1963 #19

EFFECTS OF HYPOPHYSECTOMY, GONADECTOMY AND ADRENAL CORTICAL INHIBITING DRUGS (AMPHENONE AND METOPIRONE - CIBA) ON THE REGENERATION OF THE PECTORAL FIN IN Fundulus heteroclitus (KILLIFISH)

R. A. Liversage and B. M. Burnet, University of Toronto, Cana.a

Originally Schotté, '26 and more recently Schotté and collaborators (1951-1961) have demonstrated that an intact pituitary (presumably the pituitary-adrenal axis) is essential for the initiation phases of forelimb regeneration in the adult urodele (Triturus). Contrary to this, hypophysis extirpation in urodele larvae has no inhibitory effect on the degree of forelimb regeneration; only the rate is retarded slightly (Schotté '61; Liversage, '63 [in press]).

The first experimental series is a continuation of research from the summers of 1961 and 1962 and now includes 323 young and adult, male and female, Fundulus and consists of: a) hypophysectomy followed immediately by fin amputation; b) hypophysectomy followed in 5 days (recuperation period - Liversage '59; '62) by fin amputation; and c) fin amputation followed in 5 days by hypophysectomy. Pituitary extirpation in Fundulus results in hormonal imbalances (Pickford & Atz '57); nevertheless, in these experiments, normal regeneration ensued in all cases. Pectoral fin regeneration in killifish appears, therefore, to be completely independent of the presence of the pituitary gland.

There is the possibility that partial or complete inhibition of fin regeneration (Gorbman and Bern '62) occurs during the non-reproductive period in Fundulus due to variations in the normonal background.

Preliminary experiments were undertaken, during July (1963), to test this possibility and involved: 1) gonadectomy; 2) gonadectomy and amphenone (CIBA) injections (0.7 mg/day); 3) gonadectomy and hypophysectomy; 4) amphenone injection (0.7 mg/day); and 5) metopirone injection (0.7 and 0.35 mg/day) followed by immediate or delayed (3 to 5 days) amputation in respect to injection of adrenal corticosteroid inhibitors and/or surgery.

In these preliminary experiments, fin regeneration ensued in the great majority of cases and appears to be independent of influences from the pituitary, gonads and adrenal cortices. Presumably, it is independent also of the combined influences of these endocrine organs.

Supported by grants from the National Research Council of Canada and the University of Toronto.

1963 #20

DISTRIBUTION AND EXCRETION OF AN ANIONIC DRUG IN Squalus acanthias

T. H. Maren and D. K. Maren, University of Florida, Gainesville, Fla.

Our primary question was whether a typical anionic drug is concentrated and secreted by the gill, in the same manner as shown classically by uptake of para-aminohippurate and congeners into the mammalian and fish kidney.

The drug used was 2-benzenesulfonamido-1,3,4-thiadiazole-5-sulfonamide (CL 11,366). Its pKa is 3.2 and in the dog its renal excretion and uptake closely resemble that of PAH (Travis et al., J. Pharm. and Exptl. Therap., 144, Feb. 1964). It is a powerful carbonic anhydrase inhibitor ($K_I = 5 \times 10^{-9}$ M), which enables its detection at very small concentrations (J. Pharm. and Exptl. Therap., 130:269, 1960) and allows an assessment of the physiological consequences of the distribution pattern.

One mg (3 μmoles) per kg was injected intravenously. Two hours later, the plasma pCO_2 had risen from 8 to 23 mm Hg—the typical effect of carbonic anhydrase inhibition in this species (Hodler et al., Am. J. Physiol., 183:155, 1955; Maren, Comp. Biochem. Physiol., 5:201, 1962). This lasted for 24 hours. The following gives mean (n = 3) tissue concentrations (μM) and renal clearances (ml/min) during the peak effect.

Time, hrs.	Plasma	RBC	Gill	Kidney	Urine	Clearance
2	1.0	3.3	0.5	11	300	4
6	0.4	3.6	0.2	2	1500	15

The drug is excreted nearly quantitatively by the kidney in 24 hours. U/P ratios reflect the high rates at which the fish can secrete such an anion. They are much higher than previously measured for PAH (Forster and Berglund, J. Cell. Comp. Physiol., 49:281, 1957) because the plasma concentrations of CL 11,366 are far below saturation of the renal transport system. Uptake into kidney is similar to that observed in the dog.

CL 11,366 is relatively excluded from the gill. From the known carbonic anhydrase concentrations of red cell (0.55 μM) and gill (0.63 μM), the K_I of the drug, and its concentrations in the tissues, it may be calculated that inhibition in the red cell is 99.8% complete but that in the gill it is negligible. The pCO_2 elevation is then a reflection of the red cell effect; it does not seem necessary to invoke the gill in establishment of CO_2 equilibria. Gill carbonic anhydrase may have another function; as in the nasal gland of sea gulls and the rectal gland of S. acanthias, it may be involved in NaCl secretion.

Supported by Grant NB-1297 of National Institutes of Health.

1963 #21

THE PHARMACOLOGY OF THE GILL CIRCULATION IN *Squalus acanthias*

E. Morkin, C. Shub, and A. P. Fishman, Columbia University, New York, N. Y.

The present study extends the previous observations of Burger and Bradley (J. Cell. Comp. Physiol., 37:389, 1951) on the regulation of the gill circulation of the dogfish. For this purpose, blood pressures were simultaneously recorded from the dorsal and ventral aortas of more than 30 spontaneously respiring dogfish following the administration of atropine sulfate (25 to 50 μg/Kg), norepinephrine (4 to 16 μg/Kg), acetylcholine (30 to 80 μg/Kg), or the infusion of whole blood or Ringer's solution into the caudal vein or ventral aorta. Pressures were transduced with Statham gauges and recorded with a direct-writer apparatus.

The control ventral and dorsal aortic pressures in 13 dogfish averaged 36/23,29 and 28/19,24 mm Hg, respectively; the average heart rate was 22 beats per min. After atropine, the heart rate