10. Copp, D. H., Cameron, E. C., Cheney, B. A., Davidson, A. G. F., Henze, K. G., Endocrinology, 1962, v70, 638.

11. Foster, G. V., Boghdiantz, A., Kumar, M. A., Slack, E., Soliman, H. A., MacIntyre, I., Nature, 1964, v202, 1303.

Received September 2, 1964. P.S.E.B.M., 1965, v118.

On the Suggested Ionoregulatory Role of the Teleost Caudal Neurosecretory System.* (30055)

KATSUTOSHI IMAI, JON G. STANLEY, W. R. FLEMING AND HOWARD A. BERN
Department of Zoology, Cancer Research Genetics Laboratory, University of California, Berkeley, and Department of Zoology, University of Missouri, Columbia

Since the first attempts to uncover the function in fishes of the caudal neurosecretory system and of its neurohemal organ, the urophysis, the notion has prevailed that this system is concerned with osmo(iono)-regulation(1,2). This notion has been substantiated by 3 lines of evidence in recent years: the finding of differences in serum chloride levels in freshwater teleosts subjected to deleterious concentrations of NaCl, in the presence and in the absence of the urophysis(3); the demonstration that urophysial extracts increase the Na content of goldfish by augmenting Na influx across the gill and also by effects at the renal level(4,5); and the electrophysiologic data indicating differential responses of neurosecretory neurons in the caudal system to variations in NaCl level in the blood(6-8).

In contradistinction to this evidence has been the general knowledge that urophysectomized *Tilapia mossambica* (a freshwater euryhaline teleost) can withstand not only their original freshwater environment, but can also be moved into sea water without significant mortality (Bern, unpublished; Reid, unpublished; Fleming and Stanley, unpublished). Similarly *Fundulus kansae* (Fleming and Stanley, unpublished) and *Jenynsia lineata* (Garcia Romeu, personal communication) can also survive after removal of the urophysial region. In addition, acetone-dried extracts of the caudal neurosecretory region in *Tilapia* have revealed no evidence of neurohypophysis-like activity and at best a slight atypical antidiuretic action in rats(9). Even the latter activity was essentially absent from urophysial extracts of the marine teleost, *Chaetodon miliaris* (Sawyer and Bern, unpublished; cf. 5).

In the present paper, we have pooled the data from 2 laboratories (Berkeley, Calif. and Columbia, Mo.) where experimentation has been conducted on the caudal neurosecretory system, in order to record the nature of other evidence not indicative of an osmoregulatory influence of the urophysis and its products. It seems incorrect to us to publish only the data that support a urophysial contribution to osmoregulation. The present negative data need to be considered in assessing the role of this elusive neuroendocrine apparatus.

Materials and methods. In the series of experiments performed in Berkeley, *Tilapia mossambica* weighing between 22 and 56 g were separated into 3 groups: intact, sham-operated and urophysectomized. The surgical technics have been described(3). These 3 categories of *Tilapia* were again subjected to one of the following procedures: (1) anus and urogenital orifice were ligated with a pursestring suture to prevent elimination of feces and urine; (2) a polyethylene tube attached to a small bag was sutured in place in the urogenital orifice to prevent entry of urine into aquarium water; or (3) no further operation was performed. At the beginning of the experiment all these fish were injected intraperitoneally with a salt load (1 ml per 100 g body weight of a solution containing 1238 mEq NaCl, 2.7 mEq KCl, 1.8 mEq

* Aided by NSF grants GB-1173 and GB-2264 and NIH grant 2 T1 Ca 5045.

TABLE I. Effect of Urophysectomy on Excretion of Injected NaCl by *Tilapia mossambica* in Various Experimental Conditions.

Experimental group	Operation	No. of fish	Avg body wt (g)	Chloride concentration (mEq/l) in aquarium water at hours shown					
				0	.5	1	1.5	3	6
Uninjected fish	Intact	6	29.7	.078 ±.006*	.165 ±.021	.203 ±.026	.219 ±.036	.245 ±.042	.258 ±.045
Injected fish	Intact	7	29.6	.079 ±.017	.181 ±.056	.231 ±.064	.277 ±.069	.347 ±.098	.424 ±.126
	Sham-operated	6	31.8	.072 ±.007	.154 ±.042	.191 ±.044	.226 ±.050	.291 ±.061	.359 ±.097
	Urophysectomized	8	35.1	.090 ±.033	.180 ±.042	.238 ±.060	.274 ±.076	.334 ±.088	.406 ±.077
Injected fish with anal and urogenital orifices ligated	Intact	6	30.3	.075 ±.013	.175 ±.035	.229 ±.030	.276 ±.047	.349 ±.058	.403 ±.048
	Sham-operated	6	39.2	.076 ±.007	.180 ±.014	.239 ±.026	.277 ±.027	.336 ±.036	.419 ±.033
	Urophysectomized	9	41.9	.073 ±.024	.185 ±.032	.250 ±.049	.291 ±.050	.356 ±.075	.431 ±.110
Injected fish with urine bag in place	Intact	6	34.8	.081 ±.033	.173 ±.042	.219 ±.060	.256 ±.076	.313 ±.088	.382 ±.077
	Sham-operated	6	40.5	.076 ±.003	.172 ±.017	.192 ±.015	.220 ±.017	.277 ±.031	.311 ±.025
	Urophysectomized	7	42.9	.071 ±.011	.159 ±.030	.199 ±.035	.211 ±.045	.270 ±.047	.360 ±.084

* M ± SE_m.

$CaCl_2$, and 0.24 mEq $NaHCO_3$ per liter). An additional group of 6 fish was left intact and uninjected.

Each fish was placed in an individual aquarium containing 500 ml of diluted teleost saline (1:2000, containing about .065 mEq Cl/l) per 30 g body weight. Initial readings (Table I) were made after immersion of the fish; thus, the Cl values are somewhat higher than .065. Samples (6 ml) of the water were removed at 0, .5, 1, 1.5, 3, and 6 hours after immersion of the fish, and chloride concentration was determined in triplicate with a Buchler-Cotlove chloridometer. Aquaria were aerated and kept at room temperature.

Experiments at Columbia were conducted on both *Tilapia mossambica* and *Fundulus kansae*. Urophysectomy in *Fundulus kansae* was accomplished by removal by ligature of the portion of the fish posterior to the dorsal fin. The sham-operated controls had only the tail fin similarly ligated and removed. These fish showed little healing and as a result suffered high mortality. All experiments with *Fundulus* were conducted within 48 hours after the operation.

In one group of *Fundulus* (Table II) the ligature was followed by (1) total removal of tissue posterior to ligature, or (2) total removal with some exposure of tissue anterior to ligature, or (3) non-removal of the subsequently necrotic tissue posterior to ligature. These fish were sacrificed after 48 hours, and the serum sodium was determined. In another group of *Fundulus* urine was collected from 5 urophysectomized and 6 sham-operated fish by cannulation of the urogenital papilla as described elsewhere(10). At 29 hours the salinity of the water was increased

TABLE II. Effect of Urophysectomy on Serum Sodium of *Fundulus kansae* Held in Tap Water.

Group	No. of fish	Serum sodium* (mEq/l)
Control	13	164.2 ± .7
Urophysectomized (no complications)	13	163.7 ± 1.1
Urophysectomized (tissues exposed)	10	142.6 ± 3.1
Urophysectomized (necrotic tissue)	10	156.0 ± 1.3

* m ± SE_m.

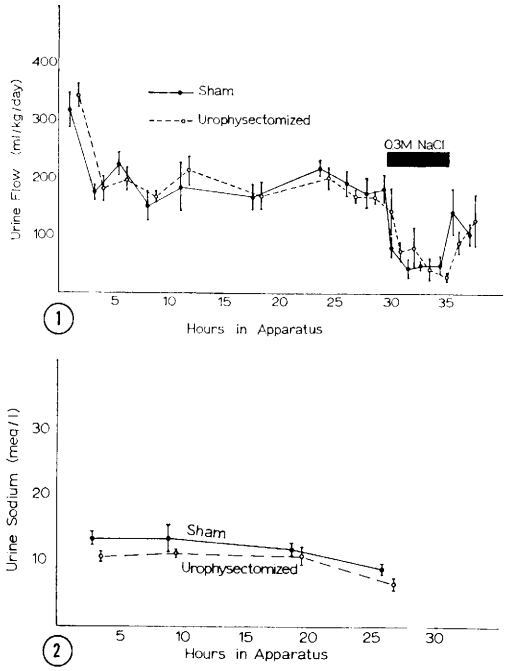

FIG. 1. Comparable urine flow in sham-operated (n = 6) and urophysectomized (n = 5) *Fundulus kansae*. Both groups of fish respond similarly by decreasing urine flow upon subjection to .3M NaCl and by increasing it when restored to their freshwater environment.

FIG. 2. Urine sodium levels in same *Fundulus* as in Fig. 1.

to 300 mM. At 35 hours the water was changed back to the original salinity. Urine sodium and rate of urine flow were determined at regular intervals (Fig. 1 and 2). Urophysectomy in *Tilapia mossambica* was accomplished by cutting off the caudal peduncle posterior to the dorsal fin. Sham-operated controls had only the tail removed. These fish healed completely in about 2 weeks and showed less than 2% mortality. Urophysectomized and sham-operated *Tilapia* were allowed to remain in a holding tank containing .018 M NaCl and about 2.5 mEq Ca^{++}/l for 2½ months. At this time, blood Na^{+} and Ca^{++} were determined, and hematocrits were performed. Other groups of similarly-held fish were transferred either to .368 M NaCl or to deionized water (both solutions containing 2.5 mEq Ca^{++}/l) for 8 days, at which time blood Na^{+} and Ca^{++} levels and hematocrit values were determined.

Observations. Table I indicates that *Tilapia* handled an injected salt load for the first 6 hours in much the same way regardless of presence or absence of the urophysis. Furthermore, regardless of the ability of the fish to eliminate feces and urine into the aquarium water, the amount of Cl excreted was essentially the same. At the end of 6 hours approximately ⅓ of the injected Cl had been excreted in some fashion, probably across the gills. It is of interest that initially the uninjected fish appeared to add as much chloride to the surrounding medium as did the salt-injected fish. However, 3 and 6 hours after the injection the aquarium water shows considerably more Cl released from the injected fish. In general, rate of excretion is lower with time, presumably as a consequence of the increasing osmotic pressure of the environmental fluid.

Table II gives the results of various types of urophysectomy on *Fundulus*. It can be seen that the mean serum Na is not decreased by a "clean" operation removing the caudal neurosecretory system. However, where tissue damage or histolysis is allowed to occur, the serum Na values are lower, presumably as a result of nonspecific injury to the organism.

Fig. 1 and 2 indicate that the urine sodium values are initially somewhat lower in urophysectomized *Fundulus* ($p=.05$) but that urine flow is unaffected. The 2 groups of fish responded similarly to exposure to hypertonic (0.3 M) NaCl solution by reducing urine flow.

Table III summarizes the effects of various external environments on Na and Ca blood levels and hematocrit in *Tilapia*. In deionized water serum levels of both Na and Ca are somewhat lower than in the fish kept in the presence of some NaCl; however, urophysectomy did not appear to result in any significant change from the controls in the environments used.

Discussion. The data presented herein represent a miscellany. Nevertheless, all information points to a lack of effect of urophysis removal in the several experimental situations studied. The euryhaline fishes used here do not appear to depend upon the caudal neurosecretory system for solution of osmotic problems, at least under the relatively short-term abnormal circumstances we have exam-

TABLE III. Effect of Various External Environments on Serum Na^+ and Ca^{++} and Hematocrit in *Tilapia mossambica*.

Environment*	Group	No. of fish	Na^+ (mEq/l)	Ca^{++} (mEq/l)	Hematocrit (% packed cells)
.018 M NaCl	Control	8	168.8 ± .8	5.6 ± .2	38 ± 1
	Urophysectomized	8	169.5 ± 1.4	5.4 ± .2	35 ± 1
.368 " "	Control	8	170.8 ± 1.0	5.3 ± .2	34 ± 1
	Urophysectomized	7	170.9 ± 1.0	5.4 ± .3	34 ± 2
Deionized water	Control	7	156.0 ± 1.7	5.1 ± .1	39 ± 1
	Urophysectomized	8	158.8 ± 1.9	4.7 ± .6	32 ± 3

* All containing 2.5 mEq Ca^{++}/l.

ined. The difference between the results attained earlier(3) and those reported here on *Tilapia* subjected to hypertonic NaCl may lie in the presumed total absence of Ca^{++} in the aquarium water in the earlier studies. Thus, the NaCl solution with no Ca^{++} would represent much greater ionic "stress" upon the fish; under such conditions, removal of the urophysis seemed to have an influence not only on chloride level but also on mortality. The fact that stickleback (*Gasterosteus*) survive well in sea water but not in isosmolar NaCl solution(11) emphasizes the importance of "ionic balance."

In the experiment involving the handling of a salt load (Table I), the fish injected with saline did not show an excretion pattern different from the uninjected fish until the third hour. One can assume that some of the excreted salt may be held in urine in the bladder, so that the aquarium chloride level may not reflect the total renal salt excretion. If the volume of urine in the bladder is small, it may not be discharged during the 6-hour period. Nevertheless, removal of the urophysis had no observable effect on chloride excretion regardless of the nature of the experimental group.

Despite the negative data presented herein, a total assessment of the physiologic contribution of the caudal neurosecretory system at the present time(12) continues to point to a regulatory role in regard to ion movements. Certainly, the data of Maetz and his collaborators(4,5) are of considerable importance; however, these authors also emphasize that the urophysial factor does not seem to play an essential role. They have found that neurohypophysial extracts have effects similar to those of urophysial extracts in the goldfish. Accordingly, the role of the urophysis in osmoregulation would appear to be a subtle one indeed; furthermore, it is possible that the caudal neurosecretory system responds only in emergency situations of a very special kind and that we have yet to delineate proper experimental situations to permit the unequivocal demonstration of its intervention. With the exception of the work on the goldfish(3,5), experimental studies conducted to date on the caudal neurosecretory system have involved euryhaline fishes. It is suggested that the caudal neurosecretory system may serve as a "fine adjustment" in osmoregulation, with the "coarse adjustment" accomplished by products of the cranial neurosecretory system(5) and/or of the adenohypophysis (prolactin: 13). In any case, it is important that investigators do not consider the osmoregulatory action of this system as established and will continue the needed diligent search for a definitive functional explication.

Summary. Urophysectomized *Tilapia mossambica* showed no significant difference in ability to handle a salt load, nor in serum sodium or calcium levels, nor in hematocrit values. Urophysectomized *Fundulus kansae* revealed no significant difference in serum or urine sodium levels, nor in urine flow. These negative results raise questions concerning the putative ionoregulatory role of the caudal neurosecretory system in fishes.

Note added in proof: Fridberg and Nishioka at Berkeley have begun an examination of the terminal spinal cord region in the long-term "depedunculated" (urophysectomized) *Tilapia*. Ordinary histologic preparations reveal the re-formation of recognizable

caudal neurohemal areas, mostly within the tissue of the spinal cord. Electronmicroscope observations indicate reorganization of the normal relationship between caudal neurosecretory axons and capillaries. Accordingly, the possibility must be admitted that urophysial function is restored in *Tilapia* kept *for long periods* after urophysectomy, and the negative data obtained should be reviewed in this context. The unexpected regenerative ability of the caudal system is now under intensive study.

1. Enami, M., in Comparative Endocrinology, A. Gorbman, Ed., John Wiley, New York, 1959, p697.
2. Bern, H. A., Takasugi, N., Gen. Comp. Endocrinol., 1962, v2, 96.
3. Takasugi, N., Bern, H. A., Comp. Biochem. Physiol., 1962, v6, 289.
4. Maetz, J., Bourget, J., Lahlouh, B., J. Physiologie, 1963, v55, 159.
5. ———, Gen. Comp. Endocrinol., 1964, v4, 401.
6. Bennett, M. V. L., Fox, S., ibid., 1962, v2, 77.
7. Yagi, K., Bern, H. A., Science, 1963a, v142, 491.
8. ———, Am. Zoologist, 1963, v3, 508.
9. Sawyer, W. H., Bern, H. A., ibid., 1963, v3, 555.
10. Stanley, J. G., Fleming, W. R., Science, 1964, v144, 63.
11. Fridberg, G., Olsson, R. Z. Zellforsch, 1959, v49, 531.
12. Bern, H. A., Yagi, K., Nishioka, R. S., Arch. d'anat. micr., 1965, in press.
13. Pickford, G. E., Phillips, J. G., Science, 1959, v130, 454.

Received November 30, 1964. P.S.E.B.M., 1965, v118.

Estrogens and Corticoids in Relation to Mammary Gland Growth in Male Rats. (30056)

DORA JACOBSOHN AND A. NORGREN
(With technical assistance of A. Hult and C. Nykvist)
Institute of Physiology, University of Lund, Sweden

Development of the mammary glands of males differs widely among species. All possible variations seem to occur, from a few ducts hardly extending beyond the nipple area and fairly large glands consisting of ducts surrounded by dense clusters of alveoli lined with large, secretory epithelial cells. The growth of the mammary glands was believed to be elicited by androgenic hormones (1,4). Since androgenic hormones circulate in the blood of males of all species, the differences in degree and type of mammary development should be accounted for. Among possible causes are variations in the sensitivity of the effector tissues and influences exerted by other hormones. Interactions between androgenic and estrogenic hormones seem of special interest in this connection (2,7,8,9).

Few attempts have been made to investigate whether a rather extensive development of the mammary glands of males (*e.g.*, rats) may be due, at least partly to concomitant action of androgens and estrogens. The chief difficulties in such investigations are 1) to deprive the animal of estrogens, which may even originate from the metabolism of androgens and 2) to find what doses result in synergistic actions of the 2 hormones(2,7,8,9). For this reason, previous studies were performed on hypophysectomized rats under experimental conditions in which the mammary glands themselves either did or did not react upon estrogens. Results of these rather complicated experiments indicated that the development of alveolar lobules is dependent on delicately balanced actions of testosterone and estrogens(8,9).

Further inquiry appeared desirable, as, among other things, possible influences of the adrenal glands could not be excluded. The present work was performed on gonadectomized (gdx) adrenalectomized (adx) rats. Because of the metabolic deficiencies present in such animals it appeared likely that the response of the mammary glands to low, though not to higher, doses of testosterone would be markedly reduced(3,5,7). Possible effects produced in the mammary glands by additional treatment with cortisone, estrone, or both, could then be suitably studied.

Experimental. Male and a few female rats