Biosynthesis and Turnover of Trimethylamine Oxide in the Teleost Cod, *Gadus morhua*  

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Liver and kidney of the teleost cod, *Gadus morhua*, contained oxygen- and NADPH-dependent monoxygenase which mediated the oxidation of trimethylamine (TMA) to trimethylamine oxide (TMAO). The microsomal monoxygenase of liver was partially characterized. The rate of enzymic TMA oxidation had its maximum at pH 8.2 and at 24 °C. The enzyme displayed Michaelis-Menten kinetics; the apparent $K_m$ value for TMA being 11 μM. All N,N-dimethyl-n-alkylamines with up to 8 carbons in the side chain were oxidized at almost the same rate. The oxidation of TMA was stimulated by octylamine and tyramine, and it was inhibited by the SH reagents $N$-ethylmaleimide and $p$-chloromercurybenzoate. Lack of inhibition by carbon monoxide and stimulation by FAD indicated that the enzyme was a cytochrome P-450-independent flavoprotein.

[14C]TMA injected intraperitoneally into cod was oxidized to [14C]TMAO. After its compartmentation the [14C]TMAO produced was excreted at a rate of approximately 0.5%/day in cod fed a TMAO-rich diet. It was inferred that high stability of body TMAO and a surplus of TMAO in their natural diet can explain the lack of endogenous TMAO synthesis encountered in many TMAO-containing marine fish.

All marine fish accumulate trimethylamine oxide in their tissues, particularly in the muscle. The content of TMAO varies within wide limits depending on factors such as species, age, season, and geographic localization. But generally, teleosts contain from 20 to 70 pmol TMAO/g, wet weight of muscle. Whereas elasmobranchs contain up to 140 pmol/g (Shewan, 1951; Groninger, 1959). Biological formation of TMAO involves oxidation of trimethylammonium catalyzed by NADPH-dependent monoxygenases:

\[(\text{CH}_3)_3\text{N} + \text{NADPH} + \text{H}^+ + \text{O}_2 \rightarrow (\text{CH}_3)_3\text{NO} + \text{NADP}^+ + \text{H}_2\text{O}\]

A TMA-oxidizing enzyme of pig liver microsomes (dimethylaniline monooxygenase EC 1.14.13.8) has been purified to homogeneity (Ziegler and Poulsen, 1978), and corresponding enzymes of a marine elasmobranch (Goldstein and Dewitt-Harly, 1973) and a marine herbivorous copepod (Strøm, 1979) have been partially characterized.

It remains puzzling that despite their high content of TMAO, most marine fish examined have low or no detectable TMA monooxygenase activity (Baker et al., 1963). A possible explanation is that TMAO found in marine fish mainly originates from their natural food; i.e. TMAO is produced by the animals which constitute the lower levels of marine food chains, such as the herbivorous copepods (Groninger, 1959; Strøm, 1979). Feeding experiments with young salmon have revealed that they depend on dietary sources of TMAO (Benoit and Norris, 1946). An additional explanation postulated for the erratic occurrence of endogenous TMAO synthesis in marine fish is a high stability of TMAO in their muscle (Forster, 1972). However, more recent measurements of the disappearance of [14C]TMAO from plasma of four elasmobranch species have indicated a high turnover rate of TMAO, ranging from 4 to 14% of total body TMAO/day (Goldstein and Palatt, 1974). A herbivorous copepod examined by us does not excrete significant amounts of TMAO under laboratory conditions (Strøm, 1979).

There is little detailed information about the oxidation of TMA and the stability of TMAO in marine teleosts. In this investigation we have partially characterized the TMA-oxidizing enzyme (monooxygenase) of cod liver microsomes and examined the in vivo turnover rate of TMAO in the teleost cod, *Gadus morhua*. For comparison we have determined the distribution of TMA monooxygenase activity in different tissues of two teleost flatfish, *Limanda limanda* and *Pleuronectes platessa*. We report that [14C]TMAO which has been compartmentalized in the body of cod disappears at a rate of approximately 0.5%/day under laboratory conditions.

**MATERIALS AND METHODS**

**Fish**—Cod, *G. morhua*, and flatfish, *L. limanda* and *P. platessa*, were caught in the vicinity of Tromsø. The fish used for studies of the monooxygenase weighed from 1.0 to 2.5 kg and from 0.3 to 0.5 kg, respectively. They were either killed immediately after capture or kept in aquaria with running seawater until used. The fish used for in vivo studies weighed from 0.3 to 0.6 kg. Unless otherwise stated, they were kept in the aquaria for 1 week without feeding prior to the injection of [14C]-labeled amines.

**Special Reagents**—[14C]dimethylamine HCl (specific activity 46.8 mCi/mmol), and [14C]TMA HCl (specific activity 3.1 mCi/mmol) were purchased from New England Nuclear. To remove nonvolatile contaminants the amines were purified by the microdiffusion technique of Conway and Byrne (1933) as described previously (Strøm, 1979). [Methyl-14C]choline CI (specific activity 52 mCi/mmol) was obtained from The Radiochemical Centre (Amersham). The reagent was alkalinized with NaOH and volatile components were removed on a rotary evaporator. The purity of all the [14C]-labeled amines was checked by ion exchange chromatography (see below). [14C]Choline (5.38 × 10⁻⁶ ± 3% dpm/g) was purchased from Packard, Downers Grove, Ill. The following chemicals were obtained from Sigma: TMA HCl, dimethylamine HCl, aniline, N,N-dimethylamine, n-octylamine, tyramine HCl, N-ethylmaleimide, p-chloromercurybenzoic acid, NADP, NADPH, NADH, FAD, glucose-6-phosphate, glucose-6-phosphate dehydrogenase (Type XV, from bakers' yeast), and glucose oxidase (Type V, from *Aspergillus niger*). Dimethylthylamine, N,N-
dimethylbutylamine, N,N-dimethyloctylamine, N,N-dimethyldecylamine, and N,N-dimethycyclohexylamine were obtained from K and K Laboratories. Histamine dihydrochloride, butyamine, and propylamine were obtained from Koch-Light Laboratories Ltd. Carbon monoxide was prepared by treating formic acid with sulfuric acid (Vogl, 1956).

Homogenization of Tissues—Samples of tissue were homogenized in 5 parts (w/v) of ice-cold 0.25 M sucrose solution (pH 7.8). Intestine, stomach, and pyloric caecum were homogenized by using an Ultra Turrax TP 18/10, whereas for gall, kidney, liver, muscle, and spleen a glass-teflon homogenizer was used. The homogenates were filtered through common cheesecloth, and the filtrates were used to detect monoxygenase activity.

Fractional Centrifugation—Liver of cod was homogenized in 10 parts (w/v) of ice-cold 0.25 M sucrose by using a glass-teflon homogenizer. Fractional centrifugation was performed as described by Reid (1967), and the procedure is summarized in the legend to Fig. 1. Low speed centrifugation was done in a Sorvall RC2-B centrifuge with a Sorvall SS-34 rotor. High speed centrifugation was done in a Beckman L5-65 ultracentrifuge with a Beckman Type Ti-60 rotor. All steps were carried out at 0 to 4°C.

Radiochemical Monoxygenase Assay—The monooxygenase was assayed routinely by its ability to form [3H]TMAO. The standard reaction mixture consisted of 150 nmol of [3H]TMA HCl (specific activity 0.5 mCi/mmol), 2.5 nmol of P450, NADPH-generating system (600 nmol of glucose-6-phosphate, 350 nmol of NADP, and 0.5 units of glucose-6-phosphate dehydrogenase), 6 μmol of MgCl2, 40 μmol of potassium pyrophosphate buffer (pH 8.2), enzyme (homogenate from 30 g of wet tissue or 0.5 to 1.0 mg protein of centrifugal fractions of liver), and water to 1.0 ml. The reaction was started with the addition of the amine and stopped with the addition of 2 ml of ethanol containing 50 μmol TMA. [3H]TMAO formed was determined by liquid scintillation counting (see below) after the removal of the rest of ['3H]TMA on a rotary evaporator under alkaline conditions (Srom, 1980). Unless otherwise stated, the unit used was nmol of TMAO formed/h at 24°C, and the standard reaction time was 20 min.

A test of monoxygenase activity in the absence of O2 was performed by adding 100 units of glucose oxidase and 50 μmol of glucose to the standard reaction mixture and flushing the reaction vessel with N2.

Spectrophotometric Monoxygenase Assay—This was performed by determining the oxidation of NADPH at 340 nm in a Beckman Model 25 spectrophotometer. The reaction conditions were as described in the assay above, except that [3H]TMA was substituted by 300 nmol of stated unlabeled amine (see text and Table I), and 600 nmol of NADPH was used instead of the NADPH-generating system. Enzyme was 1.6 mg of microsomal protein and total reaction volume was increased to 2.0 ml. The reaction was stopped by the removal of enzyme by membrane filtration (Millipore, pore size 0.45 μm). Unit used was nmol of NADPH oxidized per mg of protein per h at 24°C.

Conversion of ['3C]-Labeled Amines in Live Cod—A number of cod were injected intraperitoneally with 1.1 μCi of [14C]TMA HCl (specific activity 3.1 μCi/mmol), 2.1 μCi of [14C]trimethylamine HCl (specific activity 46.8 μCi/mmol), or 2.2 μCi of [methyle-14C]choline HCl (specific activity 22.4 μCi/mmol) in 1.0 ml of water. When fed, the fish were offered food (i.e. muscle of capelin, Malottus villousus) for the first time 5 days after the injection, and then each 2nd day. The fish were kept in aquaria with running seawater. They were killed after different times.

To extract [14C]-labeled amines the whole fish was ground by passing it twice through a meat grinder and then homogenized by using an Ultra Turrax TP 18/10. One-third of each homogenate was added to an equal amount (w/v) of 0.6 M perchloric acid, homogenized and centrifuged at 6500 x g for 10 min (4°C). The pellets were re-extracted twice with the same amount of perchloric acid and the supernatants combined. In some instances the viscera and the rest of the body were extracted separately.

[14C]-labeled amines (i.e. TMAO, TMA, dimethylamine, and choline) in perchloric acid extracts were determined by liquid scintillation counting after separation by ion exchange chromatography (see below). The pH of the extracts was adjusted to 6.5 with 6 M KOH and precipitated potassium perchlorate was removed by filtration after 2 h at 0°C. The extracts were acidified with dilute HCl and concentrated 10-fold on a rotary evaporator (30°C) and applied to the ion exchange column.

[3H]TMAO in perchloric acid extracts of fish injected with [3H]-TMAO was determined routinely by adding 1 part of 5 M KOH and 1 part of 0.1 M TMA to 2 parts of extract. Then volatile components were removed on a rotary evaporator (30°C), and the residue was dissolved in dilute HCl. Rest radioactivity was determined by liquid scintillation counting and taken as an expression for [3H]TMAO.

Ion Exchange Chromatography—To identify and separate radioactive amines a column (0.9 x 60 cm) of Zerolit 226, 100 to 200 mesh (Hopkin & Williams, Chadwell Heath, Essex, England), eluted with citrate-phosphate buffer (pH 5.0) was used (Blau, 1961). Body TMAO, TMA, dimethylamine, and choline were retarded and separated on the column. The identity of TMAO was also routinely checked by chromatography of samples treated with TiCl4 to reduce TMAO to TMAO.

Other Methods—TMAO (unlabeled) was determined by the microdiffusion technique of Conway and Byrne (1933). Protein was determined by the method of Lowry et al. (1951) using bovine serum albumin (Sigma) as standard. Succinate dehydrogenase and glucose-6-phosphatase were assayed according to Morre (1971) and Nordlie and Aron (1966), respectively. Liquid scintillation counting was performed in Hydrolux (Lumac Systems AG) using a Packard Model 4245 Tri-Carb liquid scintillation spectrometer. Counts per min were converted to disintegrations per min by use of [14C]toluene as an internal standard.

RESULTS

Localization of Monoxygenase in Cod—Homogenates of cod liver and kidney mediated the oxidation of TMA to TMAO in the presence of NADPH. The identity of TMAO produced was confirmed by ion exchange chromatography (Blau, 1961). This TMAO monoxygenase activity could not be detected in homogenates of other tissues of cod.

The livers of the cod displayed large variations in monoxygenase activity. In most individuals the activity was between 2 and 9 μmol of TMAO produced/g wet weight of liver/h in the in vitro assay at 24°C; the highest activity recorded was 23 μmol/g. The monoxygenase activity of kidney was consistently lower, i.e. about 1 μmol TMAO produced/g.

The distribution of monoxygenase activity in the centrifugal fractions of cod liver is depicted in Fig. 1. The highest specific activity, normally 100 to 300 nmol TMAO produced/mg of protein/h, was found in the fraction sedimenting between 3.3 x 104 and 3.3 x 105 g, with the highest activity recorded was 23 μmol/g. The monoxygenase activity of kidney was consistently lower, i.e. about 1 μmol TMAO produced/g.

The distribution of monoxygenase activity in the centrifugal fractions of cod liver was depicted in Fig. 1. The highest specific activity, normally 100 to 300 nmol TMAO produced/mg of protein/h, was found in the L fraction sedimenting between 3.3 x 104 and 3.3 x 105 g, with the highest activity recorded was 23 μmol/g. The monoxygenase activity of kidney was consistently lower, i.e. about 1 μmol TMAO produced/g.

FIG. 1. Distribution of monoxygenase activity in centrifugal fractions of homogenates from cod liver. Relative specific activity is percentage of total monoxygenase activity per percentage of total protein. The fractions N, M, L, and P are the materials sedimenting from 0 to 104, 104 to 3.3 x 104, 3.3 x 104 to 3.3 x 105, and 3.3 x 105 to 1.4 x 106 g, with the highest activity recorded was 23 μmol/g. The monoxygenase activity of kidney was consistently lower, i.e. about 1 μmol TMAO produced/g.
tained microsomes. Succinate dehydrogenase had its highest specific activity in the conventional mitochondrial (M) fraction, but variable amounts of this activity were also found in the L and the P fractions.

In other animals somewhat different intracellular distributions of monoxygenase activity have been recorded. The microsomal FAD-containing monoxygenase (dimethyline monoxygenase EC 1.14.13.8) of pig liver has been reported to concentrate more in the slower sedimenting microsomal fraction than in the faster sedimenting microsomal fraction (Ziegler and Pettit, 1966). The TMA monoxygenase of the marine copepod, Calanus finmarchicus, was found in the cytosol fraction (Strøm, 1980).

To investigate the properties of the monoxygenase of cod, both the microsome-containing fractions (i.e. the L and P fractions) were used. Except for the specific activity no differences were recorded between these fractions. The fractions were stored in 0.25 M sucrose solution (pH 7.8) at −80 °C. The initial freezing caused up to 20% loss in the enzymic activity, after which the preparations could be stored for several weeks without any further decrease in activity. We have had no success in solubilizing the monoxygenase with detergents; the butylamine (X-45, X-102, and N-101; Sigma) detergents destroyed the enzyme activity.

**Characteristics of Liver Monoxygenase**—The monoxygenase oxidized TMA maximally at pH 8.2 and at 24 °C. At these assay conditions the formation of TMAO was linear with time for 20 min. The oxidation of TMA was stimulated 2.5-fold with 10 μM FAD, and including CO (CO:O₂ ratio, 1:1) in the reaction vessel had no effect. These latter findings indicated that the enzyme was a cytochrome P-450-independent flavoprotein. N-ethylmaleimide (1 mM) and p-chloromercuribenzoate (0.2 mM) completely inhibited the oxidation of TMA. NADPH and NADP partially protected the enzyme against these —SH reagents.

The enzyme displayed Michaelis-Menten kinetics, and the apparent Kₘ value for TMA was 11 μM in the presence of 270 μM O₂ (concentration in buffer equilibrium with air at 24 °C) and the NADPH-generating system (Fig. 2). The apparent Kₘ value for NADPH was 160 μM in the presence of 270 μM O₂ and 150 μM TMA.

Oxidation of tertiary amines by the mammalian FAD-containing monoxygenase (EC 1.14.13.8) is stimulated by lipophilic primary amines (Ziegler and Mitchell, 1972). Therefore we have tested the influence of several primary amines on the enzymic oxidation of TMA. Pronounced effects were found for octylamine and tyramine, which inhibited TMA oxidation at low substrate concentrations and stimulated at high concentrations (Fig. 2). The monoxygenase displayed Michaelis-Menten kinetics in the presence of these primary amines, and both the Vₘₐₓ and the apparent Kₘ value for TMA were increased. The kinetics indicated that the monoxygenase did not have interacting active sites. The apparent mixed competitive and noncompetitive effects (cf. Ferdinand, 1976) of the primary amines can possibly be accounted for by their displacement of endogenous inhibitors; this is the case for the corresponding microsomal enzyme of pig liver (Ziegler and Poulsen, 1978). The following primary amines had no significant effect on the oxidation of TMA: propylamine (1 mM), butylamine (1 mM), histamine (1 mM), and aniline (5 mM).

**Substrate Specificity and Stoichiometry**—The preference of the monoxygenase for TMA as compared to larger molecular weight tertiary amines was assessed by determining the decrease in the oxidation of [¹⁴C]TMA (starting concentration, 150 μM) in the presence of an equimolar amount of an unlabeled tertiary amine (Strøm, 1980). In control experiments addition of unlabeled TMA resulted in a 50% decrease in the formation of [¹⁴C]TMAO, as would be expected. In the presence of dimethylethylamine and dimethylbutylamine, approximately 40% inhibition was observed, whereas dimethylcyclohexylamine and dimethyldodecylamine hindered the oxidation completely. Dimethylcyclohexylamine and dimethylalanine inhibited oxidation of [¹⁴C]TMA approximately 10 and 70%, respectively. The same pattern of inhibition was observed for both the liver microsomes and the kidney homogenates. These results indicated that the monoxygenase of cod had a higher affinity for some of the larger molecular weight tertiary amines than for TMA.

The liver enzyme displayed the typical stoichiometry of a monoxygenase; i.e. the consumption of NADPH equaled the formation of TMAO. In Table I are listed the relative NADPH consumption with various amines as cosubstrates, showing an almost equal oxidation rate of all N,N-dimethyl-n-alkylamines with up to 8 carbons in the side chain and of dimethylamine. Dimethyldodecylamine was oxidized at a significantly lower rate, and dimethylamine and dimethylcyclohexylamine were poor substrates for the enzyme (Table I). No oxidation of TMA could be detected when either O₂ was removed or NAD (350 μM) rather than NADPH was used in the reaction mixture.

**Turnover of TMAO in Live Cod**—After intraperitoneal injection of the precursor [¹⁴C]TMA into a number of cod, the total amounts of [¹⁴C]TMAO retained in their bodies were measured at various times. This procedure ensured that labeled TMAO was produced by the proper enzyme(s) and

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**Table 1**

| Amine                        | Relative NADPH consumption (%) |
|------------------------------|--------------------------------|
| Trimethylamine               | 100 (4)                        |
| Dimethylethylamine           | 103 ± 9 (4)                    |
| Dimethylbutylamine           | 78 ± 14 (3)                    |
| Dimethylcyclohexylamine      | 97 ± 15 (4)                    |
| Dimethyldodecylamine         | 16 ± 9 (4)                     |
| Dimethylcyclohexylamine      | 40 ± 3 (2)                     |
| Dimethylbenzylamine          | 74 ± 5 (2)                     |
| Dimethylamine                | 6 ± 3 (2)                      |

* + S.D.
followed by a normal compartmenatation of the labeled TMAO. Separate experiments revealed that injected TMA was rapidly converted. Less than 10% of injected TMA remained as such in the fish 2 days after the injections; the rest was either oxidized to TMAO or excreted. One week after the injection, virtually no [14C]TMAO remained in the fish, and only 5% of the [14C]TMAO injected was found in the fish. A typical example of the time course of the [14C]TMAO retention observed during the spring and summer season is shown in Fig. 3A. A rapid decrease in retained [14C]TMAO during the 1st week was followed by a period of very slow disappearance of [14C]TMAO. In the period from 8 to 75 days after the injection, the remaining [14C]TMAO decreased, on average, from 55 to 40% of the amount of [14C]TMAO injected. This corresponded to an excretion rate of about 0.5%/day.

The initial excretion of [14C]-labeled materials was not significantly influenced by whether the fish were fed or starved prior to the injection of [14C]TMA, but displayed seasonal changes. In all experiments started in the autumn (September) and in the winter (January to February), an average of 80% or more of injected [14C]TMA was retained as [14C]TMAO 2 to 3 weeks after the injection, whereas an average of only 60% or less was retained when the injections were performed in spring (May to June, Fig. 3). In the actual periods of autumn, winter, and spring, the seawater temperature was 8, 2, and 5 °C, respectively.

During the experiments described in Fig. 3, the cod were fed muscle of capelin (M. tholus) containing about 60 pmol of unlabeled TMAO/g, wet weight. The cod ate little during the first weeks, but by the end of the experimental period they had increased their weight by about 50%. The total amount of unlabeled TMAO per cod displayed small changes during the experimental period. The finding that the relative increase in unlabeled TMAO content is lower than the relative weight increase (Fig. 3B) can be attributed to the fact that a large portion of the weight gain was caused by growth of the liver which has a low TMAO content. (TMAO content in cod muscle is approximately 40 μmol/g, wet weight.) [14C]TMAO injected into cods was not converted into detectable amounts of perchoric acid-insoluble materials.

Injection of Choline and Dimethylamine—Choline has been claimed to be a possible precursor of TMAO in some marine animals (Bilinski, 1969, 1962; Goldstein and Funkhouser, 1972). When [methyl-14C]choline was injected intraperitoneally into cod, no labeled TMAO was found in the cod after 72 h. Dimethylamine injected was excreted within a week.

Comparative Studies—In the elasmobranch Squalus acantthias the lack of TMA monooxygenase activity in the liver coincides with the lack of [14C]TMAO oxidation in vivo (Goldstein et al., 1967). However, it was noted by Goldstein et al. (1967) that a teleost flatfish, Platichthys stellatus, oxidizes [14C]TMAO in vivo (Bilinski, 1964), but it lacks TMA monooxygenase activity in the liver (Baker et al., 1963). For comparison, we have determined the distribution of monooxygenase activity in the tissues of two species of teleost flatfish, L. limanda and P. pleatessa. These species had monooxygenase activity in the kidney, but not in the liver and other tissues; the highest activity recorded was 4 μmol TMAO produced/g, wet weight of kidney/h at 24 °C. The former fish was found to oxidize [14C]TMA injected intraperitoneally in vivo; this aspect was not investigated for the latter fish. Our data indicated that in some marine fish the TMAO synthesis takes place solely in the kidney, and it cannot be concluded that a fish has no endogenous TMAO synthesis simply because the liver is devoid of the monooxygenase.

**DISCUSSION**

The present data show that live cod synthesize TMAO by oxidation of TMA and that the monooxygenase activity of their liver is of the same order of magnitude as the activities reported for other TMAO-producing fish (Baker et al., 1963; Goldstein and Dewitt-Harley, 1973). Furthermore, in cod monooxygenase activity is also present in the kidney, indicating a more active endogenous TMAO synthesis in cod than in most marine fish. It is therefore noteworthy that the TMAO pool of cod appears to be very stable, (i.e. approximately 0.5% of [14C]TMAO formed in cod disappears per day under laboratory conditions, Fig. 3). We have previously reported that a marine copepod, Calanus finnarchicus, (containing about 140 μmol TMAO/g, wet weight) does not excrete significant amounts of TMAO (Strøm, 1979). Thus, a very slow turnover of body TMAO may be a general feature of marine animals not exposed to changes in salinity.

This concept of high TMAO stability is in conflict with the high TMAO excretion (i.e. 4 to 14% of body TMAO/day) reported for four species of marine elasmobranchs (Goldstein and Palatt, 1974). However, the latter estimates were based on measurements of [14C]TMAO disappearance from the plasma over an experimental period of 6 days after an intravenous injection. We have shown that it can take a week for [14C]TMAO to stabilize in the body of cod (Fig. 3). During this period of compartmentation of [14C]TMAO, the excretion is higher than the value of 0.5%/day recorded the following weeks. Our data are supported by the previous findings that in an elasmobranch, S. acanthias, [14C]TMAO spreads slowly from the plasma into the muscle, and that its plasma level of TMAO remains practically constant for 40 days during starvation (Goldstein et al., 1967; Forster, 1972). Furthermore, the urinary TMAO content of a teleost, Lophius americanus, displays a drastic decrease after a few hours of starvation.

**Fig. 3.** Determination of the stability of TMAO in cod fed a TMAO-rich diet. A, total [14C]TMAO content in cod after intraperitoneal injection of [14C]TMA (1.1 μCi, specific activity 3.1 nCi/μmol) expressed in percentage of total radioactivity injected (○). B, changes in total unlabeled TMAO (■) and wet weight (□) of cod expressed in percentage of values at start of experiment. Radioactive and unlabeled TMAO were determined in perchloric acid extracts of whole fish. Unlabeled TMAO content at start was calculated from the weight of fish at start and the average TMAO content (per g, wet weight) of cod caught at the same time. The fish were kept in aquaria with running seawater. The temperature was 5 °C at start and 9 °C at end of experimental period which lasted from May to August. Starting the 5th day the cod were offered food (i.e. muscle of capelin containing approx. 60 pmol unlabeled TMAO/g, wet weight) each 2nd day. Each value represents the average value of 4 or 5 cod; vertical bars represent standard deviations.
(Forster, 1972). Three other species of marine teleosts have a fairly stable TMAO content in their muscle when starved or fed a TMAO-free diet (Okaichi et al., 1959), as does cod (results not shown). It therefore seems likely that the TMAO found normally in excreta of marine fish is excess TMAO from the diet and endogenous synthesis, and that this TMAO does not equilibrate with the TMAO pool in the body. Wood (1956) has shown that the urinary TMAO of marine teleosts accounts for only a small part of total N excreted; most excess N is excreted as NH3 by the gills.

Biological N-oxidation of tertiary amines is normally catalyzed by cytochrome P-450-independent flavoproteins (Gorrod, 1978; Ziegler and Poulsen, 1978). The cod liver monooxygenase shows great resemblance with the mammalian microsomal FAD containing monooxygenase (EC 1.14.13.8) which catalyzes N-oxidation of various amines and also catalyzes S-oxidations (Ziegler and Mitchell, 1972; Ziegler and Poulsen, 1978). With respect to N-oxidation, the monooxygenase of nurse shark, *Ginglymostoma cirratum*, is also shown to have much the same properties as the mammalian enzyme (Goldstein and Dewitt-Harley, 1973).

The low specificity for TMA displayed by the monooxygenase of cod (Table I) indicates that an endogenous synthesis of TMAO is of minor importance. This is not surprising, since TMAO is very slowly metabolized and since cod must obtain a surplus amount of TMAO from the animals which constitute lower levels of marine food chains. These factors also explain the finding that some marine fish have no endogenous TMAO synthesis at all (Baker et al., 1963; Goldstein et al., 1967). On the other hand, the high affinity and specificity for TMA displayed by the soluble monooxygenase of a herbivorous copepod, *Calanus finmarchicus* (Strem, 1980), indicates that an endogenous TMAO synthesis is of major importance to animals at lower levels of marine food chains. They presumably depend on an endogenous synthesis for their supply of TMAO.

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