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.

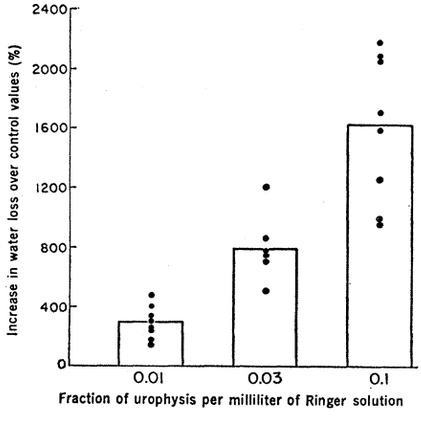

Fig. 1. Effect of varying doses of *Gillichthys* urophysis on water loss from the isolated toad bladder. Of the eight bladder preparations used for these experiments, six were subjected to the three doses in different order. Points represent individual values.

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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-

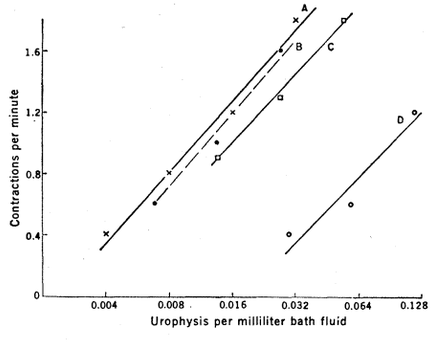

Fig. 1. Bladder-contracting activity in different preparations of trout caudal neurosecretory system, showing dose-response relationship. *A*, Urophysis, acetic acid extract; *B*, urophysis, whole homogenate in distilled water; *C*, urophysis, Ringer solution extract (supernatant); *D*, caudal spinal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

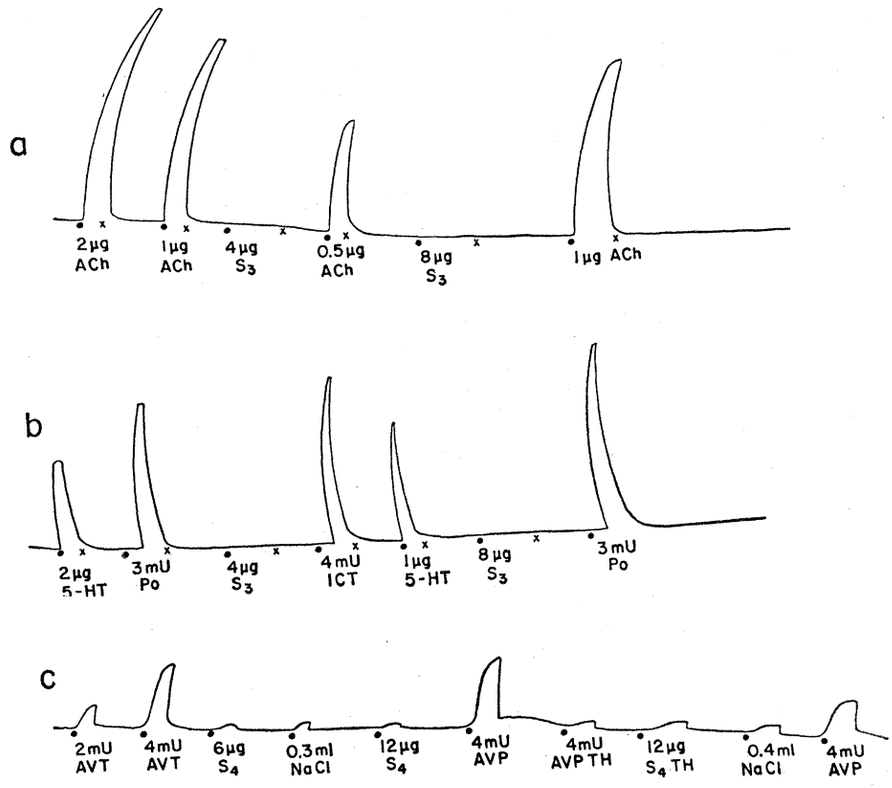

Fig. 2. Pharmacological comparison of urophysial extracts with acetylcholine, 5-hydroxytryptamine, and neurohypophysial peptides. (a) Isolated small intestine of trout. Effects of bladder-contracting doses of acetylcholine (ACh) and an extract of urophysial laboratory standard preparation (S_3). (b) Isolated rat uterus. Effects of 5-hydroxytryptamine (5-HT), mammalian oxytocin (Po), 4-serine-8-isoleucine oxytocin (ICT), and an extract of urophysial laboratory standard (S_3). (c) Rat blood pressure. Effects of arginine vasotocin, arginine vasopressin, and urophysial extract (laboratory, S_4). Abbreviations are: AVT, arginine vasotocin; AVP, arginine vasopressin; AVP-TH, sodium thioglycollate-treated AVP; S_4-TH, sodium thioglycollate-treated urophysial laboratory standard, S_4; NaCl, 0.9 percent NaCl solution. The effect of AVP is abolished by thioglycollate treatment (rupture of disulfide bond); that of the urophysial material is unaffected.

fore extraction and the use of 0.25 percent acetic acid for extraction instead of water or Ringer solution did not materially change the amount of bladder-contracting activity; acetic acid extraction seemed to increase the yield of activity (Fig. 1). As little as 1/400 of a mudsucker urophysis (equivalent to 0.05 µg or less of acetone-dried urophysis powder) per milliliter of bath fluid was enough to produce marked and reproducible responses on the isolated bladder. To ensure reproducibility of results and to enable a comparison of findings from individual experiments, and also in anticipation of further studies of the bladder-contracting activity, a laboratory standard preparation of acetone-dried *Gillichthys* urophyses was obtained. The mean weight of an acetone-dried urophysis, calculated from six batches of the laboratory standard (820 urophyses), was 21.4 ± 0.81 µg. After introduction of the laboratory standard, all dosages and experimental results were related to unit weight (µg) of the standard preparation.

Other biologically active substances which might occur in the urophysis and thus affect the isolated bladder were tested on the trout bladder and on trout and mudsucker small intestine (Fig. 2a). These included the teleost neurohypophysial hormones, 5-hydroxytryptamine, histamine, acetylcholine with and without physostigmine or atropine, adrenalin, and noradrenalin. With the exception of the neurohypophysial hormones and of adrenalin and noradrenalin, these substances also produced contractions of the bladder. When similar amounts of the substances listed above and of the urophysial extracts were applied to the isolated rat uterus (Fig. 2b) or tested on rat blood pressure (Fig. 2c), it was apparent that the trout bladder-contracting activity of urophysial extracts was associated with a substance whose pharmacological characteristics were different from those of 8-arginine oxytocin, 4-serine-8-isoleucine oxytocin (ichthyotocin, isotocin), 5-hydroxytryptamine, histamine, acetylcholine, adrenalin, and noradrenalin.

Thus the assay procedure and the laboratory standard preparation may be of use not only in the quantitative estimation of an active principle present in the teleost urophysis, but also in biological and chemical identification of this principle which may prove to be a hormone of the caudal neurosecretory system.

Several functions have been proposed for the hypothetical urophysial hormone (*3*). Most of the information available on urophysial function points to a relation with osmoregulation. However, the pressor activity described earlier (*4*) and the present bladder-contracting activity are kinetic effects. At the same time, of course, an influence on blood pressure or on bladder function could also be *ipso facto* related to osmoregulation, albeit secondarily so. Furthermore, unlike the hydrosmotic effect on the toad bladder (*1*), the kinetic effect represents a teleost principle acting upon a teleost target organ. In any case, the bioassay described herein opens the way to meaningful studies of the pharmacological, physiological, and chemical properties of the hormone or hormones of the caudal neurosecretory system. Initial findings of experiments aimed at chemical identification of the urophysial active principle(s) (*5*) support the earlier indications (*4*) that more than one active substance may be present in the teleost urophysis.

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