VIROLOGICAL AND EPIZOOTIOLOGICAL STUDIES OF FISH NEOPLASMS IN POLLUTED AND NON-POLLUTED WATERS OF THE GREAT LAKES 1976-1977

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Summary

The potential utility of monitoring neoplasia in fishes and their associated oncogenic viruses as sentinel animals for the early detection of waterborne environmental carcinogens was explored. Field epizootiological studies of Great Lakes coho salmon were made, and on increasing frequency of occurrence of thyroid hyperplasia (goiter) was found. Epizootiological studies strongly suggest that extrinsic environmental factors coupled with the low iodine environment are involved in the etiology, etc. of the disease. No evidence of virus was found in electron microscopic studies of the neoplastic thyroid tissues.

Laboratory transmission-induction trials using the brown bullhead papilloma and white sucker papilloma conducted using bottom sediments from Burlington Harbour were refractory. Cell culture studies of the white sucker papilloma and brown bullhead papilloma failed to give evidence of an in vitro transformation model system. A long term cell culture population was developed from the northern pike lymphosarcoma and epidermal hyperplasia. It is proposed to use these cells in an in vitro virus enhancement system.

Mutagen - carcinogen assays made on open lake waters with a modified Ames Test, gave statistically significant elevation of histidine revertents suggesting the presence of water-borne carcinogens. Viral enhancement studies with adenovirus in Syrian hamster cells were equivocal, although data suggest that polychlorinated biphenyls may enhance oncogenic virus transformation.

Introduction

Within the next fifty years[1], according to some forecasts, the country's population will double, and the demand for water by cities, industry, and agriculture will grow faster than the population. Today, these water uses add up to something like 350 billion gallons a day (BGD), but by 1980, by some estimates, they will amount to 600 BGD. By the year 2000, demand for water is expected to reach 1000 BGD, considerably exceeding the essentially unchanging supply of dependable fresh water, which is estimated at 650 BGD. More and more water will have to be reused, and the public health hazards of water reuse remain largely unknown.

It has been documented that our aquatic environments are fouled with chemicals and classes of chemicals which have carcinogenic potential[2]. Although there is little evidence that carcinogens in water have produced widespread cancer problems in man, it is not difficult to envisage such a possibility. Presently, because of ground water depletion, many cities are processing for drinking purposes, water which may be in excess of 30% recycled water[4]. Many inorganic and organic compounds (some known carcinogens) are not removed in current water treatment facilities[4,5]. In fact, treatment may be producing carcinogens (i.e. chlorination may produce chloroform and carbon tetrachloride, both known carcinogens)[6,8].

Of particular concern with regards to the above are the reports[9,10,11] on the possible implications of cancer-causing substances in Mississippi River water. These studies present presumptive epidemiological evidence which suggests a significant relationship between cancer mortality in white males drinking water which was obtained from the Mississippi River in the New Orleans area. The reports strongly suggest that drinking water from the Mississippi River is causally related to cancer mortality in more than one million persons in Louisiana who depend on that source for their drinking water supply. Although the results

of the New Orleans study cannot as yet be considered as conclusive evidence that cancer is in fact being caused by consuming contaminated water, these very suggestive findings must be fully taken into consideration.

It is apparent that there is an urgent need for new model systems to evaluate our aquatic environments, particularly with regards to carcinogens. In these investigations, we have made a pilot study exploring the potential utility of monitoring fishes and their associated neoplasms as indicators of waterborne carcinogens. As free living vertebrates which co-inhabit man's biosphere, they may act as a "bridge" between laboratory in vitro and in vivo testing and surveillance of human populations.

These investigations have revealed alarmingly high tumour frequencies, several of which exhibit apparent clustering in waters receiving industrial and domestic effluents and in which epizootiological studies support the role of extrinsic environmental factors[12-21]. These studies stimulate the following thought-provoking questions: Do they reflect carcinogens in the environment? Oncogenic virus and chemicals? Oncogenic viruses alone? The New Orleans "experience" gives urgency to these questions and gives credance as to the potential utility of fish as sentinel animals for the early detection of waterborne carcinogens.

In this report we summarize laboratory and field epizootiological studies to resolve the role of environmental factors and/or virus(es) in the etiology of a number of neoplastic and/or hyperplastic conditions in Great Lakes fishes which are summarized as followings. In addition, we have made pilot studies to develop in vitro probes to study the possible mutagenic and/or carcinogenic potential of Great Lakes surface water.

Transmission Trials

Field epizootiological studies of the brown bullhead and white sucker papilloma strongly suggest the role of environmental agents in enhancing oncogenic (cancer causing) virus(es) in these species[15]. Inasmuch as previous laboratory transmission trials failed, we proposed transmission studies modelled after conditions in nature where high tumor frequencies were recorded. Specifically, collections of bottom sediments (mud) were made off the industrial complexes of Burlington Harbour (area of high tumor frequency). The mud - suspected of inducing oncogenic virus(es) - was then placed in holding tanks in the laboratory. Transmission trials involving 50 fish per trial (contagion, cell free, and transplantation) of both the white sucker and brown bullhead papilloma, were made holding the experiment fish in the mud filled tanks at $10^{o}C$. One problem arising after 10 days was bacterial infections, which were overcome by intraperitoneal inoculations of chloroamphenicol. Unfortunately all fish were lost during a water breakdown at three months; although the likelihood of long term holding of the specimens seemed unlikely as they had gone off feed, presumably due to the ? mud conditions. Gross examinations of these fish revealed no evidence of tumors. At the time, winter weather had set in, and no additional specimens were available to reinitiate the transmission trials.

One problem which had plagued the field epizootiological studies of the brown bullhead papilloma was difficulty in aging the specimens. This was overcome by fixing the fins in a modified Bouin's fixative (replacing glacial acetic acid with formic acid), dehydrating, embedding in paraffin, sectioning at 8 μ and staining with hematoxyin eosin.

Brown bullheads were collected from Port Rowan on Lake Erie and the fish aged by counting growth anuli in sectioned fin rays. Figure 1 shows the age and sex distribution of the tumor within the population and that tumor frequency increases with age. No bullhead under two years of age was found with the tumor.

The tumor frequency in both males and females increased rapidly after 4 years of age. No definite conclusions can be made from this data, however, the studies suggest onset of tumor development after sexual maturity. This may suggest horizontal contact transmission during spawning of an infectious agent. The age distribution of the tumor suggests that it might take years for an overt tumor to develop. The age sex distribution reported here will be particularly valuable in epizootiological studies to resolve the geographical clustering of the tumor seen in industrial basins surveyed on the Great Lakes.

Cell Culture Studies

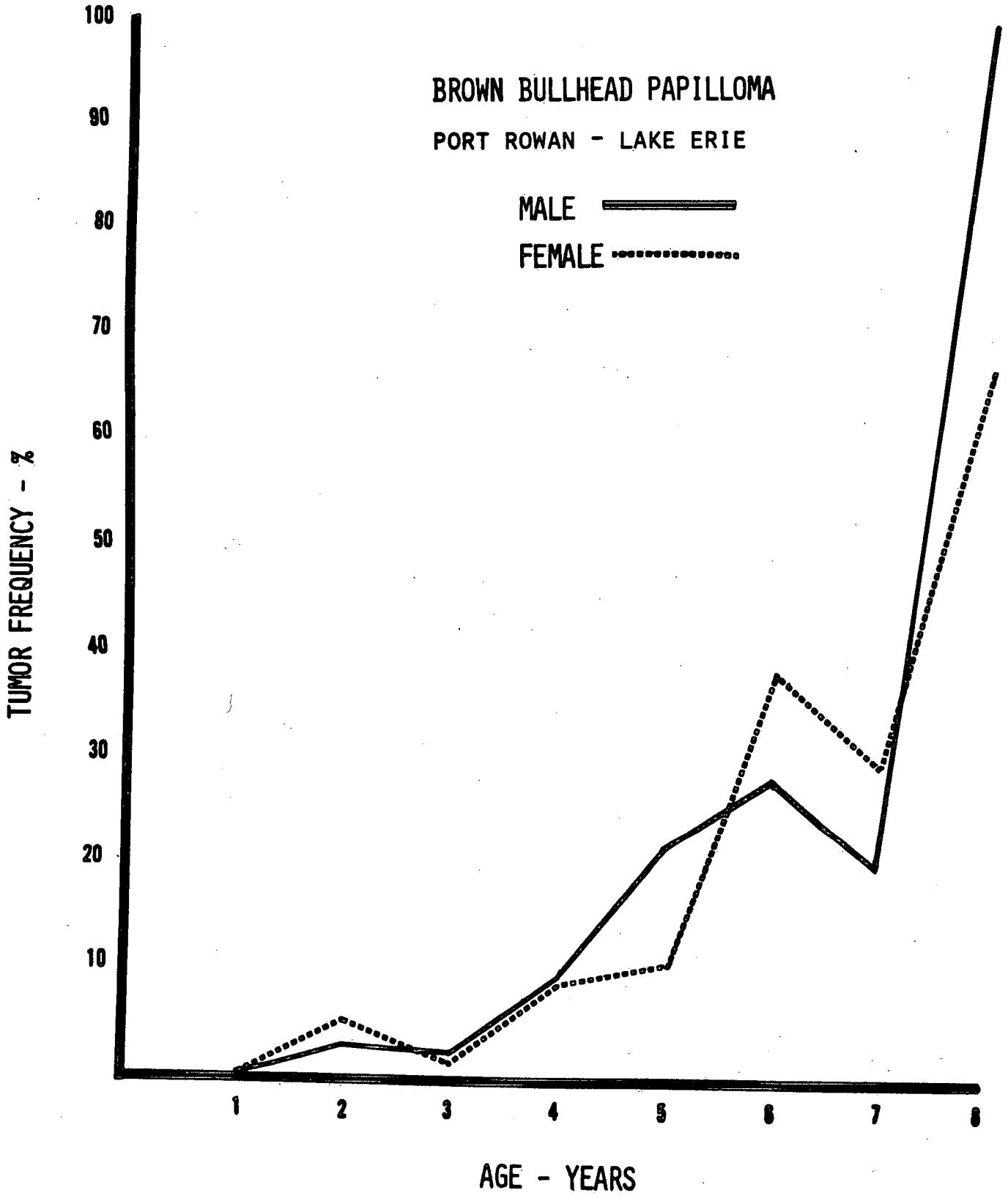
One of the major problems of environmental monitoring of agents which might enhance oncogenic virus expression is the availability of rapid, economical, and sensitive testing procedures. As an adjunct to the environmental epizootiological studies in which fishes and their associated neoplasms were monitored as sentinel animals to detect environmental factors which might enhance viral carcinogenesis, cell culture studies were initiated. An *in vitro* system has inherent advantages over *in vivo* testing in that a variety of environmental parameters may be encompassed in rapid quantitative and qualitative assays.

As an approach to this situation, cell culture studies were made with the goal being the establishment of cell lines of both normal and tumor cell populations for incorporation into virus enhancement and/or transformation assays.

Cell cultures were initiated by one of three methods (A) Explant cultures, (B) trypsin dispersed cells, and (C) static suspension cultures (lymphosarcoma). The success of the cell culture method varied from tissue to tissue and from tumor to tumor, the results of which are as follows:

Northern Pike Lymphosarcoma: A female pike with a cutaneous lesion of lymphosarcoma with muscle involvement was asceptically biopsied and the cells dispersed in Medium 199 with 30% fetal calf serum. The lymphosarcoma cells exist *in situ* as masses of individual cells which were dispersed in growth medium by gentle

FIGURE 1


BROWN BULLHEAD PAPILLOMA
PORT ROWAN - LAKE ERIE
MALE
FEMALE
TUMOR FREQUENCY - %
100
90
80
70
60
50
40
30
20
10
1
2
3
4
5
6
7
8
AGE - YEARS

pipetting. The cells grew for the first three weeks as static suspension cultures, at which time cells began to attach to the surface of the culture vessel. The attached cells assumed a spindle (fibroblast) shape vs. the round shape of the cells in suspension. The spindle-shaped cells grew and when reached confluency, were harvested using trypsin versene. This cell population (spindle-shaped cells) grew slowly for the first 6 months and were periodically split at a ratio of 1:1 or condensed at 3 week intervals. After 8 months in culture, the cells began to grow rapidly and would become confluent following a 1:3 split in 4 days.

The tumor in the pike has a tumor specific chromosome marker associated with it. The pike from which the tumor was initiated had the marker, however, the spindle-shaped cells characteristic of the outgrowth of the tumor after 10 months in vitro lost the marker. It is not known whether the culture lost the marker or whether the cell population is the result of the propagation of normal stomal cells collected in the original biopsy.

Attempts were made to grow the cells on soft agar, growth on the same being a characteristic of transformed cells; however, no growth was recorded. Reverse transcriptase assays were made on the cells which were also unsuccessful. The cultures were treated with 5-iododeoxyuridine, a treatment which has been successful in indusing a number of C-type viruses in vitro. However, no reverse transcriptase activity was detected in the treated cells. In an attempt to resolve whether the population is carrying virus (or transformed cells), the cells were injected intraperitoneally into normal northern pike; these studies are in progress.
In summary, a cell population which appears suitable for long term culture studies was derived from lymphosarcoma in northern pike. To date, no evidence of virus was detected in reverse transcriptase assays or in co-cultivation trials with the following fish cell lines (RTG-2, FHM, BB, BF-2, and GAR). No chromosome marker characteristic of the tumor was found in the cell population. Until the graft experiments are completed and/or virus is detected, it is not known whether

the cell population has the characteristics of the tumor cells.

Brown Bullhead Papilloma:

Explant and trypsin dispersed primary cultures of the brown bullhead papilloma both gave good "takes" in vitro. However, the cell populations began to lag at 10 days and all cells died out by 30 days in culture, and no long term cell cultures were achieved. Explants of the brown bullhead papilloma were co-cultivated with the brown bullhead cell line (BB) and no evidence of transformation and/or reverse transcriptase activity was detected in these cultures. The papilloma cells in co-cultivation with the BB cell line had an interesting interrelationship. The two cell populations would grow up to not make physical contact with each other. The reason for this growth phenomenon is unknown.

Carp-Epidermal Hyperplasia:

Explant cultures of the tumor in carp gave good outgrowth up to 5 days in culture, at which time the cells exhibited cytoplasmic granulation and slowly died out. The tumor cells could not take trypsinization as a means of dispersing cells for cultivation, primary cultures which were trypsinized did not attach. Co-cultivation trials of explants of the carp epidermal hyperplasia with the fathead minnow cell line did not give evidence of transformation.

Normal Carp:

Explant and trypsin dispersed primary cultures of operculum epithelium were set up from normal carp. The cells grew as spindle-shaped (fibroblast cells) and several cultures have exhibited properties indicating that they may be suitable for long term culture studies.

White Sucker Papilloma:

Primary cultures were initiated using explants and trypsin dispersed cells of the white sucker papilloma. Good takes of the primary cell cultures were obtained with both methods, the cells having the morphological characteristics of epithelial cells. After 7-10 days in culture, the cells began to die out slowly and could not be maintained. Reverse transcriptase assays were positive. The

primary papilloma cell cultures were co-cultivated with the fathead minnow (FHM) cell line and no evidence of transformation was observed nor was reverse transcriptase detected.

Normal Norther Pike:

Fingerling northern pike were sacrificed and small explants of operculum epithelium set up in flasks with Medium 199 supplemented with 30% fetal calf serum. After 7-20 days in culture, there was good outgrowth from the explants. The explants were harvested using trypsin versene as the method of cell disassociation and the dispersed cells seeded into Falcon tissue culture flasks. The cells grew slowly for 4 months, the cells having to be periodically condensed to keep the cell density up. At that time, a rapidly growing fibroblast-like cell population predominated, and to date this population of cells has been in continuous culture for 11 months. The cells appear to have good potential for long term culture (i.e. establishment of a cell line). The cells inoculated with cell free preparations of muskellunge and northern pike lymphosarcoma did not exhibit any growth pattern changes suggestive of transformation, nor was reverse transcriptase activity detected in these cells.

Chemical Screening of Environmental Carcinogens (Virus Enhancement)

It has been documented that our aquatic environment are polluted with a variety of chemicals, including carcinogens. In recent years, particular concern has been expressed concerning polychlorinated biphenyls (PCB's), a group of chemicals which have an unusually long life in nature and which have widespread geographical distribution. PCB's in the Great Lakes environment are high, and as a result commercial and/or sport fishery utilization of fishes from the lake system have been discouraged and/or banned.

Recently, an in vitro oncogenic virus enhancement assay has been developed and has been shown to be a sensitive laboratory probe for the detection of carcinogens. As an approach to elucidation of the carcinogenic and/or oncogenic

virus enhancement activity that individual environmental chemicals might have in the induction of oncogenic viruses we have undertaken investigations testing the induction of oncogenic viruses with (PCB's). Specifically, we have tested biphenyl, 3-chlorobiphenyl, 4-chlorobiphenyl, Aroclor 1242, and Aroclor 1254 in the adenovirus enhancement-assay of Casto and Diapolo[22-25]. The assay involves quantitative and qualitative virus enhancement of an oncogenic adenovirus in primary Syrian hamster cell cultures. The test procedure involves the pretreatment of cells with the test chemicals, infecting with virus, and assaying the numbers of transformed foci which are selected for by low calcium medium and growth in agar.

Three separate virus enhancement trials were made, the results of which could not be duplicated. Specifically, problems of differential toxicity of the stocks of test chemicals were encountered in the primary hamster cell cultures. Inasmuch as toxicity is a criteria for calculation of virus enhancement, no conclusions can be made at present. However, evidence of virus enhancement was detected in cells pretreated with 4-chlorobiphenyl, Aroclor 1242, and Aroclor 1254. Inasmuch as these compounds are found in high concentrations in Great Lakes fishes and in human tissues, these observations urgently need to be confirmed. In addition to the oncogenic, potential as has been documented in PCB's, we must now consider that these ubiquitous persistent compounds might enhance endogenous virus transformation resulting in tumor formation.

Ames Testing -- Water Samples

Our investigations have been concerned with the assessment and detection of waterborne carcinogens by monitoring fish neoplasia as an indicator organism. As an adjunct to these studies, we have made tests of lake waters for the presence of mutagens, carcinogens, and/or potential carcinogens as evidenced by genetic alterations in appropriate testing systems exposed to the waters in question.

In these studies we have employed the Ames bacterial system developed for the study of carcinogens. The detection of mutagenic, carcinogenic and/or potential carcinogens through the Ames Test (reversion of histidine dependent mutant strains of Salmonella typhimurium) has been reported[26,27]. The following summarizes studies made with several samples of Great Lakes waters assayed with a modified Ames Test as described below.

Ames Test: Materials and Methods

Water samples:

Water samples were collected at the following locations: Burlington Harbour, Humber River, Credit River, 16 Mile Creek, Jordan Harbour, Bronte Creek, and the Rouge River. Water samples were collected at the surface, at a depth of one foot, and at the bottom, decanted into sterile 250 ml Falcon tissue culture flasks, and transported to the laboratory. Each sample was centrifuged at 2,000 x g for 10 minutes to remove particulate debris, and the supernatant filtered through 450 nm Millipore filters. Filtrates were stored at -20°C in Falcon tissue culture flasks until analyzed.

Bacterial Tester Strains:

Histidine-dependent mutant strains of Salmonella typhimurium (TA98, TA100, TA1535, and TA1538) were obtained from Dr. Stotz, Dept. National Health & Welfare, Ottawa, Ontario. Each of the cultures was streaked on a nutrient agar plate. After incubation, single colonies were picked and grown up overnight in 10 ml of nutrient broth. This overnight culture was used to check the tester stains (i.e. crystal violet sensitivity, ampicillin sensitivity, and ultra-violet sensitivity) using the procedure of Ames et al[26,27]. After testing the reversion frequency of each strain and confirming identity by serum agglutination testing, the broth culture was seeded into 200 ml nutrient broth and was incubated overnight on a shaker. The culture was then streaked to confirm sterility, mixed with dimethylsulfoxide (14 ml/160 ml broth culture), dispersed into 2 ml sterile plastic screw cap vials, and stored at -60°C. After overnight

storage at -60°C, one vial of each strain was thawed and a viable cell count was made. The concentration of each strain was found to be approximately 5 x 10^8 organisms/ml. The frozen strains described above were used in the following assays.

Preparation of Rat Liver Homogenate Fraction (S-9)

The procedure used is similar to that of Ames and co-workers[6]. Three-month old Sprague Dawley male rats were given a single intraperitoneal injection of Aroclor 1254 suspended in corn oil (500 mg/kg body weight). The injected rats were taken off feed on the fourth day and sacrificed on day five with ether. The following procedure was carried out aseptically with chilled solution (4°C). The liver was removed, weighed, rinsed in 0.15 M KCl (1 ml/g liver weight), minced in 0.15 M KCl (3 ml/g liver weight), and homogenized with a Potter Evelen homogenizer. The homogenate was centrifuged twice at 9,000 x g for 10 minutes. The supernatant (S-9 fraction) was decanted, dispensed in 2 ml aliquots in sterile plastic screw cap vials, and stored in liquid nitrogen until use. Each batch of S-9 fraction produced was standardized for optimum mutagenesis using known carcinogens following the procedure of Ames (1975).

S-9 Mixture

One ml of the S-9 mixture consists of the following: S-9 (0.2 ml), $MgCl_2$ (15 mM), KCl (66 mM), glucose-6-phosphate (10 mM), NADP (8 mM) and $NaHPO_4$ (20 mM), pH 7.4.

Stock solutions of NADP (0.08 M) and glucose-6-phosphate (0.2 M) were prepared in water, sterilized by filtration and stored at -60°C in 2 ml sterile plastic vials. The stock salt solutions (0.33 M KCl, 0.08 M $MgCl_2$) and phosphate buffer, pH 7.4 (1.0 M) were autoclaved and stored at room temperature. The S-9 mixture was prepared freshly for each experiment and kept on ice.

Mutagens and Carcinogens

Benzo-α-pyrene (50 µg/ml; SIGMA) and 3-Methyl-cholanthrene (500 µg/ml; Eastman) were dissolved in dimethylsulfoxide in sterile glass test tubes with

screw cap and kept frozen at -20°C until used. These compounds were used as positive control for water tests.

Top agar and Minimal-glucose agar plates:

Top agar (1.0% Difco agar, 1.0% NaCl) was autoclaved and stored at room temperature in 100 ml volume. Before use, the agar was melted, cooled at 45°C and 10 ml of a sterile solution of 1.0 mM L-histidine-HCl -1.0 mM biotin was added per 100 ml agar. The petri plates (100 x 15 mm, Fisher Scientific Co.) contained 30 ml of Minimal glucose agar medium (1.5% Dacto Difco agar in Vogel-Bonner Medium E (J. Biol. Chem. 218, 1956, 97-106) with 2% glucose). The plates were prepared freshly for each assay.

Method of water assay:

The Ames testing procedure was modified to accommodate water testing. The suggestions of Dr. William Pelon of Louisiana State University Medical Center, New Oreleans, La., are gratefully acknowledged. The test protocol cited below is the test procedure used in these investigations.

Two ml of molten top agar at 45°C was mixed with the following: 0.1 ml of bacterial tester strain (stored at -60°C in DMSO), 0.5 ml of the S-9 mixture and 2 ml water sample to be tested (2.0 ml distilled water for negative control; 1.0 ml distilled water and 0.1 ml mutagen (carcinogen compounds dissolved in DMSO for positive controls)). The above contents were mixed in a sterile disposable tube (17 x 100 mm, Falcon) and immediately poured over minimal glucose agar plates. The plates were covered until the top agar hardened, then incubated at 37°C in dark for 60 hours; and the colonies counted. For each test, sterility of S-9 mixture, top agar, as well as agar base layer, was checked. The negative controls for each strain measures the normal reversion rates.

Results

The water samples collected were screened for mutagenic activity (see Table 1) using strain 1538. Several stations gave high frequencies of reversions (i.e. Humber and Jordan Rivers).

Water samples collected from Hamilton Harbour, Rouge River and Humber River were selected for intensive mutagen testing. In these studies, strains TA98, TA100, TA1535, and TA1538 were incorporated along with positive and negative controls. The assays were made on 10 plates to provide suitable data for statistical analysis[28]. The results of these tests are shown in Table 1. Of the 11 analysis made, 8 gave statistically significant (<0.001) increase in reversion frequencies.

In these investigations, only unconcentrated water samples were examined. The data suggest that compound(s) present in the water samples, capable of inducing genetic alteration among the bacterial tester strains employed, also may be present in sufficient quantities to induce carcinogenesis through somatic mutation and/or virus enhancement when such waters are consumed.

No definite conclusions can be made from these preliminary investigations, but our studies do suggest that a potential threat to consumers (human and wildlife). More extensive qualitative and quantitative investigations are necessary to resolve the public health threat. In particular, concentrates of drinking water taken from these waters should be monitored. We anticipated that mutagenic compounds would be dilute in the aquatic environment, and that the tester strains would exhibit low frequency of reversions. Because of this, statistical analysis were employed to detect significant differences, and these studies by statistical analysis indicate a high probability (<0.001) of mutagens.

If the reversions were due to factors other than mutagens in the water samples tested, the reversions should have been random among the stains. The selective nature (all tester strains gave statistically significantly increases) of these factors cannot be explained by mere chance. It is concluded that the reversions were induced and were due to substances present in the water samples collected.

TABLE 1. MUTAGENIC ACTIVITY OF LAKE WATER AS DETERMINED BY AMES TEST

	Water Samples															
	Bact. Control				Hamilton Bay				Rouge River				Humber River (1 ft. below)			
	TA98	TA100	TA1535	TA1538	TA98	TA100	TA1535	TA1538	TA98	TA100	TA1535	TA1538	TA98	TA100	TA1535	TA1538*
number of bacterial revertants per plate	30	16	21	36	40	30	55	71	72	29	34	41	43	25	25	
	25	12	31	23	45	28	46	83	60	23	28	54	46	19	28	
	17	22	22	35	46	32	54	80	41	25	23	67	40	12	23	
	26	19	25	34	46	28	49	79	58	37	22	54	43	20	18	
	20	13	31	30	31	34	57	68	64	32	26	64	44	16	25	
	22	16	29	34	33	26	41	58	63	33	25	52	39	12	26	
	25	12	19	27	27	28	41	70	68	32	21	53	33	16	20	
	25	13	17	31	43	31	38	86	69	26	25	68	38	22	26	
	19	18	24	32	48	29	40	92	59	34	34	53	31	15	20	
	24	12	25	28	43	22	42	99	66	29	24	46	36	18	22	
					<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.4< P <0.5	<0.001	<0.001	0.2< P <0.4	>0.5	

* Not tested

Thyroid Hyperplasia (Goiter) - Coho Salmon

The following summarizes progress made in the past year in studies of the etiology and epizootiology of the thyroid dysfunction in Great Lakes coho salmon. These studies were made in collaboration with Mr. R.D. Moccia (graduate student) and Dr. J.F. Leatherland of the Department of Zoology. The report is in the format that it is to be published[20].

Coho Salmon (Oncorhynchus kisutch) is a highly prized sport and commercial marine fish. In the late 1960's the species was successfully introduced into the Great Lakes[29], and has provided a spectacular multimillion dollar sports fishery to North American anglers. Since their introduction into the Great Lakes, several investigators have reported thyroid hyperplasia (goiters) in this species[19,30,31]. Recently, Drongowski and co-workers[31], and Sonstegard and Leatherland[19] have described severe hypothyroidism associated with thyroid hyperplasia in coho from Lakes Michigan and Ontario. While the hypothyroid condition (goiters) found in the coho salmon is undoubtedly due, in part, to chronic low iodine availability in the Great Lakes Basin, we report studies here which strongly suggest the involvement of environmental goiterogens (possibly pollutants) in the etiology of the thyroid disorder. In addition, our data (Table II) suggest an increase in the frequency of occurrence of overt goiters from previous years.

Sexually mature coho salmon were collected during the fall spawning runs from Lakes Michigan, Ontario, and Erie. The presence of goiters was determined by retracting the operculum of each fish and examining the base of the gill arches for evidence of swelling or nodules. Fish with one or more distinct nodules of >1 cm in diameter were recorded as having overt goiters. The frequency of overt goiters ranged from 6.3% in Lake Michigan to 47.6% in Lake Ontario, to a striking frequency of 79.5% in Lake Erie.

If low iodine availability were the sole contributing factor to goiter development, one would expect goiter frequency to be inversely proportional to

lake iodine concentration. However, Lake Michigan has the lowest level of iodine (0.9 µg/l) which is only one half that of Lake Erie (1.7 µg/l) and one third that of Lake Ontario (2.9 µg/l)[32]. Conversely, Lake Ontario has nearly 7.6 times and Lake Erie 12.6 times as high an overt goiter frequency as Lake Michigan. A nearly 13 fold difference in goiter frequency between two populations of the same species, age, and state of spawning, seems highly unlikely if iodine deficiency were the sole contributor to goiter development.

The usual response to a low iodine environment is a compensatory thyroid hyperplasia which is thought to act through a stimulation of the pituitary-hypothalamic axis due to a negative feedback system responding to low circulating hormone levels (either thyroxine (T_4) and/or triiodothyronine (T_3)[33,34,35]. Therefore, one might expect to find a correlation between low hormone levels and high goiter frequency. Our data (Table III) does not indicate such a relationship. It is of interest that coho from Lakes Michigan and Erie have extremely low levels (nearly undetectable) of thyroxine and yet represent the extremes of goiter frequency. In light of lake iodine levels previously discussed, and the fact that Lake Erie coho are from Lake Michigan egg stock, it is difficult to explain this difference in terms of a greater demand for thyroid hormones by Lake Erie fish. In addition, Lake Ontario coho had nearly 4 times the level of both T_3 and T_4 as compared to Lake Michigan fish, and yet had 8 times the goiter frequency. The data of serum hormone levels are, in some respects, at variance with data from Lakes Michigan and Ontario in previous years[19,31]. This may indicate that goiter frequency, rather than absolute hormone levels is a better criterion of thyroid dysfunction in these fish. This hypothesis is supported by the report of Sonstegard and Leatherland[19] in which they found no correlation between thyroid hormone levels and goiter occurrence in Lake Ontario coho salmon. These observations further suggest that other factors, in addition to iodine deficiency, are involved in the etiology of the goiters. With regards to the increasing goiter frequency, it appears that these factors

are becoming of greater significance. Seasonal and/or yearly fluctuations of iodine levels are highly unlikely in the Great Lakes Basin, and it is doubtful whether they could account for the annual differences in frequency observed.

The Great Lakes have been polluted with a vast array of chemicals which either alone or in concert with one another may act as goiterogens, augmenting the development of goiters in a low iodine environment. Organochlorines are suspect (i.e. PCB, DDT, Dieldrin, and Mirex) in that they have widespread distribution in the Great Lakes and have been reported to alter thyroid activity in fish[19,36], birds[38,37], and mammals[38]. There are in the literature, growing data to suggest many endocrine parallels between teleosts, birds, and mammals in the roles of thyroid hormones, which are known to be important regulators of growth and reproductive physiology in higher vertebrates[34,35]. Significant reduction in such aspects as fertility, fecundity, and growth rates, etc., could have a devastating effect on an already fragile salmon industry.

The evidence reported here suggests that the goiters in the coho salmon may be environmentally induced. Coho salmon, because of their position in the food web, may, by the process of biological magnification be exposed to waterborne and/or dietary environmental insults which are considerably higher than those to which other animals are exposed. In addition, they may be unusually responsive to environmental insults, and as such may provide a sensitive sentinel animal for the detection of environmental goiterogens. The importance of waterborne or dietary goiterogens is the fact that the fish are consumed by humans and the lake water is increasingly being utilized for drinking water. The apparent increase in goiter frequency in the coho from previous years, may reflect an increase in the levels, types, and/or effects of environmental goiterogens.

Table II

GOITER FREQUENCIES OF GREAT LAKES COHO

Lake	Year	Frequency (%)*		References
Erie	1972	44	(117)†	2
	1976	79.5	(117)	ǂ
Ontario	1975	24	(51)	4
	1971	47.6	(63)	ǂ
Michigan	1973	<1	(100)	ǂ
	1976	6.3	(111)	ǂ

* = Determined from sexually mature fish examined during fall spawning run.

† = Number of fish examined.

ǂ = These investigations.

Table III

SERUM THYROXINE AND TRIIODOTHYRONINE LEVELS IN SPAWNING COHO SALMON

Lake	Thyroxine* µg/100 ml	Triiodothyronine* ng/100 ml
Ontario	1.2 ± 0.4† (10)ǂ	636.8 ± 69.4 (10)
Michigan	0.3 ± 0.1 (10)	159.4 ± 51.4 (10)
Erie	0.1 ± 0.1 (10) (undetectable)	84.3 ± 25.9 (10)

* See method used by Sonstegard and Leatherland[14]

† = mean $\pm$ std. error of mean

ǂ = number of fish per mean

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