## RESULTS AND DISCUSSION

Plethodon cinereus is a terrestrial, lungless salamander and appears to be more sensitive to the effect of carbonic anhydrase inhibition than the mammals in earlier studies. Doses which produced no reported toxicity to pregnant female rodents were lethal to the salamander. Even the lower doses (30 and 100mg/kg) produced a high mortality rate at 10 days after initial drug injections (47% and 44% respectively). Although we have not yet explored this observed high toxicity, several explanations are immediately evident. In healthy mammals with unimpaired lung function, the chief compensation for inhibition of red blood cell carbonic anhydrase is diffusion of gaseous $CO_2$ across lung epithelium (Swenson and Maren, Resp. Physiol. 35:129-159, 1978). It is therefore, not surprising that lungless salamanders would show elevated sensitivity to a carbonic anhydrase inhibitor. Furthermore, the main route of acetazolamide excretion in all species studied thus far is the kidneys and in lower vertebrates (e.g., dogfish sharks), this is a relatively slow process (Maren, Comp. Biochem. Physiol. 5:201-215, 1962). Therefore, it is likely that high systemic levels of the drug are reached with the present protocol though we do not yet know the fate of acetazolamide in this species.

Twenty-nine of the 68 animals whose limbs were amputated survived the 50 day post-amputation period. These represented specimens receiving drug injections on days 6-8, 8-10, 11-13 and 14-16 of regeneration. These periods were selected as times when critical morphogenetic interactions, which would be comparable to those disrupted by acetazolamide in 9-10 day rodent embryos, occur (Holmes and Trelstad, op. cit). Furthermore, they cover the period which appears thalidomide-sensitive during newt limb regeneration (Bazzoli et al., J. Embryol. exp. Morph. 41:125-135). Each of the experimental animals in these groups possessed bilateral regenerates demonstrating that acetazolamide does not block the initiation of the epimorphic response nor subsequent outgrowth of the regenerate.

Morphogenesis of the regenerate progressed normally in a vast majority of the pooled regenerates. Seventy-four percent had superficially normal, 4-digit hands. An additional 23% were at late blastema to late paddle stages of regeneration which, though delayed, is within the normal range of variability for this 45-50 day regenerative period. Subsequent histological analysis of precartilage patterns in these regenerations will likely resolve some of them into specific digital pattern commitment. Two of the 58 limbs analyzed have surface indications of only three digits each. While this represents a 3% rate of gross malformation, Dearlove and Dresden (J. Exp. Zool. 196:251-262) found that over 6% of ordinary newt limbs will produce abnormal regenerates. A comparable study has not been done for this species, but a low percentage of anomalous regenerates is probable. Since one was from the 300mg/kg group and the other from a 30mg/kg group, it is unlikely that any reduction abnormality is related to a specific drug effect.

We conclude that while acetazolamide is more toxic to adult Plethodon cinereus than to adult rodents, it does not disrupt morphogenesis of regenerating salamander limbs. Since comparable doses of this drug create specific limb defects in mammalian embryos, the regenerating urodele limb may not be a good analog for limb development. It is suggested that at least some components active in morphogenetic information transfer in these two systems are unique. Further exploration of acetazolamide-induced limb anomalies may clarify these inductive mechanisms.

## FURTHER OBSERVATIONS ON TWINNING IN THE SPINY DOGFISH

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In 1976, we reported the first observation of twinning in the spiny dogfish (A.D. Woodhead, Bull. MDIBL 16:106), one instance in 270 adult female fishes samples. Although it has generally been concluded that developmental abnormalities are rare in elasmobranchs, as compared with teleosts, we found a second case of twinning this

summer, making two instances in a total 454 adult females sampled since 1976. This suggests that the phenomenon may not be as rare as previously supposed.

As was found in 1976, the twins were only attached by their "yolk sac cords" to a single common yolk sac, their bodies were quite separate one from the other; they showed no other anatomical abnormalities. The complete separation of both sets of twins indicates splitting of the embryo at the earliest stages of development. Double yolked eggs are common in birds which also have megalecithal eggs but such twins are the result of two eggs being enclosed in a single shell during passage through the reproductive tract; double blastomeres appear to be extremely rare.

The female carrying the twins was 98 cm long and weighed 6.3 kg. The twins, both male, were carried in the right uterus together with 6 other embryos (4 female, 2 male). The number of young carried was high, for a female of this size the fecundity might have been expected to range from 5 to 12 embryos (A.D.Woodhead, The Bulletin MDIBL. Vol. 16:103-106).

The twins were of the same size but were each significantly smaller than their siblings. Additionally, they were in the final stages of yolk absorption, their shared yolk sac being empty, -- by contrast, their siblings all had substantial yolk reserves remaining in the yolk sac. Size comparisons with the siblings are given below:

| | Length cm. | Weight of Body and Yolk, gm. | Body Wt. gm. | Yolk-sac Wt. gm. |
|---|---|---|---|---|
| Twins | 20, 20 | 61.2 | 30.0, 30.4 | 0.8 |
| Siblings (right uterus) | 23.5 to 24.5 | 58.2 to 61.5 | 48.4 to 54.5 | 7.1 to 12.2 |
| Mean | 24 | 60.3 | 50.9 | 9.4 |
| Siblings (left uterus) | 23.0 to 24.5 | 57.1 to 62.6 | 47.1 to 56.4 | 3.8 to 10.8 |
| Mean | 24 | 60.3 | 52.4 | 7.9 |

From the table of comparisons it appears that although the twins were each smaller than their siblings in both length and weight, if the yolk sac and body weights are combined, the total weight of the twins falls within the range of weight of their siblings (including yolk sac with body weight). It is concluded that the twins developed from an egg of the same size as the others in the oviducts but shared the yolk resource equally between themselves, resulting in smaller body size. The combined weight of the bodies of the twins is greater than the weight of the bodies (without yolk sacs) of individual siblings, for a combination of reasons. The twins had already converted all of their yolk reserves into body tissues, unlike their siblings which still had yolk reserves. Secondly, during the conversion of yolk into body tissues there is also uptake of water in utero by the growing embryos. Since the twins had converted all of their yolk into body tissues, they would have taken up a greater weight of water than their siblings, in which from 7 to 10 gms of yolk still remained in the yolk sac.

In the 1976 report of twinning in this fish, comparisons were made between the twins and only two of their siblings, when comparison was made between the combined body weight with yolk sac, the twins together were the same weight as their individual siblings. The conclusion is that the 1976 twins had similarly developed from a single egg of the same size as others in the oviducts.

In the previous report the twins still had yolk in the yolk sac comprising 36% of the combined weight of body

and yolk, and it was suggested that they might have survived to sibling birth, albeit at a smaller size. In the present case this seems less likely because the shared yolk sac was empty, -- its contents had already been used up, although the siblings were still perhaps 4 or 5 months from being born. Nothing is known about the birth of spiny dogfish, whether a female bears all her young at one time, or sequentially, when each is completely developed. In the first case, it seems that the twins would have too little yolk resource left to survive through the period until the siblings were ready for birth. In the second case the twins might have been born alive, although smaller in size than normal and possible not fully matured in their development.

## A COMPARISON OF CARDIOVASCULAR VARIABLES IN RESTING EEL POUT AND SEA RAVEN

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A few species of teleost fish possess "white" hearts, as opposed to the normal red colour of the ventricle in all other fish and vertebrates. The reason for the white appearance of the ventricular myocardium in fish such as the eel pout (Macrozoarces americanus) is a lack of intracellular myoglobin (Driedzic et al, this volume). Myoglobin is believed to facilitate $O_2$ transfer from blood in the ventricular lumen to the myocardium, as well as being a potential intracellular $O_2$ store (a high proportion if not all the myocardial $O_2$ supply is provided by venous blood in the lumen since many fish lack coronary vessels). Because of this unusual occurrence, recent work has focused on the metabolic and physiological performance of the white heart in the eel pout. One would expect physiological adaptations in fish with a myoglobin-less myocardium. For instance they may have higher venous blood oxygen tensions, or the heart may have a reduced power output, or the myocardium itself may be more tolerant to hypoxia compared to fish hearts containing myoglobin. The latter possibility has, however, been ruled out since the eel pout heart tolerates anoxia poorly (Turner and Driedzic, Can. J. Zool., 58:886-889, 1980). The two other possibilities have not been examined.

The present work reports preliminary data on blood flow and blood pressure measurements in the ventral aorta (VA) (i.e. immediately after the heart) in the resting eel pout. The oxygen tension ($P_{O_2}$) of ventral aortic blood ($P_{VO_2}$) was also measured and was used as an index of the ventricular lumen blood $P_{O_2}$. Comparisons were made with similar measurements from the sea raven (Hemitripterus americanus) which possesses a myoglobin-rich myocardium.

### METHODOLOGY

The sea ravens (n = 5; wt. = 0.9 to 2.0 kg) were obtained locally. The eel pout (n = 6; wt. = 0.8 to 1.0 kg) were transported from St. Andrews, N.B., Canada and held at MDIBL. Ventral aortic blood pressures ($P_{VA}$) and VA blood flow (cardiac output, $\dot{Q}$) measurements were not made on the same fish. An indwelling, nonocclusive polyethylene cannula (i.d. = 0.58 mm, o.d. = 0.96 mm) was used to measure $P_{VA}$ and to sample VA blood. A cuff-type electromagnetic flow probe (Biotronix) was fitted snugly around the VA for blood flow measurements. The protocol used to implant these cannulae is similar to that described by Farrell (J. Exp. Biol., in press, 1980). The fish were held in a darkened plexiglass box that approximated the fish size and received a flow-through supply of aerated sea water at 17°C, and they were allowed to recover from surgery overnight. Blood pressure and flow records were taken continuously during the day time and occasionally during the evening. The traces were only sampled at times when the fish appeared to be resting, i.e., no apparent swimming. Blood flow signals were detected with a Biotronix BL 610 electromagnetic flow meter. Blood pressure signals were detected with a Harvard pressure transducer. The blood flow and pressure signals were recorded on a Narco physiograph chart recorder after suitable amplification. Blood samples (0.3 ml) were taken from resting fish, usually at three different times for $P_{VO_2}$ and Ht