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| Title            | Water, Electrolytes, and Soluble Proteins in Cataractous Eye Lenses of Masu Salmon |
| Author(s)        | WATANABE, Yoshirou; 渡辺, 良朗; YAMADA, Juro et al.                                    |
| Citation         | 北海道大學水産學部研究彙報, 31(4), 297-305  |
| Issue Date       | 1980-11  |
| Doc URL          | <a href="https://hdl.handle.net/2115/23730">https://hdl.handle.net/2115/23730</a>  |
| Type             | departmental bulletin paper  |
| File Information | 31(4)_P297-305.pdf   |



## Water, Electrolytes, and Soluble Proteins in Cataractous Eye Lenses of Masu Salmon

Yoshiro WATANABE\* and Juro YAMADA\*

### Abstract

Amounts of water, electrolytes, and soluble proteins and the electrophoretic nature of soluble proteins in cataractous or histologically abnormal lenses of masu salmon (*Oncorhynchus masou*) were compared with those in transparent or histologically normal lenses. Cataractous lenses tended to contain more water and calcium less sodium and soluble proteins compared with transparent lenses. All the lenses which showed calcium concentrations over  $50 \times 10^{-2}$  mEq/kg lens water were opacified.

Amounts of water, inorganic ions, and soluble proteins were compared between normal lenses and four lens types of histological abnormalities. Concentrations of calcium, sodium, and potassium of normal lenses were  $19.4 \times 10^{-2}$  mEq/kg, 18.4 mEq/kg, and 81.4 mEq/kg lens water, respectively. Some type I lenses showed extremely high calcium levels. Types I and II lenses showed low potassium concentrations. Types III and IV lenses did not show any differences from the normal in the amounts of their constituents.

The disc-electrophoretic patterns of soluble proteins of normal lenses showed nine peaks. No essential differences were found between the patterns of transparent and cataractous lenses. A characteristic change in types I and II lenses was reduction of the most cathodal peak (the 9th). In type III lenses, all peaks except the 9th were low. SDS-gel electrophoretic patterns of soluble lens proteins were similar in both normal and abnormal lenses, and showed three peaks.

### Introduction

As reported in the previous paper<sup>1)</sup>, cataractous lenses of cultured masu salmon (*Oncorhynchus masou*) revealed various histological abnormalities, which were classified into four types based on the affected regions and states. According to the studies on mammalian cataracts, some lens electrolytes and soluble proteins show distinct changes in the course of lens opacification. The senile cortical cataract in man is accompanied by changes in quantities of lens inorganic ions, i.e., an extremely high concentration of sodium and a low concentration of potassium<sup>2)3)</sup>. Therefore, the opacification of the lens cortex is thought to be caused by a metabolic disorder of the lens which gives rise to the increment of sodium<sup>4)</sup>.

On the other hand, the opacification of the lens nucleus of the nuclear cataract is the result of the aggregation of soluble lens proteins called crystallin<sup>5)6)</sup>. Increase of lens calcium with the advance of opacification characterizes this cataract<sup>7)</sup>, and this is considered to be an important factor of aggregation of degenerated crystallin<sup>8)-10)</sup>. In the hereditary cataracts in mice, increases in sodium and

\* Laboratory of Physiology and Ecology, Faculty of Fisheries, Hokkaido University  
(北海道大学水産学部生理学生態学講座)

calcium and decrease in potassium were also observed with the advance of histological abnormalities<sup>11)12)</sup>. These changes of electrolytes are believed to indicate a defect of the lens epithelium which controls the exchange of materials between the lens and the aqueous humor.

In this study, water, electrolytes, and soluble protein contents were compared between normal and the four types of abnormal lenses based on their histological characteristics<sup>1)</sup>. Changes in electrophoretic nature of soluble lens proteins in affected lenses were also investigated.

### Materials and Methods

Cultured masu salmon (*O. masou*) obtained from the Mori Branch of Hokkaido Fish Hatchery were used as materials. They included yearlings (0+) and one- (1+) and two-year-old (2+) fish having transparent or opaque eyes in external appearance. The two lenses were removed from the eyeballs and washed with distilled water. One of them was used for chemical analyses and the other was subjected to the histological examination to check the type of abnormalities according to the criteria described in the previous paper<sup>1)</sup>. Some of the lenses for chemical analyses were frozen with dry ice and stored at  $-10^{\circ}\text{C}\sim-15^{\circ}\text{C}$  unless used immediately.

After measuring the wet weight, the lens was dried for 24 hours at  $90^{\circ}\text{C}$  and then reweighed for the determination of water content. The dried lens was digested by heating with a mixture of 2 ml of 60% nitric acid and 2 ml of 60% or 70% perchloric acid. The resulted product was diluted with distilled water or with 1% lanthanum chloride for the determinations of sodium and potassium or for calcium. Sodium and potassium were determined by flame-emission photometry and calcium by atomic absorption spectrophotometry, using a Hitachi 501 Atomic Absorption Spectrophotometer.

For protein assays, the lens was weighed and homogenized in 0.25M sucrose solution. The homogenate was centrifuged for 60 minutes at  $106,000\times g$  at  $2^{\circ}\text{C}$ , and the supernatant was used for the following analyses. The total amount of soluble proteins was measured by the micro-Kjeldahl method. Disc-electrophoresis was performed in 7.0% polyacrylamide gel with a Tris-glycine buffer (pH 8.3) under constant current of 4 mA/gel. SDS-gel electrophoresis was performed in 5.6% gel containing 0.1% SDS with a Tris-sodium acetate buffer containing 1 g SDS/liter (pH 7.4) under constant current of 4.5 mA/gel.

### Results

The water and soluble protein contents and the concentrations of calcium, sodium, and potassium in transparent and cataractous lenses of different ages were shown in Table 1. In transparent lenses, there seemed to be some age-dependent changes. The water content of 1+ was significantly higher than that of 0+ or 2+. An increase in calcium concentration and decreases in sodium and potassium concentrations were observed with age. The sodium concentration of 2+ (14.4 mEq/kg lens water) was significantly low compared with that of 0+ (23.2 mEq/kg). The potassium concentrations of 1+ (67.7 mEq/kg) and 2+ (75.4 mEq/kg)

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Table 1. Contents of water, electrolytes, and soluble proteins in transparent and cataractous lenses of different ages.

|                | Water %              | Calcium $\times 10^{-2}$ mEq/kg lens water | Sodium mEq/kg lens water | Potassium mEq/kg lens water | Soluble protein % wet weight |
|----------------|----------------------|--|--------------------------|-----------------------------|------------------------------|
| 0+ transparent | 52.9 $\pm$ 0.7 (10)  |  | 23.2 $\pm$ 2.8 (10)      | 85.9 $\pm$ 2.2 (10)         | 28.4 $\pm$ 0.8(10)           |
| 0+ cataractous | 58.1 $\pm$ 0.9* (15) |  |                          |                             | 25.2 $\pm$ 1.9(10)           |
| 1+ transparent | 57.4 $\pm$ 0.4** (7) | 16.5 $\pm$ 5.8 (7)                         | 21.4 $\pm$ 4.2 (7)       | 67.7 $\pm$ 5.2** (7)        | 27.4 $\pm$ 1.2 (5)           |
| 1+ cataractous | 58.4 $\pm$ 0.5 (13)  | 33.0 $\pm$ 10.7(12)                        | 18.6 $\pm$ 2.9 (13)      | 71.6 $\pm$ 1.8 (13)         | 25.4 $\pm$ 0.7(10)           |
| 2+ transparent | 54.6 $\pm$ 0.5 (8)   | 22.3 $\pm$ 5.0 (8)                         | 14.4 $\pm$ 1.6** (8)     | 75.4 $\pm$ 1.9** (8)        |                              |
| 2+ cataractous | 55.6 $\pm$ 0.5 (12)  | 149.0 $\pm$ 109.0(12)                      | 10.4 $\pm$ 1.0 (12)      | 71.6 $\pm$ 2.4 (12)         |                              |

Values are expressed as mean $\pm$ standard error.

Figures in parentheses show number of lenses.

\* Significantly different (P<0.01) from transparent lenses of the same age

\*\* Significantly different (P<0.05) from 0+ transparent lenses

were significantly lower than that of 0+ (85.9 mEq/kg). The soluble protein contents of transparent lenses were about 28% of wet weight and showed no age dependent changes.

When compared with transparent lenses of the same age, cataractous lenses appeared to be characterized by their high values of water and calcium and low values of sodium and soluble proteins. The calcium concentration was highly variable, and extremely high values were obtained in some cataractous lenses. Frequency distributions of transparent and cataractous lenses according to calcium concentration were shown in Fig. 1. The calcium concentrations of both 1+ and 2+ transparent lenses were below  $50 \times 10^{-2}$  mEq/kg lens water. The cataractous lenses, on the other hand, were divided into two groups; one below  $30 \times 10^{-2}$  mEq/kg and the other above  $50 \times 10^{-2}$  mEq/kg.

It was found in our previous

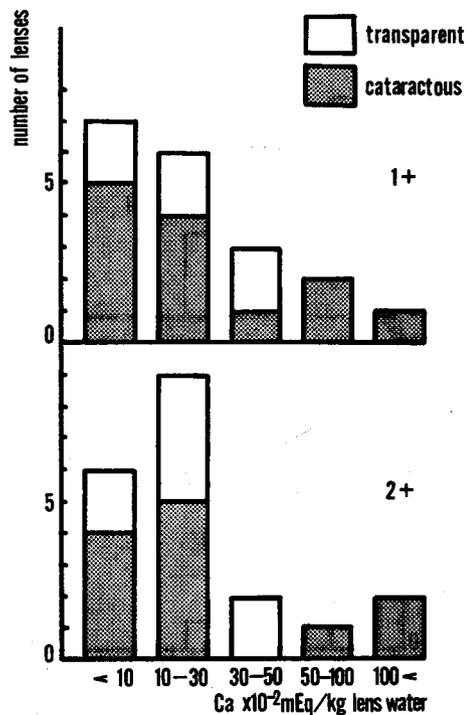


Fig. 1. Calcium concentration frequencies in transparent and cataractous lenses.

Table 2. Comparisons of contents of water, inorganic ions, and soluble proteins between normal and four types of abnormal lenses.

|        | Water %            | Calcium $\times 10^{-2}$ mEq/kg lens water | Sodium mEq/kg lens water | Potassium mEq/kg lens water | Soluble protein % wet weight |
|--------|--------------------|--|--------------------------|-----------------------------|------------------------------|
| I      | 57.8 $\pm$ 0.9(17) | 189.0 $\pm$ 106.0(13)                      | 19.0 $\pm$ 3.0(13)       | 70.4 $\pm$ 2.5(13)*         | 24.0 $\pm$ 0.7(5)            |
| II     | 56.5 $\pm$ 0.4(13) | 21.8 $\pm$ 6.2( 8)                         | 12.1 $\pm$ 1.5(10)       | 70.5 $\pm$ 2.5(10)*         | 26.3 $\pm$ 1.1(5)            |
| III    | 55.2 $\pm$ 0.7( 9) | 30.4 $\pm$ 4.8( 5)                         | 17.6 $\pm$ 3.1( 8)       | 73.4 $\pm$ 2.8( 8)          | 28.1 $\pm$ 1.1(7)            |
| IV     | 55.8 $\pm$ 0.9(15) | 17.9 $\pm$ 4.6( 6)                         | 20.0 $\pm$ 3.5(10)       | 75.0 $\pm$ 3.9(10)          | 26.1 $\pm$ 0.6(8)            |
| Normal | 55.5 $\pm$ 0.7(12) | 19.4 $\pm$ 6.6( 5)                         | 18.4 $\pm$ 2.9(11)       | 81.4 $\pm$ 2.5(11)          |                              |

Values are expressed as mean $\pm$ standard error.

Figures in parentheses show number of lenses.

\* Significantly different ( $P < 0.05$ ) from normal lenses

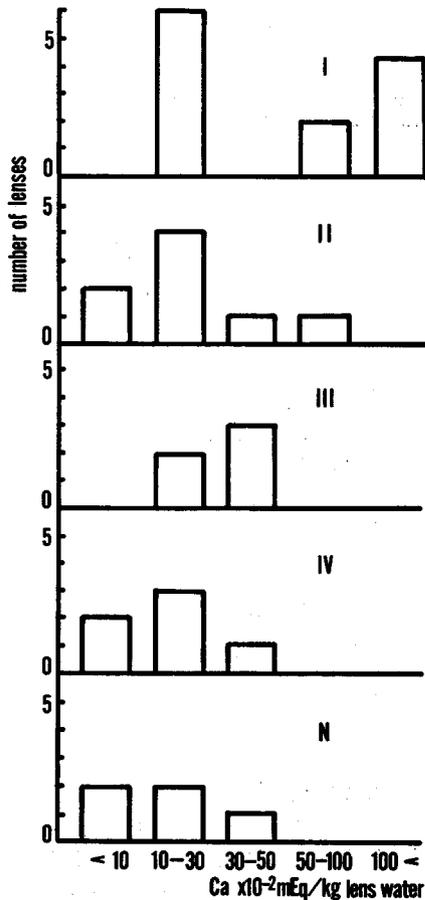


Fig. 2. Calcium concentration frequencies in normal (N) and four types of abnormal lenses.

study<sup>1)</sup> that some transparent lenses showed histological abnormalities observed in cataractous lenses. Therefore, the amounts of the major components were compared between the normal and the four histologically different types of abnormal lenses (Table 2). The lenses of type I or II which also showed the abnormalities of type III or IV were treated as type I or II.

The water content was almost the same for all lenses but was slightly high in types I and II. Though not significantly different, the average calcium concentration was extremely high in type I ( $189 \times 10^{-2}$  mEq/kg). The sodium concentration in type II was apparently low in comparison to the normal and the other types. The potassium concentration was significantly lower in types I and II than in the normal. It showed slightly lower values also in types III and IV, but other constituents in these types were at almost the same levels as in the normal. Frequency distributions of calcium concentration in the normal and the abnormal lenses are shown in Fig. 2. There were no lenses containing calcium over  $50 \times 10^{-2}$  mEq/kg in the normal

and types III and IV. Type II lenses showed nearly the same distribution pattern as the normal. On the other hand, type I lenses showed a pattern clearly different from those of other types. Lenses of type I were divided into two groups on the basis of calcium concentration; one below  $30 \times 10^{-2}$  mEq/kg and the other above  $50 \times 10^{-2}$  mEq/kg with the highest value of  $1380 \times 10^{-2}$  mEq/kg. The soluble protein level was almost the same in the normal and abnormal lenses except for type I, which showed a slightly lower value than the others.

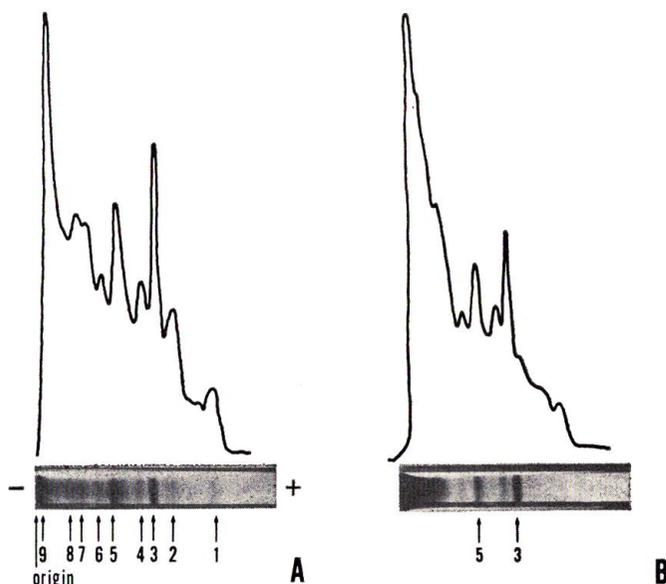


Fig. 3. Disc-electrophoretic patterns of soluble proteins of normal lenses from 1+ (A) and 0+ (B) fish. The 3rd, 5th, and 9th peaks are dominant among nine peaks. The 3rd and 5th peaks of 0+ are relatively low in comparison with 1+.

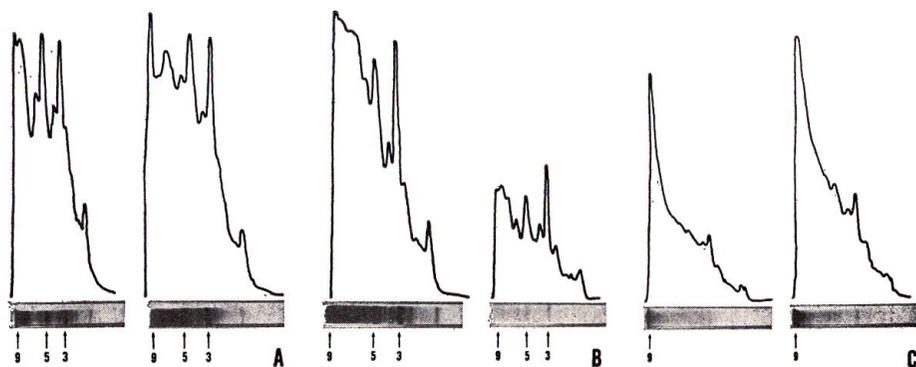


Fig. 4. Disc-electrophoretic patterns of soluble proteins of the type I (A), type II (B), and type III (C) abnormal lenses.

A typical disc-electrophoretic pattern of soluble proteins of the normal lens showed at least nine peaks, named 1st to 9th from the anode side (Fig. 3). The 3rd, 5th, and 9th peaks prevailed the others. In 0+ lenses, the 3rd and 5th peaks were relatively lower than the respective peaks in 1+ lenses. The patterns of transparent lenses did not show significant differences from those of cataractous lenses.

The disc-electrophoretic patterns of the four abnormal type lenses were compared with that of the normal lens (Figs. 3, 4). The characteristics of type I lenses were rises of the 3rd and 5th peaks and a fall of the 9th peak, causing an even height of these three peaks (Fig. 4, A). In type II, the 9th peak was not as dominant as that of normal lenses (Fig. 4, B). Most of the type III lenses were characterized by a reduction of all the peaks except for the 9th (Fig. 4, C). However, some type III lenses showed similar patterns as the normal lens. No clear differences

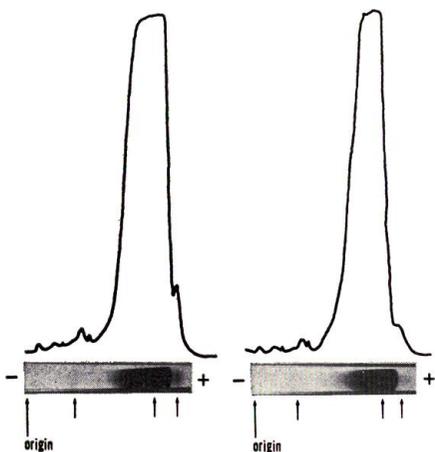


Fig. 5. SDS-gel electrophoretic patterns of soluble lens proteins of normal lenses. The central peak is dominant among three peaks.

were observed between type IV and normal lenses in their electrophoretic patterns.

SDS-gel electrophoretic patterns of soluble proteins of normal lenses were shown in Fig. 5. The central peak was predominant among 3 peaks observed. No clear changes were observed with aging, opacification, or histological abnormalities in the patterns.

### Discussion

In mammals, the lens epithelium is considered to control the exchange of inorganic ions to maintain sharp concentration gradients between the aqueous humor and the lens<sup>13,14</sup>. Concentrations of sodium, potassium, and chloride of normal rabbit lens were 38.9 mEq/kg, 188.5 mEq/kg, and 15.3 mEq/kg lens water<sup>15</sup>, while those of aqueous humor were 143.5 mEq/kg, 4.55 mEq/kg, and 109.5 mEq/kg, respectively<sup>14</sup>. Sodium and potassium concentrations of normal human lenses were reported to be 14.5 mEq/kg and 113.5 mEq/kg lens water, respectively<sup>2</sup>. Remarkable increase of sodium (210.6 mEq/kg) and decrease of potassium (21.4 mEq/kg) were observed in lenses of cortical cataract<sup>2,3</sup>. These changes in ion concentrations are thought to be brought about by functional disruption of the lens epithelium.

Sodium and potassium concentrations of normal lenses of masu salmon were 18.4 mEq/kg and 81.4 mEq/kg lens water, respectively, which are similar to the levels of normal mammalian lenses mentioned above. In masu salmon, some differ-

ences in ion concentrations are found between cataractous and transparent lenses, but these are not as distinct as observed in human cortical cataract. As reported in the previous paper<sup>1)</sup> the type III abnormalities were similar to those of human cortical cataract in histological characteristics, but the ion concentrations in this type showed little difference from the normal values. Therefore, unlike in human cortical cataract, sodium and potassium concentrations may not have much importance in this disease.

Mammalian lenses contain much soluble proteins which are classified into  $\alpha$ -,  $\beta$ , and  $\gamma$ -crystallins based on the molecular weights, charges, and antigenic specificities<sup>15)</sup>. Degeneration and aggregation of  $\alpha$ -crystallin take place with aging, resulting in a high molecular weight protein<sup>16)17)</sup>. The lenses of the human nuclear cataract contained more high molecular weight proteins and less soluble proteins than the normal lenses<sup>5)18)</sup>. Therefore, the aggregation and insolubilization of crystallins are regarded as the cause of opacification. It was also noted that an increment of calcium concentration is accompanied by the opacification of lens nucleus<sup>7)</sup>. Evidence from *in vitro* studies showed that calcium plays an important role in the aggregation and insolubilization of soluble lens proteins<sup>8)-10)</sup>.

The lenses of masu salmon also contained much soluble proteins, about 28% of wet weight. In contrast to mammalian lenses, about 70% of soluble proteins of fish lenses is reported to be  $\gamma$ -crystallin<sup>19)</sup>. Therefore, the major peak in SDS-gel electrophoretic patterns of soluble lens proteins of masu salmon is considered to be  $\gamma$ -crystallin. Zigman and Yulo<sup>20)</sup> found that the high molecular weight proteins of the dogfish lens increased with age as in the case of mammals. Since the opacification was observed only at the center of affected lenses in masu salmon, this disease may be referred to as a kind of nuclear cataract. Possibly, insolubilization of soluble lens proteins may have occurred in these lenses as in the nuclear cataract of mammals or in the old dogfish lenses. However, the electrophoretic patterns of soluble proteins did not show much differences between cataractous and transparent lenses.

Among the four types of histological abnormalities, some of the type I lenses showed extremely high calcium concentrations. As shown in Fig. 2, almost all lenses containing calcium over  $50 \times 10^{-2}$  mEq/kg belonged to type I. Lenses of this type, together with those of type II, also showed significantly low potassium concentrations. Marked increases of calcium and sodium and a decrease of potassium were pointed out in the hereditary cataract of mice<sup>11)12)</sup>, which has common histological characteristics with the type I abnormality<sup>21-24)</sup>. This shows that the type I abnormality has similar characteristics to the hereditary cataract in mice.

Gutierrez<sup>25)</sup> found nine peaks in the electrophoretic pattern of soluble lens proteins of a tuna, *Thunnus thynnus*; the most cathodal peak was of lens nucleus, and the third peak from the anode was of lens cortex. If the 9th peak in masu salmon was the nuclear protein as in *T. thynnus*, the fall of this peak in types I and II may represent degeneration of the nuclear protein. The lowered peaks at the anodal side in type III are consistent with the histological abnormalities observed only at the lens cortex, if these peaks are of cortical proteins.

In conclusion, quantitative and/or qualitative aspects of inorganic ions and

soluble proteins in cataractous lenses of masu salmon were different from those reported in mammalian cataracts. Judging from the results of chemical analyses of opacified lenses together with their variety of histological abnormalities, the cataract of masu salmon seems to have complexed pathological characteristics. These peculiarities are considered to be attributed to the nature of soluble lens proteins. Further studies of soluble lens proteins of fish in comparison with those of mammals will be needed to clarify the process of lens opacification.

### Acknowledgement

We wish to express our cordial thanks to Messrs. Masaaki UCHIYAMA and Mitsuaki ATODA, the Mori Branch of Hokkaido Fish Hatchery, who supplied us with material fish.

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