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ANOMALOUS BEHAVIOR OF ^{3}H- AND ^{14}C-LABELLED INULIN AND ^{3}H-LABELLED POLYETHYLENE GLYCOL IN INCUBATED KIDNEY TISSUE OF THE WINTER FLOUNDER, *Pseudopleuronectes americanus*.

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Renal function studies in the southern flounder, *Paralichthys lethostigma*, have revealed significant discrepancies between the measured clearances of ^{3}H-methoxy inulin and ^{14}C-polyethylene glycol: the ^{14}C-PEG clearance always exceeded, and was sometimes double, the clearance of ^{3}H-inulin (Hickman, unpublished observations, 1972). The present series of experiments was carried out to establish whether or not ^{3}H-methoxy inulin and other labelled carbohydrates used in renal function studies exhibit transtubular transport, binding, cellular penetration or other behavior that would render them unsuitable for estimates of glomerular filtration rate.

Small, freshly trawled winter flounder, *Pseudopleuronectes americanus*, 75-220 g in weight, were used in two series of experiments. In each experiment of the first series, the kidney of a freshly-killed flounder was teased into 2-5 mg fragments, rinsed in Forster balanced saline (Forster, R.P. and S.K. Hong, Jour. Cell. Comp. Physiol., 51; 259-272, 1958), and transferred to a 20 ml glass vial containing 2 ml of Forster saline to which had been added 5-15 μCi of a ^{3}H-labelled carbohydrate polymer (^{3}H-methoxy inulin, ^{3}H-PEG 1000 MW, or ^{3}H-PEG 4000 MW) and a ^{14}C-labelled carbohydrate (^{14}C-carboxyl inulin, ^{14}C-PEG 4000 MW or ^{14}C-dextran 24,000 number av. MW). The tritium and ^{14}C-labelled compounds were used in randomized combinations in different experiments. The vial was incubated at 15°C with gentle agitation for three hours.

At intervals samples of tissue were removed, weighed, dissolved in a tissue solubilizer, and prepared for liquid scintillation counting. These *in vitro* experiments measured the maximum penetration of the labelled compound into kidney tissue in the absence of glomerular filtration. Since the broken ends of tubules in this preparation close off by constriction (Kinter, W.B., Amer. Jour. Physiol., 211: 1152-1164, 1966), the distribution of the label is presumably entirely extracellular. The results of 11 *in vitro* experiments, summarized in Table 1, show that the "tissue space" or volume of distribution of ^{14}C-PEG (averaging 23.95 percent) was consistently and significantly smaller than that of ^{3}H-methoxy inulin, ^{14}C-inulin, and ^{3}H-PEG which ranged between 34.6 and 44.2 percent. A single experiment with ^{14}C-dextran yielded a space estimate of 24.2 percent almost identical to ^{14}C-PEG. In all experiments maximum space values were reached in about 20 minutes with little or no enlargement with time. Entry was rapid and the difference between the smaller tissue spaces of ^{14}C-PEG and ^{14}C-dextran on the one hand, and the larger tissue spaces of ^{3}H-inulin, ^{14}C-inulin, and ^{3}H-PEG on the other, was established before the first sample was taken at five minutes of incubation.

TABLE 1

Summary of *in vitro* incubation experiments with winter flounder kidney tubule fragments.

Compound	^{14}C PEG	^{3}H PEG	^{3}H PEG	^{14}C DEXTRAN	^{3}H INULIN	^{14}C INULIN
Av. MW	4000	4000	1000	24,000	5,500	5,500
Space Estimates§	21.5	36.2	40.3	24.2	36.9	37.8
	25.4	37.9			34.6	35.1
	24.9	38.6			40.1	36.4
	23.7				35.3	37.5
	24.5				44.2	
	23.7				41.4	
					37.1	
Average ± S.D.	23.95 ±1.37	37.56 ±1.23	40.3	24.2	38.51 ±3.50	36.70 ±1.23

§ $\left(\frac{\text{DPM/mg tissue}}{\text{DPM/}\mu\text{l medium}}\right)$

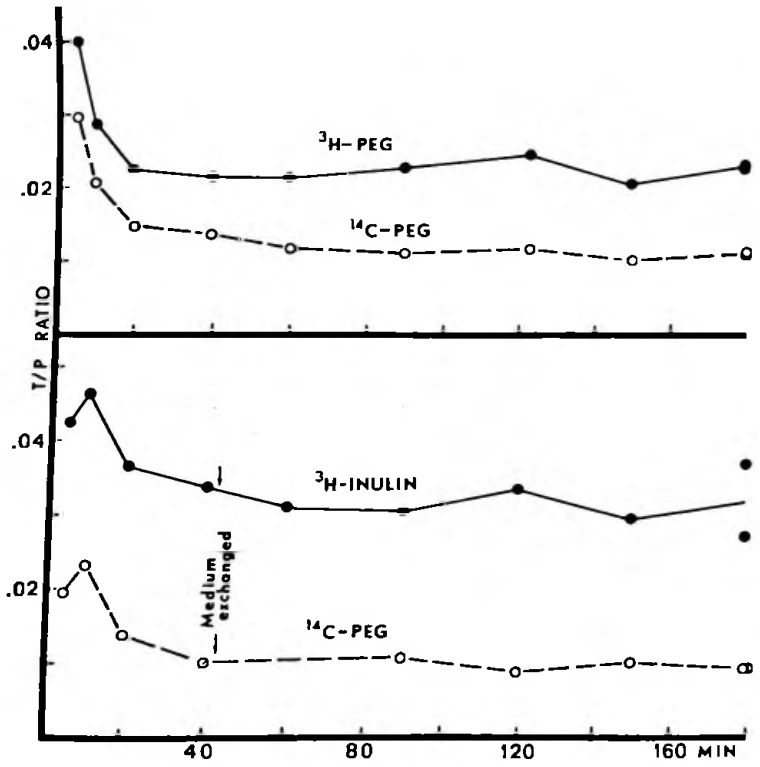

Figure 1: Tissue/plasma ratios of ^{3}H-PEG and ^{14}C-PEG (above) and ^{3}H-inulin and ^{14}C-PEG (below) in winter flounder kidney tubules incubated in unlabelled Forster medium. Isotopes injected i.v. about 20 minutes before kidney removed.

The second series of 10 experiments differed from the first in that the flounders were injected intravenously with the ^{3}H- and ^{14}C-labelled compounds and left undisturbed 15-30 minutes to allow filtration at the glomeruli and uniform dispersion throughout the extracellular space. Then the fish was killed, the kidney removed, and teased into fragments which were incubated in unlabelled Forster saline.

Samples of tissue were collected at intervals and the concentration of ^{3}H and ^{14}C measured by liquid scintillation counting. This incubation is a "wash-out" procedure that measures the amount of radioactivity trapped in the tubular lumens together with whatever activity may be present in some non-exchangeable state in extracellular and cellular spaces.

The results of this series, expressed as T/P ratios, were consistent with the first: in any comparison of ^{14}C-PEG with ^{3}H-inulin, ^{14}C-inulin or ^{3}H-PEG, the ^{14}C-PEG yielded the lower T/P ratio. Two such comparisons are shown in Figure 1. There was considerable variation in absolute T/P values in different experiments, apparently because tissue penetration was dependent on the length of the post-injection period before the fish was killed (this was not precisely timed) and differences in circulatory dispersion rates. The time-dependency of the results was dramatically illustrated by a flounder given a 12.5 hour post-injection period before sacrifice. As shown in Figure 2, the ^{3}H-inulin T/P ratio had risen to an average 1.19 as compared to the average ^{14}C-PEG ratio of 0.096, a greater

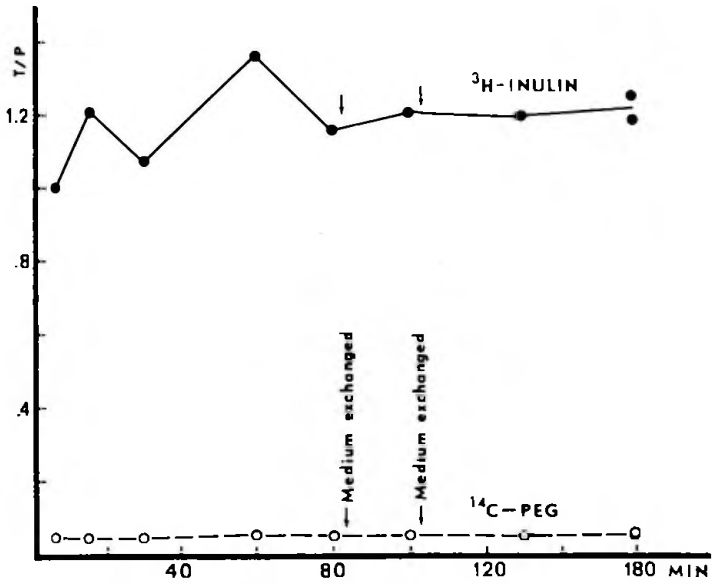

Figure 2: T/P ratio of ^{3}H-inulin and ^{14}C-PEG in winter flounder kidney tubules incubated in Forster medium. Isotopes injected i.v. 12-1/2 hours before kidney removed. Forster medium was drained and replaced with fresh medium at the two times indicated.

than 12-fold concentration difference. It is evident that the ^{3}H-inulin is firmly associated with the tissue since there was no run-out during the three-hour incubation period in unlabelled Forster medium. In this same experiment, the whole kidney tissue space 12.5 hours after injection (freshly excised, non-incubated tissue) was 174.7 percent for ^{3}H-inulin as compared to 29.6 percent for ^{14}C-PEG. In contrast, U/P ratios were 1.72 for ^{3}H-inulin and 2.44 for ^{14}C-PEG. These results convincingly show that ^{3}H-inulin is progressively accumulated in kidney tissue, whether by binding or cellular penetration, and is not quantitatively excreted by glomerular filtration. Since both series of experiments showed that ^{3}H-PEG and ^{14}C-inulin, like ^{3}H-inulin, also exhibited cellular penetration and/or binding phenomena, it seems clear that no carbohydrate polymers is ideally

suited for renal function studies in flounder. By comparison ^{14}C-PEG showed no evidence of irreversible penetration into kidney tissue.

We attempted to establish the nature of the association between these labelled compounds and flounder kidney tissue in a single saturation study, using three widely different concentrations of ^{3}H- and ^{14}C-labelled inulin and ^{3}H- and ^{14}C-labelled PEG. Although not conclusive, the results suggest that of these four compounds, only ^{3}H-PEG exhibited saturation kinetics and, hence, binding behavior. Neither ^{3}H- nor ^{14}C-labelled inulin or ^{14}C-PEG showed evidence of saturation kinetics. Thus inulin, either as such or as fructose residues, may be penetrating into cells or other non-exchangeable space, a behavior that is consistent with the absence of saturation kinetics.

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ELECTROPHYSIOLOGY OF THE PERFUSED RECTAL GLAND OF *Squalus acanthias*, IN VITRO

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Palmer first demonstrated that the rectal gland of *Squalus acanthias* perfused *in vitro* secreted a fluid whose chloride concentration was greater than that of the perfusate (Bulletin Mt. Desert Island Biol. Lab. 5:32, 1965). Hayslett and co-workers (Bulletin Mt. Desert Island Biol. Lab. Vol. 12, 1972) have extended that observation. In the present study the isolated rectal gland was perfused via its artery by the same solution used by the latter scientists: Na^{+} 281, Cl^{-} 297 and K^{+} 6 mEq. The artery was catheterized with polyethylene tubing #50. Perfusion was carried out at a hydrostatic pressure head of 85 cm H_2O resulting in a flow of about 2 ml/min. The gland was partially submerged in a petri dish held at 15 C by a thermoelectric heat-exchanger. Luminal fluid was allowed to drain by gravity through #90 polyethylene tubing either into a beaker containing perfusion-saline or collected under oil.

The electrical potential difference, PD, between the perfusion reservoir and the lumen was measured using a pair of salt bridges, polyethylene tubing #240 filled with 275 mM NaCl and 3 percent agar. One salt bridge was placed between the reservoir of perfusion saline and a reference calomel cell, "Radiometer" K401, in a beaker with 1 M KCl. The second bridge was used to establish contact with the outflow and another calomel half-cell. One end of the bridge was in a beaker containing some perfusion-saline into which solution flowed from either the arterial or luminal cannula while the other end was in 1 M KCl bathing the second calomel cell. The convention employed for the PD is such that the reference electrode is in electrical continuity with the interstitial compartment and the reported PD is the value for the lumen with respect to interstitial fluid. At the start and end of each experiment, the asymmetry PD was measured between the pair of bridges and calomel cells by allowing perfusion fluid to flow through the arterial cannula into the beaker of perfusion-saline. The mean asymmetry PD was: initial -1.4 ± 1.2; terminal -0.9 ± 1.4; and mean difference