Abspaltung der Boc-Schutzgruppe von IV mit Chlorwasserstoff/Eisessig ergab schliesslich das fluoreszenzmarkierte Hexapeptid V vom Schmp. 193–196°; UV: max λ 334 nm, Fluoreszenz: λ_{em} 535 nm (λ_{exc} 335 nm), Aminosäureanalyse nach 20 h. Hydrolyse mit 20%iger Salzsäure bei 110°:Phe 0,95; Tyr 0,72; Gly 1,05; Leu 1,00; Met 0,95; Dünnschichtchromatographie homogen im System Chloroform/Methanol/Essigsäure (80:20:1,5) und Butanol/Essigsäure/Wasser (4:1:1) auf Eastman Kieselgel-Chromatographiefolie Type K 301 V.

Physalaemin und seine C-terminale Hexapeptid-Sequenz [6–11] Lys-Phe-Tyr-Gly-Leu-MetNH_2 (VI) kontrahieren in ausserordentlich niedriger Dosis extravasculäre glatte Muskulatur und bewirken eine starke Blutdrucksenkung bei verschiedenen Spezies[9,11,12]. Dabei ist die Aktivität des Hexapeptids VI in etwa der des Physalaemins selbst gleichzusetzen[13].

Zur Testung des Dansyl-Hexapeptids V im Vergleich zur unsubstituierten Sequenz VI wurde von uns als in vitro-Modell das Meerschweinchen-Ileum und als in vivo-Modell die Blutdruckmessung am Meerschweinchen verwendet[14]. Am Meerschweinchen-Ileum diente die halbmaximale Wirkung (ED_{50}) und am Meerschweinchen-Blutdruck eine Senkung um 30% des Ausgangswertes (ED_{30}) als Vergleichsgrösse (Tabelle).

Summary. The synthesis of fluorescent labelled derivative of the N-terminal physalaemin hexapeptide [6–11], which contains a 1-dimethylaminoaphtalene-5-sulfonyl residue at the α-amino group of the N-terminal Lysine, is described. Pharmacological data of the fluorescent labelled compound as compared with the unsubstituted peptide are reported.

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X Ray-Induced Mutations of the Genetically-Determined Melanoma System of Xiphophorin Fish

We have reported previously[1] that platyfish (*Platypoecilus maculatus*) which develop from irradiated embryos reveal an increase in macromelanophore gene expression. This increase results in an overproduction of macromelanophores to premelanomas, which can be compared to that observed in F_1 hybrids between the platyfish and swordtail (*Xiphophorus helleri*). Since this increase in gene expression is inherited, we conclude that both somatic and germ cells are irreversibly altered in the same direction.

In order to examine more closely the genetics of this X ray-induced increase in macromelanophore gene expression, several crosses were made between irradiated and nonirradiated fish, the most important of which are discussed in this paper. The significance of our findings is considered with respect to the mutation theory of carcinogenesis.

Methods and results. 1. Nonirradiated females of *Sp* stocks[2] bred from fish which were irradiated as embryos (1500 R)[1] were crossed with nonirradiated males of *Sd* stocks[2] bred from nonirradiated fish. Reciprocal crosses with respect to sex and the stocks mentioned above were also made. The details of these crosses are given in Table I. All of these crosses reveal an increased expression in both irradiated and nonirradiated macromelanophore genes in the offspring (n = 242) to almost the same extent as observed in those parents having only irradiated chromosomes.

2. Males and females having half of their chromosomes irradiated were crossed with fish having none of, half of or the complete set of irradiated chromosomes. The resulting offspring (n = approximately 500) reveals a variation in macromelanophore gene expression which corresponds to the variation in the number of irradiated chromosomes present. (Table II).

3. Finally, nonirradiated platyfish of *Sp* stocks bred from fish which were irradiated as embryos (1500 R) were crossed with nonirradiated swordtails bred from nonirradiated fish. The resulting hybrids (n = 155) reveal an overproduction of macromelanophores additional to that displayed by normal hybrids.

Discussion. These crosses have excluded several hereditary constituents as possible targets of the X ray-induced alteration which results in an overproduction of macromelanophores to premelanomas.

Mutations of the macromelanophore genes themselves cannot be responsible for this alteration, since both irradiated and nonirradiated macromelanophore genes reveal an increase in expression in the offspring (Table I). Furthermore, this genetic alteration cannot involve mutations of the sex chromosomes or of cytoplasmic constituents contributed in different quantities by ovum and sperm because the increase in macromelanophore gene expression is independent of the sex of the parent contributing the irradiated chromosomes to the offspring (Tables I and II).

The correspondence of variation in macromelanophore gene expression with variation in the number of irradiated chromosomes present in the offspring (Table II) indicates that the increase in macromelanophore gene expression is due to mutations in a very large number of widely-distributed chromosomal genes. Episomal or viral particles cannot be involved in the increase of macromelanophore gene expression because one would not assume that these are transmitted to the offspring in proportions similar to those of chromosomes. Since the X ray-induced mutations of these genes always result in an increase in macromelanophore gene expression, we conclude that the affected chromosomal genes normally repress this expression. Therefore, induction of premelanomas by X-irradiation must be due to numerous mutations within a large set of specific genes, which normally controls macromelanophore gene expression. These results agree with a mutation theory of carcinogenesis which would include not only one, but numerous mutations in genes specifically concerned with control of cellular proliferation.

As a result of previous genetic experiments[3], a highly polygenic repression gene system responsible for macromelanophore gene control was found to be located throughout the chromosomes of *P. maculatus*, but to be entirely lacking in those of *X. helleri*. Replacement of platyfish chromosomes by the corresponding swordtail chromosomes, therefore, results in an enhancement of macromelanophore gene expression similar to that observed upon irradiation of platyfish chromosomes.

In addition, the genotypes resulting from the crosses presented in this paper reveal a synergistic effect of X-irradiation and hybridization on the increase of macromelanophore gene expression. The irradiated platyfish chromosomes, therefore, behave similarly to the nonirradiated ones of *X. helleri*. For these reasons, we assume that the genes damaged by X-irradiation are constituents of the repression gene system of the macromelanophore genes of *P. maculatus*. From recent cytological findings[4], it seems that the damage of this repression gene system could be related to an X ray-induced appearance of distinct chromocenters in interphasen nuclei[5].

Zusammenfassung. Die nach Röntgenbestrahlung von Embryonen von *Platypoecilus maculatus* auftretende erbliche Überproduktion von Makromelanophoren (Bildung von Prämelanomen) beruht offenbar auf einer Vielzahl von Mutationen eines über alle Chromosomen verteilten Systems von Repressionsgenen, das die für die Makromelanophorenbildung verantwortlichen Gene kontrolliert.

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Table I. Macromelanophore gene expression in crosses of nonirradiated animals of stocks bred from fish were irradiated as embryos × nonirradiated animals of the original stocks.

Parents	Offspring
a) $\overbrace{\downarrow \quad \downarrow}$ A X^{Sp} X^{Sp} – ♀ × A X^{Sd} Y – ♂ *Sp* is increased *Sd* is normal	$\downarrow \quad \downarrow$ ½A ½A X^{Sp} X^{Sd} – ♀♀ $\downarrow \quad \downarrow$ ½A ½A X^{Sp} Y – ♂♂ both the irradiated *Sp* and the nonirradiated *Sd* are increased
A X^{Sd} X^{Sd} – ♀ × $\overbrace{\text{A } X^{Sp} \; Y}^{\downarrow}$ – ♂ *Sd* is normal *Sp* is increased	$\downarrow \quad \downarrow$ ½A ½A X^{Sd} X^{Sp} – ♀♀ $\downarrow \quad \downarrow$ ½A ½A X^{Sd} Y – ♂♂ both the irradiated *Sp* and the nonirradiated *Sd* are increased
b) $\overbrace{\downarrow \quad \downarrow}$ A X^{Sd} X^{Sd} – ♀ × A X^{Sp} Y – ♂ *Sd* is increased *Sp* is normal	$\downarrow \quad \downarrow$ ½A ½A X^{Sd} X^{Sp} – ♀♀ $\downarrow \quad \downarrow$ ½A ½A X^{Sd} Y – ♂♂ both the irradiated *Sd* and the nonirradiated *Sp* are increased
A X^{Sp} X^{Sp} – ♀ × $\overbrace{\text{A } X^{Sd} \; Y}^{\downarrow}$ – ♂ *Sp* is normal *Sd* is increased	$\downarrow \quad \downarrow$ ½A ½A X^{Sp} X^{Sd} – ♀♀ $\downarrow \quad \downarrow$ ½A ½A X^{Sp} Y – ♂♂ both the irradiated *Sd* and the nonirradiated *Sp* are increased.

↓ = chromosomes characterized by this arrow are irradiated ones; A, all autosomes; *X* and *Y*, sex chromosomes; *Sp* and *Sd*, macromelanophore genes 'spotted' and 'spotted dorsal'.

Table II. Variation in macromelanophore gene expression as the result of variation in number of irradiated chromosomes

Parents		Offspring
$\downarrow$ ½N ½N increased	× N normal	$\downarrow$ ¼N ¾N (mode) variation from normal to less increased
	× $\downarrow$ ½N ½N increased	$\downarrow$ ½N ½N (mode) variation from normal to increased
	× $\downarrow$ N increased	$\downarrow$ ¾N ¼N (mode) increased

N = all chronosomes.

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[5] Supported by the Deutsche Forschungsgemeinschaft and the Stiftung Volkswagenwerk. We should like to thank Dr. G. DÖLL, Institut für Biophysik (Giessen), for his assistance in X-irradiating the fish, and Dr. J. VIELKIND, Genetisches Institut (Giessen), for valuable criticism in preparing the manuscript. These investigations include results from the dissertation of D. L. PURSGLOVE.