Effects of X-Irradiation on the Genetically-Determined Melanoma System of Xiphophorin Fish

There are many reports of the decreased proliferation of cells in all tissues as a result of extensive physiological damage caused by X-irradiation[1]. However, such treatment sometimes gives rise to accelerated proliferation, resulting in the appearance of tumors[1,2]. In order to investigate this controversial subject, we have used the in vivo system of genetically-determined melanomas of xiphophorin fish, which is produced by combining certain hereditary factors of *Platypoecilus maculatus* (platyfish) and *Xiphophorus helleri* (swordtail)[3].

In purebred platyfish, which carry a macromelanophore gene, a defined number of macromelanophores forms specific black spots on the body surface. F_1 hybrids between these 2 species show an increase in the number of these macromelanophores, resulting in the formation of large, black areas (premelanomas).

Backcross hybrids bred using the swordtail as the recurrent parent show an additional overproduction of macromelanophores in these areas, resulting in the formation of melanomas. This melanoma formation can be reduced to normal spots by repeated backcrossing with platyfish. Because the gradual replacement of platyfish chromosomes by the corresponding swordtail chromosomes results in an enhancement of the macromelanophore gene expression and the opposite replacement in a diminution, it is concluded that platyfish repression genes are capable of controlling and limiting this expression[4].

Recently, we have studied the effects of X-irradiation on xiphophorin fish having macromelanophore genes under various degrees of repression and being in various stages of development.

Conditions of irradiation. The animals were irradiated in a metal basin filled to 2 cm with water. This basin was placed 80 cm from the focus of a Röntgen Miller apparatus MG 150. X-rays were emitted at a dose rate of 22 R/min, 150 kV, 12 mA and filtered through 0.2 mm Cu and 0.5 mm Al.

Experimental animals and results. a) Genotypes having a repressed macromelanophore gene (purebred *P. maculatus* and the 4th backcross generation toward platyfish). These animals display only species-specific macromelanophore spots (Figure 1a).

Whole-body X-irradiation (100–2000 R)[5] of adult fish of this type (n = 133) has no obvious effect on the production of macromelanophore spots[6]. However, treatment (500–2000 R) of embryos and female germ cells[7] causes a uniform enlargement of the spots to thickened areas in all fish which develop from them (n = approximately 1000; Table, first row). These thickened areas resemble those premelanomas of nonirradiated F_1 hybrids (Figure 1a, b and c). Nonirradiated offspring of fish which have been irradiated as embryos also exhibit these enlarged spots (n = approximately 2000). Stocks of this phenotype have been bred successfully to the 7th generation.

This enlargement of macromelanophore spots is caused by an increase in the number of macromelanophores

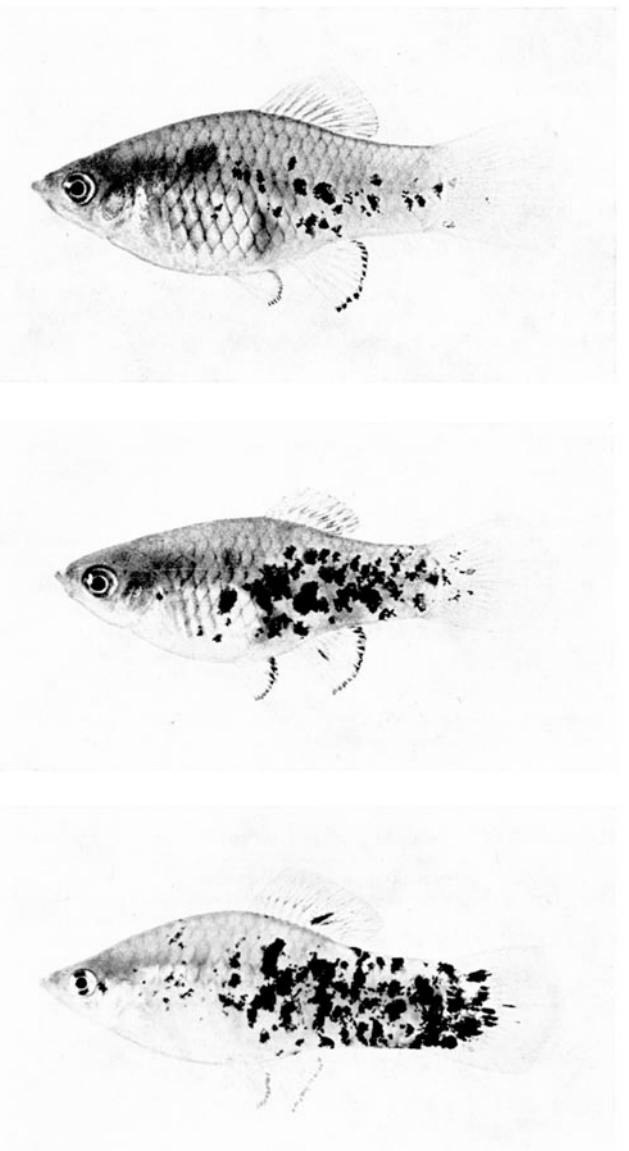

Fig. 1. a) Nonirradiated purebred *Platypoecilus maculatus* (*Sp* genotype). b) Purebred *P. maculatus* (*Sp* genotype) irradiated as embryo. c) Nonirradiated F_1 hybrid between *P. maculatus* and *Xiphophorus helleri* (*Sp* genotype).

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[5] The $LD_{50(30)}$ of adult *P. maculatus* varies around 2000 R, that of *X. helleri* around 3500 R and that of most hybrids around 3000 R.

[6] Whole-body X-irradiation (1000–2500 R) of genotypes having no macromelanophore gene (purebred *X. helleri* and certain backcross hybrids; n = 100) has no effect on the lack of production of macromelanophore spots, whether adults or embryos are treated.

[7] Sperm and spermatogonial stages present in adult males were not taken into consideration.

displayed by young animals, the macromelanophore areas of which have not yet been completely populated by these cells (Figure 2a and b). This enlargement is not to be confused with the hyperpigmentation[8] discussed by other authors[8,9], because differentiated macromelanophores of xiphophorin fish exist only in a melanized state[10].

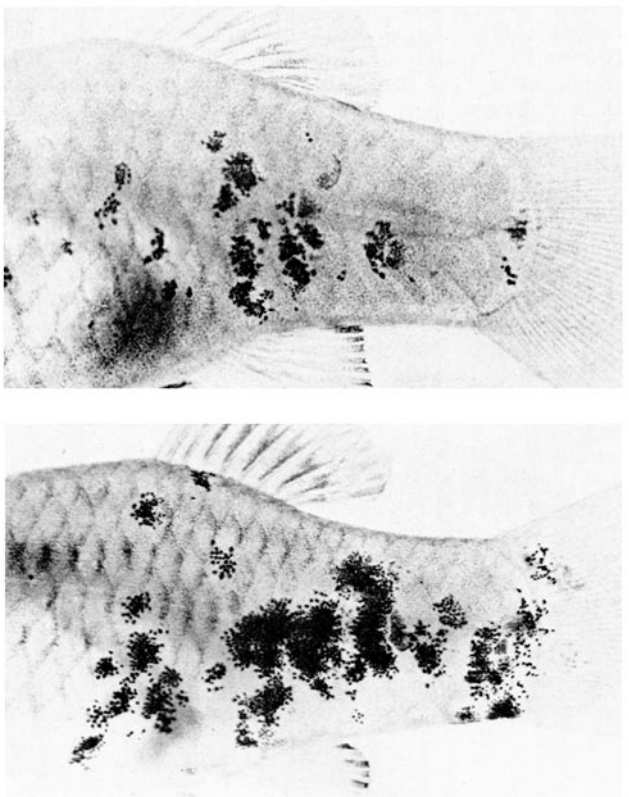

Fig. 2. a) Typical quantity of macromelanophores in a nonirradiated young purebred *Platypoecilus maculatus*. b) Increased quantity of macromelanophores in a young purebred *P. maculatus* the grandparents of which were irradiated as embryos.

Observable effects of X-irradiation on melanoma systems having various degrees of repression and being in various developmental stages

Repression gene system	Macromelanophore gene expression in adult fish		
	Nonirradiated	Irradiated	
		As adults	As embryos and germ cells in adult females
Complete	Macromelanophore spots	Macromelanophore spots (no effect)	Enhancement of spots to premelanomas
Incomplete	Premelanomas	Regression of premelanomas	Temporary enhancement of premelanomas
Absent	Melanomas	Regression of melanomas	Melanomas (no effect)

b) Genotypes having an incompletely repressed macromelanophore gene (F_1 and other hybrids). These genotypes exhibit premelanomas (Figure 1c).

Whole-body X-irradiation (150–3500 R) of adult fish of this type results in a decrease in premelanoma formation ($n = 546$). However, a slight and distinct, but temporary enhancement of the normal overproduction of macromelanophores is caused in developing fish, when these have been irradiated (1000 R) as embryos or female germ cells ($n = 152$; Table, second row). The effect on the offspring of these fish has not yet been investigated.

c) Genotypes having a derepressed macromelanophore gene (backcross hybrids having only the macromelanophore gene-carrying chromosome from the platyfish). These fish develop melanomas (see Figures in ref.[4]).

Whole-body X-irradiation (150–2500 R) of adult fish of this type causes a temporary regression or discontinuation of melanoma growth ($n = 79$; Table, third row). This observation has been supported by results showing corresponding decreases in H^3-thymidine incorporation and in the level of free amino acids in the cell pool[11]. No obvious effect on the offspring has been noticed.

Discussion. An enhancement in the production of macromelanophores is caused by X-irradiation of the earlier developmental stages of those xiphophorin fish which have a repressed macromelanophore gene. No such enhancement is observed as a result of the same treatment of the same stages of fish which have a derepressed gene. These results have led us to the conclusion that X-irradiation causes an alteration in the system of repression genes of the macromelanophore gene. Since this alteration occurs uniformly in somatic cells, as well as in all germ cells, it is thought that this repression gene system consists of common cell constituents which are present in very large quantities.

On the contrary, a regression in the production of macromelanophores is caused by X-irradiation of adults which have a derepressed macromelanophore gene. This effect is primarily due to the extensive physiological damage done to the entire melanoma system by this treatment[11]. Fish which have an incompletely repressed macromelanophore gene show both regression of premelanoma growth upon irradiation of adults and enhancement of this growth upon treatment of embryos.

Thus, the effect of X-irradiation on the melanoma system of xiphophorin fish depends on: 1. The degree to which a repression gene system is present and 2. the developmental stage of the treated organism. This suggests that reports on the effect of X-irradiation have continued to disagree because the genetic constitution and developmental stages of the systems involved have not been taken into serious consideration[12].

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Zusammenfassung. Röntgenbestrahlung von Embryonen und Keimzellen bestimmter Zahnkarpfen-Genotypen, deren Makromelanophorenproduktion durch Kontrollgene auf die Bildung kleiner Flecken begrenzt ist, verursacht bei heranwachsenden Tieren und deren Nachkommen Prämelanome. Demgegenüber hat die gleiche Behandlung dieser Entwicklungsstadien bei Genotypen, deren Makromelanophorenproduktion infolge Fehlens der Kontrollgene im Verlaufe des weiteren Lebens zur Melanombildung führt, keinen Effekt. Bestrahlung der melanomtragenden Adulten dieses Genotyps verursacht eine vorübergehende Unterbrechung des Tumorwachstums.

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Delay in Skin Allograft Rejection in Rats Grafted with Fetal Adrenal Glands

The adrenal gland is large in the mammalian fetus, decreasing later at a different rate according to the species. In mice[1] and rats[2] this involution is considerably retarded with respect to other species and the immunological system of these animals is not completely developed at birth[3–5]. In men[6] and guinea-pigs[7] the involution of the fetal adrenal gland takes place early in gestation and this is correlated with a better development of immunological functions at birth[8–10]. These facts, added to the well known functional antagonism between adrenal gland and thymus and the immunosuppressive action of adrenal glucocorticosteroids, suggest that the fetal adrenal gland might be involved in the control of the development of the immunological system. With the aim of exploring this possibility, the following experiments were performed.

Material and methods. 125 inbred male albino rats were grafted on the 10th day after birth with black skin allografts obtained from inbred male hooded rats. The hosts were treated as follows: In 22 animals, fetal adrenal glands were implanted under the skin on the 6th and 8th day after birth. The number of adrenal glands implanted was 10 in 18 cases and 5 in 4 cases. The adrenal glands were obtained from last week of gestation isogeneic fetuses. In 26 rats isogeneic adult adrenal glands were implanted under the skin on the 6th and 8th day after birth (2–4 glands per animal). 33 animals were daily injected with different doses of a suspension of 16β-methyl prednisone (from 10 to 40 µg) from the 6th day after birth until the rejection of the graft. 44 rats received the skin allograft only.

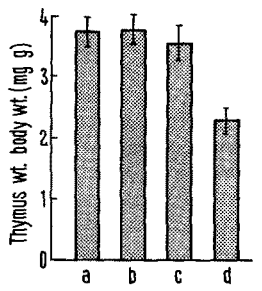
Evaluation of the success or failure of the skin grafts was based on gross inspection. Rejection of grafts was characterized by an initial take followed by inflammation, induration, hardening of the graft, and ultimate complete slough. Prolonged survival of the graft was considered to be present when the graft showed a delayed onset of the early evidence of rejection.

Histological study was performed in order to determine the fate of the grafted adrenal glands and the effect of the different treatments over the thymus. The material was composed of 7 rats implanted with 10 fetal adrenals on the 6th day of life. 7 rats daily injected by s.c. route with 30 µg of 16β-methyl prednisone. 8 rats implanted with 2 adult adrenals on the 6th day of life. 5 normal rats.

The animals were sacrificed on the 16th day of life. In the animals in which adrenals were grafted, a wide zone of skin and subcutaneous tissue corresponding to the site of the adrenal grafts and the thymus in all animals were fixed in 10% Formol, embedded in paraffin, serial sectioned at 6 µm thick and stained with Hema-

Treatment	Skin allograft survival (days) No. of animals	Less than 15	15 to 50	More than 50
Skin allograft only	44	44	–	–
Implanted with 5 fetal adrenal glands	4	3	1	–
Implanted with 10 fetal adrenal glands	18	10	1	7
Implanted with 2–4 adult adrenal glands	26	25	–	1
Daily injected with 10–25 µg of 16 MP	21	23	–	–
Daily injected with 30 µg of 16 MP	5	4	–	1
Daily injected with 40 µg of 16 MP	7	3	2[a]	2

16 MP, 16β-methyl prednisone. [a] Death before day 50th.



Thymus relative weight (thymus wt./body wt., mean S.E.) in: a) controls; b) animals grafted with 10 fetal adrenal glands; c) animals grafted with 2 adult adrenal glands; d) animals injected daily from the 6th day after birth with 30 µg of 16β-methyl prednisone.

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