ration by an equilibrating system. The maximum T/M of 0.8 was practically reached within 30 min of aerobic incubation in a balanced saline at 15° C (air as gaseous phase). This galactose transport system was markedly inhibited by phlorhizin (0.5mM) but not by 0.1mM dinitrophenol, 0.5mM ouabain or some sugars (D-glucose,2-deoxy-galactose) at a molar ratio 1:10.

1mM 2-deoxy-D-glucose-^{14}C was accumulated to a slight degree (maximum T/M 1.3). The transport of this sugar was inhibited by 0.5mM D-glucose but not affected by ouabain or D-galactose.

The transport of 2-deoxy-D-galactose was investigated using a chemical assay specific for 2-deoxy-sugars. High tissue blanks prevented a detailed analysis of this transport system. However, evidence for an equilibrating transport system and its inhibition by 0.5mM phlorhizin was obtained.

The inulin-^{3}H space at 15° C was found to be 0.4 ml/g tissue.

These preliminary data suggest that the above three sugars are transported across the basal membrane of the flounder tubular cells by at least two systems of the facilitated diffusion type.

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1969 #15

ASPARTATE METABOLISM IN TELEOST LIVER

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In teleosts, ammonia is produced in the liver and excreted passively by the gills. Interestingly enough, L-aspartate is a major precursor for ammonia in teleost liver homogenates (Salvatore et al, Comp. Biochem. Physiol. 16:303, 1965). These authors speculated ammonia arose from L-aspartate by direct deamination. It seemed worthwhile to investigate this; particularly since enzymes for direct L-aspartate deamination are thought to occur only in plants and microorganisms.

Teleost liver homogenates (20%) were prepared in o.1M K_2HPO_4-KH_2PO_4 at pH 7.4. The incubation medium contained: 10 μmoles $MgCl_2$, 10 μmoles $Na^+AsO_2^-$, and 100 μmoles K^+L-aspartate or K^+L-glutamate, in a final volume of 3.0 ml. Ammonia production was calculated as: μmoles NH_3/gram tissue/hr, at 25° C. Values are the Mean ± SE, with 4 fish in each group.

Ammonia production from L-aspartate was 14.0 ± 9.7 in the eel (Anguilla rostrata), 5.8 ± 1.2 in the short-horned sculpin (Myoxocephalus scorpius), 4.2 ± 0.4 in the long-horned sculpin (Myoxocephalus octodecimspinosus), and 2.7 ± 0.8 in the flounder (Pseudopleuronectes americanus). In all species, L-glutamate proved to be a more active substrate for ammonia production; it was 29.6 ± 10.8 in A. rostrata, 8.5 ± 1.1 in M. scorpius, 8.5 ± 1.1 in M. octodecimspinosus, and 10.0 ± 0.9 in P. americanus.

Transaminase inhibitors were studied in homogenates of M. scorpius. Isonicotinoyl hydrazide (5×10^{-3}M), semicarbizide (4×10^{-3}M), and hydroxylamine (4×10^{-3}M), completely inhibited ammonia production from L-aspartate.

Finally, if a single enzyme deaminates L-aspartate, it should be demonstrable in at least one subcellular fraction. Subcellular fractions from P. americanus and M. scorpius were isolated in 0.25 M sucrose/0.02 M Tris, at pH 7.4, by differential centrifugation. No ammonia was

produced from L-aspartate in any single subcellular fraction. However, when cytoplasmic and mitochondrial fractions were combined, ammonia was liberated. Prior dialysis against 0.02 M Tris at pH 7.4 inhibited ammonia production from L-aspartate when these fractions were combined. Apparently, a critical intermediate, perhaps α-ketoglutarate, was sequestered during dialysis.

The results suggest a transamination reaction is involved in the liberation of ammonia from L-aspartate in teleost liver. In rat liver, aspartate aminotransferase (EC 2.6.1.1) is in the cytoplasmic fraction; and glutamic acid dehydrogenase (EC 1.4.1.3) is in the mitochondrial fraction. These enzymes may also be involved in the liberation of ammonia from L-aspartate in teleost liver; particularly since all the homogenates produced more ammonia from L-glutamate than L-aspartate.

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1969 #16

PERMEABILITY AND METABOLISM OF UREA IN THE INTESTINE OF THE ELASMOBRANCH, Squalus acanthias

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This study attempted to assess the rate of penetration, content and rate of bacterial degradation of urea in the intestine of elasmobranchs. To determine the rate at which urea crosses the intestinal wall we injected two male dogfish, Squalus acanthias, with ^{14}C-urea (10 μC/Kg). After one hour, blood samples were drawn, and the intestines were ligated at the pylorus and anus, removed and flushed with 10 ml H_2O. The expressed fluid was analyzed for total and ^{14}C-urea. Comparison of the specific activities of ^{14}C-urea in plasma and intestinal fluid showed that the intestinal fluid was approximately half-equilibrated with the plasma (^{14}C-urea intestine/^{14}C-urea plasma = 0.56, 0.51) after one hour.

Intestinal fluids of dogfish were assayed for urea concentration. Dogfish which had been kept in the live car for indefinite periods of time generally had empty intestines, whereas fish fresh from the collecting boat usually contained some fluid. The fish were killed and opened and the intestines tied off at the pylorus, the rectum, and two intermediate points to produce three approximately equal segments. Each segment was then opened and as much fluid as possible withdrawn. Accurate measurement of the fluid present in the intestine was difficult. The values do give a minimum estimate, however, which might be of interest in the controversy about the drinking habits of dogfish. The total amount of intestinal fluid per fish averaged 1.3 ml with a standard error of 0.4 ml. Of eleven fish (nine of them fresh) included in this calculation, two from the live car and two fresh fish had empty intestines. We suggest that investigators who found no fluid in the intestines of dogfish from the live car were dealing with fasting fish under abnormal conditions. Conclusions drawn from such fish may have little relationship to the behavior of Squalus in the wild.

In the segment nearest the pyloric end, the mean urea concentration was 243 μmoles/ml, with S.E. = 25 and n = 7. In the middle segment, the mean was 323 μmoles/ml, S.E. = 29, n = 6.