2) Choline chloride, 5 mMo/Kgm., I. M., 3) Trimethylamine MCl (TMA), 5 mMol/Kgm., I. M.

Phlorizin had no effect on TMAO reabsorption; choline resulted in the death of the fish; TMA resulted in an increased excretion of TMAO as well as of TMA. Therefore, the effect of TMA was studied further.

Observations made on 6 dogfish during clearance experiments after a single I. M. injection of TMA revealed that in 5 of the 6 fish, TMAO reabsorption decreased markedly. Thus, the per cent of TMAO reabsorbed fell to between 27% and 82% of the filtered load without any significant change in plasma TMAO or GFR.

Associated with this change in reabsorption of the oxide was a rise in the (TMA) in plasma. The rise in excretion rate of this free amine was such that net secretion of TMA occurred. This has been assumed to be due to the constant pH gradient between plasma and urine in the dogfish which allows the free amine to diffuse into and be trapped in the acid urine as the trimethyl - ammonium ion.

The increase in TMAO excretion following TMA is to be further investigated from the standpoint of: 1) Possible synthesis of TMAO from TMA by the kidney, 2) Formation of an intermediate product from TMA which competes with or blocks TMAO reabsorption.

This study was carried out in a laboratory maintained by the New York University College of Medicine.

Development of Esterase in the Egg of Marinogammarus (Amphipoda)

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Marinogammarus finmarchicus (brown eggs) and *M. obstusatus* (red eggs) were collected from beneath Fucus on the Laboratory beach and kept in mesh trays in the laboratory in running sea water at 14° - 17°C. Eggs were removed daily from ovigerous females and raised in watch-glasses. Both species develope at essentially the same rate and hatch in 19 - 21 days.

Esterase activity was measured in terms of hydrolysis of 0.02 M alpha naphthyl acetate at pH 7.25 and 40°C. Eggs were placed in micro reaction vessels and all but 1 - 2 µl of sea water was removed. Fifty µl of 0.05 M tris buffer pH 7.25 (at 40°C) was added. The preparation was frozen and the plug of frozen buffer was rotated with a needle using the ice as a pestle to rupture the eggs. Frozen eggs retained full enzymatic activity for several weeks. To the buffer 100 µl of substrate 0.03 M was added and the tube placed in the water bath at 40°C. for 15 to 30 minutes. The napthol released was measured by a modification of Gomori's procedure. Two hundred µl of a freshly prepared mixture consisting of 5ml of 3%

Duponol containing approximately 3 mg of diazo Red ITRN salt was added to 150 µl of digest. The resulting color was read at 550 in a Lowry cuvette in a Coleman spectrophotometer using a special cuvette adapter.

Hydrolysis was proportional to time up to 75 minutes and independent of substrate concentration when less than 30% of the available substrate was hydrolysed at molarities of 0.01 to 0.04. Suitable blanks were run for preformed naphthol and non-enzymatic hydrolysis. In *M. obtusatus* the activity is quite high and there is no consistent rise during development. In *M. finmarchicus* the µg naphthol liberated per egg 15 minutes varies between 0.04 and 0.08 for the first 8 days. Between the 8th and 9th day a sudden (threefold) rise (0.26 ± .02 µg) is characteristic. Maximal activity is 2 to 3 times this value and occurs with considerable variability by the 12th to 15th day. Esterase activity increases 10 - 15 times by the time of hatching. In embryos at 12-15 days the major store of esterase is in the gut and caecae and the activity of the contents is higher than that of the cells.

In *M. obtusatus* determinations of esterase activity of separated yolky vesicles and embryonic cells showed the esterase to be distributed in both regions. The embryonic cells usually had more than half the esterase content but comprised less than half the cell volume.

Potassium Prevents Entry of Neutral Red into Yeast Cells from Acid Media

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We have investigated yeast and sea urchin eggs as laboratory material for teaching classical principles concerning the passive transfer of weak acids and bases across cell membranes. The weak base, neutral red, would be expected to traverse the membrane most rapidly as the lipid soluble non-ionized phase that is found most abundant in alkaline media. Once inside the cell, the penetrator non-dissociated dye would ionize and be trapped and would accumulate there as a relatively non-penetrator cation. Loss of dye from the medium can be detected colorimetrically when the dye is acidulated to a uniform pH.

The sea water medium for sea urchin eggs produced troublesome precipitates when alkalinized. Finally we turned exclusively to yeast which

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