

intestinal mucosal homogenates, the inhibition of H₂O absorption can be explained at the biochemical level. The toxicity of DDT in teleosts may therefore be related to disrupted osmoregulatory transport mechanisms.

Of significance in this study is the inhibition of critical events in teleost osmoregulation by concentrations of DDT which can be expected to occur in these species in the foreseeable future. Filter feeding invertebrates are among the initial marine organisms which concentrate DDT. In the extracted fat of clams (*Mya*), collected in the relatively pesticide free waters of Frenchman Bay, 9 ppm DDT has been reported by Adamson, Sullivan and Rall (Bull. MDIBL 9:2-4, 1969). It is therefore disquieting that we report having found, on several occasions, clams of the genus *Mya* approximately 0.5 to 1.0 cm in diameter, in the intestines of the winter flounder (*P. americanus*). Thus, in one step of the food chain, an invertebrate filter feeder is providing a concentrated source of DDT to a commercially important fish.

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1970 #22

TRANSPORT OF SUGARS INTO KIDNEY TUBULES OF THE FLOUNDER (*Pseudopleuronectes americanus*)

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It has been reported previously (Bull. MDIBL 9:20, 1969) that 2-deoxy-D-glucose-¹⁴C is accumulated in teased kidney tubules against a slight concentration gradient. The characteristics of this up-hill transport system have now been studied in more detail. Under standard conditions of incubation (1 h at 15 °C, air as a gaseous phase) of teased kidney tubules in an isotonic balanced saline (Forster, R. P., Science 108:65, 1948) buffered with a 5 mM TRIS-TES mixture, pH 7.2, the preparation had a mean water content of 5.5 kg H₂O/kg tissue dry wt (D.W.) (2 animals, 9 analyses). Mean value for tissue cations, in mequiv/kg D.W.: Na, 380; K, 308. Inulin-¹⁴C space: 0.377 ± 0.011 (3 animals, 11 analyses). Figure 1 shows that, in contrast with D-galactose, the rate of accumulation of 2-deoxy-glucose (2-DGLU) does not reach a steady state by 90 min incubation. In this experiment, at an external sugar concentration of 1 mM, a tissue/medium (T/M) sugar ratio of 1.1 was reached after 90 min. The mean T/M in 6 experiments was 1.25 at 60 min. The following characteristics of the transport system for 2-DGLU were determined by studying the effects of various experimental conditions on the T/M after 60 min incubation:

1. The T/M was significantly depressed by 0.1 mM dinitrophenol, establishing the metabolic dependence of the transport process.
2. Accumulation of 2-DGLU was markedly enhanced by decreasing the medium concentration of the sugar.
3. D-glucose (medium concentration 5 mM) significantly depressed the T/M for 2-DGLU; D-galactose was less effective as a competitor whereas L-glucose was ineffective.
4. Phlorhizin and phloretin (both at 0.5 mM) were equally effective in inhibiting the transport of 2-DGLU.

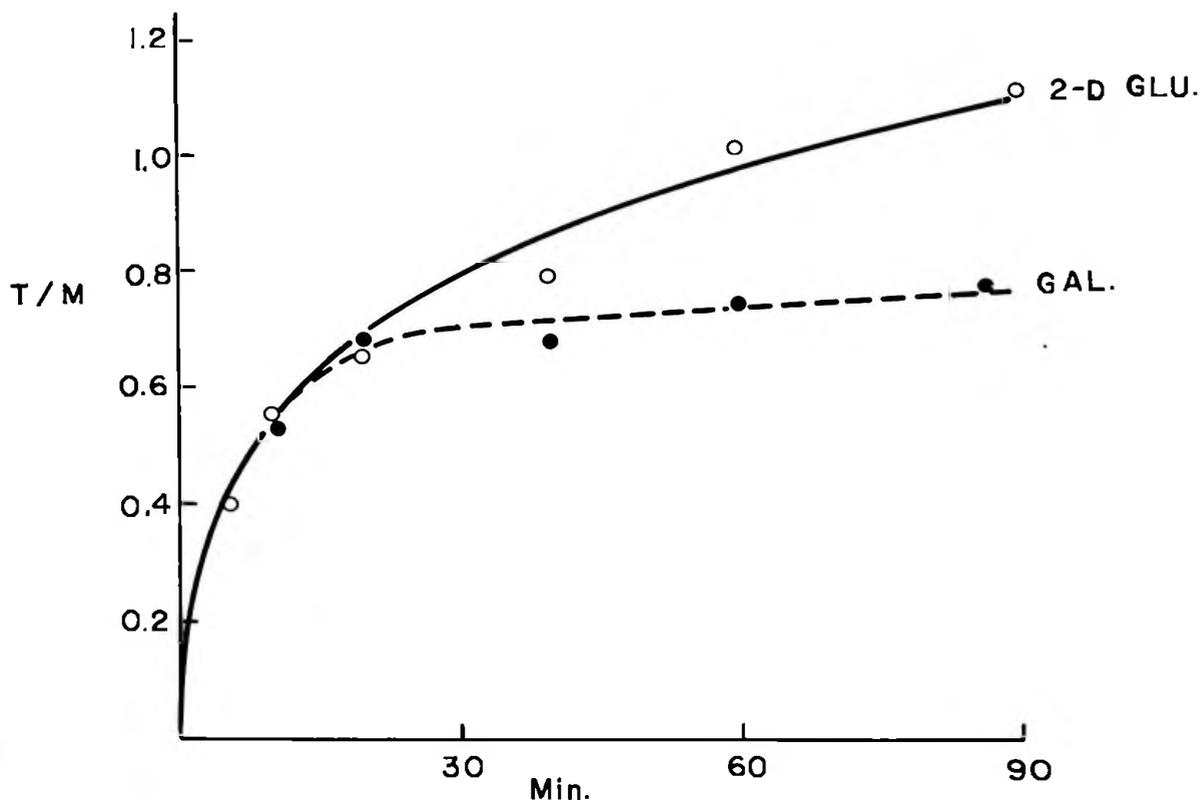


Figure 1. Entry of galactose (●) and 2-deoxy-D-glucose (○) into teased tubules of flounder kidney incubated at standard conditions (Na-saline, 15° C, air). Medium concentration of both sugars 1 mM. All values of T/M on the ordinate are the means of 3 analyses, M referring to final sugar concentrations in the medium.

5. The accumulation of 2-DGLU was not affected by the absence of external Na (Li-saline) or by the presence of 0.5 mM ouabain, indicating a Na-independent transport system. In the experiments where Li-saline was employed, most of the tissue Na had been removed by preliminary leaching of the tissue and by preincubation. Thus, the tissue accumulation of 2-DGLU observed in Li-saline could hardly be due to Na introduced into the incubating system by the tissue. Also, analyses of tissue after incubation in the presence of ouabain showed that the inhibitor was active, producing tissue of high Na and low K content.

The above observations are consistent with the view that 2-deoxyglucose is transported into the tubular cells of flounder kidney by a metabolically dependent, carrier-mediated process which is independent of external Na. These transport characteristics for 2-DGLU are in all respects identical with those observed for the transport of this sugar in slices of rabbit kidney cortex (cf. Kleinzeller et al, *Biochem. J.* 104:852, 1967; Kleinzeller et al, *Biochim. Biophys. Acta* 211:293, 1970). Since in this preparation only the peritubular face of the cells was accessible to the substrates in the medium, the results indicate that the site for the up-hill transport of 2-DGLU into the kidney tubular cells is located at the antiluminal face of the flounder kidney tubules.

L-glucose- ^{14}C and α -methyl-D-glucoside- ^{14}C did not accumulate against their concentration gradient in teased flounder kidney tubules. The T/M ratios for both sugars (medium concentration 1 mM) was only slightly higher than could be accounted for by equilibration in the inulin space (values between 0.50 and 0.59 were observed) and no significant effect of DNP (0.1 mM), phlorhizin (0.5 mM), ouabain (0.5 mM), and of other sugars (D-glucose, D-galactose and 2-deoxy-D-glucose) on the T/M ratios were found. These results suggest that no sites for the inward transport of L-glucose and α -methyl-D-glucoside are localized at the peritubular face of the flounder kidney tubules.

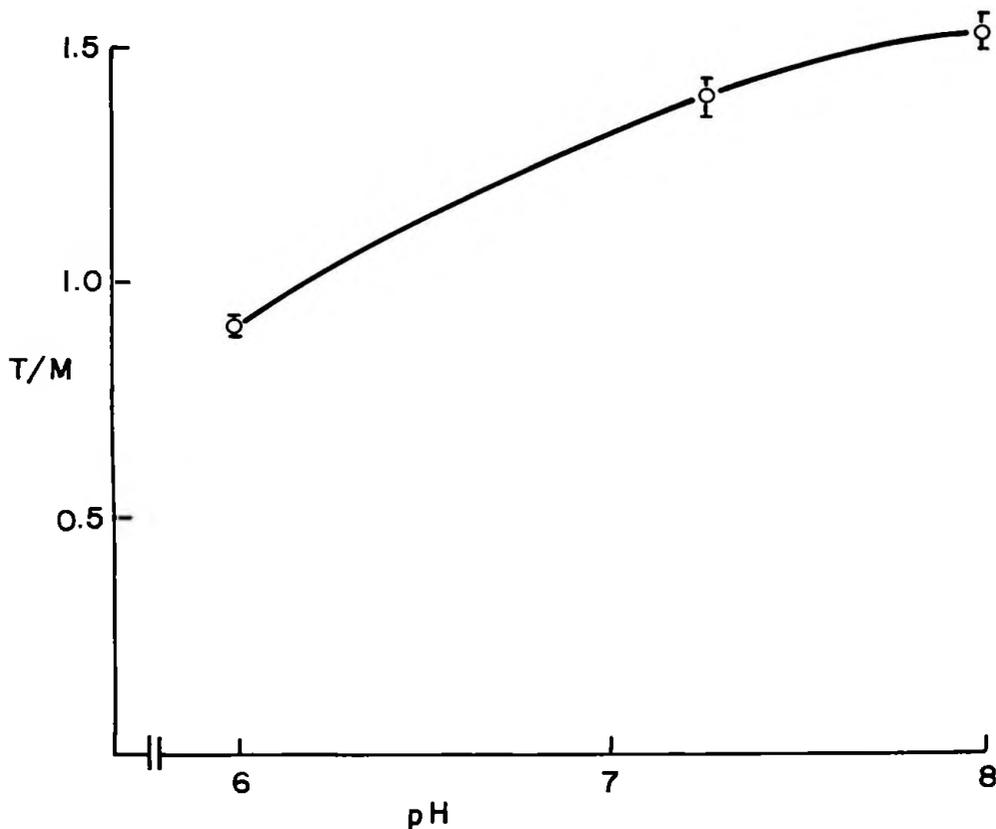


Figure 2. Effect of pH on the accumulation of 2-deoxy-D-glucose in teased tubules of flounder kidney. Experimental conditions: Na-saline containing 0.5 mM 2-DGLU and 5 mM TRIS- TES buffer system; 15°C ; air; 60 min incubation. Each value of T/M represents the mean \pm S.E. of 5 analyses.

Evidence has been summarized (MDIBL 9:20, 1969) in favor of the view that D-galactose does enter the tubular cells of flounder kidney by a mechanism of the facilitated diffusion type, localized at the peritubular face of the cells. In conjunction with Drs. W. B. Kinter and J. Pritchard, three clearance experiments were carried out to establish the transport vector of D-galactose- ^{14}C under in vivo conditions. In a total of 9 individual measurements, the clearance ratio $C_{\text{gal}}/C_{\text{inulin}}$ was 0.38 ± 0.05 (S.E.); not included in the means were two measurements on one flounder where the inulin clearance rapidly deteriorated toward the end of the ex

periment (U/P values below 2), and concomitantly, the clearance ratio increased to values higher than 1.0. The above data may be taken as evidence for a reabsorption of D-galactose from the tubular lumen against a concentration gradient. In two of these experiments, the tissue content of D-galactose was determined at the end of the clearance measurements. In both experiments, the T/plasma ratios were considerably higher than 1.0 (mean value 2.6, 9 analyses). The above data are consistent with the following model for D-galactose transport across the flounder tubular cells: Galactose is first accumulated in the tubular cells by an active transport system localized at the luminal face. Subsequently, the sugar moves in accordance with its concentration gradient from the cells to the plasma by a carrier mediated process.

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1970 #23

PHYLOGENETIC STUDY OF HEPATIC ORGANIC ANION UPTAKE MECHANISMS

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In mammals, organic anions such as bilirubin and various dyes, drugs and metabolites are rapidly and selectively transferred from plasma into the liver. The mechanism of this transfer and its selectivity are unknown. Recently, two organic anion binding proteins, Y and Z, have been isolated from the liver cell cytoplasm of the rat (J. Clin. Invest. 48:2156, 1969). The following characteristics of Y and Z support the theory that they are intracellular acceptors which facilitate hepatic uptake of various organic anions from plasma: (i) Y and Z selectively bind certain organic anions such as bilirubin, sulphobromophthalein (BSP) and indocyanine green (J. Clin. Invest. 48:2156, 1969; J. Clin. Invest. 1970, submitted); (ii) Y, the major organic anion binding protein is present exclusively in the liver (J. Clin. Invest. 48:2156, 1969); (iii) the concentration of Y increases following administration of phenobarbital, DDT and other drugs and concomitantly hepatic anion uptake is increased (J. Clin. Invest., 1970 (submitted); Proc. Nat'l Acad. Sci. 64:168, 1969), and (iv) in the newborn monkey, the concentration of Y and hepatic uptake of BSP, as measured by plasma disappearance rates, are both low and mature simultaneously (The Lancet 2:139, 1969).

A phylogenetic study was performed to investigate further the function of Y and Z. The study included an elasmobranch, the dogfish; several bony fishes; the mudpuppy, a gill-breathing amphibian which spends its entire life confined to water; four species of amphibia which possess functioning lungs and are able to exist for long periods on land; reptiles, birds and several mammals, including man. For each animal, from 2 to 6 mature individuals were studied. R. catesbiana, R. clamitans, and R. heckscherii tadpoles with and without hind limb emergence, as well as adult frogs, were separately studied.

Y and Z were identified and measured in terms of their binding of BSP in vitro (J. Clin. Invest. 48:2156, 1969). The relationship between Y and Z and hepatic organic anion uptake was investigated where possible by determining the half-life for removal of injected BSP from plasma. From 3 to 6 elasmobranchs and teleosts received a single intravenous injection of 10 mg of BSP per 100 g body weight; from 6 to 8 blood specimens were obtained in the subsequent 1 to 60 minutes; BSP content was determined after alkalization and the half-time was derived from a