These effects persisted even 1 month after dosing (Figure 1). Also, differences between experimentals and controls appeared to be greatest when ducks had access to water of lower salinity, i.e., during heavy rains (Figure 1, days 7, 8 and 10) or after transfer from 100% to 60% SW (additional experiment, not shown). Similar evidence of osmoregulatory impairment was also obtained for ducks dosed with another crude or with either of two refined oils and for herring gulls and black guillemots dosed with crude oil (Miller et al., Fed. Proc., in press, 1977).

The results presented here suggest that petroleum-induced osmoregulatory impairment in marine birds involves both a slightly reduced capacity to obtain solute-free water from 100% SW and a delayed ability to recover when water of lower salinity is available. Our preliminary results suggest that nasal gland Na,K-ATPase is one component of the avian osmoregulatory system that is affected by crude oil.

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IN VIVO RENAL TUBULAR SECRETION OF 2-DEOXY-D-GALACTOSE BY THE WINTER FLOUNDER, *Pseudopleuronectes americanus*

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The renal tubules of the winter flounder, *Pseudopleuronectes americanus*, recently have been shown to possess several distinct carrier-mediated transport systems for sugars at both the peritubular (basal) and luminal (apical) faces of the tubular epithelial cells (J. Gen. Physiol., 62:169-184, 1973; Am. J. Physiol., 231:603-607, 1976). Of particular interest was the observation that free 2-deoxy-D-galactose (2-d-Gal) and perhaps D-galactose (Gal) accumulate within tubular cells to tissue-to-medium concentration ratios (T/M) greater than 1 in vitro (Am. J. Physiol., 231:608-613, 1976). Since the teased tubule preparation exposes only the peritubular face of the tubule to the substrate, this result suggests active peritubular transport of 2-d-Gal into the cell. The ability of Gal to inhibit 2-d-Gal accumulation in vitro (and vice versa) further supported a carrier-mediated peritubular transport mechanism (Am. J. Physiol., 231:608-613, 1976).

Since presentation of substrates in vivo exposes both luminal (via filtration) and peritubular (via peritubular capillaries) faces of the tubular cells, in vivo renal clearance experiments provide a measure of the net transport of that substrate. In conjunction with in vitro data such as that cited above, clearance data permits evaluation of the relative contributions of luminal and peritubular transport to the overall handling of a given substrate (Am. J. Physiol., 231:603-607, 1976). In the studies reported below, we have utilized clearance techniques to evaluate the importance of peritubular transport to net 2-d-Gal excretion in vivo and to examine the specificity of 2-d-Gal transport.

Details of the clearance methods utilized here have been previously reported (Am. J. Physiol., 231:603-607, 1976). Doses of ^{14}C-2-d-Gal and ^{3}H-polyethylene glycol (^{3}H-PEG) were 25 µmol/kg (2 µCi) and 250 mg/kg (5 µCi), respectively. Where used, inhibitor concentrations were 2.5 mmol/kg for Gal and D-glucose and 2.5 µmol/kg for phlorizin. Resulting plasma levels were 5-50 µM 2-d-Gal, 0.5-3 mg/ml PEG, 5-10 mM glucose, and 5-10 µM phlorizin. Total, free, and phosphorylated

2-d-Gal were determined in plasma, urine, and kidney tissue according to the method of Kleinzeller (J. Gen. Physiol., 62:169-184, 1973).

Results of nearly 100 clearance measurements in 17 fish are summarized in Table 1. Unlike any other sugar previously studied in flounder or in mammals, 2-d-Gal showed net secretion in vivo. The clearance of 2-d-Gal ($C_{2\text{-d-Gal}}$) was significantly greater ($P < 0.001$) than the glomerular filtration rate (GFR), as measured by the clearance of PEG (C_{PEG}). On the average, the clearance ratio (i.e., $C_{2\text{-d-Gal}}/C_{PEG}$) in these fish was nearly 4. Kidney tissue demonstrated extensive 2-d-Gal accumulation, with a tissue-to-plasma ratio (T/P) of 56 for total sugar (Figure 1). Much of this sugar was phosphorylated (T/P = 37), but T/P free 2-d-Gal was still over 19. In similar experiments on two aglomerular goosefish, *Lophius americanus*, 2-d-Gal urine-to-plasma ratios (U/P) were 3to 7 times PEG U/P. However, in the goosefish U/P 2-d-Gal never reached unity. Similarly, although the goosefish kidney did accumulate 2-d-Gal, T/P were far lower, 7 for total sugar and 3 for free 2-d-Gal. Thus, secretion, if present in the goosefish, must be much more limited than in the flounder.

TABLE 1

CONTROL 2-DEOXY-D-GALACTOSE[a] CLEARANCE DETERMINATION

	$\bar{x}$	(± SEM)
Fish Weight (kg)	0.295	(0.026)
Urine Flow (ml/hr)	0.281	(0.065)
Plasma PEG[a] (mg/ml)	1.97	(0.40)
Urine PEG (mg/ml)	4.13	(1.40)
U/P PEG	1.67	(0.30)
Clearance PEG (ml/hr)	0.409	(0.113)
Plasma 2-d-Gal (μM)	22.30	(3.30)
Urine 2-d-Gal (μM)	85.70	(16.34)
U/P 2-d-Gal	4.84	(0.88)
Clearance 2-d-Gal (ml/hr)	0.940	(0.185)[b]
Clearance Ratio[c]	3.78	(0.66)
Number of Fish	17[d]	—

[a]Abbreviations: 2-deoxy-d-galactose = 2-d-Gal; polyethylene glycol = PEG.

[b]$P < 0.001$ vs clearance PEG (i.e., GFR) (paired t test).

[c]Clearance 2-d-Gal/clearance PEG.

[d]A total of 93 clearance determinations in 17 fish.

The effects of galactose on 2-d-Gal clearance and on its tissue accumulation are shown in Figures 1 and 2. When galactose was given at the same time (i.e., T_o) as the labeled 2-d-Gal and PEG, the clearance ratio was unity throughout the experiment. Thus, 2-d-Gal excretion could be totally accounted for by filtration alone and secretion was abolished. Tissue accumulation was

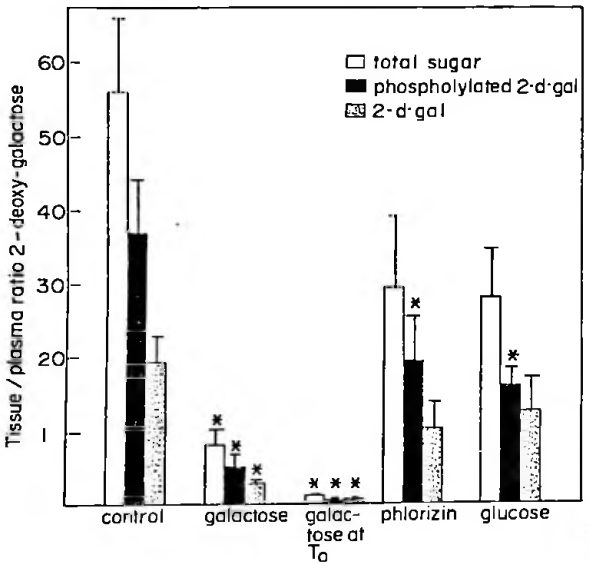

Figure 1. Terminal tissue-to-plasma ratios (T/P) for 2-deoxy-D-galactose (2-d-Gal) after clearance measurements and i.v. injection of other sugars. T/P are given for total 2-d-Gal, phosphorylated 2-d-Gal, and free unmetabolized 2-d-Gal. Conditions are as described in the text. Values shown are the means ± SEM (Bars). Four individual determinations were made in each of 6 fish for control and galactose, 4 fish for phlorizin and glucose, and 2 fish for galactose at T_o. * = $P < 0.05$ vs control.

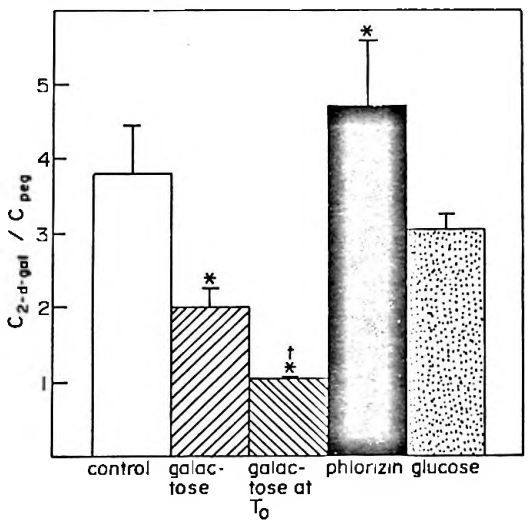

Figure 2. Effects of galactose, phlorizin, and glucose on the clearance of 2-deoxy-D-galactose ($C_{2\text{-d-Gal}}$). Results are expressed as the clearance ratio of $C_{2\text{-d-Gal}}$ over C_{PEG} (the GFR). Values given are the mean ± SEM (Bars). Ninety-three clearance periods in 17 fish are summarized in the control value. Other values are: Galactose = 20 determinations in 4 fish; galactose at T_o = 8 in 2 fish; phlorizin = 19 in 3 fish; and glucose = 8 in 2 fish. * = $P < .05$ vs control. † = P 0.05 vs galactose.

similarly depressed, with T/P total sugar of only 1.32 and 0.70 for free 2-d-Gal (Figure 1). In a second set of experiments, 4 to 6 2-d-Gal and PEG clearance measurements were made prior to Gal injection. Under these conditions, the clearance ratio ($C_{2\text{-d-Gal}}/C_{PEG}$) fell 50-75% within 30-60 min after Gal injection. The average clearance ratio after Gal in these experiments was 2.01, which was significantly different from both control and Gal at T_o experiments (Figure 2). Tissue values were also intermediate between control and T_o injection values, with a T/P for total 2-d-Gal of 8 and for free 2-d-Gal, 2.9 (Figure 1). It would appear that under these conditions, 2-d-Gal accumulates during the control period and slowly diffuses into urine after Gal blocks secretory peritubular transport, both reducing tissue sugar and maintaining limited "secretion" of 2-d-Gal.

Results following similar experiments (i.e., 4-6 control periods followed by injection of inhibiting sugars) using phlorizin and glucose as "inhibitors" were less conclusive than the galactose studies, but are nevertheless informative. Phlorizin significantly increased ($P < 0.05$, paired t test) the clearance of 2-d-Gal (Figure 2). Glucose similarly increased the clearance ratio over control values in the same animals, but not to statistically significant levels. Both phlorizin

and glucose significantly decreased tissue sugars, with values approximately 50% of control levels (Figure 1). This increase in secretion coupled with a decrease in tissue content suggests that phlorizin may be inhibiting reabsorption of 2-d-Gal. Such a mechanism is certainly possible in light of the extensive phlorizin-sensitive reabsorption of Gal previously described in the flounder *in vivo* (Am. J. Physiol., 231:603-607, 1976). Glucose too inhibits Gal reabsorption in the flounder, and may, like phlorizin, also inhibit 2-d-Gal reabsorption.

In summary, 2-d-Gal shows extensive net secretion by the winter flounder in vivo. Secretion is accomplished via a peritubular carrier-mediated mechanism which may be blocked by galactose. Results using phlorizin and glucose as inhibitors suggest that 2-d-Gal may also be reabsorbed by an apical transport mechanism of lesser magnitude, perhaps via the carrier which mediates galactose reabsorption.

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BILE SECRETORY FUNCTION IN ISOLATED PERFUSED LIVER OF THE LITTLE SKATE, *Raja erinacea* II

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Preliminary studies (Bulletin, MDIBL, 1974) of bile secretory function in the isolated perfused skate liver performed at ambient temperature (22-25°C), demonstrated a linear rise in bile production with increasing perfusion pressure, which was presumably related to hydrostatic filtration through "leaky" intercellular junctions into the bile canaliculus. We therefore modified the previously described technique by maintaining the perfusate at 12-15°C by circulation through a coil immersed in a refrigerated bath. Additionally, the portal vein was perfused through a large glass cannula (inside diameter 2.5-3.0 mm) and the liver was inverted on a perforated petri dish to avoid hilar compression of the portal and biliary system by the weight of the liver and thus to maximize perfusate flow.

Perfusate pressure was varied from 1.5 to 2.0, 2.5, 3.5 and 5.0 cm of H_2O and resulted in a linear increase in perfusate flow as seen in previous experiments at ambient temperature. However, perfusate flow rates were 3 to 4 times greater at each given perfusion pressure increment. Bile flow rates varied considerably but increased with increasing perfusate pressure from 2.2 ± .84 $\mu l\ hr^{-1}\ g^{-1}$ liver at 1.5 cm to 4.17 ± 2.17 at 5.0 cm. There was no relation between perfusate flow and bile flow. Bile/plasma ratios of ^{3}H-inulin (N=5) or ^{14}C-inulin (N=4) also demonstrated considerable variation between experiments, but tended to increase toward unity during the study (0.50-0.92 after 4 hours).

Despite these fluctuations in bile flow and inulin permeability, the isolated perfused skate liver efficiently removed ^{35}S-bromsulphalein (BSP) and ^{14}C-sodium taurocholate (NaTc) from the perfusate. The initial hepatic uptake of ^{35}S-BSP (5 μCi, specific activity 50.2 μCi/mg) demonstrated a $T_{½}$ of 13.5 ± 2.6 min in 9 experiments as compared to a $T_{½}$ of 16.6 ± 2.6 min for the plasma disappearance of BSP in the free swimming elasmobranch (Am. J. Physiol., 230:974, 1976). Although hepatic uptake of ^{35}S-BSP was virtually complete by 2 hours in the isolated perfused skate liver (98.2 ± 0.6% of the administered dose), only small amounts appeared in bile by 4-5 hours. In contrast, 200-400 mg of NaTc, which was also removed efficiently by the isolated perfused skate liver by 2 hours (92.5 ± 5.9% of the administered dose), was largely recovered in bile