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Effects of Algae Extracts from New York/New Jersey Coastline, USA on Cultured Mammalian Cells

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Production of natural substances by algae from the marine environment that have toxic effects on humans and other organisms has been well established. Perhaps best known among these is the neurotoxin produced by the dinoflagellate Gonyaulax polyedra which causes red tides in tropical waters (Atlas 1984). However, many of these substances are also antibacterial, antiviral and/or antineoplastic (Scheuer 1990). The toxic nature of these substances, which include phenols, terpenoids, acrylic acid, and compounds containing bromine, has made many of them unfit for development for pharmaceutical purposes (Burkholder and Sharma 1969).

The development of alternatives to animal testing have allowed researchers to test various compounds of natural or synthetic origin for toxicity to cells in culture. This growth of in vitro toxicity testing is due to the advantages of lower cost, faster results and greater potential for standardization (Frazier et al. 1989). In vitro toxicity testing has become a system of choice for the testing of consumer products and potential pharmaceuticals. Cell culture systems from a variety of mammalian species can be used to show changes on a cellular or biochemical level, after exposure to various concentrations of chemicals. The survival of living cells after exposure to chemicals can provide useful information related to toxicity of test compounds (Naughton et al. 1989).

The survival of mammalian cells in culture is dependent upon continued DNA replication followed by cytokinesis. Most normal or mildly transformed cells will attach to a glass or polystyrene surface and move to fill all possible surface area by mitosis. This in vitro locomotion of cells can be altered by serum deprivation

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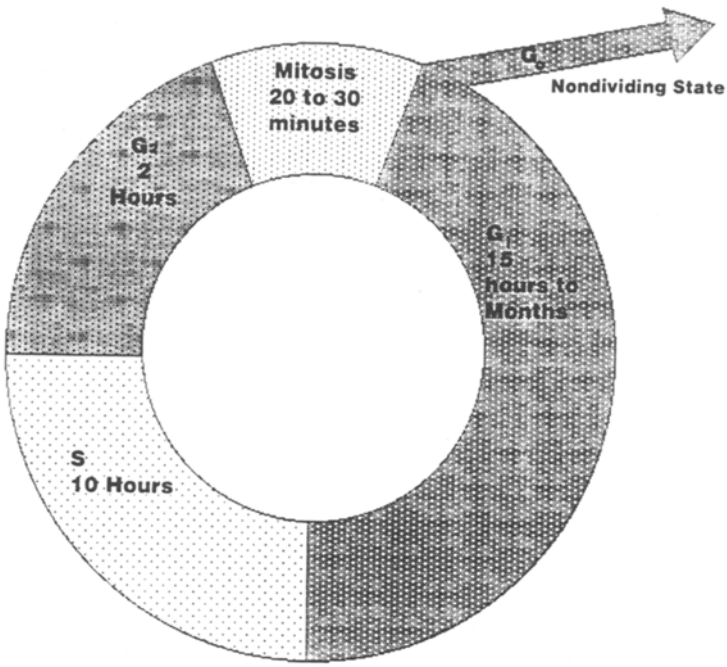

Figure 1. Graphic representation of cell cycle showing G_o brought on by serum deprivation.

which moves cells from the cell cycle G phase to G_o (Figure 1). Mitosis can be resumed by entry back into the cell cycle after this serum dependent physiological stress by returning cells to serum rich media (Paternoster et al. 1981).

Cell attachment and spreading over available surface is observed microscopically, and the morphology of cells is described in qualitative terms. Studies involving time lapse video photography have shown that alterations in normal cell locomotion during times of toxic exposure can be measured by electrical current between cell and substrate (Giaever and Keese 1992). One can view the results of exposure to toxic materials by the failure of cells to move through mitosis and reach confluence.

Lustigman et al. (1992) have shown that certain marine algae from the Atlantic coast of Northern New Jersey/New York have well defined antimicrobial activity. This gives promise for possible new natural sources of antimicrobial pharmaceuticals for human health since these extracts of Phaeophyta were previously seen to display bactericidal activity on human clinical bacteria (Lustigman and Brown 1990). In this study, we report the effects of two of these more promising antimicrobial extracts on cultured Chinese Hamster Ovary Cells. An <u>in vitro</u> technique involving

cultures was used for determination of cell toxicity. The recovery of mitosis/locomotion after monolayer scraping of confluent cultures and observation of plating efficiency of these cells were used as markers in preliminary testing of known antimicrobial algae extracts. This study was performed in a test system in which serum levels were altered to mimic a variety of degrees of physiological stress.

MATERIALS AND METHODS

Chinese Hamster Ovary (CHO) cells grown in Ham's nutrient medium F-12 purchased from GIBCO, Grand Island, New York. Ham's F-12 was enriched with 1 ml Glutamine, and 10 ml Fetal Bovine Serum. Penicillin/Streptomycin and Kanamycin were added in 1 ml amounts to prevent bacterial and fungal contamination. All procedures were performed in an aseptic manner and all flasks and pipettes were sterile and disposable. CHO cells were subcultured after one week of growth and allowed to grow to confluence in Lab-Tek chamber slides with two separate compartments on a glass slide (Figure 2). Upon reaching confluence, monolayers were scraped free of cells in .2 cm x .2 cm outlined squares. Ham's F-12 was then freshly prepared again with .25%, 1%, 5% and 10% serum. Regrowth rates in control cultures of varying serum levels were observed and compared with cultures in the same serum percentages with algae extract. These samples of seaweed were collected monthly from March to September 1988, at three locations: two in Sandy Hook, N.J. and one at Montauk, L.I., N.Y.

Experimental chamber slides were exposed to extracts of either Fucus spirilis or Fucus vesiculosis in predetermined concentrations of 40 ug/ul. All algae extracts used in this study were separated into 10 g portions and blended with water in a Waring blender for 10 minutes. Samples were centrifuged for 10 minutes at 10Xg. The supernatant was filtered and allowed to evaporate until dryness. The total dry weight was obtained. The extract was resuspended in the appropriate solvent to give a final concentration of 40ug/uL. According to the method reported by Lustigman (1992), these samples were resuspended in water and applied to filter paper disc (2 cm in diameter). Both regrowth percentage in scraped boxes and plating efficiency were determined for CHO controls as well as CHO cells exposed to sections of disc containing 10 ul/ug of extract from F. spirilis and F. vesiculosis. All tests were performed in chamber slides containing media with .25%, 1%, 5% and 10% serum. Chamber slides were incubated at 37° C with 10% CO_2 in balanced air

Figure 2. Chambers for monolayer growth and scraping.

for 24 hours. Cellular regrowth or wound healing was recorded as percent confluence of each .2 cm x .2 cm box. Percent attachment (plating efficiency) was also examined after 24 hours. Phase contrast microscopy was used throughout this study.

RESULTS AND DISCUSSION

The data in Table 1 show that under physiological conditions determined by percent fetal bovine serum, CHO cells show variable ability to "heal" the wounded or scraped area of confluent monolayers under the influence of algae extracts. In repeated experiments F. spirilis shows less toxicity as determined by greater plating efficiency and ability to move by mitosis into areas free of attached cells than F. vesiculosis. As shown in figures 3 and 4 both control and F. spirilis extracts show close to 100% restoration of confluence of CHO cells in highest serum amounts. F. vesiculosis exposure never reached significant restoration of confluence. These fibroblast-like cells are stimulated to return to the mitotic cycle by the in vitro wound. This appears to mimic the in vivo healing of wounds by the release of fibroblast growth factor (Rifkin and Moscatelli 1989). Below 1% serum, cells are forced into G_0 and removed from the active cell cycle. These data might also suggest that small increases seen in restoration of cell attachment in "wound" areas in the low serum cultures are due to nutrient potential of the extracts themselves (Scheuer 1990).

Lustigman (1992) showed significant zones of inhibition when algae extracts from both F. spirilis and F. vesiculosis were used on lawns of Micrococcus imfimus,

Table 1. Percent confluence and percent cell attachment in scraped area of chamber slides.

	Fucus spirilis		Fucus vesiculosis	
SERUM CONFLUENCE LEVEL	% CONFLUENCE WITH ALGAE	% CONFLUENCE CONTROL	% CONFLUENCE WITH ALGAE	% CONTROL
10%	60	>90	35	>85
5%	15	>90	25	>55
1%	15	>80	15	>30
0.25%	5	>50	0	>20
SERUM LEVEL	% ATTACHED WITH ALGAE	% ATTACHED CONTROL	% ATTACHED WITH ALGAE	% ATTACHED CONTROL
10%	65	>90	45	>95
5%	20	>90	30	>85
1%	35	>90	20	>70
0.25%	10	>25	8	>25

a marine bacterium. Extracts in methanol, chloroform and water all showed inhibition. Bhakhuni and Silva (1974) also point to F. spirilis and F. vesiculosis as members of the Fucales displaying bactericidal activity. They attribute this activity to the significant concentrations of polyphenols known to be excreted by these brown algae which damage integrity of the cell membrane. The results of in vitro tests on mammalian cells do not, however, show dangerous toxicity from the extracts of F. spirilis when compared to results using F. vesiculosis. These results suggest that further studies should be performed on extracts of F. spirilis to study mutagenic or carcinogenic influence on cultured cells. Negative tests of mutagenicity could point more positively to possible use of this purified extract as a future antimicrobial agent for human or veterinary use.

Percent attachment or plating efficiency of cells is shown in table 1. These data show that algae extracts generally cause a decrease plating efficiency of cells. Only F. spirilis appears to allow close to normal plating of CHO cells in the richer 10% serum concentration as compared to F. vesiculosis. Plating or the lack of it as seen through return to monolayer confluence is quantified by observation using phase contract microscopy (Figures 3 & 4).

These extracts produced by brown algae of the New York/New Jersey coast clearly have varying

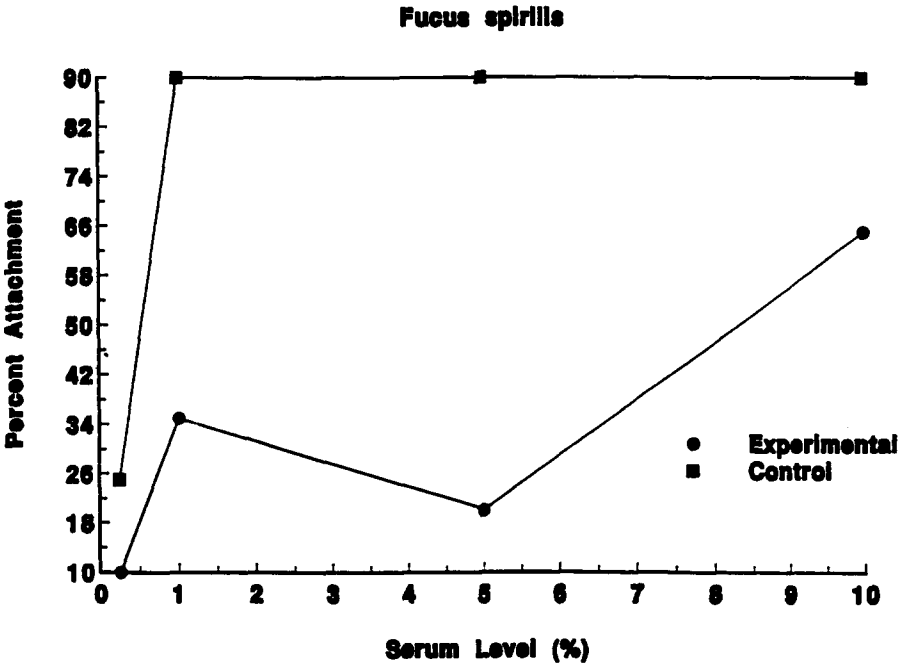

Figure 3. Attachment of CHO cells after exposure to extract of Fucus spirilis.

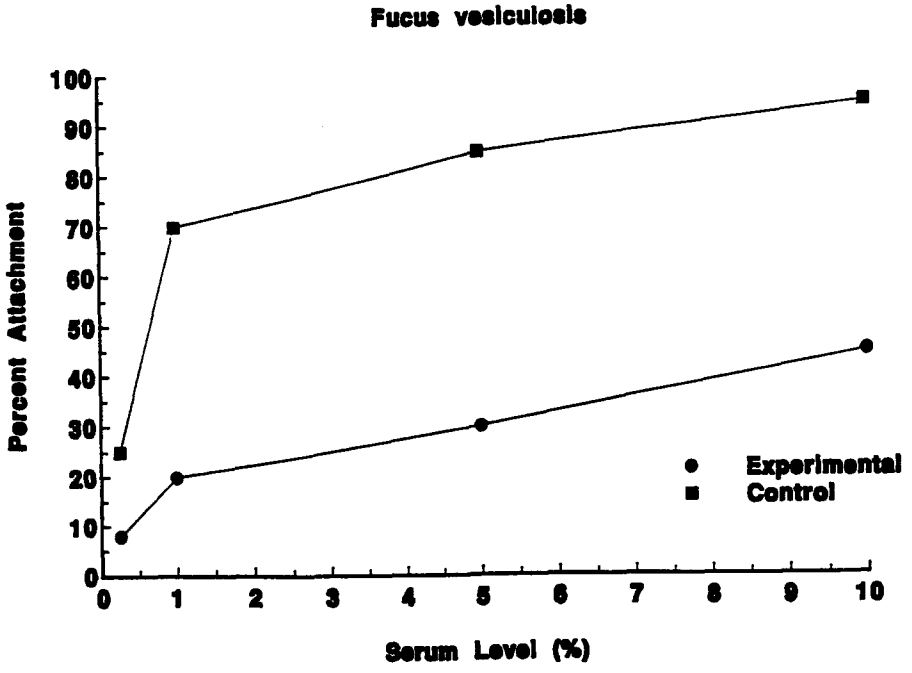

Figure 4. Attachment of CHO cells after exposure to extract of Fucus vesiculosis.

antimicrobial activity. This study has been a preliminary attempt to use the in vitro alternatives to animal testing to study the varying toxicity of these substances in mammalian systems. These tests were used for pre-screening of promising future pharmaceuticals derived from the marine environment. The information derived can be used to make a judgment regarding progress to the next stages of in vitro testing, i.e., mutagenicity and carcinogenicity (Goldberg and Frazier 1989). The results of these in vitro and future studies should give a rapid, cost efficient indication of the potential value of pharmaceuticals from untapped marine flora.

REFERENCES

Atlas, R (1984) Microbiology. MacMillan Publ. Co., New York 10033, 432.

Bhakhuni, DS, Silva, M (1974) Biodynamic substances from marine flora. Bot Mar 17:40-51.

Frazier, JM, Tyson, CA, McCarthy, C, McCormick, JJ, Meyer, D, Powis, G, Ducat, L (1989) Contemporary issues in toxicology: Potential use of human tissues for toxicity research and testing. Toxicol Appl Pharmacol 97:387-397.

Giaevre, I, Keese, CR (1992) Toxic? Cells can tell. Chemtech 22:116-125.

Goldberg, AM, Frazier, JM (1990) Alternatives to animals in toxicity testing. Sci Am 261:24-30.

Lustigman, B, Brown C (1990) Antibiotic production by marine algae isolated from the New York/New Jersey Coast USA. Bull Environ Contam Toxicol 46:329-335.

Lustigman, B, Lee, L, Thees, N, Masucci, J (1992) Production of antibacterial substances by macroalgae ofthe New York/New Jersey Coast USA Bull Environ Contam Toxicol 49:743-749.

Naughton, GK, Jacob, L, Naughton, BA (1989) A physiological skin model for in vitro toxicity studies. In Alternative methods in toxicology, Vol 7. Edited by AM Goldberg, pp 183-189. Mary Ann Liebert, New York.

Paternoster, ML, DiLorenzo, AM, Holland, MJ (1981) Effects of cells density and serum-limitation on purine salvage in intact human skin fibroblasts. In Vitro 17:107.

Rifkin, DB, Moscatelli, D (1989) Recent developments in the cell biology of basic fibroblast growth factor. J Cell Biol 109:1-6.

Scheuer, PH (1990) Some marine ecological phenomena: chemical basis and biomedical potential. Science 248:173-177.

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