TRIMETHYLAMINE OXIDE IN FISH MUSCLE III. THE ORIGIN OF TRIMETHYLAMINE OXIDE IN MARINE FISH MUSCLE

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(Received March 13, 1959)

Trimethylamine oxide (TMO) has been found widely distributed in marine fish but more scanty or absent in fresh water fish. Several factors, such as diet, excretion mechanism of nitrogenous compounds and osmotic pressure of body fluids or surrounding medium, have been suggested to be responsible for the one-sided distribution of the base, but little evidence has been obtained on this matter¹⁾. Recently, OGILVIE and WARREN²⁾ have reported that TMO may originate in muscle tissue of killifish, *Fundulus heteroclitus*, as a result of two processes, one exogenous and one endogenous in origin.

In our previous works, the occurrence of TMO in two species of fresh water fishes, goldfish³⁾ and eels⁴⁾, was studied, and the base was confirmed to be entirely of exogenous origin. The similar feeding experiment was extended to marine fishes, using carp meat as a TMO-free basal diet. The present paper deals with the results on the globefish, *Fugu niphobles* JORDAN et SNYDER, the filefish, *Monacanthus cirrhifer* TEMMINCK et SCHLEGEL, and the jack mackerel, *Trachurus japonicus* TEMMINCK et SCHLEGEL.

Experimental

Globefish and filefish were kept in our laboratory in the tank of circulatory system, which was described in the previous paper³⁾, and jack mackerel in the tank of running sea water at the Misaki Marine Biological Station of the University of Tokyo. The temperature of sea water was not controlled in the experiments on globefish and jack mackerel, and ranged from 19° to 24°C in the former and from 24° to 26°C in the latter, while it was adjusted to 22°C in the experiment on filefish. The preparation of diet and the feeding were similarly carried out as in the previous works³⁾, and the fishes were kept in excellent condition and had a good appetite.

The bases, TMO and trimethylamine (TMA), were determined by the modified DYER method⁵⁾ and on some specimens also by the cis-aconitic anhydride method slightly modified by SASAKI et al.⁶⁾ Total nitrogen in trichloroacetic acid extracts of the ordinary muscle of jack mackerel was also determined by the micro KJELDAHL method. Analysis was done on the muscle of each individual except the red muscle

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of jack mackerel, which was obtained from 2 or 5 fish and pooled.

Results

1. *Globefish*.

The fish which were 7.8~9.7 cm in length and 15.5~29.9 g in weight, were obtained from the Kamakura Aquarium at where they had been kept on a diet of fish meat in running sea water. On arrival at the laboratory, the fish were divided into three groups. The first one was fed with the basal diet, the second one the basal diet containing 1% TMO and the last one was kept on fasting. Four fish were removed for analysis at intervals and the results are shown in Fig. 1. The average concentration of TMO in the muscle is 75 mg% at the beginning and the same level is maintained when the fish are on the TMO-free diet for a period of twenty-five days or on fasting for ten days. On the other hand, the level steadily rises up while the fish are on the diet containing TMO and the mean value is 98 mg% on the seventh days and 113 mg% on the fourteenth day. Thus, it is clearly shown that TMO in food is retained in the muscle and that the muscle TMO is maintained in a relatively constant level, even when the base is not supplied from the outside. No appreciable amount of TMA was recognized in all individuals and this is true for filefish and jack mackerel.

Fig. 1. Trimethylamine oxide in the muscle of globefish.

2. *Filefish*.

The fish were 7.3~9.5 cm in length and 14.7~30.5 g in weight. The fish, which had been kept on fasting for four weeks at the Misaki Marine Biological Station, were divided into two groups and the one was fed with the basal diet and the other continuously kept on fasting, both at the station for a further period of one week. Then, the fasting group was removed for analysis and the remainder was transferred to the laboratory in Tokyo. There the fish were again divided into two groups and the one was successively fed with the basal diet and the other the diet containing 1% TMO for ten days, which was followed again by the basal diet for twenty days. As shown in Fig. 2, similar results are obtained as those on globefish. The average concentration of TMO in the muscle is about 33 mg% in the fish which have been kept on fasting for four weeks, and this level is maintained by the fish kept on fasting for a further period of one week and also by those fed the TMO-free diet for a further period of seventeen days. The concentration, on the other hand, becomes four times

higher when fish are on the diet containing the base for ten days and it is maintained without any decrease during the successive feeding of the TMO-free diet for twenty days.

Table 1. Values obtained by the picrate and cis-aconitic anhydride methods on the TMO content (mg%) in the muscle of filefish.

A: Fish fed the basal diet for one week.

B: Fish of the twenty-seventh day in Fig. 2.

Fish No.	A		B	
	Picrate method	Cis-aconitic anhydride method	Picrate method	Cis-aconitic anhydride method
1	22.5	23.2	126.6	123.4
2	26.4	28.0	105.5	95.0
3	38.9	40.3	97.9	91.0
4	46.6	48.4

Fig. 2. Trimethylamine oxide in the muscle of filefish.

Some specimens were analyzed by both picrate and cis-aconitic anhydride methods, to ascertain the reliability of TMO estimation. The results are summarized in Table 1. The values obtained by two methods coincide each other fairly well.

3. *Jack mackerel.*

The fish, 10.5~12.7 cm in length and 17.1~37.0 g in weight, were preliminarily fed with the jack mackerel meat for one week in running sea water at the Misaki Marine Biological Station. Then the fish were divided into three groups as describ-

ed above. The two groups were kept the diets, as shown in Fig. 3 and the other on fasting for three weeks. The results, which are presented in Fig. 3, are considerably different from those of globefish and filefish. After the preliminary feeding of jack mackerel meat for a week, the average content of TMO is 217 mg% in the ordinary muscle and 147 mg% in the red muscle. In the both muscles of the fish fed with the TMO containing diet, there occurs no appreciable change of the TMO level, while it decreases to 170 mg% in the ordinary muscle and to 82 mg% in the red muscle when fish are on TMO-free diet for two weeks. On the other hand, the level of TMO in the ordinary muscle of fasting fish rises steadily up to 321 mg% in three weeks, whereas the effect of starvation is not appreciable in the red muscle. Total nitrogen in the

extracts of the ordinary muscles was also analyzed, as shown in Fig. 4. The curves show that the amount of extractive nitrogen is maintained in a relatively constant level throughout the feeding experiment.

Fig. 3. Trimethylamine oxide in the muscle of jack mackerel.

Fig. 4. The total nitrogen in the extracts of the ordinary muscle of jack mackerel.

Discussion

The metabolism of TMO in the muscle of three species of marine fishes, globe-fish, filefish and jack mackerel, is found significantly different from that in fresh water fishes. The most striking difference exists in the long maintenance of a relatively constant and fairly high level of TMO, even when the supply from the outside is cut off by feeding fish with the TMO-free diet or by keeping them in starvation. The presence of a system to maintain the attained level or to secure the lower limit level of the base may be one of reasons why the marine fishes generally contain a large amount of TMO in the muscle and may lead to the suggestion that the base exerts some physiological function in marine fishes.

The observed significant rise in TMO in the muscle of globe-fish and especially of filefish when they are on the diet containing TMO may suggest that some of the base in marine fish is exogenous origin. The accumulation is much larger than that of fresh water fish. On the other hand, it is demonstrated that the TMO level in the muscle of jack mackerel is not influenced by the ingestion of TMO and this may be partly due to the history of test animals.

In addition, another striking difference is seen between the two tetrodon fishes and the jack mackerel in the change of the muscle TMO during the starvation. The steady rise of TMO level in the fasting jack mackerel, the phenomenon also observed on killifish by OGILVIE and WARREN²⁾, is very interesting and may provide an evidence for the endogenous formation to TMO in these fishes. In this case, the percentage of TMO nitrogen to the total extractive nitrogen rises from 9.6 to 14.8% in three-week fasting, as the amount of the latter does not change during the period. The red muscle reveals the slightly different pattern of change in TMO content from the ordinary muscle.

TMA was not detected in all specimens, differing from the case of goldfish reported by the present authors and of the red muscle of frigate mackerel, *Auxis tapeinosoma* BLEEKER by KAWABATA⁷⁾.

Some individual variation of the TMO content was observed in the specimens analyzed at the same time as shown in figures, perhaps due to the unevenness in size of test animals, but it would exert little or no influence on conclusions in the present study.

The picrate method, a most common determination method of TMA in fish muscle, has been known to respond to other amines than TMA, but the values obtained by this method agreed well with those by the cis-aconitic anhydride method, which is considered to be specific to tertiary amines.

Summary

The most striking difference in the metabolism of TMO between fresh and marine fishes was found in the long maintenance of a relatively constant and fairly high level of the base by the latter, even when the supply from the outside was cut off. Some of TMO in the muscle of marine fish was also confirmed to be of the exogenous and of the endogenous origins.

Acknowledgement

The authors wish to express their thanks to Prof. Y. SUMIKI for his kind advice, and are indebted to Prof. Y. OSHIMA, Messrs. N. ISHIWATA and F. YASUDA in obtaining fishes.

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