that a shunt pathway was short-circuiting the potential. Morphologically this shunt may correspond to either extracellular channels between the myocardial cells or else to the longitudinal raphe of the heart where the myocardial cells form a seam from which arises the pericardium.

Numerous micropunctures of myocardial cells with a glass microelectrode demonstrated an intracellular resting potential of -65 ± 5 mV. When high concentrations of KCl were applied to the extraluminal surface of the heart, the resting potential was unaffected. With high concentrations of KCl intraluminally however the myocardial cells promptly depolarized. On the basis of these observations we constructed an equivalent circuit for the myocardium. The component of the circuit had the following values: $R_m = 1000$ ohm-cm^2, $R_s = 220$ ohm-cm^2, and $E_m = -65$ mV.

In addition to confirming more accurately our impression that the tunicate myocardium shares most of the major electromechanical properties of mammalian myocardium our experiments have more precisely characterized the nature of the tunicate myocardium. In particular excitation of the inner (luminal) membrane only of this preparation is coupled to the contractile process whereas the outer (extraluminal) membrane is relatively inert. The graded contractile response is unique among the myocardial preparations studied so far. Supported by USPHS HL 13288 - 03 and HL 16152 - 01.

1973 #51

COMPARISON OF RENAL CLEARANCE OF SEVERAL GLOMERULAR MARKERS IN WINTER FLOUNDER

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The previously reported observation of significant differences between inulin and ^{14}C-polyethyleneglycol (PEG) clearances in southern flounder (Hickman *et. al.* Bull. MDIBL 12:47-50, 1972) and the demonstration of cellular accumulation of ^{3}H-inulin, ^{14}C-inulin, and ^{3}H-PEG but not ^{14}C-PEG in kidney and other tissues of winter flounder (Hickman *et. al.* and Schmidt-Nielsen *et. al.* Bull. MDIBL 12:99-103, 1972) have raised a serious question of whether inulin is a reliable measure of glomerular filtration in renal clearance studies in fish. In the present investigation this problem was further studied by comparing the clearance of four compounds namely non-labelled inulin, ^{3}H-inulin, ^{14}C-PEG, and creatinine in winter flounder.

The flounder were kept in aquaria equipped with running sea water for four to 14 days before experimental use. Procedures for handling the fish were essentially those of Maack and Kinter (Am. J. Physiol. 216:1034-1043, 1969). Bladder urine was collected for three to four consecutive two-hour periods through a polyethylene catheter (PE 90) inserted into the urinary papilla and tied securely in place. Gentle pressure was applied externally over the bladder at hourly intervals to assure expression of urine not spontaneously voided. Blood samples, 0.4 ml., were obtained by caudal vein puncture at the beginning, midpoint, and end of each experiment. Plasma concentrations at the midpoint of each urine collection period were determined by interpolation. All test compounds were obtained commercially, ^{14}C-PEG (mol. wt. 4000) from New England Nuclear Corp. (Boston), ^{3}H-inulin from Amersham-Searle Corp. (Chicago), the non-labelled inulin, and creatinine

from Nutritional Biochem. Corp. (Cleveland). Samples of urine and plasma were dissolved in Bray's solution and counted in a Nuclear-Chicago automatic scintillation counter. Inulin in the urine and $ZnSO_4$ plasma filtrates was determined by the method of Walser *et. al.* (J. Clin. Invest. 34:1520-1523, 1955), creatinine by the alkaline picrate method of Folin and Wu (J. Biol. Chem. 38:81-110, 1919).

To establish the optimal route of administration of the compounds the rates of absorption were determined in three fish given doses of ^{3}H-inulin, creatinine and non-labelled inulin simultaneously by i.v., i.m., and i.p. injection. It was found that the plasma level of inulin steadily increased to a maximum within four hours after i.p. injection, then remained relatively steady over the next

TABLE 1

COMPARISON OF THE RENAL HANDLING OF NON-LABELLED INULIN, ^{14}C-PEG AND ^{3}H-INULIN IN WINTER FLOUNDER. INULIN DOSE 300 mg/kg; ^{14}C-PEG AND ^{3}H-INULIN GIVEN IN TRACER AMOUNTS.

FISH No.	WT. kg.	INULIN (nonlabelled)		14C-PEG		3H-INULIN	
		U/P	UV/P ml/hr.	U/P	$\dfrac{U/P_{PEG}}{U/P_{IN}}$	U/P	$\dfrac{U/P3_H}{U/P_{IN}}$
I. Inulin and ^{14}C-PEG i.p. 16 hrs. prior to collection							
1	0.245	1.56 ± 0.21 (3)*	0.479 ± 0.04	1.40± 0.15	0.956± 0.05		
2	0.220	2.81 ± 0.41 (4)	0.92 ± 0.11	2.60 ± 0.42	0.92± 0.016		
3	0.325	2.50 ± 0.24 (3)	0.59 ± 0.117	3.01± 0.32	1.20± 0.033		
II. Inulin and ^{14}C-PEG i.m. 2 hrs. prior to collection							
4	0.255	0.975 ± 0.01 (3)	0.196 ± 0.06	1.20± 0.12	1.23± 0.117		
5	0.450	1.72 ± 0.225 (4)	0.496 ± 0.05	1.67± 0.008	0.97±		
6	0.285	1.59 ± 0.23 (3)	0.278 ± 0.07	1.55± 0.21	0.98± 0.017		
III. Inulin and ^{3}H-inulin i.p. 20 hrs. prior to collection							
7	0.605	1.24 ± 0.03 (3)	1.25 ± 0.147			1.39 ± 0.07	1.12 ± 0.06
8	0.455	1.70 ± 0.22 (4)	2.86 ± 0.60			1.68 ± 0.04	0.91 ± 0.03
9	0.395	1.29 ± 0.16 (4)	0.75 ± 0.11			1.27 ± 0.17	0.98 ± 0.05

*Mean ± S.E. The number in the parenthesis denotes the number of 2 hr. collection periods.

28 hours. Creatinine was also rapidly absorbed following i.m. injection reaching a maximum plasma level within one hour which was followed by a seven to eight hour period of decline. All subsequent experiments presented here were performed either 16-24 hours after i.p. injection or two hours after i.m. injection of the test compounds.

Nine experiments were performed in which the clearance of non-labelled inulin was compared with either ^{14}C-PEG or ^{3}H-inulin. Results are presented in Table 1. The renal clearance of inulin was highly variable among different animals ranging from 0.14 to 3.8 ml/hr. in 53 fish used in these and other studies but was quite constant in individual fish. The inulin U/P ratios, also ranged widely from 0.88 to 2.81 but were likewise constant in individual fish. No apparent difference was detected in the renal clearance of non-labelled inulin, ^{14}C-PEG and ^{3}H-inulin; the mean U/P_x to U/P_{inulin} ratios were 1.04 for ^{14}C-PEG (20 observations in 6 fish) and 1.01 for ^{3}H-inulin (11 observations in three fish). It thus appears that regardless of differences in the distribution volume in kidney and other tissues of winter flounder these compounds are equally suitable for use as glomerular markers in this species.

Table 2 presents data on the renal clearance of creatinine in winter flounder which was found

TABLE 2

RENAL HANDLING OF CREATININE IN WINTER FLOUNDER

FISH No.	WT. kg	INULIN* U/P	INULIN* UV/P ml/hr.	CREATININE* U/P	CREATININE* $\dfrac{U/P_{creatinine}}{U/P_{inulin}}$
1	0.250	1.44 + 0.115 (4)**	0.43 + 0.03	14.27 + 2.62	9.70 + 1.12
2	0.245	1.45 + 0.125 (4)	0.38 + 0.14	13.50 + 0.84	9.37 + 0.65
3	0.325	1.00 + 0.13 (4)	0.207 + 0.06	19.00 + 0.39	19.50 + 1.92
4	0.605	1.23 + 0.02 (4)	1.22 + 0.01	11.19 + 0.58	10.65 + 1.65
5	0.455	1.85 + 0.09 (4)	2.85 + 0.60	10.80 + 1.43	5.77 + 0.55
6	0.395	1.29 + 0.16 (4)	0.747 + 0.11	6.44 + 0.48	4.20 + 0.53
7	0.245	1.56 + 0.21 (4)	0.478 + 0.04	7.67 + 1.10	5.03 + 0.78
8	0.220	2.98 + 0.37 (4)	0.92 + 0.11	22.85 + 2.15	7.76 + 0.51

* Inulin, 300 mg/kg and creatinine, 300 mg/kg, were administered i.p. 16-24 hrs. prior to collection, except in fish No. 7 and 8, they were administered i.m. 2 hrs. prior to collection.

** Mean + S.E., The number in the parenthesis denotes the number of 2 hr collection periods.

to be much greater than that of inulin. The mean U/P_{cr} to U/P_{inulin} ratios ranged from 5.03 to 19.5 indicating net tubular secretion of creatinine.

Data presented here clearly show that there is no significant difference in the renal clearances of non-labelled inulin, ^{3}H-inulin, and ^{14}C-PEG in the winter flounder. In contrast creatinine undergoes net tubular secretion.

Recently Knoefel, *et. el.*, observed an interesting phenomenon in the distribution of inulin in the dog (Life Sciences 1973, in press). They found that when non-labelled inulin was given simultaneously with ^{3}H-inulin the distribution volumes (V_d) of the two compounds were identical and steady but when tracer ^{3}H-inulin was given alone, without non-labelled inulin as a carrier, the V_d was always greater than before and slowly increased with time. The explanation was offered that when tracer amounts of inulin were injected a relatively large fraction of the drug was bound to tissues, resulting in a larger calculated V_d, when large doses of non-labelled inulin were injected with ^{3}H-inulin, the binding sites were saturated with a sufficiently small fraction of the total amount that binding no longer had a demonstrable effect on the calculated V_d of either compound. This finding may be relevant to the apparent discrepancy between the inulin clearance data reported here for winter flounder and that previously observed in southern flounder or to the results of tissue accumulation studies.

1973 #52

THE RATE OF AQUEOUS HUMOR FORMATION IN *Squalus acanthias*

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In connection with determining the accession rates of electrolytes from plasma into the aqueous humor of dogfish (Maren, *et. al.,,* this Bulletin), a knowledge of the volumes and rates of production of this fluid was important. Therefore an attempt was made to measure these rates by the steady state dilution of inulin perfused through the anterior chamber. This technique has been used previously to measure aqueous formation rates in the cat eye (Invest. Ophth. <u>6</u>: 76-83, 1967) where values were obtained which agreed with those from other methods.

Male and female dogfish weighing from 1.5 to 3.0 kg were placed in a trough-like wooden holder. The spiracles were perfused with sea water (16°C) at a rate of 1.5 L/minute. The eye was anesthetized with Xylocaine Jelly®. The lateral postlimbal conjectiva was grasped with a tweezer and two 22-gauge needles for the inflow and outflow of perfusate were introduced into the anterior chamber. The positions of the needles were varied from fish to fish. The needles were connected by polyethylene tubing to glass syringes driven by an infusion/withdrawal pump. One or two 5-mm long pieces of the needle stylus were pushed through one of the needles into the anterior chamber. These "iron bars" could then be rotated inside the aqueous chamber by means of a magnet held at