We have developed a new modification of the hybrid sucrose gap voltage clamp technique which eliminates or compensates for each of these difficulties. Essentially, a similar technique as that described by Morad and Orkand (J. Physiol. 219:167-189, 1971) was used with several new modifications: 1) a "guard gap" compartment which catches extracellular leakage current; 2) overshoot compensation technique for extracellular resistance; 3) electrostatic shielding between the current passing electrode and high impedance preamplifier input. This technique enables measurement of membrane current-voltage relations which are "instantaneous" within two msec. The membrane conductance at rest and during the plateau is measured as the slope of these current-voltage relations.

Our experimental results in the frog ventricle are in accord with the classical observation that the ionic conductance is higher at rest than during the plateau phase of the action potential. However an unexpected result from these experiments was that the conductance was constant during the entire plateau and rapid repolarization phases unlike Weidmann's experiment which seemed to show a decreasing conductance during the time course of the plateau. Figure 1 shows voltage clamp pulses applied at different times during the plateau and at rest and the calculated membrane conductances associated with each time.

It can be clearly seen that the conductance is constant during the plateau and then rises two-fold at rest. These results were essentially unchanged in the presence of tetrodotoxin (10^{-6}M) which is known to block the primary sodium inward current. These results have important implications as to the nature of ionic transport in this excitable membrane. Since well-documented net Na^+, Ca^{+2}, and K^+ effluxes occur during the plateau large permeability changes must be occurring In other excitable systems (nerve and skeletal muscle) such permeability changes are accompanied by corresponding conductance changes since the movement of the various ionic species are independent of each other. The constant conductance observed during the plateau of the myocardial action potential must mean that as potassium permeability increases to repolarize the membrane the sodium and/or calcium permeability must be decreasing by exactly the correct amount to explain the constant total conductance. This relation between the changes in specific ionic permeabilities suggests that a cross-ionic interaction may occur in the membrane. A possible molecular mechanism for such an interaction might be a multi-ionic carrier which controls the ionic movements. For instance a counter transport of Ca^{++} and K^+ would be an attractive hypothesis to explain repolarization of the membrane. The counter-transport carrier concept, while well-known to "transport workers," is foreign to the electrophysiological community.

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EXCRETION RATES OF TRIMETHYLAMINE OXIDE IN ELASMOBRANCHS

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All elasmobranchs maintain relatively high concentrations of trimethylamine oxide (TMAO)

in their body fluids for osmoregulatory purposes. However elasmobranchs differ in their ability to synthesize this end-product. Thus the nurse shark and lemon shark synthesize TMAO endogenously while the spiny dogfish and little skate do not. It is unusual to find such dramatic differences in metabolism within such a closely-related group of organisms. This suggests that there may be basic differences in the manner in which TMAO is handled by different elasmobranchs. In the present study we determined and compared the rates of loss of injected ^{14}C-TMAO from elasmobranchs known to differ in their ability to synthesize TMAO.

Spiny dogfish *(Squalus acanthias)* and little skates *(Raja erinacea)* were caught by handline and otter trawl respectively off Mt. Desert Island. Nurse sharks *(Ginglymostoma cirratum)* and lemon sharks *(Negaprion brevirostris)* were captured by handline in the vicinity of Bimini, Bahamas. Experiments on the first two species were performed at the Mount Desert Biological Laboratory in aquaria maintained at 12-14°C while investigations on the latter two species were conducted at the Lerner Marine Laboratory, Bimini in aquaria maintained at approximately 27°C.

Fish were injected intravenously with approximately 5μC^{14}-TMAO/Kg. Blood samples were drawn from a caudal vessel four to 24 hours after injection and at 24-hour intervals for six days. On the third day of the experiment lemon sharks, nurse sharks, and spiny dogfish were anesthetized with hexobarbital (15 mg/Kg, i.v.) and placed on an operating table; their gills were continuously perfused by flowing sea water. A muscle biopsy (approx. 0.2g) was taken through an incision (approximately 3 cm long) made along the dorsal midline surface. The wound was filled with Gelfoam and sutured closed. The entire procedure took about 15 minutes. The fish maintained spontaneous branchial movements during the operation and recovered from the anesthesia within five to 10 minutes after return to the aquarium. Muscle samples (as well as other tissue samples in the dogfish) were taken after anesthetization of the fish on the last day of the experiment (Day 6).

Plasma samples were analyzed for chemical TMAO and ^{14}C-TMAO as described previously (Goldstein, Hartman and Forster. Comp. Biochem. Physiol. 21:719, 1967). Tissue samples were analyzed for ^{14}C-TMAO by dissolving 100 mg of the tissue in one ml Protosol (N.E. Nuclear), suspending the mixture in 15ml of either Aquasol (N.E. Nuclear) or a toluene based cocktail Omniflour (N.E. Nuclear), and counting the mixture in either a Packard Tricarb or Nuclear Chicago liquid scintillation counter. Samples were corrected for quenching with the aid of an external standard. Chemical TMAO was assayed by microdiffusion after the tissue samples had been dissolved in concentrated KOH and neutralized with perchloric acid.

Based on certain simplifying assumptions a mathematical model was developed to determine rates of loss of TMAO from an equilibrated internal compartment. The equations of the model were solved to give:

(1) $C_2 = C_2(0)\exp\left(\frac{-K+}{V_2}\right)$ where C_2 is concentration of ^{14}C-TMAO $\left(\frac{\text{dpm}}{\text{ml}}\right)$ in the internal compartment at time +(days), $C_2(0)$ is the concentration of ^{14}C-TMAO (dpm/ml) in the compartment at +=0, K is a permeability coefficient (ml/day) and V_2 = volume of internal compartment in which TMAO is distributed (ml). Loss rates are calculated as $-K/V_2$ (loss coefficient) and expressed as day^{-1}.

TMAO excretion in skates was measured directly after the final blood samples were taken from the fish. The fish were placed in plastic aquaria containing 3L of aerated sea water and maintained

at 12-14C. Water and plasma (derived from blood taken from a caudal vessel) samples were dissolved in a mixture containing 15 ml Aquasol and 1 ml Protosol and counted in a liquid scintillation counter. These values (corrected for quenching) were used to calculate total excretion rates of TMAO by use of the equation

(2) μmoles TMAO excreted /Kg· min = dpm ^{14}C-TMAO excreted /Kg· min ÷

$$\frac{\text{dpm } ^{14}\text{C-TMAO/ml plasma}}{\mu\text{mole TMAO/ml plasma}}$$

Figure 1 shows the pattern of disappearance of ^{14}C-TMAO from the plasma of four elasmobranchs injected intravenously with ^{14}C-TMAO. Following the first day after injection the rates of

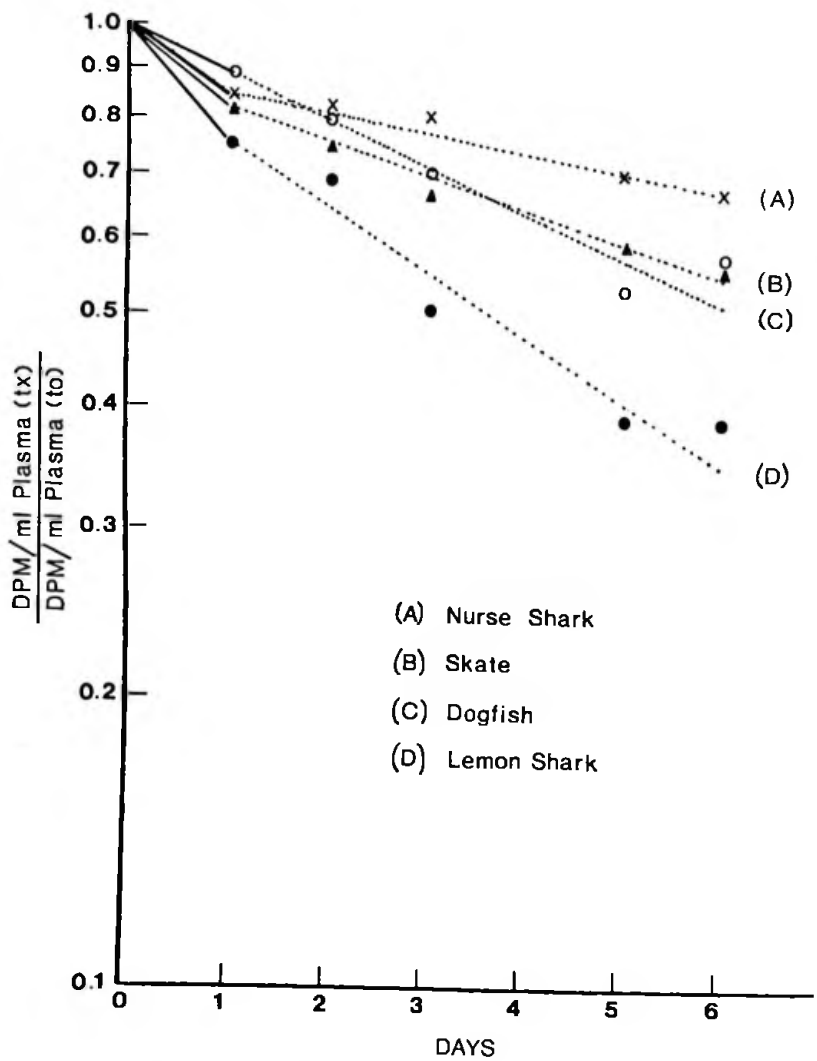

Figure 1. Loss of ^{14}C-TMAO from plasmas of four elasmobranch fishes. Values are means of five fish per group. The slope of each dotted line is K/V_2 determined by fitting equation 1 (see text) to data points for each species.

disappearance follow first order kinetics. These data suggest that after a 24-hour equilibration period the loss of ^{14}C-TMAO from the plasma is due to exit of the compound into a single compartment assumed to be the external environment and that filling of internal body compartments has reached a steady state. Support for this hypothesis was obtained by demonstrating that the concentration of ^{14}C-TMAO in the muscle relative to that in plasma remained constant between three and seven days in all fish except the lemon shark. Furthermore we measured these same relative concentrations (^{14}C-TMAO in muscle/^{14}C-TMAO in plasma) from one to three days after injection of the tracer into nurse sharks and found that the relative concentration of the compound was unchanged during this period. Since muscle accounts for the bulk of the fish outside of extracellular fluid we assumed that ratio of ^{14}C-TMAO concentration in tissues to that in plasma was not changing significantly during the six day experimental period and that disappearance of ^{14}C-TMAO from plasma represented loss of the isotope into the external environment.

Loss coefficients of ^{14}C-TMAO for the four elasmobranchs employed in this study are shown in Table 1. The magnitude of these coefficients varied from .044 in the nurse shark to .138 in the

TABLE 1

^{14}C-TRIMETHYLAMINE OXIDE LOSS COEFFICIENTS IN ELASMOBRANCHS

Fish	Body Wt. (kg)	Loss Coefficient (day^{-1})	Correlation Coefficient (r)
Little Skate	0.85 ± .07	0.078 ± .022	0.94 ± .02
Spiny Dogfish	1.90 ± .13	0.104 ± .022*	0.92 ± .02
Lemon shark	1.24 ± .04	0.138 ± .040	0.98 ± .00
Nurse shark	1.79 ± .43	0.044 ± .018*	0.92 ± .02

Values are means ± S.E. of five fish per group. *Mean for nurse shark significantly different from that of dogfish ($P<.05$).

lemon shark. The skate and dogfish were intermediate being .078 and .104 respectively. Assuming that these coefficients reflect rates of loss of TMAO to the external environment then in the four elasmobranchs studied TMAO excretion ranged from approximately four percent to 14 percent of total body TMAO per day. The higher value observed in the lemon shark is undoubtedly an over estimate because as indicated above their muscle compartment continues to load with ^{14}C-TMAO even after six days. To test validity of this method for calculating excretion rates we compared the loss coefficient obtained by this method in the skate with the rate of excretion of TMAO measured directly in the same fish. The rate of excretion of TMAO was found to be 0.51 ± .07 mmoles/Kg · day. If one assumes that the average concentration of TMAO in skates is 26 mmoles/Kg (plasma value) then this rate of excretion represents 2.0 percent of total body TMAO. This value is somewhat lower than the loss coefficient for TMAO determined in the skate (7.8 percent, Table 1). The dis-

crepancy could be due to either a) difference in excretion rates of TMAO under the two conditions (free swimming versus confinement in a 3L aquarium); b) in complete equilibration of TMAO in the internal compartment; c) both. Nevertheless, the lack of correlation between endogenous TMAO biosynthesis and the magnitude of the loss coefficient strongly suggests that the ability of some elasmobranchs (lemon and nurse sharks) but not others (spiny dogfish and little skate) to synthesize TMAO is not related to differences in excretion rates.

In a separate study we measured and compared ^{14}C-TMAO and total TMAO concentrations in plasma, liver, kidney, muscle, skin, and cartilage. Table 2 shows these concentrations and the relative

TABLE 2

CONCENTRATIONS OF ^{14}C-TMAO AND TOTAL TMAO IN PLASMA AND ORGANS OF DOGFISH, *Squalus acanthias*

Organ	^{14}C-TMAO $\left(\frac{\text{dpm/g organ}}{\text{dpm/ml plasma}}\right)$ x100	TMAO (μmole/ml or g)	Relative Specific Activity (S.A. Organ/S.A. plasma x100)
Plasma	-	71 ± 5	-
Skin	54 ± 4	35 ± 3	110
Muscle	40 ± 8	138 ± 6	21
Cartilage	80 ± 11	21 ± 5	271
Liver	125 ± 14*	40 ± 7*	222
Kidney	82 ± 9	53 ± 10	110

Values are means (± S.E.) of five fish per group. *Value corrected for water content (30%).

specific activities of ^{14}C-TMAO in these organs. The steady-state specific activities were significantly higher than plasma in liver and cartilage and lower as indicated above in muscle.

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1973 #21

FITNESS IN THE HERMIT CRAB *Pagurus acadianus* WITH REFERENCE TO *Hydractinia echinata*

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Jensen, 1970 (Ophelia 8: 135-144) has shown that European hermit crabs *Pagurus bernhardus* have a preference for mollusc shells colonized by the hydroid *Hydractinia echinata* and Wright, 1973 (Nature 241: 139-140) has suggested that the possession of commensal hydroids may be an important