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.

This work was supported by grant from NSF GB-27495 Al.

[1]T.S.T. Chen is a Kentucky Heart Association Postdoctoral fellow.

1973 #52

THE RATE OF AQUEOUS HUMOR FORMATION IN *Squalus acanthias*

Per J. Wistrand and Anthony B.C. Talalay, Department of Medical Pharmacology, University of Uppsala, Sweden; and Department of Pharmacology and Therapeutics, University of Florida College of Medicine, Gainesville, Florida

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. 6: 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

three cm from the eye. Fluorescein was added to the perfusion fluid so that under ultraviolet light adequate mixing of aqueous humor could be observed. At the perfusion rate of 13.6 µl/minute used in many of the experiments the fluorescein "cloud" travelled the distance, ~10 mm, from the tip of the inflow needle to the tip of the outflow needle in eight minutes. During this transit the fluorescein was found to fill the whole of the pupil and the anterior chamber. Thus no laminar stream between the two needles was observed. Inulin-14_c in a shark Ringer solution, containing 80 units per ml of heparin, was perfused at different rates through the anterior chamber and the outflow perfusate was collected into the withdrawal syringe. For the initial one hour of the experiment the perfusion rate was kept high to facilitate mixing and equilibration. This rate was then decreased to the desired rate for the rest of the experiment. The outflow was usually collected in half or one-hour periods, depen-depending on flow rate. The total duration of the experiment varied between one and five hours.

Some fish were brought back to the live car and were used again the next day for a similar experiment on the second eye. Inulin radioactivity of the perfusion fluid of the inflow and outflow sides was determined using a liquid scintillation system. Aqueous humor formation rates were calculated using the standard equation derived by Heisy *et. al.* (Am. J. Physiol. 203:775-781, 1962).

The mean values of the aqueous formation rates are seen in Figure 1. The aqueous humor is produced at a constant rate of about 1 µl/min during the four and a half hours of perfusion. Attempts to measure the volume of aqueous humor by injecting inulin into the eye and measuring its dilution were not successful, yielding values clearly higher than observed when aqueous fluid was withdrawn carefully with a syringe. From the latter procedure we estimate the volume to be 0.25 ml, whence the rate constant for fluid production is .004 min^{-1}, about one-third what is found in mammals (Davson, The Eye, 2nd edition, Academic Press, 1969).

The inulin perfusion technique might overestimate the rate of production of aqueous humor, due to diffusion of inulin into the tissues adjacent to the aqueous humor, e.g., the vitreous body. It

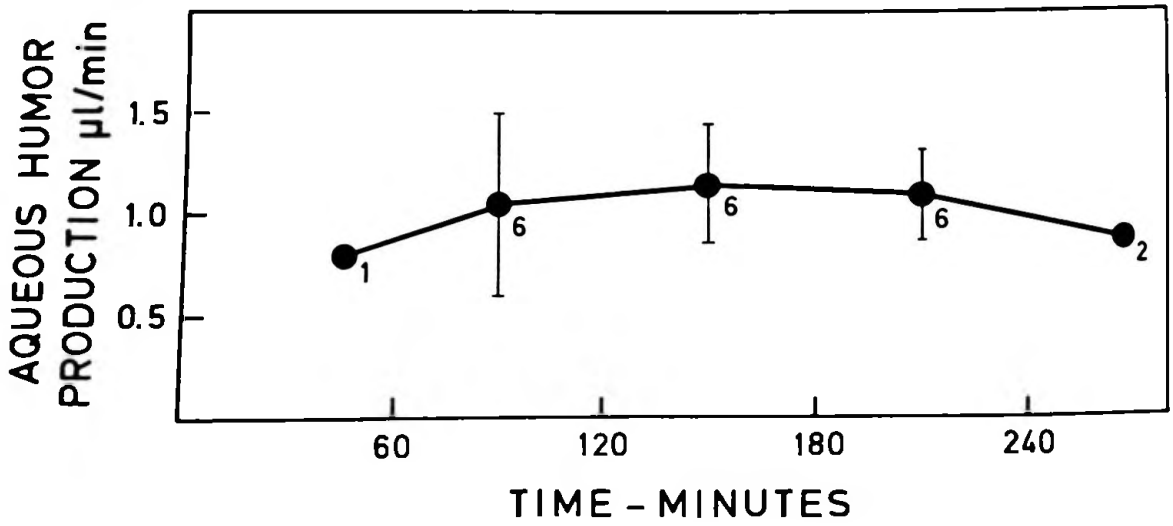

Figure 1. Aqueous humor formation rates in dogfish. Inulin perfusions were performed in eight eyes of six dogfish for four and one half hours. Perfusion rates were varied between 68 and 3.4 µL/min. Vertical bars indicate ± 1 s.e.m., numbers represent number of eyes.

has been reported (Bull. MDIBL 12:90-91, 1972) that when cerebrospinal fluid secretion was measured in the dogfish by the steady state marker dilution technique the use of larger less diffusible markers such as blue dextran and serum albumin appeared to give values for secretion one-half of those obtained when inulin was employed (Comp. Biochem. Physiol. 12: 171-177, 1964). However the conditions in the eye might be more favorable for the use of inulin since our data do not indicate an appreciable loss of marker from the perfusate by diffusion. The values for the formation rate did not change despite a 20-fold variation in the perfusion rates.

An attempt was also made to record the intraocular pressures of the dogfish eye. These were measured in two fish swimming in a 25-gallon tank of sea water. The fish were restrained under water with a rubber diaphragm loosely fitted around the trunk. A 20-gauge needle was introduced into the anterior chamber and connected to a water manometer (inner bore 2.5 mm) by a 15 cm piece of polyethylene tubing. The system was filled with heparinized dogfish Ringer solution. The pressures were read after they had stabilized and were found to be 1.0 and 0.4 cm H_2O in the two fish. These very low pressures are roughly equivalent to venous pressure in this species.

This work was supported by NIH grant GM AI 16934 to Dr. Thomas Maren and by grant 04R-4154 to the author from the Swedish Medical Research Council.