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Evaluation of Environmental and Hereditary Factors in Carcinogenesis, Based on Studies in *Xiphophorus*

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Abstract: Neoplasia in *Xiphophorus* can be classified into a) a large group that is triggered by carcinogens; b) a large group triggered by promoters; c) a small group that develops "spontaneously" following interpopulational and interracial hybridizations; and d) a small group that develops "spontaneously" following germ line mutation. The process leading to susceptibility for neoplasia is represented by the disintegration of gene systems that normally protect the fish from neoplasia. Hybridization is the most effective process that leads to disintegration of the protection gene systems. Environmental factors may complete disintegration and thus may trigger neoplasia. It is discussed whether the findings on *Xiphophorus* may also apply to humans.

In cancer research emphasis is being placed today on physical and chemical carcinogens which we receive from our polluted environment. But the most important factors determining whether or not these agents will induce neoplasia are those that we inherit as genes from our ancestors. These genes have been evolved by mutation, selection and genetic drift in our ancestry. Some of them code for neoplastic transformation, while others protect us from cancer but may fail to work in a certain percentage of individuals. Except for some domestic animals, no animal species has such a high tumor incidence as found in the human species.

To study the environmental and hereditary factors of carcinogenesis experimentally one needs a suitable animal model. *Xiphophorus* is such a model.

Xiphophorus, including platyfish and swordtails, is a viviparous fish which was introduced to cancer research about 50 years ago when Kosswig (*1*), Gordon (*2*), and Häussler (*3*) found that certain hybrids of these fishes develop melanoma (*4*). We started our research on cancer using this animal model in 1957 (for review see Refs. *5* and *6*).

The Genetic Relatedness of Different Taxonomical Groups of Xiphophorus

Xiphophorus inhabits rivers, lakes, brooks, ponds, and pools in Central America, reproduces in closed populations, and has evolved into innumerable, phenotypically distinguishable races which are isolated geographically or ecologically. Those races in which phenotypic differences are more pronounced have been considered as species,* although all xiphophorine fish can be hybridized and all hybrids are fertile. The degree of the relationship between these groups is at the subspecies level, even between the platyfish (*Xiphophorus maculatus*, *X. variatus*, *X. xiphidium*, *etc.*) and the swordtail (*Xiphophorus helleri*, *X. montezumae*, *X. signum*, *etc.*). The basis for this taxonomic concept is the normal pairing of the chromosomes in hybrids during meiosis, the sequential conformity of satellite DNA (*9*) and the low degree of enzyme polymorphism (*10–12*). The different groups of *Xiphophorus*, known as species in the literature, actually are populations or races, comparable to the populations and races of the human species.

Insensitivity of Purebred Populations to Carcinogens

Although thousands of individuals of *Xiphophorus* from many isolates have been collected by several investigators no tumors have been detected. This indicates that neoplasia appears to develop only very exceptionally in the wild populations. Furthermore, in the progeny of wild populations, which in the case of *X. helleri* and *X. maculatus* have been bred since 1939 (about 80 and 120 generations, respectively), no tumors have occurred. These purebred wild populations also are highly insensitive to carcinogens such as X-rays, benzo(a)pyrene, and N-methyl-N-nitrosourea (MNU). About 10,000 purebred animals have been treated (*13–17*), but so far practically none have developed neoplasia. We assume that these animals have evolved stringent protection mechanisms against cancer.

Sensitivity of Hybrid Populations to Carcinogens

In contrast to the insensitive wild populations of *Xiphophorus* and their purebred descendants, hybrid populations derived from members of different purebred populations are sensitive to carcinogens. Eighteen out of 470 F_1-hybrids (3.8%) developed neoplasia following treatment with X-rays or MNU, respectively. Tumor incidence increases dramatically in the following hybrid generations (F_2–F_{24}): 582 out of 4,439 (13%) animals developed a large variety of neurogenic, epithelial, and mesenchymal neoplasia following treatment with X-rays or MNU, respectively (Table 1; Fig. 1a–c; Refs. *13–18*). Many of these hybrids developed multiple tumors. The same is true for the domesticated ornamental xiphophorine fish, which actually are also hybrids. We have designated these neoplasms as "carcinogen-triggered" neoplasms.

* Rosen (*7*), for instance, has listed 16 species, and Radda (*8*), 17 species.

TABLE 1. MNU- and X-ray-induced Neoplasms in an Experimental Hybrid Population of *Xiphophorus*

	MNU (%)	X-rays (%)
Melanoma (benign)	128 (2.88)	93 (2.70)
Melanoma (malignant)	104 (2.34)	34 (1.00)
Neuroblastoma	56 (1.26)	7 (0.20)
Squamous cell carcinoma	2 (0.05)	0
Epithelioma	10 (0.23)	6 (0.17)
Carcinoma (low differentiated)	3 (0.07)	4 (0.11)
Carcinoma (high differentiated)	2 (0.05)	5 (0.14)
Adenocarcinoma (kidney)	4 (0.09)	2 (0.05)
Adenocarcinoma (thyroid)	2 (0.05)	3 (0.07)
Papilloma	5 (0.12)	0
Hepatoma	3 (0.07)	1 (0.03)
Fibrosarcoma	82 (1.85)	6 (0.17)
Rhabdomyosarcoma	16 (0.36)	2 (0.05)
Lymphosarcoma	1 (0.02)	0
Reticulosarcoma	1 (0.02)	0
Total	419	163

One year after treatment: 4,439 survivors (compiled from Refs. *13–18*).
Five hundred and eighty-two out of 4,439 (13.11%) hybrids developed neoplasia, 87% of the hybrids were sufficently protected.

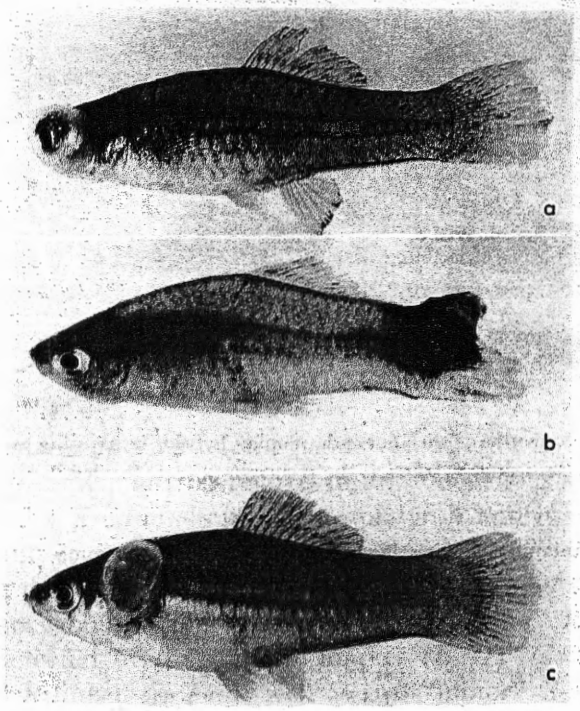

FIG. 1. (a) Neuroblastoma, (b) carcinoma, (c) fibrosarcoma; induced by MNU-treatment in *Xiphophorus* hybrids (from experiments of M. Schwab (a, b) and C.-R. Schmidt (c)).

Genetic Disintegration of Protection Mechanisms against Cancer in Hybrids

One approach to an understanding of the genetic basis of the protection mechanism against cancer, or its failure, comes from an experiment running continuously in our laboratories for breeding of animals that "spontaneously" develop melanoma, without any treatment (Fig. 2).

Crossing of a spotted platyfish with a nonspotted swordtail results in F_1-hybrids that develop benign melanoma instead of spots. Backcrossing of the F_1-hybrids using the swordtail as the recurrent parent results in offspring (BC_1), 50% of which exhibit neither spots nor melanoma while 25% develop benign melanoma (like the F_1), and 25% develop malignant melanoma. Further backcrossing of the fish (not shown in Fig. 2) carrying benign melanoma with the swordtail results in a BC_2 that exhibits the same segregation as the BC_1. The same applies for further backcrosses (see Table 2). Backcrossing of the fish carrying malignant melanoma

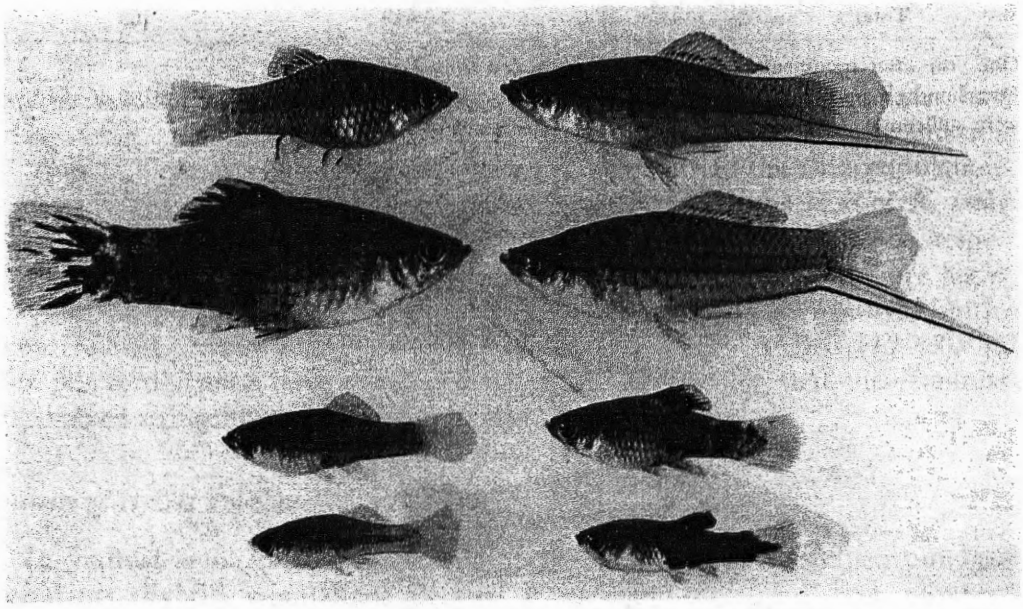

FIG. 2. Scheme of production of melanoma-developing hybrids by crossing and backcrossing of *X. maculatus* × *X. helleri* using *X. helleri* as the recurrent parent; for details see text.

TABLE 2. 1 : 1 Segregation of Animals Carrying Benign and Malignant Melanoma

Spotted stock of platyfish	BC— generation	R_{Diff}/— (benign)	—/— (malignant)
Spotted (Sp)	1-4	173	207
Spotted dorsal (Sd)	1-12	1,358	1,344
Spotted dorsal mutant (Sd′)	1-3	1,050	1,030
Stripe-sided mutant (Sr′)	1	263	250
Lineatus mutant (Li′)	1	112	119
Total		2,956	2,950

with the swordtail results in a BC_2 in which 50% of the animals do not develop melanoma, while the remaining 50% develop malignant melanoma.

As opposed to the crossing experiments described above, backcrossing of the melanoma-bearing hybrids using the platyfish as the recurrent parent results in a gradual suppression of neoplasia in the following generations (Fig. 3).

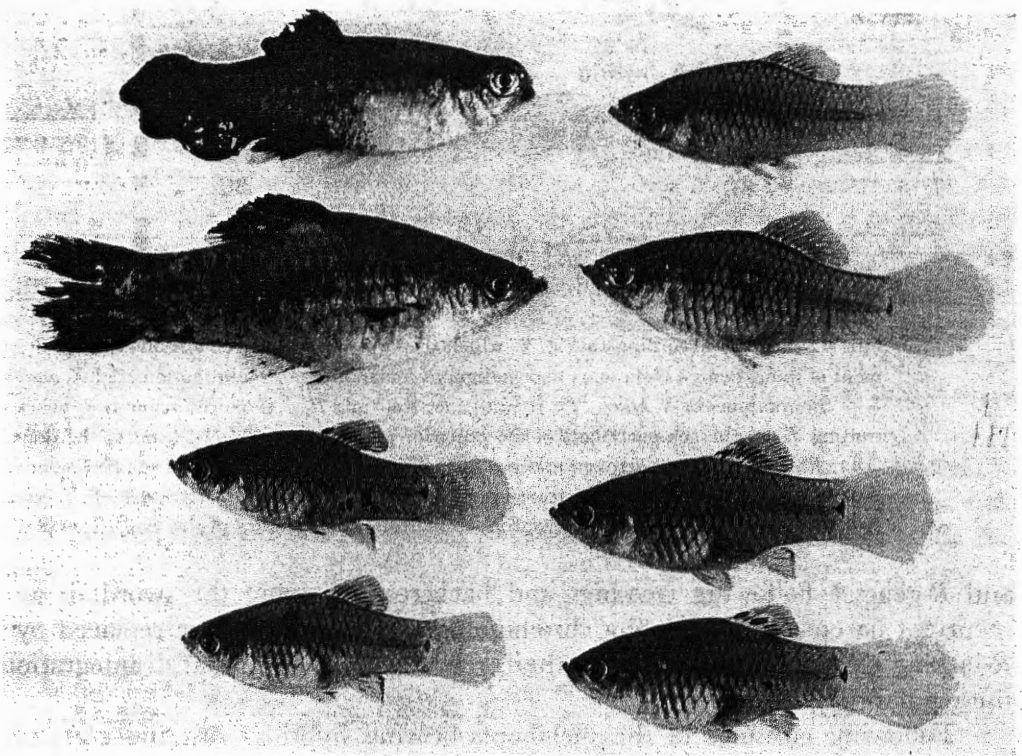

FIG. 3. Scheme of suppression of melanoma by backcrossing a melanoma-bearing *X. maculatus*/*X. helleri* hybrid with *X. maculatus*; for details see text.

These results, with the inclusion of cytogenetic findings, were interpreted as follows (see Fig. 4):

The genetic information for neoplastic transformation is encoded in a "tumor gene" (*Tu*), that might be related to an endogenous virus (*19*). *Tu* is located at the end of the X-chromosome of the platyfish and is normally under the control of linked and/or nonlinked regulating genes (*R*) (*20–22*). In the platyfish of the experiment described above, the *R*-gene that actually suppresses tumor formation is the homozygous nonlinked "differentiation gene" R_{Diff} (*5, 23, 24*) which can easily be detected by the esterase marker (*Est-1*) closely linked to R_{Diff} (*25, 26*). A "major" *R* linked to *Tu* as well as two "minor" *R*-genes which suppress melanoma formation compartment-specifically in the dorsal fin (R_{Df}) and the posterior part of the body (R_{Pp}) are mutated to R', R_{Df}', and R_{Pp}', respectively, and can no longer suppress *Tu*. Further *R*-genes also present in the system are not taken into consideration. On the other hand the swordtail lacks both the corresponding *Tu*-

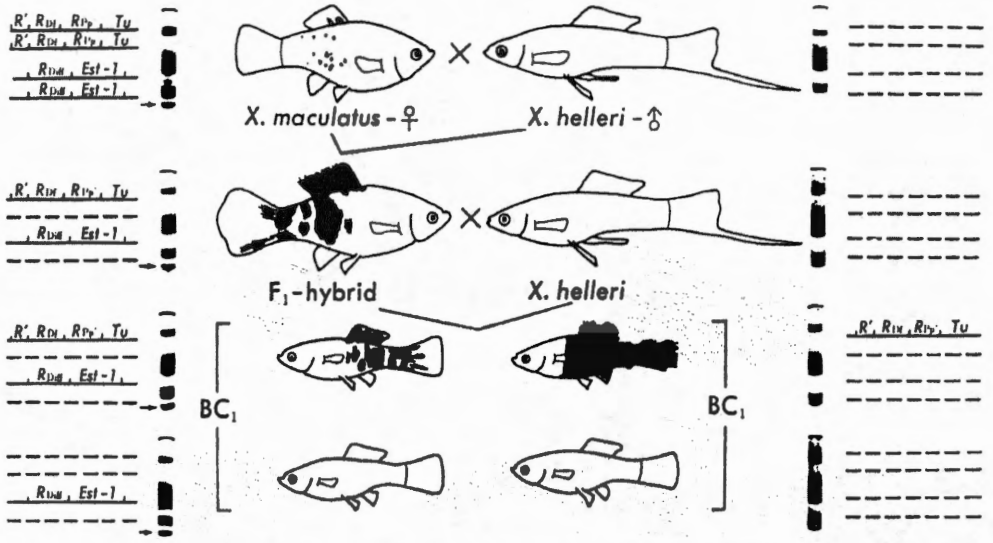

FIG. 4. Scheme according to Fig. 2, which displays the genetic conditions for the development of spots, benign melanoma and malignant melanoma. —— chromosomes of *X. maculatus*; ---- chromosomes of *X. helleri*. *Tu*, tumor gene; R_{PP}' and R_{Df}', impaired regulating genes controlling *Tu* in the compartments of the posterior part of the body (P_P) and of the dorsal fin (Df); *R'*, impaired compartment nonspecific regulating gene; R_{Diff}, regulating gene controlling differentiation of neoplastically transformed cells; *Est-1*, locus for esterase-1 of *X. maculatus* (see arrows; polyacrylamide gel electrophoresis from homogenates of the eye) (from Ref. *29*).

and *R*-genes.* Following crossings and backcrossings using the swordtail as the recurrent parent, the *R*-carrying chromosomes of the platyfish are replaced by the *R*-lacking chromosomes of the swordtail, resulting in the gradual disintegration of the regulating gene system for *Tu*.

Following crossings of the melanoma-bearing hybrids with the platyfish as the recurrent parent (see Fig. 3), the *R*-carrying chromosomes are re-introduced into the descendants, resulting in a reconstruction of the original regulating gene system that suppresses the activity of *Tu*.

"Spontaneous" development of melanoma as well as its suppression following the appropriate crossing procedures were found in several experimental hybrid populations derived from different purebred populations of different geographical or ecological origin. Genetic analysis showed that the *R*-gene systems suppressing *Tu* are population-specific (*13*). The same applies, in principle, for the "spontaneous" development of neuroblastoma and thyroid carcinoma also found in the experimental hybrid populations (*6*).

Thus the genetic protection mechanisms against cancer have evolved independently in the different populations or races and, consequently, become dismantled if chromosomes derived from different populations are combined in the hybrids.

Since the mode of the "spontaneous" occurrence and disappearance of these

* Several copies of *Tu* may be present in all individuals but it is part of gene complexes that are not expressed in these experiments (*6*, *13*).

tumors in the different generations of fish broods appears to show similarities to the mode of the spontaneous occurrence and disappearance of certain tumors in generations of human families, we would like to designate them as "familial" neoplasms. Familial neoplasms are restricted to very few hybrid broods as compared to the carcinogen-triggered tumors.

Disintegration of Protection Mechanisms against Cancer as a Precondition for the Development of Carcinogen-triggered Neoplasia

In order to disclose the genetic basis for the development of those neoplasms which must be triggered by a carcinogen, we have modified the experiment shown in Fig. 4 to the experiment shown in Fig. 5: the $R'/R_{Df}'/R_{Pp}'/Tu$-chromosome was replaced by the $R/R_{Df}/R_{Bs}'/Tu$-chromosome, the "major" R of which is nonmutated and active. Since this R is inherited along with Tu, neoplasia does not develop spontaneously in the hybrids. Following treatment with carcinogens, those hybrids carrying the R/Tu-chromosome turned out to be susceptible to carcinogen-triggered neoplasia (*27*); those lacking R_{Diff} (determined by the esterase) are highly susceptible. We assume that the R present in the system becomes impaired or is deleted by the carcinogen in a somatic cell thus giving rise to nepolasia.

The majority of the carcinogen-triggered neoplasms (see Table 1 and Fig. 1) is presumably due to such a single mutation event in a particular somatic cell. This is derived from the fact that these neoplasms appear as foci of cells in the fish tissues.

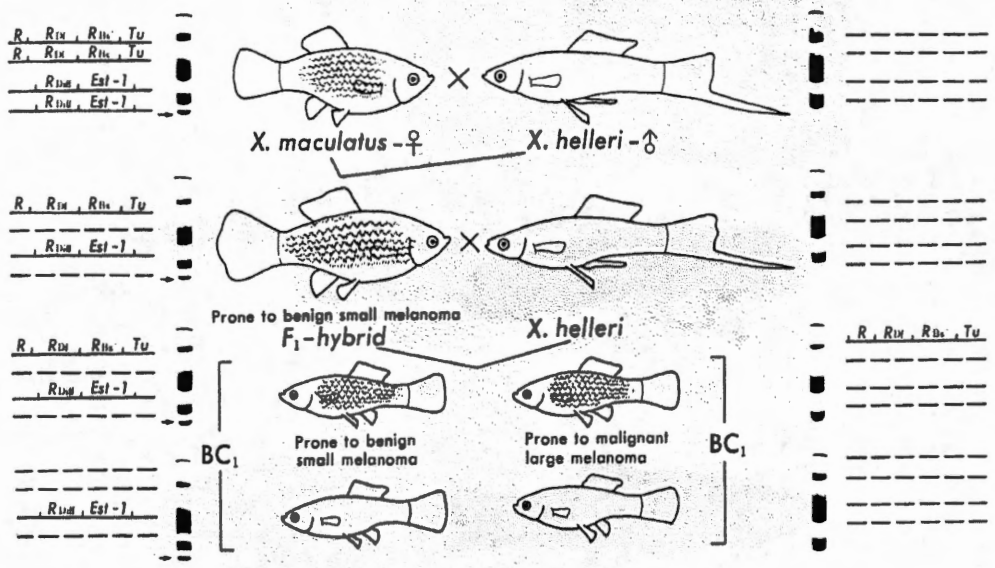

FIG. 5. Crossing scheme displaying the procedure for obtaining fish of genotypes prone to benign melanoma or to malignant melanoma following carcinogen treatment. Abbreviations as in Fig. 4. R_{Bs}': impaired regulating genes controlling Tu in the compartment of the body side (Bs); for details see text (from Ref. *27*).

Disintegration of Protection Mechanisms against Cancer by Germ Line Mutation

Examining 66 structural changes (deletions, duplications, translocations) of different X- and Y-chromosomes in the germ line of different *Xiphophorus* stocks (*20*) we found a mutant in which the *Tu* is detached from its linked *R*-genes and is translocated to an autosome. As a consequence of this event melanoma development starts in the tail fin of 14-day-old embryos, extends to all areas of the developing embryo and the young fish is thus building a lethal "whole body melanoma," which reflects the genuine effect of the completely derepressed *Tu* on the melanophore system (Fig. 6).

We have also selected laboratory stocks carrying other germ line mutation-conditioned melanomas which are restricted to certain compartments of the fish.

Tumors that are related to chromosomal rearrangements in germ line cells are inherited according to a dominant Mendelian trait. We therefore designate them as "Mendelian inherited" neoplasms. Mendelian inherited neoplasia in *Xiphophorus* is not restricted to hybrids but was also found exceptionally in the purebred progeny from X-irradiated fish of wild populations. Broods containing animals which develop Mendelian inherited neoplasms are very rare as compared to broods containing carcinogen-sensitive animals that may develop carcinogen-triggered neoplasms.

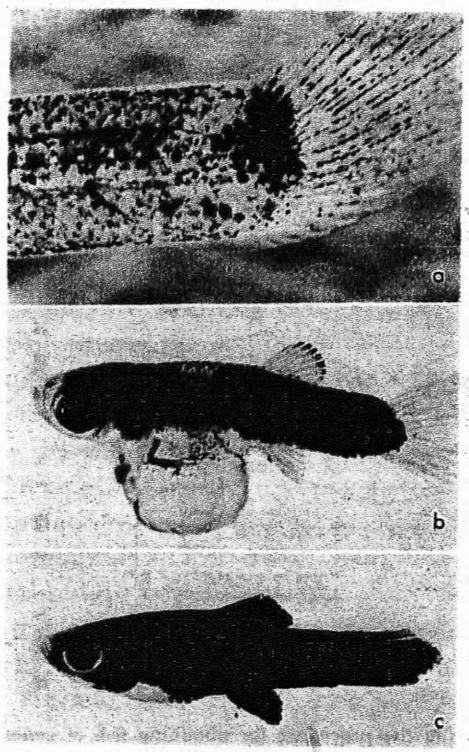

FIG. 6. Tumor gene-mediated melanoma conditioned by germ line mutation. (a) Tail of a 14-day-old embryo (3 mm in length) exhibiting some T-melanocytes at the peduncle of the tail fin. (b) 18-Day-old embryo (4 mm in length) exhibiting a whole body melanoma. (c) Neonate (6 mm in length) exhibiting a whole body melanoma (from Ref. *32*).

Disintegration of Protection Mechanisms against Cancer by Promotion of Cell Differentiation

From fish carrying *Tu* but obviously lacking all the *Tu*-specific *R*-genes (following hybridization or germ line mutation) we bred several stocks that are still incapable of developing melanoma spontaneously. In these fishes differentiation of pigment cells *via* neural crest cells, chromatoblasts, stem(S-)melanoblasts, intermediate(I-)melanoblasts, advanced(A-)melanoblasts, melanocytes and melanophores (see Fig. 8), is almost completely delayed at the S-melanoblast stage which is not yet capable of neoplastic transformation (*28–31*). Those cells entering the stage of I-melanoblasts, which is the only stage capable of neoplastic transformation, become neoplastically transformed. Immediately thereafter they become terminally differentiated and are removed by macrophages. In these animals both the pretransformational delay of cell differentiation and the post-transformational terminal cell differentiation represent the only protection against cancer that remains in the system.

Chemical and biological agents, such as methyl-testosterone (*31*), cyclic AMP (*32*, *33*), corticotropin (*32*), 12-O-tetradecanoyl phorbol-13-acetate (TPA) (*34*), 2-4-dinitrochlorobenzol (DNCB) (*35*), and BrdUrd (*36*), as well as general environmental changes, such as a decrease in the temperature and an increase in the salinity of the water in the tank, promote the differentiation of large amounts of

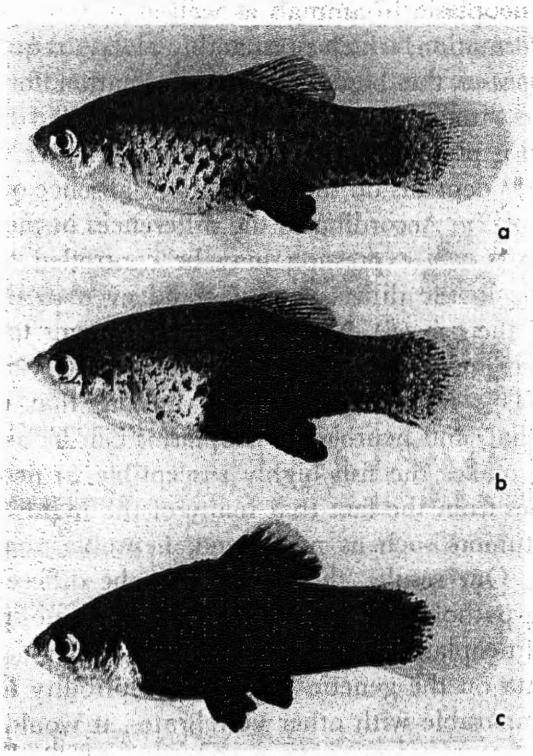

FIG. 7. Melanoma triggered presumably by endogenous steroid hormone (see text). Melanoma formation started when the fish became the dominant male in the swarm. (a) Beginning of melanoma development. (b) 6 weeks later. (c) 12 weeks later.

noncompetent cells to competent ones, which subsequently become neoplastically transformed. Thus promoters of cell differentiation appear as promoters of neoplasia, provided the *Tu* is derepressed and cell differentiation is delayed at the precompetent stage. Carcinogens such as X-rays, UV, and MNU, which are powerful mutagens, may also trigger cell differentiation in these animals like methyltestosterone, cyclic AMP, corticotropin, TPA, *etc.*, which certainly are not mutagens.*[1]

Promoters of differentiation stimulate neoplasia in a large variety of hybrid genotypes (*31*, *37*). We assume that this is also the case if the promoters are produced endogenously. Evidence comes from melanomas that develop "spontaneously" during maturity, preferentially in males that are sexually highly active (Fig. 7) (*13*). Probably these melanomas are triggered by steroid hormones (*31*, *37*).

Thus, agents that are not carcinogens may overcome the protection mechanism that acts *via* delay of differentiation. The resulting neoplasms are designated as "promoter-triggered" in this paper.

The Common Genetic and Cellular Basis of Mesenchymal, Epithelial, and Neurogenic Neoplasms

While the development of mesenchymal, epithelial, and neurogenic neoplasms is mostly considered to be independent, it appears that regardless of what causes cancer, the process leading to neoplasia in animals as well as in humans is always the same, *i.e.*, neoplastic transformation, which presumably always requires specific genetic information. In *Xiphophorus* the basic genetic information for neoplastic transformation comes from the "tumor gene" *Tu* which is inherited through the germ line of all individuals and is present in all somatic cells. Whether *Tu* becomes expressed as a deleterious gene*[2] depends on the inactivity or absence of genes that normally suppress the activity of *Tu*. According to the differences in mesenchymal, epithelial, and neurogenic tissues, *Tu* expression may be controlled by different sets of regulating genes specific to the different tissues and even to the different compartments of the tissues of the fish (*6*). Nevertheless there seems to exist a superior genetic mechanism that controls *Tu* independently from the specific tissue in which it may be expressed. This is derived from the finding that carcinogen-triggered mesenchymal, epithelial, and neurogenic neoplasms can be assigned to a particular chromosome which makes the fish highly susceptible to neoplasia (see Fig. 5). Further evidence comes from the fact that many of the highly susceptible individuals develop multiple tumors such as melanoma, neuroblastoma, rhabdomyosarcoma, and epithelioma. Our results, therefore, unify the different kinds of cancer in *Xiphophorus*, and the pathological peculiarities of the tissue-specific neoplasms appear as accessories of neoplasia (*21*, *38*, *39*).

While we do not have data on the genetic basis of susceptibility to neoplasia in humans and none even comparable with other vertebrates, it would be worth-

*[1] We have developed test systems in which we can distinguish between the initiating and promoting activity of a carcinogen, (see Ref. *31*).

*[2] We assume that *Tu* has important functions in ontogeny which at present are not known (*6*).

while to examine whether the unity of neoplasia suggested in the *Xiphophorus* model may be valid for neoplasia in general.

The Common Basis of Neoplasia Etiology

It is generally accepted that neoplasia in humans can be classified according to etiology into three groups: a) a large group of "carcinogen-dependent" neoplasia (almost 50% of all tumors), *e.g.*, lung cancer; b) a large group of "endocrine-dependent" and "digestion-related" neoplasia (also almost 50% of all tumors), *e.g.*, breast, prostatic, and colon cancer; and c) a small group of neoplasms in which genetic factors are supposed to be involved, *e.g.*, retinoblastoma, meningioma, melanoma, *etc.*

In principle the same classification presents itself in the *Xiphophorus* model, comprising (a) the "carcinogen-triggered," (b) the "promoter-triggered," and (c) the "familial" and the "Mendelian inherited" neoplasms.

While these etiological groups of neoplasia are considered to be independent in humans (and other mammals), we found in our model that they are closely related to each other by the genetic and cellular basis of their development. This will be explained by means of the different etiological types of melanoma, on which the most data are available (Fig. 8).

Genotypes showing a high susceptibility to the induction of neoplasia by carcinogens carry a *Tu* that is repressed by only one *R*-gene (see Fig. 5). If this *R* remains unchanged, or becomes impaired or deleted in a noncompetent cell like an A-melanoblast (Fig. 8, on the left and Aa), no melanoma will develop. If, however, this *R* becomes impaired or deleted in a competent or precompetent cell,

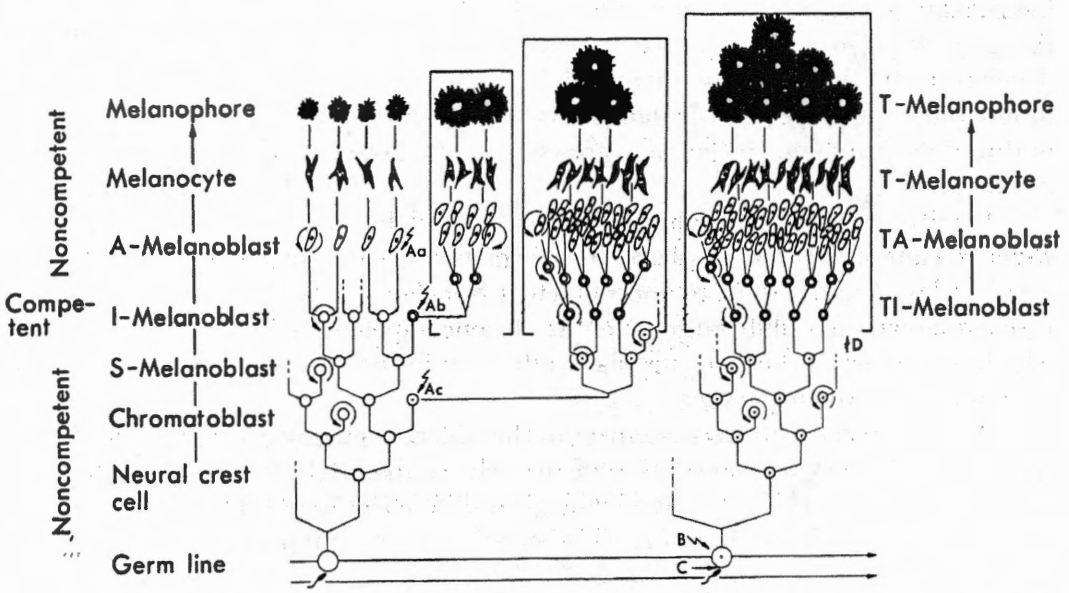

FIG. 8. Differentiation of normal and of neoplastically transformed pigment cells in different etiological types of neoplasia; for details see text.

melanoma develops. Depending on the stage of differentiation of the cell in which the mutation occurs, and depending on its potency in diffferentiation and proliferation, the descendants of that cell have a different fate, which opens it to the different etiological types of neoplasia mentioned above (compare Ab, Ac, B/C, D in Fig. 8).

Derepression of *Tu* may be induced by mutation of the *R* in an I-melanoblast (Ab in Fig. 8). This cell is competent and becomes neoplastically transformed. Following the processes involving cell division and cell differentiation, the transformed cells (T-cells) form an easily detectable small cell clone that gives rise to neoplasia. The origin of such a carcinogen-triggered neoplasm is unicellular, and the melanoma grows exclusively by proliferation.

The *R* may also mutate in an S-melanoblast (Ac in Fig. 8). This cell is not yet competent. It still remains untransformed and may multiply over a long latent period as a normal stem cell. Later on, those descendants reaching the stage of competence by differentiation are simultaneously transformed. After some cell divisions, paralleled by progress in cell differentiation, they become visible as a large cell clone consisting of hundreds or thousands of dividing TA-melanoblasts and T-melanocytes, which give rise to melanoma.

Those S-melanoblasts which do not further differentiate may reproduce identically throughout the further life of the fish and may serve as a permanent source of I-melanoblasts, which then become neoplastically transformed. The origin of such a carcinogen-triggered melanoma is multicellular, although it can be traced back to a single mutational event in a somatic cell. The melanoma grows by both permanent transformation and proliferation of the descendants of the mutated cell.

Derepression of *Tu* may also be induced by mutation (B in Fig. 8) or by hybridization-conditioned elimination of the *R* (if it is nonlinked; C in Fig. 8) in the germ line. As a consequence "Mendelian inherited" or "familial" melanoma develops in the progeny "spontaneously" as soon as noncompetent S-melanoblasts differentiate to competent I-melanoblasts. The origin of such a melanoma is highly multicellular, and the melanoma grows by both permanent transformation and proliferation.

Finally, the *Tu*, although already derepressed by mutation or elimination of *R*-genes, cannot mediate neoplastic transformation because pigment cell differentiation is delayed in the stage of noncompetent S-melanoblasts (D in Fig. 8). In this case promoters may shift large amounts of noncompetent cells to competent ones, which subsequently become neoplastically transformed by *Tu*, and give rise to promoter-triggered melanoma.

A comparison of the development of the different etiological types of neoplasia shows that the genetic constitution of the cells is absolutely the same in all cases. Morphological, pathological, and cytological differences found in these melanomas (*e.g.*, size, invasiveness, chromosomal aberrations), are epiphenomena of the basic event, *i.e.*, neoplastic transformation (*38*, *39*).

The Significance of Hybridization for Susceptibility to Neoplasia

Xiphophorine fish do not develop neoplasia following any one of the tumor-inducing methods alone. They develop neoplasia only if different methods are combined, involving a) elimination of *R*-genes by hybridization, b) germ line mutation of *R*-genes by mutagens, c) shift of noncompetent cells to competent ones by promoters, and d) somatic mutation of *R*-genes by mutagens. The events leading to neoplasia represent a multistep process. It depends on the experimenter to determine the succession of the different steps, and it is easy to see that the last step that completes the multistep process determines the etiological type of neoplasia, *i.e.*, a) familial neoplasia, b) Mendelian inherited neoplasia, c) promoter-triggered neoplasia, and d) carcinogen-triggered neoplasia.

As we discussed in previous section the majority of the neoplasms in *Xiphophorus* is represented by carcinogen-triggered and promoter-triggered tumors, and these are also about 90% of all human neoplasms. In the fish system the steps that precede the trigger do not occur in the neoplasia developing animals but in those of the preceding generations which were still more or less insusceptible to neoplasia. Germ line mutations and interpopulational or interracial matings in the preceding generations are the events that contribute to the disintegration of the genetic protection mechanisms. Out of these events, germ line mutations are less important

TABLE 3. Neoplasms in Animals

Drosophila laboratory stocks	Various neoplasms (*45*)
Solenobia hybrids	Various neoplasms (*46*)
Xiphophorus hybrids	Various neoplasms
Girardinus laboratory stocks	Promoter-triggered melanoma (*47*)
Ornamental guppy strains	Carcinogen-triggered hepatoma (*48*)
Orange medaka	High incidence of hepatoma (*49*)
Domesticated trout	Aflatoxin-induced liver tumors (*50*)
Salvelinus hybrids	Fibrosarcoma (*51*)
Domestic carp	Neuroepithelioma (*52*)
Ornamental hybrid carp	Ovarian neoplasia (*53*)
Lake Ontario hybrid carp	Pollution-conditioned gonadal tumors (*54*)
Goldfish	Erythrophoroma (*55*)
Bufo calamita/viridis hybrids	Chordomas (*56*)
Musk duck/mullard hybrids	Gonadal tumors (*57*)
Peacock/guinea fowl hybrids	Gonadal tumors (*58*)
Improved breeds of fowl	Leukosis (*59*)
Mus musculus/M. bactrianus hybrids	High tumor incidence (*60*)
Laboratory mice strains	Various neoplasms
Hybrids of mice strains	High tumor incidence (*61*)
BALBc/NZB hybrids	50% Plasma cell tumors (*62*)
Blue ribbon mice	100% Mammary tumors (*63*)
Sprague Dawley/Long Evans rat hybrids	Increased tumor incidence (*64*)
Domestic dogs	Various neoplasms (*59*)
Boxer dogs	Very high tumor incidence (*65*)
Domestic cats	Various neoplasms (*59*)
Sinclair swine	Melanoma (*66*)
Lippizaner horses	100% Melanoma (*67*)

than hybridization because they are always rare or become repaired, respectively, as compared to crossings that are easily accomplished. On the other hand, somatic mutation and promotion cannot contribute to the disintegration of protection mechanisms against cancer in the germ line but can only complete this disintegration. Thus the majority of the neoplasms in *Xiphophorus* belongs to those types that are triggered by carcinogens or promoters, like their counterparts in humans.

The phenomenon of introducing susceptibility to neoplasia through hybridization is not limited to *Xiphophorus*. Susceptibility to neoplasia has been observed in a large variety of plant hybrids, especially in cultivated plants, that are mainly bred by hybridization methods. Hybrids of cabbage, lilies, tobacco, tomatoes, calanchoe, thorn-apple, poplar, *etc.* are well-known examples (*40–44*). Furthermore, many examples can be cited from the animal kingdom (see Table 3). It appears that: (a) in animals from wild populations neoplasia is difficult to induce and the incidence of "spontaneously" developing neoplasia is low, while (b) in hybrids as well as in domestic and laboratory animals (that actually are also hybrids) neoplasia is easily inducible and the incidence of "spontaneously" developing neoplasms is high (see also Ref. *59*). This does not exclude that there are populations in the natural habitat that overlap and hybridize, thus giving rise to a hybrid population that is susceptible to neoplasia. The high tumor incidence in certain Pacific flatfish populations could be interpreted in this sense (*68*).

While we do not have data on the relation between hybridization and cancer in human beings comparable to the data on plants and animals, it is interesting to speculate whether the many facts on tumor incidence in humans that do not agree with the concept of the primacy of environmental factors in carcinogenesis (*69–71*) can be explained by interpopulational and interracial hybridization in preceding human generations. Certainly, interpopulational and interracial human mating may have occurred at any time in any place. Because of the high mobility of humans as compared to other mammals one should also expect high values of heterogeneity. Various estimates based on enzyme variation showed that heterogeneity in humans is comparable to that of domestic animals such as cats, but is about six times greater than that of two species of (wild) macaques (*Macaca fuscata* and *M. cyclopis*), about ten times greater than that observed in the large wild mammals such as elk, moose, polar bear, black bear and elephant seal, and about twice as great as that of most feral rodents studied so far (see reviews and discussions in Refs. *72–74*). On the basis of these data and on the assumption that tumor incidence in humans is related to interpopulational and interracial hybridization like that of domestic animals, one could explain why humans have a high incidence of neoplasia as compared to animals in wild populations.

There may be also differences in the frequency of such hybridizations in humans, which might be due to geographical, ecological, political, ethnological, religious and other exogeneous factors. Of course, it is very difficult or even impossible to determine the heterogeneity of a recent human population in terms of biological measures.

Nevertheless there are at least some data on chromosomal heteromorphisms in human populations that might be useful for estimates of heterogeneity within

and among different populations. According to such estimates it appears that, for instance, Japanese populations exhibit a low degree of Q- and C-band chromosome heteromorphisms, whereas Americans have a much higher degree of this heteromorphism, with blacks having more prominent heteromorphisms than whites (*75*, *76*). One is tempted to assume that this chromosomal heteromorphism reflects the differences in the degree of the heterogeneity between the Japanese and white and black U.S. populations.

It is interesting to note that the ratio of prostatic cancer in Japanese, U.S. whites and U.S. blacks is reported as 1: 30: 60 (*77*, *78*). Such differences cannot be explained by environmental carcinogenic influences, which certainly differ only in a low degree. They also cannot be explained by racial differences; natural selection will not favor one race and discriminate against another but will work against susceptibility to cancer in all populations and races. We suggest that these differences in tumor incidence are due to different degrees of interpopulational and interracial matings in nations, which might dismantle protection mechanisms against cancer as it does in *Xiphophorus*.

Similar explanations might be conceivable, for instance, for the differences in tumor incidence between African blacks and American blacks, which is 1: 3.

On the same basis one could explain the independence of tumor incidence from changes in the environment. For instance, in the area of West Germany, where environmental conditions changed dramatically in the beginning of this century, no change in tumor incidence (standardized for age) can be detected (*69*, *70*). This indicates that the frequency of individuals being insusceptible or susceptible to cancer remained constant, presumably by virtue of an unchanged interpopulational mating behavior. The considerable low differences in tumor incidence between polluted and nonpolluted areas in the U.S.A. might be also explainable by an overall constant frequency of susceptible and insusceptible individuals due to a constant degree of heterogeneity. On the other hand, the extreme low tumor incidence of active Mormons and Seventh-Day Adventists, as compared to the total U.S. white population (*79*) might be due rather to the biological homogenicity of their population (which favors insusceptibility to cancer) than to environmental factors. The same could apply for the low tumor incidence in Japan as compared to that of other industrial nations.

Breast and colon cancer in humans, which represent a very high percentage of total neoplasia, has been found to be highly correlated to animal fat intake in 39 nations (*80*), and it has been proposed that low animal fat intake is responsible for a low, while high animal fat intake is responsible for a high incidence of these neoplasms. The order of the nations is headed (low fat intake, low tumor rate) by Thailand, the Phillippines, Japan, and Taiwan, continues to Czechoslovakia and Austria and ends with the Netherlands, the U.S.A., Canada, Denmark, and New Zealand (high fat intake, high tumor rate). It has been shown, however, that tumor incidence in the Dutch is twice as high as that in the Finns, though both have the same fat intake. The same is true if we compare the Swiss (high tumor incidence) with the Polish (low tumor incidence, but same fat intake). The Danes have an extremely high animal fat intake and an extremely high incidence of breast cancer.

If one compares, however, the population of Copenhagen with that of rural Denmark one finds that fat intake in Copenhagen is much lower than in rural Denmark while urban Danes have a higher tumor incidence than rural Danes.

This is not to say that fat intake will have no influence on the incidence of breast and colon cancer, however it becomes clear that fat intake alone cannot explain the differences in tumor incidence in the different nations. In our opinion it might be extremely valuable to investigate how much effect hybridization may have on the frequency of neoplasia in our highly developed nations that certainly are melting pots of mankind in contrast with those that consist of genetically more homogeneous populations. Similar ideas were expressed many years ago by W. E. Heston (*81*).

Environmental Factors versus Hereditary Factors

Genetic studies have identified hereditary factors that operate in only a small group of human neoplasms (see previous section) while epidemiological studies suggest a predominant role of environmental factors (*e.g.*, cigarette smoke tar, UV, X-rays, asbestos, many chemical and biological substances, life style, *etc.*) in the causation of the majority of neoplasms in humans (*77*, *78*, *82*, *83*). Emphasis is being placed on the detection of these environmental factors, and expectations are being raised that cancer will become a rare disease if these factors are removed from the environment. Apart from it being impossible to remove all carcinogenic factors from our environment, statistical studies have raised doubts that these expectations will become totally true (*69*, *70*).

Based on our studies on *Xiphophorus* we suppose that environmental factors represent only the tip of an iceberg in the multistep process of the causation of neoplasia. The most important steps leading to neoplasia, *i.e.*, those that bring about susceptibility, are supposed to be hidden in our ancestry. Hybridization of members of different populations as well as germ line mutations (possibly facilitated by hybrid dysgenesis; Refs. *84*, *85*) might have disintegrated the genetic mechanisms that originally evolved to protect us from the deleterious activity of the genetic information for neoplastic transformation, which in the controlled state seems to be a normal part of our genome. During the life of a human being they have to retain their function for over 70 years while those of *Xiphophorus* must operate for 2 years only. From *Xiphophorus* we know that the entire regulating gene system which makes the fish insusceptible to neoplasia over their lifespan is polygenic, and susceptibility is raised step by step as the regulating gene system becomes dismantled. For humans one is tempted to suggest the same principle that leads to susceptibility. The complete protection gene system operating on the level of gene regulation, cell differentiation, proliferation, immune surveillance, *etc.*, however, must be more polygenic in orders of magnitude as compared to that of *Xiphophorus*. If the protection gene system has been disintegrated in the ancestry enough genes still remain to protect us from cancer through a certain part of our life. In the course of life, however, they might become impaired one after the other

in the descendants of a certain cell, and mutation of the last regulating gene may trigger neoplasia.

Thus, the state (complete or dismantled) of the protection mechanisms against cancer which we inherit from our ancestors determines whether we are sufficiently protected from cancer or whether environmental factors (carcinogens or promoters) may trigger neoplasia in earlier or later years of our lifespan.

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