to the non-striated muscle cells of the vessel wall. The axons terminate in channels or invaginations of the muscle cell without specialized junction areas. Microtubules 260 Å in diameter are present in the axons along with a variety of sizes (0.2 to 0.3 microns in diameter) of dense cored vesicles. The dense cores are aggregates of smaller (250 Å) dense particles. In the occasional cell bodies of neurones which are encountered the cytoplasm contains large numbers of the characteristic vesicles in close association with an elaborate Golgi region from which the vesicles appear to arise.

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1966 #16

THE OCCURRENCE OF MITOCHONDRIA IN MATURE ERYTHROCYTES OF Myxine glutinosa

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In the circulating blood in the gills of the hagfish the erythrocytes have normal mitochondria as identified in the electron microscope. Not all sections passing through the nucleus show mitochondria but most show one and about 10% show 3-5 mitochondria per section. No ribosomes were observed.

In contrast the mature erythrocytes of Squalus acanthias show polyribosomes but no mitochondria.

In both species there are other vesicles in the erythrocyte cytoplasm and in some of the these myelin forms have a superficial resemblance to mitochondrial cristae.

In Myxine pinocytotic vacuoles occur at the cell membrane.

1966 #17

ON THE ORIGIN OF TRIMETHYLAMINE OXIDE (TMAO) IN THE SPINY DOGFISH, Squalus acanthias

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This investigation is part of a general study on the comparative biochemistry of nitrogen metabolism, especially as related to environment. Trimethylamine oxide (TMAO), which appears to play an osmoregulatory role in elasmobranchs, is found in high concentrations in the body fluids of the dogfish, Squalus acanthias. The source (endogenous vs. exogenous) of this nitrogenous compound is unknown.

The level of TMAO (approximately 70 μmoles/ml) in the plasma of dogfish maintained in live cars remained relatively constant for weeks even though the fish were not fed and stomachs were found to be empty (this study and Cohen, Krupp and Chidsey, Am. J. Physiol. 194:229, 1958). The ability of whole dogfish and isolated liver preparations to synthesize TMAO from radioisotopically labeled precursors was tested. No counts were detected in TMAO after incubating either liver homogenates or liver slices with C^{14}-trimethylamine for 1-3 hours at room temperature under conditions permitting the detection of as little as 1% of the counts incorporated into TMAO.

The assay procedures were sensitive enough to detect a rate of TMAO synthesis corresponding to the rate of excretion of this compound by the dogfish (approximately 0.1 mmoles/Kg x day). These results indicate that TMAO is not synthesized in the dogfish, at least via the pathway known for other species (Bilinski, J. Fish, Res. Bd. Canada, 21:765, 1964). These results do not rule out the possibility of alternate routes of synthesis.

Similar results were obtained when choline-methyl-C^{14} was incubated with liver slices. Choline-C^{14}-methyl chloride and C^{14}-trimethylamine were not converted (less than 10%) to TMAO when these compounds were injected intravenously into fish and the plasma sampled daily for 4 days. Labeled trimethylamine was rapidly excreted but only 1% of the injected choline was collected in the urine in 24 hours.

The constant plasma levels observed during weeks of starvation is probably the result of two factors—the active reabsorbtion of TMAO in the dogfish kidney and the large pool of TMAO in the muscle. Permeability of muscle of the dogfish to TMAO was relatively low. As shown in Table 1, the specific activity of C^{14}-TMAO in muscle 2 days after intravenous administration of

Table 1

CONCENTRATION OF LABELED AND UNLABELED TMAO IN PLASMA, MUSCLE, RED CELLS AND LIVER OF DOGFISH INJECTED WITH C^{14}-TMAO

Fish (1.5-3.5 Kg) were injected intravenously with C^{14}-TMAO (approximately 1.5 μc), maintained in live cars without feeding, and sacrificed either at 2 or 20 days.

Exp. no.	Days	Tissue	TMAO (μmoles/ml plasma or tissue water)	C^{14}-TMAO (cpm/ml plasma or tissue water)	Specific activity (cpm/μmoles TMAO)	% plasma specific activity
1	20	Plasma	45	730	16.2	
		Muscle	154	764	5.0	31
		RBC	58	783	13.5	83
2	20	Plasma	78	1550	19.9	
		Muscle	216	904	4.2	21
		RBC	85	1540	18.1	91
3	2	Plasma	76	4727	62.2	
		Muscle	225	745	3.3	5
		RBC	64	632	9.9	16
		Liver	87	3680	42.2	68

C^{14}-TMAO was only 5% of that in plasma. Even this small percent can be accounted for by C^{14}-TMAO in extracellular space of dogfish muscle (15%). Red blood cells were 16% equilibrated with plasma, and liver 68%. After 20 days, the specific activity in muscle was 20-30% of that in plasma and RBC about 80-90%. It is apparent that muscle and even RBC pick up and presumably release TMAO very slowly. TMAO was not bound to muscle protein. The slow release of TMAO from muscle probably accounts for the small amounts of TMAO lost by the gills and kidneys.

Indirect evidence was obtained for the low permeability of teleost muscle to TMAO. Muscle of longhorn sculpin (Myoxocephalus scorpius) contained significant concentrations of TMAO (ap-

proximately 60 μmoles/ml tissue water) when the level in plasma was less than 1 μmole/ml.

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1966 #18

THE INHIBITION OF SPONTANEOUS MOTILITY OF SMOOTH MUSCLE IN THE SPINY DOGFISH, Squalus acanthias, AND IN THE SEA ANEMONE, Metridium dianthus BY SULFHYDRYL REACTANTS

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It has been established that thiol reagents can inhibit the contractility of striated muscle (Weber and Portzehl, Advanc. Protein Chem. 7:162, 1952). A similar sulfhydryl group dependence has been demonstrated for contractility and spontaneous motility of mammalian smooth muscle (Goodman and Hiatt, Biochem. Pharmacol. 13:871, 1964).

The present research involving the spiny dogfish, Squalus acanthias, and the sea anemone, Metridium dianthus, was initiated as part of a comparative study to determine whether sulfhydryl group dependence is one of the biochemical characteristics common to all smooth muscle.

Squalus acanthias. Strips of smooth muscle (5 mm by 40 mm 0.5 gm wet wt) were cut from various regions of the gastrointestinal tract, washed with sea water, then washed and mounted in Ringers solution modified to contain in meq per liter: NaCl, 256; KCl, 10; $CaCl_2$, 6. The tissue was maintained at 12° in the photoelectric muscle contraction device previously described (loc. cit.). Spontaneous isotonic motility characteristics were continuously recorded under constant load (0.6 gm) before and after the addition of test compounds.

Marked rhythmic spontaneous motility was demonstrated for all parts of the gastrointestinal tract. Strips of distal stomach were highly sensitive to cholinergic compounds including acetylmethylcholine. The terminal region of the spiral valve was found particularly active in this system although far less sensitive to acetylcholine. After equilibration for 30 minutes strips cut from the region of the last turn of the spiral demonstrated regular, periodic isotonic contractions for periods as long as 48 hours without extraneous stimuli or added source of energy. Normal mean frequency of contraction was 1.4 cycles per minute with linear displacement about 5% of strip length.

As in the case of mammalian small intestine, a variety of reagents which react with protein sulfhydryl groups caused marked inhibition of spontaneous motility (Table 1). However, in the dogfish, generally higher concentrations were required for inhibition. Cysteine, homocysteine, or reduced glutathione can negate the inhibitor effects of sulfhydryl group reactants. When compounds with no reactivity toward thiol groups were added, such as glycine or serine, there was no effect on the contractile pattern.

Metridium dianthus. Strips of parietal muscle of the stalk (5 mm by 40 mm) were cut transversely from the body wall midway between the oral and pedal discs without anesthesia. Strips were trimmed and mounted in the above modified Ringers solution maintained at 10-12°. Spontaneous rhythmic isotonic contractions with loads of from 0.6 to 2.5 gm were recorded for as long as 48 hours on one strip with no added source of energy. The "normal" activity of Metridium dianthus in the present Mg^{++} free Ringers solution differs markedly from that of Metridium