000 rad sufficient for complete inhibition of cell multiplication. Doses in the range of 10^6 rad resulted in complete cell death in five of eight cultures; recovery of cell multiplication in the remaining three cultures occurred about 2 to 3 weeks after irradiation. After recovery from irradiation, all cultures were serially transferred at intervals of 1 to 3 weeks for a total of at least six transfers. At each transfer, 0.1 ml of the ILEM cell suspension was transferred to 1.9 ml of fresh, enriched medium. In all instances, cell density of about 200,000 cells per milliliter was attained at the termination of the experiment.

In parallel experiments, ILEM cells irradiated with similar doses while suspended in isotonic saline showed the same response to radiation as those suspended in enriched medium. On the other hand, unirradiated ILEM cells, suspended for 3 hours in enriched medium previously irradiated with 10^6 rad, propagated at the same rate as those suspended in unirradiated medium. Enriched medium showed no protective effect on normal Lich-1 cells. In order to determine whether this phenomenon of extreme resistance to irradiation was in any way related to the source of radiation (cobalt-60 γ-rays compared to 250-kv (peak) x-rays), ILEM cells were irradiated by the same x-ray generator, and the factors used were those employed for normal Lich-1 cells (with the exception of a target-culture distance of 20 cm, above 10,000 r). No significant effects on growth characteristics were demonstrated with graded x-ray exposures ranging from 1,000 to 130,000 r.

The mechanism for the development of this unusual radioresistance in ILEM cells is not known. It is improbable that the radioresistance is due merely to the quantitative decrease in DNA content of these cells; on the basis of general relationships between radiation lethality and cellular DNA content reported for various cells and viruses (*9*), a 90-percent reduction in cellular DNA alone should result in only a 10- to 20-fold increase in the radiation dose required to kill cells. Chemical radioprotective agents in vitro, on the other hand, have thus far been shown to cause only a two- to fourfold increase in radioresistance (*10*). Radioresistant mutant strains of cultured cells and bacteria have been isolated, but these show a maximum increase of tenfold in radioresistance, and there is nothing to suggest that the mechanism of radioresistance in these instances is related to that observed in ILEM cells (*11*). The fact that infection with the lipovirus has produced a line of cells whose radioresistance is increased 1000-fold suggests, rather, that some basic alteration in a radiosensitive "target" in the cell has taken place as a result of changes induced by this cytopathic agent.

It appears that in ILEM cells doses up to 100,000 rad do not significantly affect the basic metabolic functions governing cellular synthesis and growth. However, the primary effect of ionizing radiation in most rapidly dividing cultured cells is the inhibition of mitosis, and the mitotic process itself is normally very sensitive to ionizing radiation. Certain differences have been observed in the mechanism of cell division in ILEM cells. First, preliminary studies indicate that the multiplication of ILEM cells is not delayed by colchicine at a concentration of $10^{-4}M$. (Division of Lich-1 cells is inhibited at the concentration of $10^{-6}M$.) Secondly, it has been shown that the division time of the ILEM cell is only about 6 minutes, as compared to 15 to 20 minutes for Lich-1 cells, though their cell-doubling times are roughly similar (*3*). Possibly, infection with the lipovirus has sufficiently altered the process of cell division such that it is no longer sensitive to ionizing radiation, and cell death results from a different mechanism requiring considerably higher radiation doses.

Further work is needed to elucidate the metabolic changes which give rise to radioresistance in lipovirus-altered cells. A system in which such gross alterations in cellular radiosensitivity can be selectively produced, however, offers another means of studying the mechanisms of radiation damage in human cells.

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Trematode Parasitism and Polymorphism in a Marine Snail

Abstract. Velacumantus australis *is a common Australian snail which harbors several larval trematodes. Many populations have a small proportion of banded shells. Analysis of three samples from a coastal lake shows that banded snails are less likely to harbor larval trematodes than unbanded snails.*

The Australian mud whelk *Velacumantus australis* occurs in large numbers on sandy mud flats of many coastal lakes, estuaries, and sheltered bays on the eastern and southern coasts of Australia. It commonly harbors several species of larval trematodes, and the incidence of infection in adult snails of some populations is as high as 50 percent (*1*).

Many populations have a small proportion of individuals with banded shells, in which a white band about 1 to 2 mm wide is present in the lower part of each whorl. Banding is found in snails of all ages, including very young juveniles which do not carry larval trematodes. Banded snails are scattered randomly among the other snails.

Three samples of *V. australis* were collected from different areas of Lake Burril, 145 miles (233 km) south of Sydney, during December 1964 and January 1965. A total of 25,155 snails were collected, of which 3.92 percent had banded shells. The frequency of banded snails tended to be highest in the youngest juveniles and lowest in old adults.

Random samples of 815 banded and

Table 1. Numbers of banded and unbanded snails infected with larval trematodes.

Condition	Banded	Unbanded
Infected	46	140
Uninfected	769	1415
Total	815	1555

1555 unbanded snails of various ages were dissected and examined for larval trematodes. Representatives of the families Heterophyidae, Echinostomatidae, Philophthalmidae, and Schistosomatidae were present. Heterophyids accounted for 80 to 81 percent of the infections in both banded and unbanded snails. Small juveniles are not infected. The results of the dissections are given in Table 1. They show that the incidence of larval trematodes in banded snails (5.64 percent) is significantly less than that in unbanded snails (9.00 percent). ($\chi^2_1 = 8.34$; $p = .01$ when $\chi^2_1 = 6.64$).

Banding is probably genetically controlled, and it would appear that a lowered susceptibility to larval trematode infection is associated with it. Parasitized snails are usually sterile and may have a shortened length of life (*2*). Other evidence suggests that banding may be a balanced polymorphism (*2*); if this is so, parasitism is partly responsible for its maintenance.

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Gymnodinium breve: Induction of Shellfish Poisoning in Chicks

Abstract. *Oysters exposed to laboratory cultures of the fish-killing dinoflagellate,* Gymnodinium breve, *are toxic when fed to chicks. The ecological significance of this result is interpreted in relation to the scarcity of well-documented reports of shellfish poisoning in higher animals from those areas of the Gulf of Mexico in which* G. breve *"blooms" occur.*

Ecological and epidemiological studies have strongly implicated two dinoflagellates, *Gonyaulax catenella* (*1*) and *G. tamarensis* (*2*) as causes of shellfish poisoning along the Pacific and Atlantic coasts of North America. Studies with cultures have clearly established *G. catenella* as a specific agent for shellfish poisoning along the Pacific coast (*3*). Despite the periodic occurrences of catastrophic mortalities of various marine animals associated with "blooms" of dinoflagellates (red tides) in the Gulf of Mexico (*4*), the incidence of shellfish poisoning in humans definitely traceable to eating molluscs from this region is strikingly low. At least two fish-killing dinoflagellates, *Gymnodinium breve* (*5*) and *Gonyaulax monilata* (*6, 7*) occur in this area.

Our interest in reasons for the infrequency of reports of shellfish poisoning from the Gulf area has led us to consider several possibilities: (i) failure of molluscs to feed on these dinoflagellates; (ii) failure of toxin (or toxins) to accumulate in molluscs; (iii) lack of susceptibility of higher vertebrates to the particular toxin (or toxins); and (iv) infrequent occurrence of toxic dinoflagellate "blooms" in areas of shellfish (mollusc) production.

Recently, in perhaps the only well-established case on record, *G. breve* has been thought to cause human illness. In December 1962 several persons suffered a mild illness, suggestive of a paralytic poisoning, after eating oysters taken from Sarasota Bay, Florida, during the occurrence of a red tide (*8, 9*). Extracts of oysters from the suspected batch were toxic to kittens and mice (*9*). Moreover, McFarren and co-workers extracted a ciguatera-like poison from the oysters, clams, and *G. breve* cultures. Eldred and co-workers observed a rough correlation between the toxicity of oyster and clam extracts to mice and concentrations of *G. breve* at several locations along the west coast of Florida during 1963 (*8*).

However, it has been noted that "blooms" of dinoflagellates, including species of *Gonyaulax*, may coincide with *G. breve* "blooms" in the implicated area (*10*). Thus the evidence is primarily circumstantial and has only associated *G. breve* with an unusual occurrence of shellfish poisoning. The need for controlled studies to determine the toxicity of molluscs exposed to a single species of dinoflagellate suggested this work. The results of three such experiments are presented in this report.

In our studies assays were conducted by controlled feeding of oyster (*Crassostrea virginica*) tissues to test animals rather than injecting or feeding extracts, since the use of extracts of such tissues might circumvent the influence of some natural process on a potential toxin. The molluscs were held in the laboratory in noncirculating, aerated aquariums containing sea water for 1 to 4½ days before use. We used mass unialgal cultures of *G. breve* grown in artificial sea water media (*7, 11*) at 25° ± 1°C and approximately 8100 lu/m² (750 ft-ca) illumination.

Experimental oysters in 2 to 4 liters of culture were exposed to from 1.2 ×

Table 1. Toxicity to chicks of oysters exposed to *Gymnodinium breve.*

No. chicks	Mean weight (g)	Type of oysters fed to chicks	No. *G. breve* per gram of chick*	Time (hours) to	
				Equilibrium loss†	Death
		Experiment No. 1			
2	100	Control	0		
2	100	Exposed	1×10^4	6, 17	
		Experiment No. 2			
2	60	Control	0		
2	60	Exposed	3×10^5	3, 10	10, 22
		Experiment No. 3			
4	33	None	0		
4	33	Control	0		
4	33	Exposed	9×10^4	1½, 2, 2½, 5	6, 6½, 8, 17

* Calculated as follows:

$$\left(\text{No. } G.\ breve \text{ filtered} \times \frac{\text{wt. exposed oysters fed per chick}}{\text{total wt. exposed oysters}}\right) / \text{ chick wt.}$$

† Birds in this condition were lying on side or back.