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CHANGES IN CATALASE ACTIVITY OF THE TISSUES AND BLOOD
OF "MASU", *ONCORHYNCHUS MASOU*, WHEN TRANSFERRED
FROM FRESH-WATER TO SEA WATER

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There have been many reports on studies of osmoregulation in salmon fry, parr and smolt.¹⁾ As possible regulators of salt and water balance in fish, some hormones such as those of the thyroid, the gonads, the interrenals and the pituitary glands have been investigated.²⁾ However, it is presumable that some enzymes in tissues and blood might also be related to osmoregulation in fish. In previous papers, the author has reported that the kidney catalase activity of the salmonid fishes is much higher than that of the typical fresh-water ones,³⁾ and that in *Oncorhynchus masou* the catalase activity of the liver, kidney and blood of the smolt is significantly higher than that of the parr, at least, in the time of the seaward migration.⁴⁾

The present report gives the results of an experiment performed to find whether some changes of the catalase activity of the tissues and blood in the smolts of *O. masou* occurred when they were transferred from fresh-water to sea water. As the experiment was done at the time of the seaward migration of this species, it may be said that the smolts used were a physiologically marine form.^{5,6)}

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MATERIALS AND METHODS

In the present experiment were used the smolts of *O. masou* which had been reared in a fresh-water pond. The experiments were performed twice in two years. The first experiment (Exp. No. 1) was done during July 24-August 5, 1953; this period is a little past the active season of the seaward migration in the smolt of this fish. The second experiment (Exp. No. 2) was performed on June 2-3, 1954; this time just corresponds to the active season of seaward migration. In spite of that the date of Exp. 1 was later than that of Exp. 2, the fishes of the former were smaller in size than those of the latter. The body weight was 16-38g and the standard length 11.5-14.7cm in Exp. 1, and the body weight 28-53g and the standard length 13.5-16.4cm in Exp. 2. The fishes were acclimatized in dilute sea water. The periods of such acclimatization were 26 hours in Exp. 1 and 3.5 hours in Exp. 2. The dilute sea water in Exp. 1

was about 40% sea water, and its σ_{15} was 1.009. In the case of Exp. 2, sea water was dropped slowly into fresh-water during 3.5 hours, in which water the fishes were acclimatized gradually; the final σ_{15} of this dilute sea water was 1.006. Then the fishes were transferred to sea water. The σ_{15} of sea water used was 1.024 in Exp. 1 and 1.025 in Exp. 2. In both the acclimatization and experiment, aquaria of about 21 liters in Exp. 1 and of about 29 liters in Exp. 2 were used. Into these respective aquaria were transferred 17 fishes in Exp. 1 and 31 fishes in Exp. 2; the water was constantly aerated and renewed every other day. The water temperature was varied from 14.6° to 15.6°C in Exp. 1, and from 10.5° to 11.0°C in Exp. 2. In both experiments, the fishes were fed sparingly with dried and ground crustacean larvae in the aquarium. Although the cause was not clear, the fishes of Exp. 1 fed well, while those of Exp. 2 ate little. As the control fishes, smolts were used which were collected from the pond in which they had been reared. However, examinations were made to ascertain whether or not the catalase activity in tissues and blood changes when the fishes are confined several days to a small aquarium. Table 1 shows results, however, for parrs of *O. masou*, which

Table 1. Influence on the catalase activity in the tissues and blood of the parr of *Oncorhynchus masou* when confined two weeks to a fresh-water aquarium with running water; July 29–August 12, 1953

Tissues and blood Group	Catalase activity M±standard error of the mean*						No. of fishes examined
	Liver	Kidney	Blood	Gill	Stomach	Muscle	
Pond	76±2.7	16±1.8	19±2.1	1.6±0.35	0.55±0.164	0.66±0.017	6
Aquarium	61±2.1	13±2.2	16±0.8	2.4±0.68	0.97±0.196	0.32±0.076	5
P**	<0.005	>0.3	>0.2	>0.3	>0.1	<0.005	

* Standard error of the mean = $\sqrt{d^2/n(n-1)}$

** Probability was calculated from the table of Student's t, using the equations:

$$t = \frac{m_1 - m_2}{\sqrt{SE_1^2 + SE_2^2}} \quad \text{and} \quad n = (n_1 - 1) + (n_2 - 1),$$

where m_1 and m_2 represent the two means, SE_1 and SE_2 the two standard errors, and n_1 and n_2 are the number of fishes from which the respective means are obtained.

were confined two weeks to a fresh-water aquarium through which running water was flowing. It will be seen that the significant falls were found only in the catalase activity of the liver and the muscle. Possibly, similar falls might be observed in the smolts if examined.

The methods of enzyme preparation, and of the estimation and calculation of catalase activity are the same as those of the previous experiments.^{3,4)} That is, the catalase activity was calculated in k per dilution rate of tissue wet weight g, except that of the blood where it was calculated in k per dilution rate of the blood volume sampled.

RESULTS

The results obtained are given in Tables 2 and 3. The changes of the catalase activity in the liver, kidney and blood are illustrated in Figures 1 and 2.

Liver. In Exp. 1 the catalase activity increased at 6 hours in the dilute sea water to a highly significant degree ($P < 0.001$). However, in both experiments no significant changes of the catalase activity were found after transference of the fishes from the dilute sea water to sea water.

Table 2. Changes of the tissue catalase activity in the smolts of *O. masou* in dilute sea water and in sea water (Exp. No. 1); July 24-August 5, '53

Time Tissues	Catalase activity M \pm standard error of the mean					No. of fishes examined
	Liver	Kidney	Gill	Stomach	Muscle	
6 hrs in dilute sea water <i>P</i>	69 \pm 0.2 <0.001	18 \pm 1.8 —	1.9 \pm 0.13 —	1.3 \pm 0.27 >0.7	0.20 \pm 0.034 >0.2	5
12 hrs in sea water <i>P</i>	63 \pm 3.0 >0.1	13 \pm 0.7 <0.001	2.2 \pm 0.05 >0.1	0.63 \pm 0.184 <0.05	0.26 \pm 0.142 >0.6	4
24 hrs in sea water <i>P</i>	63 \pm 4.1 >0.2	17 \pm 1.3 >0.5	2.1 \pm 0.38 >0.6	0.79 \pm 0.150 >0.05	0.14 \pm 0.017 >0.1	5
48 hrs in sea water <i>P</i>	62 \pm 3.3 >0.3	17 \pm 0.9 >0.4	3.7 \pm 1.78 >0.3	0.90 \pm 0.149 >0.1	0.58 \pm 0.200 >0.3	4
12 days in sea water <i>P</i>	59 \pm 1.2 >0.6	16 \pm 0.7 >0.05	2.4 \pm 0.25 >0.1	2.0 \pm 0.32 <0.05	0.22 \pm 0.026 >0.3	8
Control (Pond)	58 \pm 1.7	18 \pm 0.7	1.9 \pm 0.17	1.2 \pm 0.12	0.34 \pm 0.120	8

Table 3. Changes of the catalase activity in the tissues and blood of the smolts of *O. masou* in sea water (Exp. No. 2); June 2-3, '54

Time in sea water Tissues and blood	Catalase activity M \pm standard error of the mean			No. of fishes examined
	Liver	Kidney	Blood	
3 hrs <i>P</i>	53 \pm 7.1 >0.6	9.4 \pm 1.95 >0.1	15 \pm 3.0 >0.5	5
6 hrs <i>P</i>	53 \pm 3.8 >0.4	8.5 \pm 1.63 <0.05	16 \pm 2.9 >0.7	5
12 hrs <i>P</i>	46 \pm 3.1 >0.4	11 \pm 1.7 >0.3	15 \pm 0.7 >0.2	5
18 hrs <i>P</i>	41 \pm 3.4 >0.05	12 \pm 0.5 >0.3	16 \pm 1.6 >0.6	6
Control (Pond)	49 \pm 2.8	13 \pm 0.8	17 \pm 1.6	9

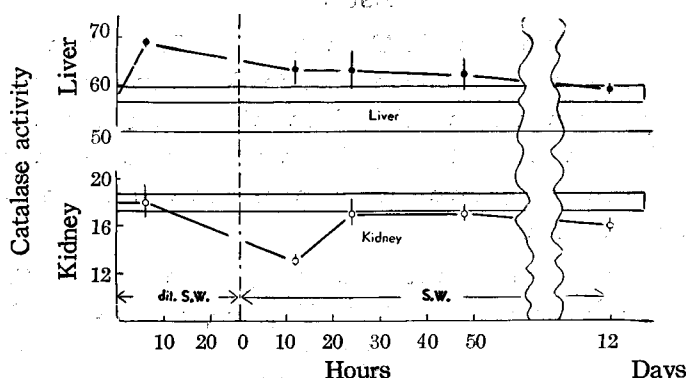


Fig. 1. Changes in the catalase activity of the liver (●) and kidney (○) in the smolts of *O. masou* in dilute sea water and in sea water (Exp. No. 1); each bar indicates the standard error of the mean in the experimental fishes and the height of each rectangle shows that in the respective control.

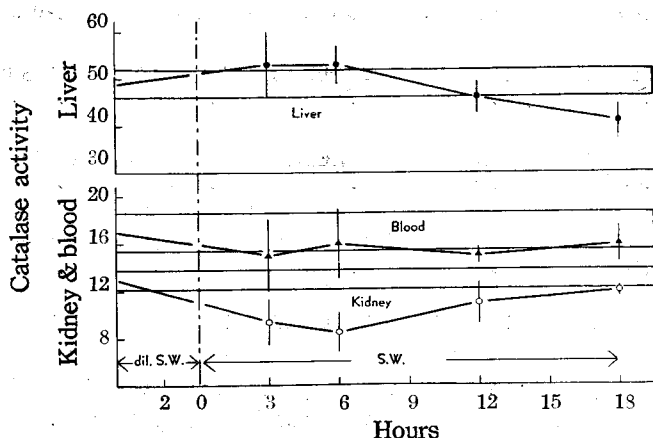


Fig. 2. Changes in the catalase activity of the liver (●), kidney (○) and blood (▲) in the smolts of *O. masou* in sea water (Exp. No. 2); each bar indicates the standard error of the mean in the experimental fishes and the height of each rectangle shows that in the respective control.

Kidney. In the dilute sea water, no significant changes were found in the catalase activity in both experiments. However, in sea water the catalase activity fell at about 6-12 hours and returned to the normal level after about 12-24 hours. The depression was highly significant ($P < 0.001$) in Exp. 1 and probably significant ($P < 0.05$) in Exp. 2.

Blood. The changes in the catalase activity of the blood were examined only in Exp. 2. No significant changes were found, at least, within 18 hours, though the individual

variations were large during the initial 6 hours in sea water.

Stomach. No significant changes of the catalase activity were found at 6 hours in the fishes in the dilute sea water. However, the enzymatic activity decreased at 12 hours in the fishes in sea water, and the depression was probably significant ($P < 0.05$). Even after 24 hours in sea water, the catalase activity appeared to retain the low level, though the fall in this period was probably non-significant ($P > 0.05$). After 48 hours in sea water the catalase activity appeared, at first, to approach the normal level. However, it increased conversely after 12 days in sea water, and the increase was probably significant ($P < 0.05$).

Gill and muscle. In tissues of both gill and muscle, no significant changes of the catalase activity were found either in the fishes kept in sea water or in those in dilute sea water.

DISCUSSION

It has been reported that Atlantic salmon smolts can survive direct transfer to sea water.^{1,7,8)} Similarly, the smolts of *O. masou* could be transferred from fresh-water to sea water directly. However, a method of acclimatization in the dilute sea water was adopted taking the natural migration of the fish into consideration, and as the time for that acclimatization 3.5 and 26 hours were selected in the present experiments.

As described in the previous papers,^{3,4)} the physiological significance of the catalase activity in the tissues and blood is not clear at present. However, it is noticeable that the kidney catalase activity depressed at about 6-12 hours and returned to the normal level after about 12-24 hours when the smolts were transferred from fresh-water to sea water; these times just agree with those of both the marked increase and return to the normal level in the body chloride content of the chum salmon fry in sea water.⁹⁾ Further, in this connection it is very suggestive that changes of the structure of the tubules, unusual increase of the secretion in them, and agglomerated appearance of melanin in the connective tissue cells have been observed in the kidney of goldfishes which were reared several days in dilute sea water.¹⁰⁾

No significant changes were found in the blood catalase activity. That may be ascribed to the evidence that this enzyme exists mainly within the blood cells being protected by their membranes.^{11,12)} However, as is clear judging from the values of the standard error of the mean, the standard deviations of the blood catalase activity are considerably large, at least, at 3-6 hours in sea water, though the changes were not significant. Similarly large fluctuations of the enzymatic activity could also be found in such tissues as those of liver and kidney in the initial several hours in sea water.

The depression of the stomach catalase activity may be associated with the fact that the fish drinks sea water. Contrary to expectation^{1,9,13)} no significant changes were found in tissues either of the gill or of muscle. It is not clear at present whether that is

actually the case or whether small changes but large relatively to the very low levels of these tissues may occur which could not be estimated by the present method.

SUMMARY

The smolts of *Oncorhynchus masou* were transferred from fresh-water to sea water after being acclimatized in dilute sea water. On these fishes, the changes of the catalase activity in the tissues and blood were examined at various time intervals.

1. The liver catalase activity showed a significant change at 6 hours after transference of the fishes to the dilute sea water. However, no significant changes were found in transferences to sea water.

2. In sea water, the kidney catalase activity of the experimental fishes decreased significantly at about 6-12 hours and returned to the normal level at about 12-24 hours.

3. Any significant changes of the blood catalase activity were not found. However, a large fluctuation of the enzymatic activity was observed at the initial 3-6 hours in sea water.

4. Significant depression of the stomach catalase activity was found at 12 hours in the experimental fishes in sea water.

5. In tissues of both the gill and muscle, no significant changes were found.

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