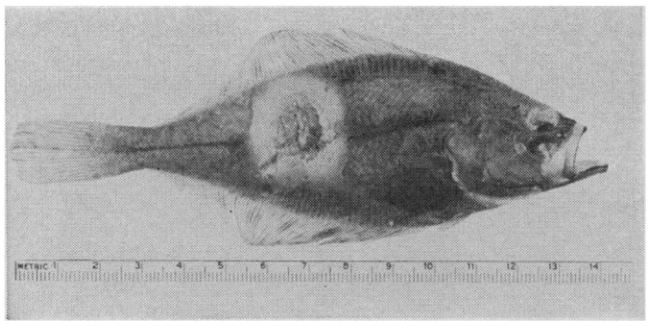

Fig. 1 (above). Epidermal papilloma on the right (pigmented) side of a flathead sole, *Hippoglossoides elassodon*, with a warty center and plaque-like edge.

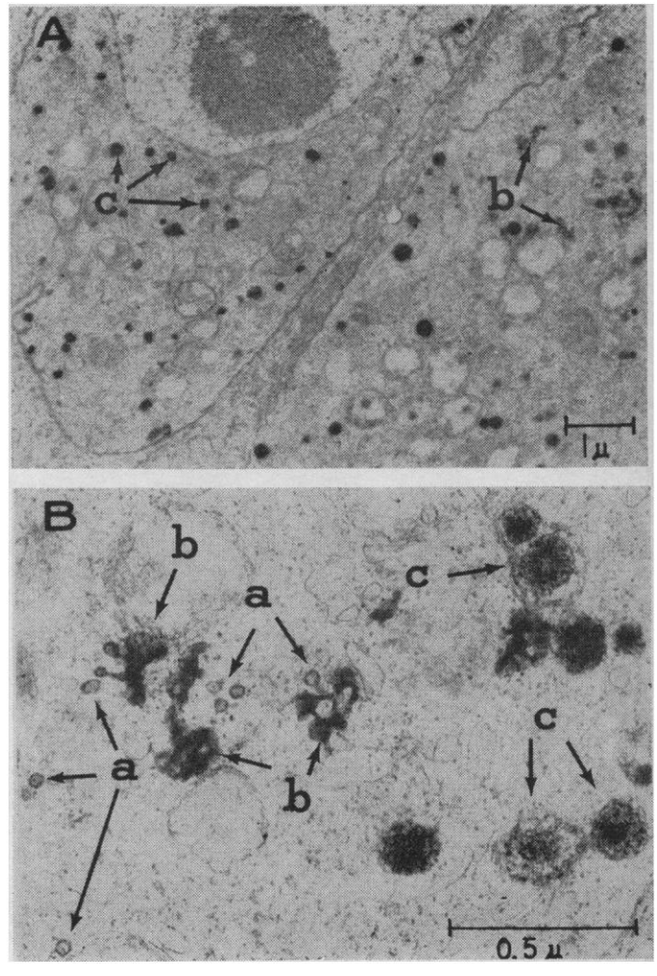

Fig. 2 (right). *A*, Electron micrograph showing parts of several epithelial cells of an epidermal papilloma: homogeneous bodies (*b*) and granular bodies (*c*). The smaller virus-like particles shown in Fig. 2*B* are not resolvable at this magnification (× 9000). *B*, Electron micrograph of part of an epithelial cell of an epidermal papilloma: virus-like particles (*a*), homogeneous bodies (*b*), and granular bodies (*c*). (× 50,000)

poorly shown in tissue sections. Although its nature remains unknown, this granular body may be related to the virus-like particle mentioned above; both types of particles occurred simultaneously in neoplastic cells, but were never found in normal epidermis. Suggestive transitional forms between the two types of particles were sometimes observed, suggesting that the granular body is a developmental form of the smaller particle of virus-like morphology. Neither type of particle was ever found in the nuclei of tumor cells.

Epidermal papillomas transmissible by viruses or cell-free filtrates have been described in several species of mammals, including cottontail rabbits, cattle, horses, and man (*4*). We can find no proven instance of virus-transmitted epidermal papillomas in fish. Lymphocystis disease (*5*, *6*), apparently transmissible by a virus, produces characteristic tumor-like masses in marine and freshwater fish, but the tumors are thought to be composed of giant, connective tissue cells; their neoplastic nature is questionable. "Fishpox," epidermal hyperplasia of fishes, and epidermal papillomas of fishes have been thought to be of viral etiology (*6*). It should be pointed out that the histopathology of these diseases, especially of fish papillomas, is similar to that of the epidermal papillomas of the flathead sole. Epidermal papillomas on flathead sole that were kept in the laboratory during our study appeared to be progressive; furthermore they did not regress as carp-pox has been reported to do (*6*).

The morphology of the two types of particles described herein is unlike that of any known virus, including the Shope papilloma virus of rabbits (*7*), the common wart virus of humans (*8*), and the lymphocystis virus of marine and freshwater fish (*9*). In the Shope papilloma and common wart, the virus particles are located within the nuclei of epidermal cells at the base of the neoplasm; near the surface, however, the particles are observed throughout the cell. In the epidermal papillomas of the flathead sole, the particles described herein were found only in the cytoplasm, suggesting that virus-like particles in the cells of epidermal papillomas can be located in the nucleus or cytoplasm, and that the location varies in different species. The virus-like particles and associated granular bodies in the epithelial cells are ultrastructurally very different from melanin granules of the flathead sole (*10*). Finally, it is recognized that the virus-like particles may bear no causal relation to the epidermal papillomas.

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Antibody Plaque Formation by Normal Mouse Spleen Cell Cultures Exposed in vitro to RNA from Immune Mice

Abstract. *Suspensions of normal spleen cells from nonimmune mice were treated in vitro with RNA extracted from spleen cells from donor mice immunized 4 days previously with sheep erythrocytes. Subsequent incubation of the RNA-treated cells in tissue culture medium at 37°C for several days resulted in a marked increase in the number of localized zones of hemolysis ("antibody plaques") in relation to the number of viable cells plated in agar containing sheep erythrocytes and complement. Nonimmune cells maintained in tissue culture medium did not form plaques after incubation with either RNA from immune mice or ribonuclease-treated RNA from immune mice, or with RNA from nonimmune donor mice, or from donors immunized with chicken erythrocytes or bovine serum albumin.*

The localization of hemolysis in semisolid medium is the basis of a method for rapid estimation of the number of antibody-secreting cells present in a suspension of lymphoid cells (*1*). This procedure has been used successfully to study the kinetics of hemolysin formation by lymph node and spleen cells from experimental animals after primary or secondary immunization with sheep erythrocytes (*1*, *2*).

Cohen and Parks recently reported that spleen cells from normal B_6AF_1 mice acquired hemolysin-forming activity after incubation in vitro with RNA extracted from spleens of isologous donor mice immunized with sheep erythrocytes (*3*). They observed a three- to fivefold increase in the number of antibody plaques formed by nonimmune cells plated in agar immediately after 30 minutes incubation with RNA from immune donors. Before incubation, there were on the average 20 to 25 plaques formed with the total number of spleen cells plated from five nonimmune mice. After treatment with splenic RNA from 20 immunized donor mice, the number of plaques increased to an average of 90. Prior treatment of RNA from immune donors with ribonuclease inhibited this effect.

Spleen cells from nonimmune NIH albino mice also acquire hemolysin-forming activity after brief incubation with RNA extracted from spleen cells from donor NIH mice immunized with sheep erythrocytes (*4*). The number of specific antibody plaques formed by normal spleen cells increased 10- to 20-fold after treatment with RNA from immune donors. Maximum plaque formation occurred after treatment with RNA obtained 4 days after donor immunization. RNA obtained from donors immunized with bovine serum albumin or treated with actinomycin D prior to and after injection with sheep red blood cells did not induce plaque formation in normal cell suspensions (*4*).

This report confirms and extends the foregoing observations and describes results obtained with tissue cultures of normal mouse spleen cells after treatment in vitro with RNA from donor mice immunized with sheep erythrocytes. For these experiments, spleen cell suspensions from normal NIH mice were incubated in vitro with: (i) RNA extracted from spleen cells from mice immunized with sheep red blood cells; (ii) ribonuclease-treated or deoxyribonuclease-treated RNA from immune donors; and (iii) RNA from nonimmune donors or from donors injected with an unrelated antigen such as bovine serum albumin or chicken erythrocytes. The immune donor mice were 6- to 10-week-old NIH males injected intraperitoneally with 0.1 ml of a 20 percent suspension of fresh, washed, sheep red blood cells in buffered saline. Four days later, at the peak of antibody plaque formation, serum samples were obtained by retro-orbital puncture. Hemagglutinins and hemolysins to sheep erythrocytes were determined by standard serial dilution procedures with a microloop technique with 0.025 ml volumes of both serum dilutions and 0.5 percent sheep red blood cells (*5*).

Those mice with serum titers of 1:512 or higher were killed, and their spleens were immediately frozen at −60°C with dry ice. Prior to freezing, spleen samples, approximately 5 mm^3, were chosen at random and used for preparation of cell suspensions to determine the number of antibody-forming cells per million viable nucleated donor spleen cells (*1*). RNA was extracted from the frozen spleens as follows (*6*). The spleens were weighed and suspended in three volumes of cold 88 percent phenol containing 4.3 mg of bentonite powder per milliliter. To this mixture was added an equal volume of cold 0.02*M* phosphate buffer, *p*H 7.2, containing 0.01*M* sodium EDTA (ethylenediaminetetraacetate). The mixture of spleen cells, phenol, and buffer was homogenized at 0°C for 10 minutes with a high-speed VirTis tissue homogenizer. The aqueous phase was separated by centrifugation at 2400*g* for 15 minutes in the cold and was freed of phenol by five extractions with equal volumes of cold ethyl ether. Two volumes of absolute ethanol were added to the concentrated extract. The precipitate was recovered by centrifugation at 1200*g* for 10 minutes in the cold. The resulting pellet was suspended in 5.0 ml of cold sterile phosphate buffer. The RNA concentration was estimated by ultraviolet spectrophotometry and by the orcinol reaction (*7*).

As controls, 40 μg of ribonuclease or deoxyribonuclease was added to 1.0 ml samples of extract, and the mixtures were incubated at 26°C for 30 minutes and then chilled in an ice bath. A separate 1.0-ml sample was heated at 100°C for 10 minutes. As additional control preparations, RNA-rich material was extracted from spleens from nonimmunized normal mice and from mice injected 4 to 6 days previously either with chicken erythrocytes (0.1 ml of a 20 percent suspension injected intraperitoneally) or with bovine serum albumin (BSA) (0.2 ml of an emulsion of 10.0 mg of