Trimethylamine Oxide in Fish Muscle

I. The Origin of Trimethylamine Oxide in Goldfish Muscle

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The distribution and post-mortem change of trimethylamine oxide (TMO) in aquatic animals have been extensively studied, but its metabolism in fish is still obscure. On the origin of TMO, several hypotheses have been advanced; BALDWIN¹⁾ regarded it as a detoxification end-product of ammonia and KUTCHER and ACKERMANN²⁾ as a methylation and oxidation product of lower cleavage compounds in protein degradation. A part of TMO was also supposed by DYER³⁾ to be derived from ingested choline or allied compounds. Against these assumptions on the side of the endogenous metabolic origin, REAY⁴⁾ suggested that TMO originates from the food and is simply retained in the muscle. A sole experimental evidence on this problem was given by BENOIT and NORRIS⁵⁾, who clearly demonstrated by a feeding test that TMO in muscle of young salmon is of an exogenous origin.

The object of this work has been to obtain some information about the metabolism of TMO in fish and the present paper deals with the results on goldfish, *Carassius auratus*.

Experimental

The fish were kept in a tank (A) connected by a siphon (B) with a filter tank (C), as shown in Fig. 1, which has been devised and developed by the present authors⁶⁾. In this system, the filtered water was circulated (20~30 l/hr.) from C back to A by an air-lift pump (D) simultaneously with aeration. The water in A was accordingly introduced by B to the bottom chamber (E) of C and forced to pass upwards through a sand filter (F) provided above E. The water flow of this type extended the durability of the filter, as the greater part of feces and food particles from A settled on the bottom of C in innocent form and consequently the sand layer was hardly stopped up with debris. The filter, moreover, served as an efficient biological filter and made it possible to keep both marine and fresh water animals in good condition, such as goldfish, eels, globefish and baby clams. A set consisting of a tank of 40 litres capacity with a filter tank of 10 litres was chiefly used. The maximum weight of goldfish which could be held was about 20 g. per 1 l of the water. Goldfish, the

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body weight of which ranged from 8 to 16 g. were used as test animal. The water temperature was not controlled and ranged from 20° to 24°C.

The basal diet was prepared by combining two parts of minced and boiled carp meat with one part of 30% gelatin solution as a binder and the mixture was stored in a refrigerator. In every preparation, the basal diet was checked up on the absence of TMO and trimethylamine (TMA). The diet containing TMO was obtained by in-

corporating recrystallized $\text{TMO} \cdot 2\text{H}_2\text{O}$ to the basal diet before cooling. The cake was cut into small pieces, which were given in a slight excess to fish three to four times every day, unless otherwise stated.

The fish removed from tanks for analysis were immediately killed and the muscle was carefully collected. Usually, five grams of the pooled muscle from three to five individuals were homogenized and extracted with trichloroacetic acid solution and TMO was determined together with TMA on the extracts

according to the modified DYER method by the present authors⁷. The fish and the muscle were ice-cooled, otherwise TMA formed during the treatment in the muscle containing TMO.

Fig. 1. Diagram showing the main part of fish and filter tanks.

Results

1. Fish fed a diet containing 5% TMO

Thirty-five goldfish preliminarily fed the basal diet for a week were divided into two groups. The one was fed a diet containing 5% TMO and the other the basal diet, both for one hour *ad libitum*. The fish took diet at the rate of about 8% of body weight. After feeding, they were placed in other vessels and three individuals were removed for analysis at intervals. Results are shown in Fig. 2. The muscle is TMO- and TMA-free while the fish are on a TMO-free diet, but already two hours after the ingestion of TMO it contains the base, the concentration of which is highest in specimens of the sixth hour and becomes lower thereafter. In

Fig. 2. TMO and TMA in the muscle of goldfish fed a diet containing 5% TMO. A; The TMO-free diet. B; The diet containing 5% TMO. C; Fasting.

accordance with the decrease in TMO concentration, TMA appears in the muscle.

2. Fish continuously fed a diet containing 1% TMO

After being kept on the basal diet for five days, the fish were fed a diet containing 1% TMO for a further period of six days. A control group was also provided as mentioned above. The fish took diet at the rate of 10% of body weight per day for the total period. Judging from the curve in Fig. 2, specimens were removed four hours after the beginning of feeding of the day. As shown in Fig. 3, the muscle of fish receiving a TMO-free diet is again confirmed to be TMO- and TMA-free. While the fish are on a diet containing 1% TMO, the relatively constant level of TMO and TMA is maintained, but the bases rapidly disappear in the following fasting period of two days. In another experiment, in which the feeding of a diet containing 1% TMO was followed by that of the basal diet instead of fasting, the rapid disappearance of the bases was also recognized.

Fig. 3. TMO and TMA in the muscle of gold fish continuously fed a diet containing 1% TMO.

3. The formation of TMA from choline

The fish preliminarily fed the basal diet for a week were divided into four groups and placed in separate tanks. The three groups were fed the basal diet containing respectively each 5% TMO, betaine and choline chloride for one hour *ad libitum* and the last one the basal diet similarly. The pooled muscle from three individuals was analysed for TMO and TMA four and twenty-four hours later. The results are summarized in Table 1. Figures in the

Table 1. TMO and TMA in the muscle of goldfish fed TMO, choline chloride or betaine.

Supplements	None	TMO		Choline chloride		Betaine	
Dose per individual mg	0	21		25		28	
Hours after ingestion	24	4	24	4	24	4	24
TMA mg%	0	0.55	1.10	0	2.28	0	0
TMO mg%	0	4.16	2.64	0	0	0	0

table show that the pattern of TMO and TMA formation in the muscle of fish fed TMO is similar to that seen in Fig. 2, whereas the level of the former is slightly low and the appearance of the latter is earlier. None of both bases originates from ingested betaine and only TMA from ingested choline twenty-four hours later. It may be noted here that goldfish were very sensitive to TMA-HCl in the food, and the authors failed to feed fish a diet containing even 0.5% TMA-HCl.

Discussion

BENOIT and NORRIS confirmed that the muscle of young salmon fed the liver does not contain TMO, whereas on the diet consisting of 50% liver and 50% pecten or 50% liver and 50% salmon meal it accumulates a considerable amount of TMO, and concluded that the oxide present in pecten and salmon meal is retained in the muscle. Strictly speaking, this conclusion may be a hasty one, because their work did not exclude the possibility of the TMO formation from some other constituent present in pecten or salmon meal, but not in the liver, although this is not likely to happen. And in such an experiment, it is desirable to use the same basal diet with or without the addition of a substance in question. In this experiment, therefore, the diet was strictly defined.

The result in this work was analogous to that of BENOIT and NORRIS, and it is confirmed that some of ingested TMO appears in the goldfish muscle, which is TMO-free while the fish are on a TMO-free diet. It is, moreover, newly found that the metabolism of TMO in the goldfish muscle is very rapid and TMA forms several hours later. The rapid rise and fall of TMO in the muscle, as shown in Figs. 2 and 3, may suggest that there might occur a considerably large fluctuation of the TMO content even in a day, and that a relatively constant level of the bases seen in Fig. 3 would be due to the fixed sampling at a definite time. In spite of the ingestion of a large dose, the level of muscle TMO was rather low, and the authors suppose that there is a system to control the upper level of it, as DYER⁹⁾ did. The rapid disappearance and low level of muscle TMO may suggest that this base is physiologically of little importance to goldfish.

The late appearance of TMA in accordance with the decrease of TMO may indicate the metabolic fate of the oxide present in the muscle and some of TMO is supposed to be reduced to TMA before being excreted. The small quantity of TMA, which was determined in many fresh water fish by ANDERSON and FELLERS⁸⁾, might be of this nature. The reduction of the oxide by muscle tissue was also observed, as mentioned above. In addition, it was also recognized when the homogenized muscle of TMO-free goldfish was incubated with TMO.

DYER and WOOD⁹⁾ assumed that some of TMO may originate from choline or other trimethylammonium bases by analogy from their results on bacteria, but this is found not applicable to goldfish, because no appreciable quantity of TMO formed in the muscle, whereas TMA was detected twenty-four hours after the ingestion of choline (Table 1).

Summary

While goldfish were on a TMO-free diet consisting of carp meat and gelatin the muscle was TMO- and TMA-free, but it retained a small amount of the base from

the food for a short period. In the muscle of fish fed the oxide, TMA also appeared later, and this may suggest together with other evidences that some of TMO is reduced to TMA before being excreted. Only TMA formed in the muscle from ingested choline and none of both bases from betaine.

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