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Oncozoons and the Search for Carcinogen-Indicator Fishes

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This essay attempts to bring into perspective the importance of hereditary as well as environmental factors as potential causes of neoplasms in feral fishes. Concepts delineated by Knudson regarding hereditary cancers in man and experimental animals will probably be found operative in certain demographic units (oncozoons) among feral fishes bearing neoplasms. Hereditary factors include: antioncogenes (regulatory genes), which act as suppressors of neoplastic expression in homozygous, heterozygous, or hemizygous states, as well as constitutional (structural) genes that influence carcinogen activation or deactivation through enzyme gene products, genes that influence immunologic responses, and gene abnormalities that favor spontaneous or induced mutations.

The two major classes of genes (oncogenes and antioncogenes) that influence the manifestation of cancers appear to operate through different mechanisms, but conceivable interactions have not been widely investigated, especially in tumor enzootics among feral fishes. Some explorations have been undertaken in the laboratory by Anders and collaborators in studies of suppressor genes (antioncogenes)and the cellular *sarc* gene (an oncogene) in melanophoromas in platyfish-swordtail hybrids and backcrosses. Some feral fish oncozoons that exhibit features of hereditary oncodemes as seen in man have been tentatively identified here as candidate systems to be studied more intensively in laboratories, particularly using cytogenetic analysis and breeding methods.

In the search for carcinogen-indicator fish species in feral habitats, the traditional approach has been to survey fish populations with the aim of first finding enzootics of fish neoplasia. Advances in understanding carcinogen metabolism and the pharmacokinetics of carcinogens in fishes suggest an alternative approach, outlined herein, that could strengthen the rationale for using neoplasms in feral fishes as indicators of environmental carcinogens in aquatic environments.

This volume is appropriately focused on mechanisms of pollutant action, and it has held steadfastly to that theme. We see from other papers that mechanisms can be of critical importance to our understanding of why particular carcinogens are selective for certain fish species and for particular cell phenotypes within a given species. Absent, however, has been consideration of the possible role(s) of hereditary factors in carcinogenesis in fishes. The reason is all too obvious: very little has been done in the way of investigating hereditary factors in relation to cancers in feral fishes.

This omission can be disadvantageous in two ways. First, it can obfuscate the need to examine the known enzootics of neoplasms in fishes with a view toward sorting out the environmental from the hereditary factors involved. Currently the prevailing viewpoint seems to be that most or all of the clusters of neoplasms in certain species in certain habitats are related to environmental factors, chiefly xenobiotics. This view may turn out to be correct, but at present we cannot on the basis of evidence answer the question: How is it known that some of these neoplasms are not largely related to hereditary phenomena?

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Second, the omission of hereditary studies deprives us of the advantages such studies can afford in analysis of mechanisms. In point of fact, evidence now available concerning many human cancers indicates that both hereditary and environmental cancers find their expression through abnormalities in identical loci of genetic regulatory material (*1*). This evidence has already been of value in prompting investigations that show the chromosomal locations of some of the genes (antioncogenes) which, in normal individuals, suppress or preclude development of specific neoplastic histotypes. It is of importance to learn how these antioncongenes work, e.g., whether independently of oncogenes, through antagonisms with oncogenes, or in concert with oncogenes.

The sorting-out exercise to which I refer has long been a central theme in human cancer epidemiology. Little by little, "cancer environmentalists" have succeeded in identifying about a half-dozen viruses and perhaps 30 or so chemicals able to cause human cancers. In addition, ionizing radiation and ultraviolet radiations have long been recognized to cause some human cancers. For most of the cancers involved, these agents exert their effects through changes in genetic material in somatic cells.

On the other side of the picture are the so-called fam-

ilial or hereditary cancers. About 50 types or syndromes are now well or fairly well characterized (*1*). These hereditary cancers also come about through alterations of genetic material, but the initial step usually takes place at least one generation earlier, and in the germ line rather than in somatic cells. What is inherited is a predisposition to cancer, since one step of a two- or several-step process is deleted.

From this storehouse of information on human cancers, can we see some patterns that might facilitate a preliminary sorting-out of those clusters of fish cancers that are caused by environmental agents from those that have a predisposing hereditary component? Fortunately, Knudson has already done much of the conceptual spadework for us in a paper published just about a year ago (*1*). In that paper Knudson uses the term "oncodeme," whence the origin of the term "oncozoon" that appears in this essay. Knudson defines oncodemes to be "demographic units with different expectations of cancer, depending on environmental and hereditary variables." Surely there is nothing revolutionary in this concept. It is a general restatement of the nature vs. nurture principle that pervades all of biology. But it offers an insightful way of classifying hosts bearing cancers of identical histological types and biological behaviors. Interestingly, Anders has offered a strikingly similar way of classifying tumor-bearing hosts, based on his and others exhaustive studies of the *Tu* oncogene and its regulatory gene (antioncogene) in Xiphophorin fishes (*2*).

Knudson identifies four implications inherent to the somatic mutational theory of cancer (*1*).

1. Spontaneous mutations should produce some irreducible "background level" of cancer; i.e., there will always be some cancers, even if all xenobiotic environmental oncogens are removed. For us, specifically, this means that not all the neoplasms we see in feral fishes are related to environment. Further, some small proportion of the neoplasms in clusters that are related to environment represent the spontaneous background component. In practical terms, this means beware of studies in which the control populations show absolutely no cancers.

2. Agents that can change the host genome by mutagenesis or by addition or deletion of genetic material should increase this background incidence. Knudson notes that most investigators now believe that most (some 80%) of the world's human cancer befalls this group. Can we presume that a similar percentage of fish cancers also belong in this category?

3. Abnormalities that favor either spontaneous or induced mutations should impose an elevated risk of cancer. This group is best exemplified by xeroderma pigmentosa—actually a group of disorders in which various aspects or steps of DNA repair processes are faulty. The group of cancers belonging to this category is relatively small, but it is suspected that many cancers in group 2 are also influenced by factors belonging to group 3. Do any of the known fish cancers belong to this group?

4. Inheritance of an initiating mutation should strongly predispose to cancer. This group, Knudson notes, should be at very high risk, especially if the number of steps leading to cancer is small. In this group, the number of steps is reduced by one, leaving the remaining steps to be made by spontaneous mutations or by environmental oncogens such as chemicals, viruses, radiation, or physical agents operative in fiber carcinogenesis and solid state carcinogenesis. The prime example of this group is hereditary retinoblastoma. Predisposition to the neoplasm is inherited as a dominant character, but the neoplasm occurs only if both of two recessive alleles are affected. Hence, two steps are required: one may be hereditary, while the second results from mutation, deletion, or recombination events causing dysfunction of the second allele in a somatic cell. In many (about 60%) of the cases of retinoblastoma, both alleles are rendered dysfunctional by spontaneous or environmental agent-induced mutation, deletion, or recombination. Because the $13q^+$ allele protects against retinoblastoma, it has been classified as an antioncogene by Knudson. Of the 50-odd hereditary cancer types identified in man, not all are as clearly explained at present as retinoblastoma. Do any of the known fish cancers belong to this oncozoon?

Before attempting to answer the questions above, I would like to point out that extension of Knudson's concept of oncodemes would lead to the creation of many more (in fact, almost limitless) oncodemes if one were to assign different numbers and potencies of mutagens and carcinogens, as well as multiple antioncogenes, to the equation. As shown in Figure 1, four levels of environmental factors interacting with four levels of hereditary factors in all possible combinations yields 16 oncodemes. However, each of these oncodemes does not carry a unique risk factor. If we assume additive qualities of the environmental and hereditary factors, only seven equal-risk groups are formed, and within some of these the environmental component can be high, low, or intermediate, and the same is true for the hereditary component. I present this only to emphasize that the problem of identifying oncodemes or oncozoons can be made extremely complex.

Let us return now to the matter of sorting out environmental feral fish cancers from the rest. We may think of this as a process of triage, since we have only minimal information to go on, and we want to give priority of attention to enzootics of environmental origin because we can theoretically do something about them.

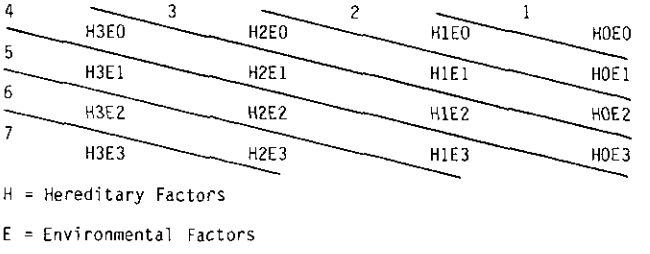

FIGURE 1. Equal-risk groups of oncozoons.

The exercise would be completely a matter of guesswork if we did not have an important resource to draw from: the human experience with hereditary and environmental cancers. What then are the earmarks of environmental vs. hereditary cancers, that have been learned from human studies? They are listed in Table 1.

As another aid toward distinguishing between hereditary and environmental cancers, we may make cautious use of Knudson's comparison of properties of oncogenes and antioncogenes in human cancers (Table 2) (*1*). The caution is that antioncogene inactivity cannot be equated with hereditary cancers because, as in the retinoblastoma paradigm, the nonhereditary forms entail the same mechanisms as the hereditary forms. However, Knudson notes that "... no constitutional abnormalities of oncogenes have yet been described, so that a test of the idea that either germinal or somatic mutation of oncogenes could lead to cancer has not yet been possible." Since there are currently no known examples, except in experimental transgenic mouse models (*3*) of hereditary cancers in which the primary event is oncogene activation, evidence of oncogene activation in a neoplasm may tentatively be interpreted to indicate a nonhereditary nature of that neoplasm.

Armed with the above points of difference between environmental cancers and hereditary cancers, let us attempt a "sort" on the cancers in fishes. We will deal only with the four main groups implicated by Knudson, as we certainly lack the precise factual knowledge needed to deal with oncozoons any more finely subdivided in terms of mixtures of hereditary and environmental factors.

Into Knudson's group 1 would fall those cases cataloged in the Registry of Tumors in Lower Animals which do not currently seem to belong to clusters associated with any locality, specific habitat, particular species or hybrid, or some restricted gene pool. They are of a wide variety of histotypes, and most of them are presumably caused by "background" mutations. They belong to the irreducible baseline of cancers that will always be with us. If we had detailed data on karyotypes, phenotypes, and familial distribution, we could probably reclassify some of them into hereditary and nonhereditary groups. As it is, they are of value to us chiefly because they do represent the evidence for "background" cancers.

In Knudson's oncodeme (oncozoon) group 2, I have placed the examples of clusters or enzootics that appear, on the basis of criteria reviewed above, to fit reasonably well (Table 3). Prime examples, though occurring in nonferal fish, are the hepatic tumors induced in rainbow trout by aflatoxins and several other known carcinogens, which I need not review here. Nephroblastomas induced in rainbow trout by dimethylnitrosamine (*4*) also belong in this group, although there appears to be a sizeable background level of this histotype in that species. Many specimens of nephroblastoma cataloged at the Registry of Tumors in Lower Animals came from hatchery rainbow trout that were reared in the post-aflatoxin contamination era and were not known to be exposed to any carcinogen (*5*). One must ask if there may not be a strong hereditary component in some of these tumors, as there is in a large compartment of nephroblastoma (Wilms' tumor) in man. It might be revealing to examine nephroblastomas in feral fishes and their hosts' nonneoplastic cells to determine whether chromosomal anomalies are present in the tumors and/or the germlines of the hosts.

Esocid lymphomas are another clear-cut example of environmental cancers. They occur at high prevalences in northern pike throughout the range of this species, except in England, a noteworthy exception (*6–9*). Tumor-bearing populations are found in habitats in which there is no evidence of chemical pollution, and a retrovirus has been extracted from the tumor cells (*10*). Its reverse transcriptase has an optimal activity at temperatures lower than those of mammalian and avian retroviruses (*11*). The tumor cells carry a unique karyotypic "signature" consisting of 1 submetacentric marker, 1-minute marker, and three to five pairs of smaller-than-normal chromosomes, set within a mode of 50, whereas the normal karyotype consists of 50 acrocentric chromosomes (*12*). Epizootiological studies suggest the disease is transmitted by fish-to-fish contact during spawning activities (*13,14*). It makes its first

Table 1. Features of environmental vs. hereditary cancers in man.

Environmental	Hereditary
Common in the aggregate; also common for many specific histotypes and specific exposures	Uncommon in the aggregate, rare for each histotype
Associated with habit, "way of life," diet, geographic location, occupational exposure, socioeconomic groups, certain virus infections, etc. Focal distribution	Absence of focal distribution except in limited or circumscribed gene pools
Broad variety of histotypes	Narrower variety of histotypes. Nearly 25% involve central or peripheral nervous systems or neurocristal derivatives
Tend to occur in older age groups (long latent period)	Tend to occur in early age groups, with exceptions
Usually no familial pattern	Familial pattern

Table 2. Comparison of oncogenes and antioncogenes in human cancer.[a]

Oncogenes	*Antioncogenes*
Gene active	Gene inactive
Specific translocations	Deletions or invisible mutations
Translocations not hereditary	Mutations hereditary and nonhereditary
Dominant	Recessive
Tissue specificity may be broad	Considerable tissue specificity
Especially leukemias and lymphomas	Solid tumors

[a] From Knudson (*1*).

Table 3. Oncozoons probably of environmental type among feral fishes.

Tumor clusters	Site	References
Lymphomas, northern pike and muskellunge	U.S.A., Canada, Ireland, Sweden, Finland, U.S.S.R.	(*6–14*)
Liver tumors, several species of flatfish	West and East Coast estuaries, U.S.A.	(*15–22*)
Liver tumors, Atlantic tomcod,	Hudson River estuary	(*24,25*)
Liver tumors, sauger, and walleyes	Torch Lake, MI	(*28*)
Liver tumors, bullheads	Black River, OH	(*20,47*)
Liver tumors, bullheads	Fox River, IL	(*64,65*)
Liver tumors, hagfish	Gullmar Fiord, Sweden	(*27–29*)
Liver tumors, white suckers	Deep Creek Lake and Pleasant Valley Lake, MD	(*33,34*)
Chromatophoromas, Japanese croakers (nibe, koichi)	Kii Peninsula and Seto Inland Sea, Japan	(*62*)
Dermal fibromas, walleyes	Lake Oneida and other locations	(*66*)
Orocutaneous papillomas, white suckers	Lake Ontario	(*40,67*)
Cutaneous papillomas, freshwater drum	Lake Erie	(*67*)
Orocutaneous papillomas, bullheads	Delaware and Schuylkill Rivers	(*68*)
Orocutaneous papillomas, bullheads	Fox River, IL	(*64,65*)
Orocutaneous papillomas, European eels	Estuaries northeastern Europe	(*58–61*)
Orocutaneous papillomas, bullheads	Chlorinated sewage pond	(*69,70*)
Orocutaneous papillomas, bullheads	Black River, OH	(*27*)
Orocutaneous papillomas, bullheads	Buffalo River	(*71*)
Leiomyomas, testis, yellow perch	South Bay, Lake Huron	(*72*)

appearance in young adults. The tumor histotype fits with Knudson's generalization that leukemias and lymphomas are more commonly related to environmental factors such as viruses, radiation, and chemical mutagens, any of which can enhance oncogene activity, than to inherited factors.

The discoveries, during the past decade, of high prevalences of liver tumors in pleuronectid fishes of several species, first on the west coast and more recently on the east coast of the U.S., consistently in association with urban domestic and industrial waste pollution, have provided some of the most convincing examples of environmental oncozoons that are available among feral fishes (*15–22*). As seen in this symposium, investigations have already gone far toward identifying a formidable catalog of known carcinogens in the environments and tissues of the affected fish, and metabolites of some of these carcinogens have been demonstrated in the bile. Taken in the aggregate, the evidence that environmental factors are responsible for the flatfish liver tumors approaches, in kind, the evidence that cigarette smoking is a cause of lung cancer in man. Evidence for specific or nonspecific chromosomal translocations not present in the germ cell line and for specific oncogene activation is not yet available in relation to the flatfish liver tumors. It must be kept in mind that evidence does exist that heredity can predispose to development of "spontaneous" liver tumors in mice (*23*).

For the liver tumor clusters in tomcod (*24,25*), bullheads (*26,27*) sauger and walleyes (*28*), the epizootiological evidence is less strong, for a variety of reasons reviewed by Mix (*29*), but in my opinion it is sufficient to place these groups among the environmental oncozoons until proven otherwise.

Hepatic neoplasms in hagfish from the PCB- and chlorinated pesticide-polluted waters of the Gullmar Fjord in western Sweden (*30*) have been included in Table 3, although Mix (*29*) argues that the evidence for environmental chemical causation is weak. Time and space do not allow a full discussion of this issue, and the reader is referred to the original reports (*30–32*).

There is insufficient evidence to justify inclusion of a cluster of cholangial neoplasms in white suckers from Deep Creek Lake, MD (*33,34*), in Table 3. It has been listed there only because the report represents the first to describe hepatic neoplasms in feral fishes, either marine or freshwater, and perhaps served to stimulate subsequent studies of neoplasia in feral fish from polluted waters. Recently Black (*35*) has observed additional examples of liver neoplasms in white suckers from tributaries of the Great Lakes. In his experience, as well as in that of the Deep Creek Lake study, tumors have been found only in the oldest and largest fish collected, a finding consistent with environmental causation.

To my knowledge, we have no oncozoon among fishes that would correspond to Knudson's oncodeme 3, exemplified by conditions such as xeroderma pigmentosa and the chromosome breakage syndromes (Bloom's syndrome, Fanconi's anemia, ataxia-telangiectasia, and glutathione reductase deficiency). It is unlikely that hosts belonging to this oncozoon will be discovered until extensive use of cytogenetic studies and DNA repair defects come into play in studies of feral fishes bearing neoplasms.

In Table 4 are listed some of the clusters of neoplasms that would seem to be good candidates for membership in Knudson's oncodeme (oncozoon) of hereditary cancers. Most, but not all, are neoplasms of neural or neurocristal origin. This is in keeping with Knudson's observation that about 25% of the presently known human hereditary cancers are of neural or neurocristal origin, but taken alone does not represent hard evidence.

The melanophoromas in platyfish-swordtail hybrids and backcrosses, so thoroughly studied by Gordon (*36,37*) and in more recent years by Anders and collaborators (*2,38*), have not been included in Table 4, as this is a laboratory system and not a "natural experiment" in feral fishes. The system seems to fit well into the hereditary cancer oncozoon, and has even provided evidence that suggests the *Tu* regulatory gene (antioncogene) of the platyfish may, in this instance, act through suppression of c-*sarc* (*Tu*) oncogene activity (*2*). The

Table 4. Oncozoons postulated to be of hereditary type, predisposed to by germline abnormalities involving antioncogenes.

Tumors and hosts	References
Neuroblastoma-ependymoblastoma and maligant Schwannoma in coho salmon, hatcheries	(*55,56*)
Nerve sheath tumors in snappers, Caribbean Sea	(*50*)
Nerve sheath tumors in bicolor damselfish, Florida reefs	(*51–53*)
Gonadal tumors of carp and goldfish, Lake Ontario	(*40,41*)
Ameloblastomas in chinook salmon, brown trout, and others. (Highly multicentric)	(*73–75*)
Nephroblastomas in American eels (2 cases), striped bass (2 cases), and other species	(*5*)
Retinoblastomas (only 3 cases, each in a different species)	(*57*)
Gastroenteropancreatic neuroendocrine tumors in hagfish, Gullmar Fjord, Sweden	(*31,32*)

situation appears to parallel that in human retinoblastoma, where N-*myc* oncogene activity is enhanced in the tumors (*39*).

Though little is yet known about the hereditary and cytogenetic features of gonadal tumors in carp and goldfish hybrids (*40,41,42*), the mere fact that similar tumors do not occur in the parent species suggests an hereditary influence, in the absence of strong evidence for environmental carcinogens. Chromatophoromas in goldfish (*43*) and gonadal tumors in nishikigoi carp bred under artificial conditions (*44,45*) offer additional systems to be investigated, as do the chromatophoromas reported in three individuals of the Gulf killifish (*Fundulus grandiformis*) bred in a commercial hatchery (*46*). Anders has pointed out (*2*) that inbreeding favors germ line-determined tumors by increasing the probability of introducing an accessory oncogene, while hybridization may have the same effect, but operates through the mechanism of deleting a regulatory gene (antioncogene).

The nerve sheath tumors described in goldfish from populations with limited gene pools by Schlumberger (*47–49*), and the nerve sheath tumors in gray snappers (*50*) and in bicolor damselfish (*51–53*) living under nonpolluted feral conditions are of special interest because these groups appear to represent potential experimental models of human von Recklinghausen's neurofibromatosis, one of the best examples of an hereditary disease, strongly predisposing to neurofibrosarcoma. Schmale (*54*) has found evidence that he interprets as pointing to a transmissible cause of neurofibromatosis in damselfish, but until that evidence is consolidated and reinforced, cytogenetic and breeding studies of this system seem worth pursuing.

A particularly interesting set of tumors yet to be investigated from the hereditary aspect is the neuroblastoma with tendency to differentiate toward ependymoblastoma, described by Meyers and Hendricks (*55*) in fingerling coho salmon from hatcheries. This neoplasm has most of the earmarks of hereditary cancers, but has not been investigated cytogenetically or in breeding experiments. The neoplasm has been found in fish from widely separated hatcheries in the U.S. and one example is reported in a fish exported to Japan in the embryonated egg stage, then reared there in well water free of known carcinogens and untreated by chlorine (*56*). The investigators further discovered that in the same population of fish, malignant Schwannomas (neurilemmomas) appeared when the salmon reached young adulthood (*56*). The incidence of neuroblastoma/ependymoblastoma was estimated at 12/100,000 by Meyers and Hendricks (*55*). This is in the same order of magnitude as the incidence of hereditary retinoblastoma in children (2/100,000), and it is likely that a relatively high incidence in the coho salmon is due to the practice of using only small numbers of breeder fish to produce millions of offspring. A single carrier of a mutant antioncogene similar to the $13q^{rb}$ could introduce the mutant gene into an "unnaturally" large proportion of the total offspring reared in a hatchery. I am inclined to agree with the investigators, who favor an hereditary nature of the neuroblastomas/ependymoblastomas and malignant Schwannomas in coho salmon (*56*). This tumor system begs to be brought into the laboratory for cytogenetic and breeding analyses, as well as for study of oncogene activity and possible interrelationships between oncogenes and antioncogenes.

Retinoblastomas are listed in Table 4, but have not been found in fishes in sufficient numbers to offer opportunity for study of their possible hereditary origin. Only three examples are in the Registry of Tumors in Lower Animals at the Smithsonian Institution, each in a different species (*57*). It is not known whether any of these represents a hereditary form of the disease, analogous to 35 to 40% of the total cases of retinoblastoma.

In Table 4 I have included, with a query, the cases of gastroenteropancreatic (GEP) neuroendocrine tumors reported by Falkmer in hagfish (*31,32*), for two admittedly weak reasons: (a) there is a resemblance of these cases to a syndrome of hereditary cancers in man known as multiple endocrine neoplasia (MEN), type 1; and (b) the prevalences of these multiple neuroendocrine neoplasms in hagfish from the polluted and control areas are about equal and are approximately the same as the incidence of MEN type 1 in man. Further, the structural genes for the peptidergic hormones have been highly conserved in evolution, according to Falkmer and Grimmelikhuijzen (*32*), and presumably also their corresponding regulatory gene(s) (antioncogenes).

Some outstanding examples of enzootic neoplasms in feral fishes have been included in Table 3 although the evidence available suggests that both hereditary and environmental factors may be operative. For example, both viral and chemical causes of the highly prevalent oral papillomas (benign neoplasms) in European eels have been proposed (*58–61*), but there is also reason to postulate that hereditary factors of a broad constitutional sort (such as structural genes for carcinogen-activating enzymes) may be involved. American eels, a very closely related species, apparently are not subject to the disease. Similarly, the melanophoromas in Jap-

anese croakers (nibe) appear to be associated with polluted habitats, but related croakers in the same habitats have lower prevalences (*62*).

What, then, have we learned from the above sorting-out exercise that is of value in the search for carcinogen-indicator fish species? The following points deserve thought.

None of the features listed in Tables 1 and 2, which may be of help in distinguishing between predominantly hereditary and predominantly environmental oncozoons, is sufficient in itself to make a conclusive decision. However, if several features favoring one or the other type of oncozoon are present, there is inferential reason to use this evidence as a guide in deciding what investigations should be undertaken next. As a hypothetical example, a cluster of tumors of neural or pigment-cell origin, occurring at an early age in fish of a particular species or hybrid cross, or within a limited gene pool, and in a habitat remote from any obvious xenobiotic pollution, might well be classified tentatively as an hereditary oncozoon. Cytogenetic and breeding studies would be indicated before making an exhaustive, costly search for environmental carcinogens.

Relatively high prevalence (which means as little as 1% or greater) of one or more tumor histotypes in a feral species in a habitat where there is no reason to suspect constrictions on the gene pool, and where known xenobiotic pollution exists, favors an environmental etiology and justifies expenditure of effort toward identifying carcinogens and/or their metabolites in the habitat, food supply, and tissues and body fluids of the species concerned. Adding to the level of probability of belonging in this category would be tumor siting in liver and/or kidney, organs that are involved in detoxification and elimination of genotoxic carcinogens. Further increasing the weighting in this direction would be neoplasms in more than one species occupying the same habitat, and sited in the same organ, e.g., liver. A preponderance of tumors in the older age groups also favors placement in this category. It is interesting that, in recent years, hepatic neoplasms and nephroblastomas are among the commonest neoplasms being found in feral fishes.

We are far from being in a position to presume that in fishes, as in human beings, neural tumors comprise a disproportionate compartment of those tumors related to hereditary predisposition. Nevertheless, in view of the conserved nature of much genetic information during evolution, this is a premise worth attention and test. Surely not all neural and neurocristal neoplasms are hereditary, for as Knudson points out, any and every tumor type can theoretically result from either a combination of hereditary and nonhereditary events or from nonhereditary events alone. In man, it currently appears that for unknown reasons certain tumor types are more likely to have a hereditary component than others. When these tumor types occur in fishes, perhaps a red flag should be raised to remind us to think about hereditary factors. I have already cited the examples of melanophoromas, neurilemmoma/neurofibromatosis, neuroblastoma/ependymoblastoma, gastroenteropancreatic neuroendocrine tumors, retinoblastoma, and nephroblastoma. Only in the melanophoromas in platyfish-swordtail hybrids has a distinct hereditary factor been demonstrated as yet. It seems likely that each species will have its own peculiar set of tumors to which it will be hereditarily predisposed. In that event, it may become appropriate to coin some term such as "oncotaxons." I suspect that Schlumberger had thoughts of this sort in mind when he wrote his classic, but too-little-read paper titled "Tumors characteristic for certain animal species" (*63*).

The most powerful tools used to sort out hereditary cancers from others in man have been family history analysis, cytogenetic techniques, cytogenetic techniques combined with marker linkage studies, and, more recently, deletion mapping by analysis of polymorphisms in restriction enzyme-digested fragment lengths. These methods have not been applied to fish tumor studies except in the platyfish-swordtail melanophoroma system (*2*) and, with respect to cytogenetics only, in the esocid lymphomas (*12*). The methods are difficult to apply in studies of feral fishes, but improved methods of maintaining and breeding fishes in the laboratory or in hatcheries may usher in a new era.

Let us now consider the group of fish tumors that I have placed in the environmental oncozoon, to see how the experiences with them can instruct us in the search for carcinogen-indicator fish.

Historically, the experience has always started with a search for neoplasms in fish in some particular location where pollution of broad or totally undefined kind and extent was thought to exist, largely on the basis of knowledge of xenobiotic input. After unusually high prevalences of tumors in certain fishes were found, then chemical analyses of water, sediments, gastrointestinal contents, and tissues of the hosts were performed (sometimes), in an effort to find the cause(s).

During a quarter of a century of this pattern of searching, a great deal has changed. Most conspicuous, perhaps, has been the development and deployment of exquisitely sensitive and accurate methods of detection and identification of xenobiotic compounds, both organic and inorganic. Equally important have been the advances in dissecting out the mechanisms of action of chemical carcinogens and the metabolic conversion of potential carcinogens to proximal carcinogens. With the help of bacterial assays for mutagens, direct and indirect acting mutagens, many also carcinogens, have been identified. Carcinogens not previously recognized as such have also been identified in increasing numbers through rodent and fish bioassays. Pharmacokinetic studies have revealed portals of entry, tissue distribution, detoxification, and carcinogen activation sites, and excretory pathways and rates for many xenobiotics. Synergisms and antagonisms between xenobiotics have been discovered. Adducts of carcinogens with target cellular DNA, RNA, and proteins have been demonstrated and immunological methods developed for their detection. The roles of viral and cellular oncogenes have

been quite extensively delineated. In short, what has happened in the above areas of investigation is what this volume is about. What is the significance of it all?

I submit that we are approaching the point, if we have not already arrived, where it may be possible to "fix the odds" of finding carcinogen-indicator fishes by going at the problem from the causes side, rather than from the effects side. In so doing, we would be testing the predictive value of the science now available. Stepwise, the procedure would run somewhat as follows.

(1) Select an aquatic habitat in the near downstream vicinity of urban domestic and/or industrial waste outfalls.

(2) Analyze the water column, suspended and bottom sediments, and planktonic and invertebrate levels of the food web for presence of known mutagens and carcinogens, either direct- or indirect-acting.

(3) Sample the fish population to determine which species are available in sufficient numbers as candidates for carcinogen indicators.

(4) Select those species of fish whose lifestyles and feeding habits should subject them to exposure to the mutagens/carcinogens, either via water column or via contact with sediments, or via ingestion of sediments and foods containing the identified mutagens/carcinogens. Where possible, select species that spawn in and do not migrate extensively from the selected habitat.

(5) Analyze the tissues and body fluids of the selected species for content of precarcinogens, ultimate carcinogens, and mutagens corresponding to those identified in the environment. The most relevant tissues and fluids to be studied include liver, kidney, gills, skin, depot fat, ova, serum, bile, and urine.

(6) Collect large samples of the populations of the species of fish selected in step 5. Perform complete necropsies on these fish, including histologic examination for confirmation of grossly visible neoplasms and identification of microscopic neoplasms or preneoplastic lesions.

(7) Repeat step 6 in a control ("clean") habitat ecologically as similar as feasible to the contaminated habitat, and compare tumor prevalences for fish of the same species and of comparable sex and age (or size) distribution.

(8) If tumors of specific histotype(s) are found at statistically significant higher prevalences than in fish of the same species from the control habitat, an oncozoon has been identified. It now becomes pertinent to examine the possibility that this is an hereditary oncozoon.

(9) To the limited extent they are applicable, the features listed in Tables 1 and 2 as characteristic of hereditary tumors and of antioncogene involvement are used to arrive at a presumptive judgment as to whether the oncozoon has an hereditary component of the type associated with dysfunctional antioncogenes. Especially useful in this step is cytogenetic analysis to determine whether a specific visible chromosomal anomaly is present in normal cells of the tumor-bearing hosts, in the tumor cells only, or in both. If found in both, such an anomaly points to an hereditary factor responsible for loss of antioncogene function. Absence of any specific chromosomal anomaly, however, does not eliminate the possibility of an invisible mutation that may be present in antioncogenes both in the host cells and in the tumor cells.

(10) Provided step 9 reveals little or no evidence that the oncozoon has a predisposing hereditary factor, it may be assumed the oncozoon is predominantly environmental in type. At this point the initiation of another sorting-out process is indicated: that of sorting out the causal xenobiotic(s) from all the others usually present in polluted habitats. But that is another story.

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