Table 1. Viscosity parameters for water.

log A	B (°K)	T_0 (°K)
−3.54	222	149.4 (*5*)
−3.54	224	148.4 (*6*)
−3.58	226	149.4 (*6*)
−3.54	222	150 (*4*)

method for deriving T_g, which gives an appreciably higher value.

The viscosities of many liquids over broad temperature ranges can be accurately represented by the equation (*2*)

$$\log \eta = \log A + B/(T-T_0) \quad (1)$$

where η is the viscosity at temperature T, and A, B, and T_0 are constants. The extrapolation by this equation of the viscosities of glass-forming liquids to T_g, as determined by volumetric or calorimetric measurements, gives log $\eta_g = 13 \pm 1$ (*2*, *3*). For example, for glycerol (*2*), log $A = -4.76$, $B = 950$, and $T_0 = 132$°K. Substitution of these values in Eq. 1, with log $\eta_g = 13$, gives $T_g = 185$°K, the correct value as cited by Yannas.

The viscosity parameters for water have been determined in several independent investigations giving essentially the same values (Table 1).

Using Eq. 1, with log $\eta_g = 13$, we obtain $T_g = 162° \pm 1$°K, which is appreciably higher than $127° \pm 4$°K, the value derived by Yannas. This result suggests that T_g for glycerol-water solutions might go through a minimum somewhere above a water weight-fraction of 0.4, the upper limit of Yannas' measurements. If we consider the correlation between viscosity and structure due to H-bonding which has been demonstrated for pure water (*4*), a possible minimum in the constant-viscosity temperature T_g has interesting implications regarding the structural variation as a function of composition in supercooled glycerol-water solutions.

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30 December 1968

Teleostean Urophysis: Stimulation of Water Movement across the Bladder of the Toad Bufo marinus

Abstract. *An effect of material from the caudal neurosecretory system of a teleost on the isolated toad bladder is described. Urophysial breis from* Gillichthys mirabilis *result in dose-related water movement across the bladder. As little as one one-hundredth of a urophysis induces a threefold increase in osmotic water movement.*

The caudal neurosecretory system of fishes and its neurohemal organ in teleosts, the urophysis, form an endocrine apparatus whose function has proven remarkably elusive (*1*, *2*). Recently a water-retaining effect in toads was reported (*3*). The isolated toad urinary bladder was examined to determine whether it played a role in this water-retention effect and whether the degree of response was dose-dependent and could therefore be used in the development of an assay. Previous reports had indicated little or no hydrosmotic influence of urophysial material, as can be seen with various neurohypophysial peptides (*2*, *4*). The present report describes a pronounced, dose-related response of the toad bladder to urophysial preparations.

Table 1. Effects of 0.1 of a urophysis (UH) from *Gillichthys* per milliliter and equivalent amounts of abdominal spinal cord (SC) on water loss in milligrams by isolated toad bladders.

First period	Second period	Third period	Fourth period	Fifth period
10.3	143.7 (UH)	4.5	5.3 (SC)	4.9
6.8	128.2 (UH)	4.9	4.0 (SC)	4.1
10.3	21.5 (SC)	6.6	152.2 (UH)	5.1
8.5	8.8 (SC)	5.4	122.8 (UH)	4.4

The toads (*Bufo marinus*) were obtained from Hawaii and kept in the laboratory at 22°C. The procedure for the preparation of toad bladder as described by Bentley (*5*) was followed with certain modifications. A hemibladder, tied to the end of a glass tubing, was filled with 1 ml of an amphibian Ringer solution (*5*) (*p*H 8.2 to 8.3), immersed in 30 ml of the same solution, and allowed to equilibrate for 3 to 4 hours. For the last hour of equilibration, the inside solution was changed to 20 percent Ringer solution, and the outside solution was replaced by fresh Ringer. Before weighing, the bladder was suspended inside a balance and immersed in Ringer in a funnel (also inside the balance) connected to a reservoir by rubber tubing. When the bladder was to be weighed, the Ringer solution was drained from the funnel by lowering the reservoir. This setup has the advantage of leaving a constant amount of water adhering to the surface of the preparation at the time of weighing.

The bladder preparation was weighed before and after 30-minute periods to determine water loss. Urophysial and other materials were added to the external solution 10 minutes before each test period; intervening 30-minute periods served as controls. Each hemibladder was subjected to a maximum of three experimental periods alternated with four control periods. After each 30-minute period, there was an interval of about 30 minutes during which the bladders were rinsed by refilling and placing in fresh solution. During all these periods and during rinsing, the bladder contained 1 ml of 20 percent Ringer solution and was immersed in 30 ml of undiluted Ringer solution at room temperature.

Urophyses from mudsuckers (*Gillichthys mirabilis*) were homogenized in Ringer solution with a glass homogenizer. Whole homogenates were used throughout. *Gillichthys*, a Pacific Coast goby (20 to 30 g) is rather easily collected in tidepools and is a hardy euryhaline fish.

A good dose-response relation was obtained with three doses of the urophysial preparations, given in increasing or decreasing order (Fig. 1). As little as 0.01 of a urophysis, equivalent to about 0.2 μg of acetone-dried urophysis powder (*6*), per milliliter of Ringer solution increased water loss to about three times the control values. Doses of 0.03 and 0.1 of a urophysis per milliliter resulted in increases of approximately 8- and 16-

fold, respectively. Preparations of abdominal spinal cord had little or no effect (Table 1).

Failure of earlier experiments (*2*, *4*) to demonstrate a hydrosmotic effect may be due to different urophysial preparations or to differences in the test animals. Urinary bladders of *Bufo marinus* from Florida showed much lower responses to *Gillichthys* urophyses, compared with *Bufo marinus* from Hawaii. However, the most probable reason for failure to observe the hydrosmotic effect may have been the short test periods used in previous studies. When the water loss was determined for each 15 minutes of a 45-minute experimental period, the weight loss for the first 15 minutes was just slightly higher than control values; the weight loss for the second 15 minutes was much higher than the first; and the last 15 minutes gave values lower than the second. In addition, in this study, bladders were subjected to urophysial material for 10 minutes before being tested.

Other agents (such as acetylcholine, catecholamines, serotonin, and bradykinin) possibly present in nervous tissue do not stimulate water across the toad bladder (*7*). Neurohypophysial peptides are, of course, highly active; however, the delayed response to the urophysial preparation indicates some characteristics different from those of the neurohypophysial peptides. Although the urophysial hydrosmotic effect is necessarily to be viewed as a pharmacological one (no urophysis or homologous organ is present in amphibians or in any other tetrapods), this activity may reflect an important osmoregulatory influence as yet to be delineated in teleosts. At the moment, it appears that this effect is not due to the same factor responsible for teleost bladder contraction (*6*). Nevertheless, the utility of the effect in detecting and assaying a biologically active substance from the caudal neurosecretory system is evident.

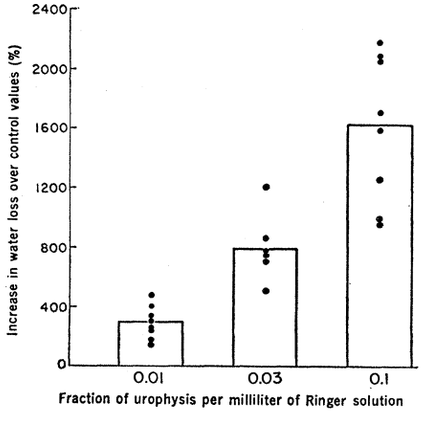

Fig. 1. Effect of varying doses of *Gillichthys* urophysis on water loss from the isolated toad bladder. Of the eight bladder preparations used for these experiments, six were subjected to the three doses in different order. Points represent individual values.

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Teleostean Urophysis: Stimulation of Contractions of Bladder of the Trout Salmo gairdnerii

Abstract. *A new activity of the teleost caudal neurosecretory system is described. Extracts of the urophysis of the mudsucker* Gillichthys mirabilis *and of the trout* Salmo gairdnerii *cause rhythmic contractions of the isolated urinary bladder of the trout. The dose-related response provides the basis for a quantitative bioassay of this urophysial principle.*

In determining whether urophysial extracts influenced water movement across the isolated fish urinary bladder, comparable to the effect seen on the toad bladder (*1*), it was noted that the trout bladder showed rhythmic contractions upon exposure to such extracts (or to homogenates of fresh urophysis in Ringer solution). In view of the continued need for a reliable biological assay for any active principle or principles which may occur in the teleost urophysis, this phenomenon was further analyzed. It is now possible to define this kinetic activity with considerable precision and to propose a quantitative method for assay. The new activity of the caudal neurosecretory system is discussed, and the utility of the bioassay procedure is outlined.

Urinary bladders were dissected from rainbow trout (*Salmo gairdnerii*) immediately after decapitation and immersed in trout-Ringer solution (*2*). The distal (cloacal) end of the bladder was attached to a suitable extension fitted to a Statham or a Bionix pressure gauge, and the proximal (renal) end was tied off. The internal space in the strain gauge and the bladder was filled with a 1 to 5 dilution of trout-Ringer solution, the bladder was immersed in an organ bath containing 10 ml of trout-Ringer solution at room temperature, and contractions were recorded on chart paper with a Beckman or a Bausch and Lomb amplifier-recorder. Aqueous or acetic acid extracts were added to the organ bath, and responses (contractions per minute) were measured for 5 minutes; the bath was then rinsed out twice with the Ringer solution. A good log dose-response relation was obtained when urophysial extracts from the trout or from the mudsucker (*Gillichthys mirabilis*) were used (Fig. 1).

Drying of urophyses in acetone be-

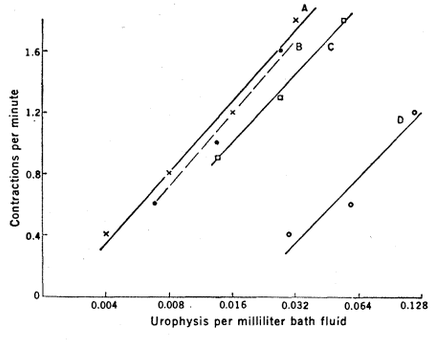

Fig. 1. Bladder-contracting activity in different preparations of trout caudal neurosecretory system, showing dose-response relationship. *A*, Urophysis, acetic acid extract; *B*, urophysis, whole homogenate in distilled water; *C*, urophysis, Ringer solution extract (supernatant); *D*, caudal spinal cord, whole homogenate in Ringer of area containing cell-bodies of caudal neurosecretory neurons; no bladder-contracting activity is present in abdominal spinal cord.