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Renal Excretion of Tetracycline in the Aglomerular Toadfish, *Opsanus tau.** (28737)

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The renal excretion of tetracycline by the dog and man has been claimed to be affected by glomerular filtration alone(1,2). However, Sullivan and Fanelli(3) have adduced evidence in the dog that tritiated tetracycline is excreted by the renal tubules in addition to filtration at the glomeruli when the clearance is corrected for the amount of free drug in the plasma water relative to the creatinine clearance. The mechanism of renal excretion was attributed to nonionic diffusion(4). In the course of this work is was deemed of considerable interest to know whether or not tetracycline could be excreted by the aglomerular kidney which is characteristic of certain marine teleosts. The natural choice, therefore, fell upon the aglomerular toadfish *Opsanus tau* because of its ready availability and extreme hardiness in captivity. This fish is an excellent experimental animal in which to study the process of renal tubular excretion(5).

Methods. Thirty-two quantitative urine collection periods were secured from 20 toadfish weighing 155 to 440 g. All toadfish were obtained from Great South Bay, L. I., and housed in running sea water or in full strength artificial sea water. Tritiated tetracycline‡ (specific activity 5.1 μc/mg) in doses of 5 to 50 mg/kg was administered intramuscularly in a small volume of distilled water after obtaining control blood and urine samples. In most cases a control blood sample was omitted as it was found that radioactive background counts from control toadfish plasma were very uniform from animal to animal. Stress on the hemodynamics of the fish were thereby reduced to a minimum. After a period of 4 to 6 hours during which tetracycline became distributed through the body fluids a polyethylene catheter was inserted into the urinary papilla and secured in place. The free end of the catheter tubing was plugged and anchored to the anal fin by 2 loose ties. Urine collection periods were 5 to 18 hours in duration. A blood sample was secured at the end of the urine collection period by puncture of the caudal vessel. Under these conditions the rate at which the plasma concentration of tetracycline falls is so slow that a blood sample drawn at the end of the period suffices for calculation of urine

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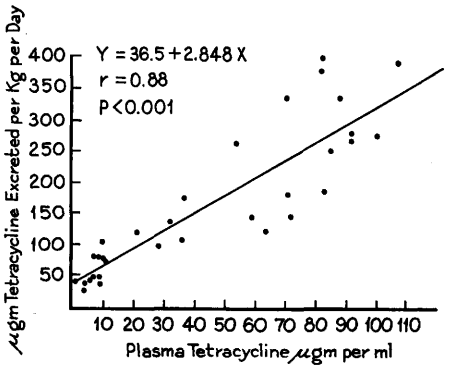

FIG. 1. Relationship between urinary excretion of tetracycline and total plasma concentration in the toadfish.

to plasma ratios. This is borne out by 2 experiments in which the plasma concentration of tetracycline after absorption did not decrease by more than 15% in a succeeding 24-hour period. However, blood samples taken at the end of a collection are considered to contain a minimal plasma tetracycline concentration for that period. In some instances a second and higher dose of tritiated tetracycline was injected in order to study the renal excretion at low and high plasma levels. The second urine collection period was begun 4 to 6 hours later and another blood sample was drawn at the end of the period. Radioactive counting of tritium was performed using a Packard Tri-Carb Liquid Scintillation Spectrometer employing an internal standard. The counting procedure followed was that of Takesue *et al*(6). Rate of excretion of tetracycline was calculated as the product of the urinary concentration times the urine flow in ml/kg/day and has the dimensions of μg/kg/day.

Results and discussion. Fig. 1 portrays a mass plot of the rates of urinary excretion at various plasma levels of tetracycline. The rate of renal excretion of tetracycline increases in a linear manner with the plasma concentration. Assuming the relationship depicted in Fig. 1, the correlation between tetracycline excretion and plasma concentration is 0.88 and is significantly different from zero ($P<0.001$). The regression line is also given. There is no evidence for an excretion maximum at the higher plasma levels as observed for excretion of phenol red(7) and creatinine(8) in the toadfish. In this limited study it is not known whether the rate of renal excretion of tetracycline is related to urine flow as these fish were presumably in a state of "laboratory diuresis" because of experimental manipulation(9). This is evidenced by the fact that both urinary and plasma chlorides progressively increased from handling toadfish in a manner simulating the renal excretion experiments.

Urine to plasma tetracycline concentration ratios ranged from 0.44 to 0.88 (mean 0.59) and there was no consistent trend in these values at any plasma concentration of tetracycline. Since the fraction of tetracycline free in the plasma water was unknown these ratios are somewhat equivocal.

Attempts to correlate urinary excretion of tetracycline with urine pH to ascertain whether or not the antibiotic in this species was excreted by nonionic diffusion were unsuccessful. Urine pH values indicated that the pH does not appear to be rigidly fixed at pH 5.75 as is the case for the dogfish and marine sculpin(10) but varies from approximately 6.5 to 7.5. Intraperitoneal injections of sodium sulfate or sodium bicarbonate did not alter the urine pH from control values.

The data reported indicate that tetracycline is excreted by the aglomerular kidney by a diffusion process in the direction of peritubular blood to tubular urine. The mechanism is passive in that there is a direct relation between total plasma concentration and rate of tubular excretion and, in this respect only, gives evidence against an active tubular secretory process. However, since no information is available regarding the binding of tetracycline to plasma proteins the presence of competitive excretory phenomena or of transtubular pH gradients and potential differences in this species, this interpretation must be viewed with caution. Since the toadfish kidney is entirely devoid of glomeruli (11) it is obvious that a glomerulus is not necessary for excretion of this antibiotic.

Summary. Tritiated tetracycline, after injection into the aglomerular toadfish appears to be excreted by a diffusion process. It has

been shown that the aglomerular renal tubule is permeable to a highly complex molecule such as tetracycline.

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Isolation of Human Plasma Prothrombin of High Specific Activity by Gel Filtration.* (28738)

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In previous studies(1), ultracentrifugal analyses of preparations of human plasma prothrombin of high specific activity indicated that the homogeneity of the protein as judged from the schlieren pattern was dependent upon the pH and ionic strength of the solvent. In addition, sedimentation velocity studies in a partition cell demonstrated that the activity of the zymogen sedimented at a rate greater than that of the majority of the protein. Since there was no distinct component in the schlieren pattern which could be attributed to the prothrombin activity, it was concluded that the specific activity of this zymogen must be considerably higher than previously suspected.

The present communication describes attempts further to fractionate these preparations by gel filtration, using solvent conditions in which the difference in molecular dimensions between the active zymogen and non-zymogen protein would be maximal and hence afford the most suitable conditions for fractionation and purification. The results obtained support previous speculations that human plasma prothrombin has a specific activity at least twice that of the bovine species.

Materials and methods. Prothrombin was isolated from freshly collected human ACD plasma by a procedure previously described (1). Prothrombin activity was measured by the 2-stage method of Ware and Seegers(2), using Lot 3B NIH thrombin as reference. Protein concentration was determined by the method of Daughaday *et al*(3), as well as by a micro-modification of the biuret method of Henry *et al*(4). Specific activity measurements are expressed in terms of NIH units/mg protein.

Sephadex G-75 (medium), G-100 and G-200 gel columns were prepared as outlined by Flodin(5). Column dimensions were 1.3 × 30 or 1.3 × 60 cm. As suggested by Flodin (5), effluent volumes are expressed as the elution volume (V_e); this is the volume of eluant required to move the peak concentration from the top to the bottom of the gel bed. This necessitated a correction for the volume contained in the drip point of the

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