RELATION BETWEEN THE PIGMENTATION AND PTERIN DERIVATIVES OF CHROMATOPHORES DURING DEVELOPMENT IN THE NORMAL BLACK AND TRANSPARENT SCALED TYPES OF GOLDFISH (*CARASSIUS AURATUS*)¹

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Received March 23, 1960

FLUORESCENT substances of pterin type have been known to exist abundantly in the colored portions of the adult skin of Amphibia and Pisces, while they can scarcely be found in the ventral white portions. These substances were shown to be greatly accumulated in the chromatophores of bullfrogs (HAMA and OBIKI 1959). In Cyprinidae, the violet fluorescent pterins are the main components as reported by ZIEGLER-GÜNDER (1956). The chemical nature of these substances has been studied by several investigators (POLONOVSKI, BUSNEL and PESSON 1943; HÜTTEL and SPRENGLING 1943; TSCHESCHE and KORTE 1951, 1953; HIRATA, NAWA, MATSUURA and KAKIZAWA 1952; KORTE 1954; MATSUURA, NAWA, GOTO and HIRATA 1955; NAWA, MATSUURA, GOTO and HIRATA 1955; ZIEGLER-GÜNDER 1956; TSCHESCHE and GLASER 1958). Our reports on Cyprino-pourpre A1, Cyprino-pourpre A2 and Cyprino-pourpre B, which were found in the adult skin and scales in carp and normal red scaled goldfish (*Ryûkin*), are relevant to this. We showed that these substances corresponded to isoxanthopterin, 7-hydroxybiopterin and isoxanthopterin-6-carboxylic acid, respectively. Besides these substances, another violet fluorescent substance of pterin type, Cyprino-pourpre C, has been found in their larvae. All of these substances appear approximately in accordance with the first pigmentation of chromatophores such as melanophores or xanthophores in the course of development (HAMA, GOTO and KUSHIBIKI 1952; KUSHIBIKI, HAMA and GOTO 1954; MORI and MATSUMOTO 1956, 1957). These facts suggest a close relationship between the pigmentation and pterin in chromatophores. To clarify further such relationship, it is desirable to employ different types of one species which differ in the mode of pigmentation. For such a purpose, the normal black and the transparent scaled types of goldfish, which differ genetically in their pigmentation, were used. This paper deals with the difference between these two types in the degree of pigmentation and in pterin contents during development.

MATERIALS AND METHODS

The materials used are the offspring by the inbreeding of homozygous normal black scaled fish and of homozygous transparent scaled fish, respectively. Homo-

¹Contribution No. 19 from Biological Laboratory, Keio University.

zygous fish have been segregated by self-breeding heterozygous transparent scaled fish "Sanshiki-démékin" (Calico telescope-eyed goldfish).

Normal development of chromatophores in the two different types of goldfish

Homozygous normal black scaled type (tt): At room temperature of about 21°C, the eye cup becomes pigmented 36 (33–40) hours after spawning. In about ten hours, melanophores appear along the ventral border of myotomes and blood vessels, then in the head and trunk regions. After this time, the melanophores increase in number and extend over all of the surface of the body. After the appearance of melanophores, guanophores appear first on the eye. Subsequently, xanthophores appear, at first on the dorsal surface of the head, shortly before hatching. About 3.5–4.0 days after spawning, the embryo begins to hatch out. Thus, the larva assumes a gray or brownish colored appearance. About 40 days after hatching, the larva is transformed into adult form, being colored a velvet black. It is in this stage that "Ryûkin" assumes a red colored appearance as a result of depigmentation of larval melanophores. The depigmentation of larval melanophores, as such, is presumed to occur in the normal black scaled type fish in which the shape of adult melanophores is quite different from that of larval ones. In the adult, the melanophores and xanthophores become distributed richly on the dorsal side with fewer numbers towards the ventral side where they are generally lacking. The guanophores are most prominent on the ventral surface where they give a dead white appearance to the abdomen.

Homozygous transparent scaled type (TT): Until about ten days after hatching, when the larva reached 6 to 7 mm in length, pigmentation of this type cannot be clearly distinguished from the *tt* type, the chromatophores differentiating to the same degree in both types. After ten days, both types can be distinguished through their different colors, the precocious depigmentation taking place in the transparent scaled type fish. First of all, the xanthophores begin to depigment along the dorsal region, and their depigmentation extends antero-posteriorly until all xanthophores completely disappear within a few days. The larva then becomes gray in color. Shortly after the beginning of depigmentation of xanthophores, the guanophores in the eye begin to disappear. Almost at the same time as the blackening of the eyeball, the guanophores of peritonium also disappear. Finally, the melanophores, which continued to increase in number at a slow rate (compared with the *tt* type) until about 12–13 days after hatching, begin to depigment, and their melanin granules disappear from the entire surface in a few days. Thus, the larva becomes quite transparent.

Chemical nature of pterins found in goldfish

Various names were proposed in our previous papers for the pterins isolated from the larvae and adult skins of frog and fish by the use of paper chromatography. Some of these have been clarified in their chemical nature (HAMA 1953; HAMA and GOTO 1954, 1955; HAMA, GOTO and KUSHIBIKI 1954; KUSHIBIKI *et al.* 1954; MORI and MATSUMOTO 1956, 1957; FIDLER and WOOD 1957; TSCHESCHE and GLASER 1958; GOTO and HAMA 1958; HAMA and OBIKA 1958; FORREST, HAT-

FIELD and VAN BAALEN 1959; HAMA, GOTO, OBIKA and MATSUMOTO 1959; VISCONTINI and MÖHLMANN 1959; HAMA, MATSUMOTO and MORI 1960; MORI, MATSUMOTO and HAMA, unpublished data). The nomenclatures, chemical natures and mutual relations of fluorescent substances found in the goldfish are given in Tables 1 and 2 and in Figure 1. Among these substances, the violet fluorescent substances are the characteristic and main components in Cyprinidae as described before. These data also confirm that there is no difference in the natures of respective pterins among the normal black and red scaled types and in carp. The detail will be published in a forthcoming paper.

TABLE 1

Chemical natures of the fluorescent substances appearing in goldfish

Nomenclature	Chemical structure	Color of fluorescence
Cyprino-pourpre A1 (Rana-chrome 4)	isoxanthopterin (2-amino-4, 7-dihydroxypteridine)	violet
Cyprino-pourpre A2	7-hydroxybiopterin (Ichthyopterin)	violet
Cyprino-pourpre B	isoxanthopterin-6-carboxylic acid	violet
Cyprino-pourpre C	?	violet
Cyprino-rouge	?	red
Rana-chrome 1	biopterin (2-amino-4-hydroxy-6-(1', 2'-dihydroxypropyl)-pteridine)	blue
Rana-chrome 3	?	blue
Rana-chrome 5	2-amino-4-hydroxy-6-carboxypteridine	blue
Xanthopterin	2-amino-4, 6-dihydroxypteridine	bluish green
Rhacophoro-jaune	2-amino-4, 6-dihydroxy-6-lactyl-6, 7-dihydropteridine*	yellow
Riboflavin		yellow
FMN	flavin mononucleotide	yellow
FAD	flavinadenine dinucleotide	yellow

* Postulated formula proposed by FORREST *et al.* (1959). See also FIDLER and WOOD (1957) and VISCONTINI and MÖHLMANN (1959).

TABLE 2

Maximum wavelength in ultraviolet absorption and fluorescence spectra of violet fluorescent pterins found in goldfish

	Ultraviolet absorption spectra				Fluorescence spectra	
	0.1N NaOH		0.1N HCl		McIlvaine's buffer pH 5.0	
	Max. 1 (m μ)	Max. 2	Max. 1 (m μ)	Max. 2	Beckman type (m μ)	Farrand type (m μ)
7-Hydroxybiopterin (Cyprino-pourpre A2)	256	342.5	289	344	416	400
Isoxanthopterin (Cyprino-pourpre A1)	253	339	285	340	410	387
Cyprino-pourpre C	258	339	285	343	415	397

FIGURE 1.—Mutual chemical relation among violet fluorescent pterins found in goldfish.

Extraction of fluorescent substances

Extraction was carried out on the whole egg, embryo and larva and on the skins and scales in the adult. In the embryo and larva, pterins are found abundantly in the skin part. The materials of respective stages were washed carefully with redistilled water, blotted and weighed. The fluorescent substances were obtained by the following methods. (1) Extraction with trichloroacetic acid: The materials were homogenized in a volume of ten percent trichloroacetic acid equal to 20 times the fresh weight. The homogenate was heated thrice in a water bath for 15 minutes at 70°C and then, after cooling, centrifuged at 3000 rpm for five minutes. The combined supernatant fluid was washed six times with one half the volume of ethyl ether to remove the trichloroacetic acid. The ether layer, separated by centrifugation, was discarded at each washing. The resulting aqueous extracts were reduced to about 1.0 or 0.5 ml over concentrated H₂SO₄ under lower pressure. (2) Extraction with ethanol: The extraction was carried out following a slight modification of the method of AWAPARA (1948). This method used originally to extract free amino acids is suitable for obtaining easily extractable or unstable pterins, e.g., Cyprino-pourpre C. The materials were homogenized in a volume of absolute ethanol equal to 20 times the fresh weight. To the homogenate, redistilled water was added to a concentration of 70 percent. This ethanol solution was heated thrice in a water bath for 15 minutes at 80°C, then centrifuged. Chloroform, equal to three times the resulting supernatant fluid, was added to remove the protein and fat soluble substances. The aqueous solution containing the fluorescent substances, which was separated by centrifugation after vigorous shaking, were reduced to about 0.5 ml *in vacuo*.

Fluorimetry

Among the main components of pterins in goldfish, isoxanthopterin-6-carboxylic acid is found in very small amounts so that the quantitative estimation was restricted to 7-hydroxybiopterin (Ichthyopterin), isoxanthopterin and Cyprino-pourpre C. The fluorimetric estimation (MATSUMOTO and MORI 1958) was carried out on the materials of respective developmental stages. The individuals in the process of depigmentation were variable in growth so that exceptionally large or small ones were avoided. The fluorescent substances extracted by method

(1) were purified by paper chromatography. The concentrated extracts were developed twice by *n*-propanol-1% NH_4OH (2:1) by the ascending method and dried by aeration in the dark room. The substances were eluted with redistilled water, from the respective homogenous band of paper, by a descending method in a dark moist chamber for about 12 hours. The elutes, after adjusting its volume, were mixed with an equal volume of M/10 McIlvaine's citric acid-phosphate buffer, pH 5.0. The Hitachi fluorimeter (light source: mercury vapor lamp SHL-100 UV; primary filter UV-D2; secondary filter Hitachi #46 (460 $\text{m}\mu$)) was used. The fluorescent intensity of 1.0 μg per ml of quinine sulfate standard in 0.1N H_2SO_4 was equivalent to that of 0.508 μg per ml of 7-hydroxybiopterin and 0.551 μg per ml of isoxanthopterin in McIlvaine's buffer solution, pH 5.0, respectively. As Cyprino-pourpre C has not yet been clarified in its chemical structure, the fluorescent intensity was represented by that of quinine μg in the above mentioned condition. The recovery rate was 72.2 percent for 7-hydroxybiopterin and 73.5 percent for isoxanthopterin in a system of the liver of goldfish and 7-hydroxybiopterin or synthetic isoxanthopterin of suitable concentration.

RESULTS

Pterin fraction in the larvae and in the adult skin of normal black (tt) and transparent (TT) scaled types

The detection and identification of fluorescent substances were carried out mainly by paper chromatography. Developing solvents were as follows: *n*-propanol-1% NH_4OH (2:1), *n*-butanol-alcohol-water (4:1:1), *n*-butanol-acetic acid-water (4:1:1), *n*-propanol-pyridine-water (5:3:2), three percent sodium citrate, five percent acetic acid, three percent NH_4Cl , five percent Na_2HPO_4 and three percent urea. It seems advisable to explain the results by division of them into the following four stages with melanophores being adopted as a marker for convenience: (1) melanophore-free stage, (2) early melanophore stage, (3) late melanophore stage and (4) adult form and adult (Tables 3 and 4 and Figure 2). (1) *Melanophore-free stage*: The eggs and embryos from early morula to the stage immediately before the pigmentation at eye cup were examined. Two yellow fluorescent substances were detected and were identified as FMN and FAD, respectively, in an excellent developing solvent, 2,4-lutidine-*n*-butanol-water (4:1:1) (YAMAGIRI 1957), or by a hydrolytic treatment of them. No qualitative or quantitative differences in these substances were found in the two types. No pterins were found.

(2) *Early melanophore stage*: This stage covers the period from the first melanophore pigmentation at eye cup to the hatching stage. As described before, no difference was found with reference to the pigmentation in the two types. Paper chromatographic assay confirms the same sorts and quantitative similarity of pterins from the size and intensity of fluorescing spots in both types. Simultaneously, with the first appearance of pigmentation, blue, yellow and violet fluorescent pterins appeared successively. These three corresponded to biopterin (Rana-chrome 1), Rhacophoro-jaune and 7-hydroxybiopterin (Cyprino-pourpre

TABLE 3

Developmental stages and experimental conditions with reference to the normal black (tt) and transparent (TT) scaled types

Developmental stages (Age at about 21°C)	Whole body length (mm)	Number of individuals used for paper chromatography*
(1) <i>Melanophore-free stage</i>		
Morula (2.5 hours after spawning)	0.6	500
Gastrula (8 hours after spawning)	0.6	500
Trunk formation (20 hours after spawning)	0.6	500
(2) <i>Early melanophore stage</i>		
Eye cup becomes pigmented. (36 hours after spawning)	0.6	500
Hatching (3.5–4.0 days after spawning)	4.0–4.2	500*
(3) <i>Late melanophore stage</i>		
Five days after hatching (Both types cannot be distinguished.)	5.0–5.5	250
15 days after hatching (Both types can be distinguished through the pigmentation.)	8.0–9.0	250*
40 days after hatching (Depigmentation is not complete in the TT type.)	14–15	20*
(4) <i>Adult form and adult</i>		
40 days after hatching (Depigmentation is complete in the TT type.)	14–15	20*
Two years old	55–60	3

* Also for quantitative estimation.

A2), respectively. Subsequently, Cyprino-pourpre C and, a little later, isoxanthopterin (Cyprino-pourpre A1) appeared. The appearance of the last corresponded approximately to the period where guanophores appeared. Here, it is noted from the phylogenic standpoint that the sequence in which these pterins successively appear is definite notwithstanding the difference of species, e.g., the red scaled type of goldfish, carp or *Tribolodon hakuiensis* (a kind of rudd, Cyprinidae).

(3) *Late melanophore stage*: This stage covers the period from the two-day stage to the 40-day stage after hatching. In the 40-day stage, the larvae are transforming into the adult chromatophore coloration, the *tt* type being heavily pigmented in black and the *TT* type slightly pigmented or already completely depigmented. In this section, only pigmented larvae were selected. Four violet, three blue, two yellow and one red fluorescing spots were detected on the chromatogram. The four violet corresponded to isoxanthopterin, 7-hydroxybiopterin, isoxanthopterin-6-carboxylic acid and Cyprino-pourpre C, the three blue to biopterin, Rana-chrome 3, and 2-amino-4-hydroxy-6-carboxypteridine, the two yellow to ribo-

TABLE 4

Rf values of fluorescent substances in the normal black (tt) and transparent (TT) scaled types (developed at 17°C, in the dark, ascending method)

Spot number in Figure 2	a	Rf values in various solvents*				Identification
		b	c	d	e	
1	0.38	0.34	0.40	0.78	0.75	Cyprino-pourpre C
7	0.20	0.14	0.18	0.60	0.56	7-Hydroxybiopterin
9	0.13	0.14	0.16	0.30	0.32	Isoxanthopterin
13	0.02	0.01	0.06	0.38	0.59	Isoxanthopterin-6-carboxylic acid
3	0.34	0.36	0.30	0.67	0.65	Biopterin
5	0.26	0.27	0.24	0.52	0.52	Rana-chrome 3
10	0.10	0.03	0.12	0.46	0.49	2-Amino-4-hydroxy-6-carboxypteridine
2	0.36	0.51	0.31	0.32	0.32	Rhacophoro-jaune
4	0.31	0.57	0.19	0.33	0.32	Riboflavin
6	0.24	0.01	0.04	0.57	0.54	FMN
12	0.09	0.09	0.01	0.45	0.38	FAD
8	0.14	0.07	0.29	0.42	0.42	Xanthopterin
11	0.09	...	0.09	...	...	Cyprino-rouge

* Solvents: (a) *n*-Propanol-1% ammonia (2:1); (b) 2,4-Lutidine-*n*-butanol-water (4:1:1); (c) *n*-Butanol-acetic acid-water (4:1:1); (d) three percent NH₄Cl; (e) five percent sodium citrate.

FIGURE 2.—Chromatogram showing the kinds of pterins in larva and adult of normal black (tt) and transparent (TT) scaled types. Solvent: *n*-Propanol-1% NH₄OH (2:1), at 17°C in the dark, ascending method. Color of fluorescence: b = blue; bg = bluish green; r = red; v = violet; y = yellow. Broken circle: faint fluorescence.

flavin and Rhacophoro-jaune, the one red to Cyprino-rouge, respectively. Whether the last is a pterin remains unsettled. The kind of spots was similar in both types, but the amount of pterins was in much less degree in the *TT* type, the fluorescing intensity of respective spots being proportional to the degree of pigmentation. During depigmentation, violet fluorescent substances disappeared in the following sequence: namely, Cyprino-pourpre C, a little later isoxanthopterin, and subsequently 7-hydroxybiopterin.

(4) *Adult form and adult*: The adult form denotes the *TT* type completely depigmented 40 days after hatching. The chromatogram in adult form differed qualitatively in its figure in both types. 7-Hydroxybiopterin, isoxanthopterin and isoxanthopterin-6-carboxylic acid were the pterins which could be found in the *tt* type as well as in the red scaled type—Cyprino-pourpre C being absent in their adults. So Cyprino-pourpre C is a substance which is found only in their larval life. The *TT* type is characterized by the complete absence of these pterins. However, if a large amount of skin is used, three blue, two yellow and one bluish green fluorescent substances can be unfailingly detected in the *tt* type fish. The three blue correspond to biopterin, Rana-chrome 3 and 2-amino-4-hydroxy-6-carboxypteridine, the two yellow to riboflavin and Rhacophoro-jaune, and the one bluish green to xanthopterin. Xanthopterin is scarcely found in the adult skin of the red scaled type fish. Only a trace of these substances is present in the *TT* type fish.

Quantitative estimation of isoxanthopterin, 7-hydroxybiopterin and Cyprino-pourpre C in the normal black and transparent scaled types

The estimation was carried out in the larvae at hatching, at 15-day and at 40-day stages after hatching. The contents and their mutual relation of 7-hydroxybiopterin, isoxanthopterin and Cyprino-pourpre C in a single fish in both types are given in Table 5 and Figure 3. As described before, Cyprino-pourpre C alone cannot be represented with a molar concentration. As the table and figure indicate, the quantity of pterins is proportional to the degree of pigmentation. At hatching stage, no difference was found in the two types. This datum is in accordance with the morphological observation of similar pigmentation in the two types. At the 15-day stage, a clear difference between the two types was found. In fact, the larvae of both types can be clearly distinguished by their characteristic pigmentation. At the 40-day stage, the *tt* type fishes are heavily pigmented and the *TT* type are perfectly depigmented or in the process of depigmentation. The estimation in the latter type, then, was done by dividing them into two kinds on the basis of being completely or incompletely depigmented. Remarkable differences were found in the two types. The quantitative difference in pterins in the *TT* type corresponded to that of pigmentation of the two groups. From the above description, it can be concluded that there exists an apparent correlation between the degree of pigmentation and the quantity of pterins.

TABLE 5

Quantitative estimation of violet fluorescent pterins in the postembryonic larvae of the normal black (tt) and transparent (TT) scaled types

Type	Number of individuals (wet weight mg)	Number of experiments	Contents per individual				
			7-Hydroxybiopterin		Isoxanthopterin		Cyprino-pourpre C
			10 ⁻³ μ g quinine	10 ⁻⁶ moles*	10 ⁻³ μ g quinine	10 ⁻⁶ moles*	10 ⁻³ μ g quinine
Hatching stage							
<i>tt</i>	500 (561 $\pm$ 2)	2	95 $\pm$ 3†	0.19	15 $\pm$ 1	0.05	11 $\pm$ 2
<i>TT</i>	500 (568 $\pm$ 3)	2	99 $\pm$ 4	0.20	12 $\pm$ 3	0.04	9 $\pm$ 1
15-day stage after hatching							
<i>tt</i>	250 (535 $\pm$ 7)	2	824 $\pm$ 8	1.66	94 $\pm$ 4	0.29	72 $\pm$ 4
<i>TT</i>	250 (568 $\pm$ 8)	2	432 $\pm$ 13	0.87	45 $\pm$ 11	0.14	29 $\pm$ 2
40-day stage after hatching							
<i>tt</i>	20 (386 $\pm$ 10) (heavily pigmented)	3	3107 $\pm$ 96	6.25	386 $\pm$ 57	1.19	574 $\pm$ 23
<i>TT</i>	20 (383 $\pm$ 40) (incompletely depigmented)	3	192 $\pm$ 19	0.38	57 $\pm$ 11	0.18	38 $\pm$ 5
<i>TT</i>	20 (519 $\pm$ 28) (completely depigmented)	2	103 $\pm$ 12	0.21	0	0	0

* Calculation formula:

Molar concentration of 7-hydroxybiopterin or (isoxanthopterin) per individual = $\frac{\mu\text{g quinine of 7-hydroxybiopterin or (isoxanthopterin) per individual}}{\text{individual}} \times 2.01 \cdot 10^{-6}$ or $(3.07 \cdot 10^{-6})$

The standard pterin solutions used for this calculation were prepared by the spectrophotometric method using TSCHECHÉ's data on synthetic 7-hydroxybiopterin as a basis for Cyprino-pourpre A2 ($\log \epsilon = 4.16$ in 0.05N NaOH at 342 m μ , mol wt 252.2) and employing synthetic isoxanthopterin (mol wt 179.1) for Cyprino-pourpre A1.

† mean $\pm$ standard deviation.

DISCUSSION

Among the various kinds of chromatophores, guanophores are almost devoid of pterins in the ventral white skin of the frog as well as in goldfish. It will be true to say that the presence of 7-hydroxybiopterin, isoxanthopterin and isoxanthopterin-6-carboxylic acid in the adult skin of red scaled goldfish is due to the xanthophores (allophores). Whether these substances, or some of these, exist also in the melanophores has not yet been demonstrated. At any rate, the above considerations are concerned with the adult chromatophores, and not the larval ones. The presence of the larval melanophores, which depigment during development (as clearly seen in the red scaled type fish), has been recognized by many workers (FUKUI 1927; GOODRICH and HANSEN 1931). On the contrary, the behavior attendant upon development of xanthophores has been scarcely reported. It has been reported that the difference between the erythrophores, characteristic of adult, and the xanthophores depends solely on the color of pigments within the

FIGURE 3.—Graph of contents of violet fluorescent pterins in a single fish of normal black (*tt*) and transparent (*TT*) scaled types.

* Slightly modified.

cell (PARKER 1948). According to our experience, red or orange pigments are present in every xanthophore of the adult, but not in those of larva. On the basis of this difference and owing to the fate of xanthophores in the larval stage of transparent scaled type fish, it is rather reasonable to discriminate between the two kinds of larval and adult types in xanthophores as well as in melanophores.

As described before, the numbers of melanophores, guanophores and xanthophores in the transparent type increase at a slow rate up to 10–13 days after hatching, followed by depigmentation (some difference in days is found). Thus, the fish becomes quite transparent. GOODRICH and HANSEN (1931) have previously clarified the difference of depigmentation between the red scaled and transparent scaled types by determinating the change in number of larval melanophores in the course of development. The graph presented by them is presented in modified form in Figure 3. This figure is concerned with melanophores, but the same holds approximately true for the xanthophores from our experience.

It is clear from the data of GOODRICH and HANSEN (1931) that the pterin contents are nearly proportional to the number of melanophores. Whether the pterins are related to either melanophores or xanthophores or to both has not been demonstrated. To obtain a decided conclusion, the localization of pterins in the chromatophores in question should be cleared up. From the above descriptions, it is suggested that gene action has its effect on the simultaneous disappearance of pigmentation and pterins in the chromatophores in the *TT* type fish. In fact, at the depigmentation of guanophores in this type, a conspicuous guanase activity was demonstrated by one of the authors (KAJISHIMA 1960).

SUMMARY

A relation between pigmentation and pterin contents in chromatophores was investigated employing the normal black scaled (*tt*) and transparent scaled (*TT*) types of goldfish which differ genetically in the mode of pigmentation during development.

1. The process of appearance and depigmentation of melanophores, guanophores and xanthophores in the course of development was described.

2. The pterin fractions in the course of development was examined by paper chromatography. Simultaneously, with the first pigmentation in the embryos, pterins such as bipterin (Rana-chrome 1), Rhacophoro-jaune, 7-hydroxybipterin (Cyprino-pourpre A2, Ichthyopterine), Cyprino-pourpre C, isoxanthopterine (Cyprino-pourpre A1) and isoxanthopterine-6-carboxylic acid (Cyprino-pourpre B) appear in the same way in the two types. Whereas the violet fluorescent pterins of 7-hydroxybipterine, isoxanthopterine and isoxanthopterine-6-carboxylic acid continue to remain in the adult skin of the *tt* type, no pterins are found in the transparent *TT* type. Cyprino-pourpre C is found only in the larva.

3. The fluorimetric estimation of the three main components in the larval life, 7-hydroxybipterine, isoxanthopterine and Cyprino-pourpre C, was compared at respective stages of development in both types. No difference was detected at hatching stage where the embryos assume morphologically the same pigmentation. A clear difference was found at the 15-day stage where the embryos can be distinguished through pigmentation. Large amounts of pterin were found in the *tt* type fish (heavily pigmented), while little or no pterins were found in the *TT* type fishes which were depigmented incompletely or completely. These data indicate a close relation between the degree of pigmentation and the pterin contents of the chromatophores.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to PROFESSOR ADRIEN ALBERT (The Australian National University, Canberra) for his critical reading and his help in the preparation of the manuscript. We also wish to acknowledge the kind supply of the isoxanthopterine by PROFESSOR ADRIEN ALBERT and DR. SABURO NAWA (National Institute of Genetics, Mishima).

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