Studies on a Blue-Green Serum Pigment of Eel—IV.

Seasonal Variation of Concentration of Pigment in Serum*

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The serum of eel is, unlike those of other fishes, blue-green due to the presence of a pigment. We have clarified so far that the pigment is a complicated chromoprotein having small quantities of biliverdin and sugar, together with considerable amounts of lipid^{1,2)}. The amino acid and sugar compositions rather resemble those of β_1 -lipoprotein in human serum³⁾.

In the course of these studies, we had many chances to observe the color of individual samples of eel serum. And it came under our notice that the color tone seems to vary considerably with the season of catch, and also with the degree of growth to some extent.

The present study was undertaken to find a clue to elucidation of the physiological function of the chromoprotein by surveying in detail the variation of concentration of the pigment.

Experimental

Material: Three specimens of each of cultured and wild eels were used per month throughout a year. The cultured eels were supplied from Shizuoka Pref., while the wild ones mainly from Ibaragi Pref., unless otherwise indicated. The eels were transported to our laboratory alive. Blood was individually collected by heart puncture, and after standing for clotting, was centrifuged to separate serum. Four individuals of catadromous silver eel descending towards the sea were kindly supplied at Fishery Lab., The Univ. of Tokyo, Ikawazu, Aichi Pref. The serums were prepared as above and transported to our laboratory with ice.

The color of serum, together with body weight, total length, sex, and gonad weight of all the eel specimens are given in Tables 1~2.

Methods of analysis:

(1) Starch-gel electrophoresis: According to the previous paper⁴⁾ starch-gel electrophoresis was conducted of the serum samples. The starch-gel was prepared in 0.033 M tris-borate buffer, pH 8.4, in 14×26×0.6 cm, to which six samples usually were

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applied at one time. The runs were performed at a constant current of 23 mA for 7 hrs. at 4°C, using 0.33 M tris-borate buffer, pH 8.4, as the bridge solution. After the run, the gel was horizontally sliced into two parts. The one was subjected to general stain for protein with amido black 10B, and the other to specific stain for lipoprotein or glycoprotein as follows. Lipoprotein was stained with sudan black B according to the method of SWAHN⁵⁾. As for glycoprotein, the staining method of DRILHON *et al.*⁶⁾ originally used for paper electrophoresis was applied with some

Table 1. Specifications of cultured eels used.

Date of sampling	No.	Body weight (g)	Total length (cm)	Sex	Gonad weight (g)	Color of serum
1966 May 24	1	208	53	♂	0.2	Blue-green
	2	161	46	♀	trace	"
	3	120	43	♀	"	"
June 24	1	252	54	♀	"	Lightly blue-green
	2	130	42	♀	"	Blue-green
	3	110	43	♂	"	"
July 12	1	270	54	♀	"	Blue-green*
	2	177	49	♂	"	"
	3	85	39	♀	"	Blue-green
Aug. 18	1	124	42	♂	"	Blue-green
	2	120	44	♀	"	Lightly blue-green
	3	105	40	♂	"	Blue-green
Sept. 21	1	207	49	♀	"	Lightly blue-green
	2	185	50	♂	"	"
	3	135	44	♀	"	"
Oct. 25	1	283	56	♀	0.3	Lightly blue-green
	2	223	53	♀	0.3	Blue-green
	3	160	47	♀	0.4	Lightly blue-green
Nov. 21	1	180	49	?	trace	Lightly blue-green
	2	175	48	?	"	Blue-green
Dec. 16	1	242	54	♀	0.4	Yellow-green
	2	197	51	♀	trace	Blue-green*
	3	123	44	♀	"	Lightly blue-green
1967 Jan. 12	1	151	43	♀	"	Blue-green
	2	127	45	♂	"	Lightly blue-green
	3	84	42	♀	"	Blue-green
Feb. 18	1	254	59	♀	0.7	Lightly blue-green
	2	220	50	?	trace	Yellow-green
	3	103	47	?	"	Blue-green
Mar. 28	1	213	52	♂	0.1	Lightly blue-green
	2	171	54	♂	trace	"
	3	84	40	♀	"	Blue-green
Apr. 24	1	194	52	♀	"	Lightly blue-green
	2	172	48	♀	"	"
	3	103	42	?	"	Blue-green

* Slightly tinged with red, probably owing to hemolysis.

Table 2. Specifications of wild eels used.

Date of sampling	No.	Body weight (g)	Total length (cm)	Sex	Gonad weight (g)	Color of serum
1966	1	282	54	♀	0.5	Blue-green*
May 24	2	68	38	?	trace	Blue-green
	1	296	57	♀	0.8	Yellow-green
June 24	2	259	53	♀	0.7	"
	3	101	42	?	trace	Blue-green
(July)		(Not available)				
	1†	189	51	♀	0.5	Yellow-green
Aug. 18	2†	187	51	♀	0.8	Green
	3†	172	51	♀	0.6	Blue-green*
	1	298	56	♀	trace	Yellow-brown
Sept. 21	2	101	41	♂	"	Green
	3	100	43	♂	"	"
	1	453	62	♀	8.8	Green
Oct. 25	2	265	56	♂	0.6	Yellow-green
	3	125	44	♂	0.2	"
	1†	297	58	♀	5.0	Blue-green*
Nov. 21	2†	290	56	♀	5.3	Yellow-green
	3†	235	54	♂	0.4	Blue-green*
(Dec.)		(Not available)				
1967	1	220	54	♀	1.6	Blue-green*
Jan. 12	2	171	52	♀	1.1	Lightly green
	3	161	50	♂	0.2	"
	1	297	59	♀	1.5	Lightly blue-green
Feb. 18	2	217	54	♀	0.7	Yellow-green
	3	134	48	?	trace	"
	1	350	61	♀	0.8	Yellow-green
Mar. 28	2	182	50	♀	trace	Green
	3	77	41	♂	"	"
	1	69	38	?	—	Blue-green
Apr. 24	2	48	35	?	—	"
	3	43	33	?	—	"
1966	1	493	69	♀	12.2	Colorless
Nov. 25	2	403	65	♀	7.0	"
(Catadromous	3	263	58	♂	0.8	"
eels)	4	263	57	♂	0.9	"

* Slightly tinged with red, probably owing to hemolysis.

† Eels supplied from Aichi Pref.

†† Eels supplied from Tottori Pref.

modifications; The gel was dipped for 15 mins. with shaking, in 0.05 M sodium metaperiodate solution. After rinsing with running distilled water for 5 mins. to stop the reaction, the gel was stained with the Schiff's reagent (fuchsin-sulfurous solution) to a dense violet, and then decolorized with 0.1% sodium sulfite solution warmed at about 40°C.

(2) Determination of chromoprotein concentration in serum: After making transparent by glycerol immersion, the amido black stained gel was subjected to densitometry, and the relative proportion of the chromoprotein component was determined based on the resultant curve. The concentration of chromoprotein was calculated using its relative proportion thus obtained and the total protein concentration of the serum as analyzed by a modified Biuret method of GORNALL *et al.*⁷⁾

Results and Discussion

The serum of cultured eel usually was blue-green with some yellowish-green exceptions, and that of wild eel was yellowish-green to green, while the serum of small wild eel less than 70 g was blue-green in most cases. Furthermore, we confirmed the observation by OIDE *et al.*⁸⁾ that the serum of catadromous eel is colorless and transparent.

Fig. 1. Starch-gel electrophoretic patterns of eel serum protein (0.033 M Tris-borate buffer, pH 8.4).

Table 3. Seasonal variation of blue-green chromoprotein concentration in eel serum.

Date of sampling	No.	Cultured eels			Wild eels		
		Serum protein concentration (%)	Chromoprotein		Serum protein concentration (%)	Chromoprotein	
			Relative proportion in serum (%)	Concentration (mg%)		Relative proportion in serum (%)	Concentration (mg%)
1966 May 24	1	3.23	21.0	679	5.23	19.0	994
	2	4.33	23.2	1,010	4.02	15.2	611
	3	4.46	19.6	876	Not available		
June 24	1	5.46	15.3	836	4.69	14.2	666
	2	4.92	19.4	955	5.23	16.7	874
	3	4.69	14.0	656	4.92	15.3	754
July 12	1	5.84	21.0	1,230	Not available		
	2	6.07	27.0	1,640			
	3	6.69	25.0	1,680			
Aug. 18	1	5.37	24.5	1,320	8.00	23.0	1,840
	2	5.78	19.1	1,100	6.38	19.3	1,230
	3	5.62	28.7	1,620	7.23	15.9	1,150
Sept. 21	1	5.84	12.7	741	6.07	14.7	882
	2	4.38	15.2	665	5.00	12.6	630
	3	4.73	13.1	619	5.46	18.4	1,010
Oct. 25	1	6.93	—	—	6.38	9.9	631
	2	5.85	—	—	6.15	—	—
	3	4.77	17.8	849	5.46	11.5	629
Nov. 21	1	5.23	—	—	6.95	11.7	814
	2	5.23	15.4	806	5.93	—	—
	3	Not available			6.95	—	—
Dec. 16	1	5.78	10.2	580	Not available		
	2	5.62	—	—			
	3	5.54	9.2	509			
1967 Jan. 12	1	5.16	7.7	398	5.78	9.8	566
	2	4.54	13.2	599	5.93	10.3	610
	3	4.62	6.9	318	4.78	13.4	641
Feb. 18	1	4.77	—	—	5.46	—	—
	2	5.23	5.2	272	3.23	—	—
	3	4.23	—	—	4.31	—	—
Mar. 28	1	5.20	10.4	541	6.10	10.7	652
	2	5.00	—	—	6.70	—	—
	3	5.50	15.2	836	5.70	12.7	724
Apr. 24	1	5.04	—	—	5.62	—	—
	2	5.02	—	—	5.63	—	—
	3	4.81	—	—	4.14	—	—
1966 Nov. 25 (Catadromous eels)	1	Not available			3.70	0	0
	2				4.77	0	0
	3				4.15	0	0
	4				3.93	0	0

— Data were not available because of insufficient separation of the chromoprotein from other components.

In starch-gel electrophoresis, the eel serum protein is separated into at least 15 components as photographically shown in the preceding paper¹⁾. Fig. 1 schematically illustrates a typical example of the amido black stained electropherogram and its densitometric tracing, together with schematic patterns of lipoprotein and glycoprotein-specific stains.

The chromoprotein corresponded to the component No. 11 in the amido black stained electropherogram. This component was positive in lipoprotein as well as glycoprotein stains. In Table 3 are tabulated the total protein concentration, together with the relative proportion and concentration of chromoprotein, for all the serum samples analyzed. The total protein concentration of serum of both cultured and wild eels fell mostly within a range of 4~7% throughout a year, showing some increase in summer and some decrease, especially with the cultured eels, in winter. In the case of catadromous eels, the values were clearly lower than those of sedentary eels collected in the same month.

Fig. 2 graphically illustrates monthly fluctuations of chromoprotein concentration of both cultured and wild eels. There seems to be no significant difference between both sexes, as seen in this figure. The chromoprotein concentration remarkably increases in summer and decreases in winter, with both cultured and wild eels. This tendency is particularly clear with the cultured eels. The seasonal fluctuation of the pigment concentration could not be covered by some fluctuation of the total

Fig. 2. Seasonal variation of chromoprotein concentration in eel serum.

Fig. 3. Relation between the chromoprotein concentration in serum and the body weight for the cultured and wild eels used.

protein concentration of serum as shown in Table 3.

In the next place, was examined the relation between the chromoprotein concentration and body weight. As shown in Fig. 3, the plotted points were so dispersed that no definite relation between them can be found. The similar relation was investigated as to the cultured and the wild eels which were obtained in the same month. For the sake of convenience, those points of the cultured and wild eels in the same month are linked with solid and broken lines, respectively. Shape and inclination of the resultant curves were also too multifarious to indicate any relation. Thus, there may be no significant relation between the chromoprotein concentration and the body weight of eel.

As seen in Fig. 2 or 3, in the midsummer months, July and August, both cultured and wild eels gave very high chromoprotein concentrations ranging from 1,100 to 1,800 mg%, which account for approximately 1/4 of the total of serum protein.

During the period from December to February, however, the concentration with cultured eels markedly decreased to less than 400 mg%, occupying only 1/3~1/5 of the highest value in summer. In the case of wild eel, the decrement was moderate to such an extent that no specimen of less than 500 mg% concentration was found in winter. As a whole, the variation with the wild eels seems rather moderate, the chromoprotein concentration being generally lower in summer and higher in winter, compared to the corresponding value with the cultured eels.

In the case of cultured eel, the supply of foodstuff usually is set about at April or May, being gradually increased to the maximum in the summer season when the water temperature of culture ponds is highest. In October the supply is reduced, and completely stopped in November. Accordingly, the seasonal variation of the chromoprotein concentration with the cultured eels seems to be clearly consistent with that of foodstuff supply just mentioned. Taking into consideration the facts

Fig. 4. Relation between the chromoprotein concentration in serum and the maturation degree of gonad for the wild eels and catadromous eels.

that the amino acid and sugar compositions rather resemble those of β_1 -lipoprotein in human serum⁸⁾ whose role is considered to be the transport of lipid⁹⁾, and that the proportion of lipid moiety in the whole molecule of the pigment varies from preparation to preparation¹⁰⁾, the remarkable rise of chromoprotein concentration in summer may be attributed to the necessity of transport of mass lipid introduced in blood. Thus, the difference in the variation of pigment concentration between the cultured and wild eels might be explained by the difference of food supply. In contrast to the case of cultured eel, the food supply is not artificially controlled for wild eels and, in addition, the river water is, even in winter, warm enough to stimulate them to take some food.

The relation between the maturation degree of gonad and the chromoprotein concentration was examined. Since almost all the cultured eels used in the present study were 1 or 2 years old and sexually immature, the data on wild eels and catadromous eels were analyzed here (Fig. 4). As described above, the serums of catadromous eels were almost colorless and transparent. The complete absence of the chromoprotein was confirmed by starch-gel electrophoresis for the four serum samples examined here (refer to Table 3). In the other cases, no definite correlation seems to exist between the gonad maturation and the chromoprotein concentration, though a roughly hyperbolic distribution of the plotted points is conceived.

The disappearance of pigment in the serum of catadromous eels seems to be related with the fact that they usually take little food while their gonads are rapidly getting close to maturity¹¹⁾. The chromoprotein might have been consumed up for the gonad maturation.

Summary

The eel serum was monthly examined for the blue-green chromoprotein concentration throughout a year. The concentration was neither sex- nor growth-dependent. A seasonal variation of the concentration was found for both cultured and wild eels: The concentration was highest in July and August, 3~5 times as much as that in winter. The seasonal variation was clearer in the case of cultured eel, roughly consisting with that of the amount of foodstuff given. With the catadromous eels, a complete disappearance of the chromoprotein was confirmed.

Based on these results, the physiological role of the chromoprotein was discussed.

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