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β-Alanine uptake by *Amphioxus* (*Branchiostoma*)—Inhibition by furosemide*

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CERTAIN diuretic agents are not renal specific in their actions. For example, we have demonstrated that ethacrynic acid and chlorothiazide inhibit transport by the small intestine.[1] In the course of studies on the phylogenetic development of transport systems,[2] we have investigated the effects of diuretics on adult *Amphioxus* (*Branchiostoma caribaeum*). This fish-like member of the subphylum Cephalochordata, of about 5 cm length, is widely known because of the many basic features it shares with higher chordates (dorsal hollow nerve cord, notocord, pharyngeal gill slits).[3] We have found that this organism concentrates a number of non-alpha amino acids, from a bathing medium of sea water, apparently against a concentration gradient. Of interest is not only the uptake of compounds such as β-alanine by *Amphioxus*, but also the inhibition of this event by a diuretic agent, furosemide. Furosemide (4-chloro-*N*-(2-furylmethyl)-5-sulfamoylanthranilic acid) is a known active natriuretic and diuretic agent in man and animals. (References to recent studies are given by Leme *et al.*[4])

The sea water in which the *Amphioxi* were shipped was filtered and aerated with 95% O_2 and 5% CO_2. The sodium content of the water was 355 m-Equiv. In some cases, the sea water was fully or partially saturated with furosemide at 30° prior to aeration. Each *Amphioxus* was preincubated for 45 min in 7 ml of the fluid at 30° in an oscillating water bath. β-Alanine-1-^{14}C was then added to produce a concentration of approximately 1×10^{-5} M.

A small aliquot of fluid was removed initially, and another aliquot after an additional incubation of 1 hr, for liquid scintillation counting. By the disappearance of radiolabel, entry into *Amphioxus* was calculated. That β-alanine entry had taken place (and that significant decarboxylation was not occurring) was shown by recovery experiments. In these latter studies, after incubation in the radiolabeled fluid, the *Amphioxi* were transferred to sea water and incubation was carried out for an additional hour. The bathing fluid was then sampled to determine if any radiolabel had leached out. *Amphioxi* were subsequently homogenized in 5 ml sea water, the mass was centrifuged (Servall SS-1 centrifuge at greatest speed), and the supernatant was counted for radioactivity.

After incubation of *Amphioxus* in 1×10^{-5} M β-alanine, there was considerable disappearance of radiolabel from the bathing medium. The final ratio of (radioactivity per unit weight of *Amphioxus* to radioactivity per unit volume of bathing solution) was over 40:1 in some cases. Reincubation of whole *Amphioxus* in unlabeled sea water washed out only 3 per cent of the counts that had been lost from the original medium. Autoradiographic studies (NTB_2 film) showed considerable radioactivity in the exterior surface of *Amphioxus* with some activity in the gut. The addition of furosemide to the bathing medium decreased the uptake of β-alanine by *Amphioxus*. This decrease was approximately proportional to the furosemide concentration (Fig. 1).

Homogenization of *Amphioxus* in sea water revealed 84 per cent of the radioactive counts, which had disappeared from the original bathing fluid, to be in the clear supernatant after centrifugation. This supernatant fluid was chromatographed, as were the original bathing fluids and standards of β-alanine-1-^{14}C. By utilizing butanol:acetic acid:water (4:1:1, v/v/v) and Whatman No. 1 paper in the ascending direction, each of the fluids revealed only one peak of radioactivity ($R_f = 0{\cdot}32$) which was identical to the standards. Dialysis of the *Amphioxus* homogenate against 5 times its volume of sea water permitted 75 per cent of the radioactivity to pass into the outer bathing fluid within 3 hr. The majority of β-alanine-1-^{14}C within *Amphioxus* was thus in an unchanged chemical form and capable of being freed by dialysis of the homogenate.

The mechanism of inhibition of β-alanine uptake by furosemide has yet to be determined. It is not

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an osmotic effect, since 1×10^{-3} M urea, sucrose or ethacrynic acid did not decrease β-alanine accumulation by *Amphioxus*. (Furosemide is soluble in sea water to the extent of approximately $1{\cdot}4 \times 10^{-3}$ M.) Uptake inhibition might be secondary to an effect of furosemide on sodium transport.

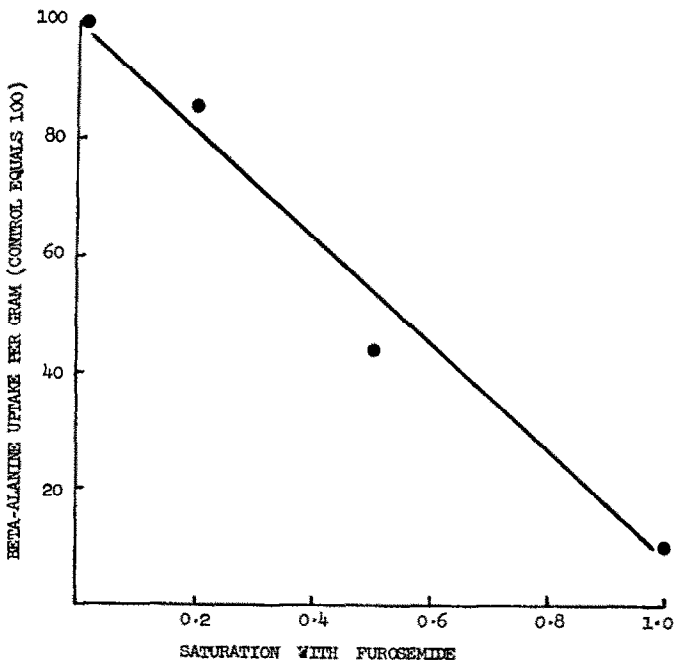

FIG. 1. Effect of furosemide saturation of sea water on β-alanine uptake by *Amphioxus*. By 1·0 is meant full saturation (approximately $1{\cdot}4 \times 10^{-3}$ M furosemide). Each point is the mean of 3 animals.

(We have some evidence for a decreased ^{22}Na flux.) These initial observations suggest that *Amphioxus*, and perhaps other sea forms, may provide an interesting approach to a comparative study of diuretic agents.

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