We believe that the data presented on total Mg^{++} levels in erythrocytes are the first from any teleost fish during migration from freshwater into seawater. The functional significance of the changes remains to be determined.

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1 Ebel, H., and Gunter, T., J. clin. Chem. clin. Biochem. *18* (1980) 257.
2 Sasayama, Y., et al., Comp. Biochem. Physiol. *74A* (1983) 577.
3 D'Costa, M., and Pel-Tak-Cheng, Clin. Chem. *29* (1983) 519.
4 Nie, N. H., et al., Statistical package for the social sciences. McGraw-Hill, Inc., New York 1975.
5 Miles, H. M., and Smith, L. S., Comp. Biochem. Physiol. *26* (1968) 381.
6 Speich, M., Bousquet, B., and Nicolas, G., Clin. Chem. *27* (1981) 246.
7 Walser, M., Ergebn. Physiol. *59* (1967) 185.

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Somatic growth in hypophysectomized pituitary-homografted rats is promoted by prolactin

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Summary. Hypophysectomized male rats bearing a homograft of two adenopituitaries under the kidney capsule showed a significant increase in b.wt as compared to hypophysectomized non-homografted animals. Radioimmunoassay of growth hormone (GH), ACTH, α-MSH, β-endorphin and prolactin (PRL) revealed that only the latter was highly increased in the plasma of hypophysectomized homografted rats. These animals showed also increased levels of plasma corticosterone. However, daily injection of corticosterone failed to promote somatic growth in hypophysectomized non-homografted rats. These results suggest that PRL, and not other hormonal factors, promotes somatic growth in hypophysectomized homografted rats, and stress the concept that only PRL is secreted in significant amounts by pituitary homografts.
Key words. Prolactin; somatic growth; hypophysectomy; corticosterone; pituitary hormones.

It has been reported that prolactin (PRL) stimulates somatic growth in various species, including amphibians[1,2], reptiles[3] and birds[4]. In these species, PRL increases food consumption and promotes feeding behavior[5]. A stimulation of somatic growth has also been described in hypophysectomized pituitary-homografted rats[6–8]. These pituitary homografts are believed to secrete high amounts of PRL and little, if any, of the other hypophyseal hormones[9,10]. Although the rat implanted with extra anterior pituitary glands under the kidney capsule has been widely used as a model of chronic hyperprolactinemia, its hormonal status has not been fully characterized[11]. Various authors have described a progressive decrease in growth hormone (GH) plasma levels in hypophysectomized animals made hyperprolactinemic by adenopituitary homografts[12,13]. As hyperprolactinemic rats have been reported to exhibit also high levels of corticosterone[14,15], the present experiments were undertaken to investigate whether somatic growth in hypophysectomized homografted rats is indeed promoted by PRL or by other hormonal factors.

Male rats of the Wistar strain, weighing about 180 (± 20) g, were hypophysectomized by the trans-auricular route under ether anesthesia. During the same surgical session, the animals received a homograft of two adenopituitaries from animals of an identical strain under the kidney capsule. Control rats were sham-hypophysectomized and/or sham-homografted. All animals were kept at room temperature (21 °C) under a light-dark cycle (lights on between 08.00 and 20.00 h) with food and water available ad libitum. Water containing NaCl 0.9% was given to hypophysectomized rats. The completeness of hypophysectomy was checked after sacrifice.

A group of hypophysectomized and non-hypophysectomized non-homografted rats were injected s.c. daily with corticosterone (1 mg/kg, in corn oil) or with the vehicle. Somatic growth was checked by weighing the rats every week for 6 weeks after operation. At the end of this procedure, all animals were killed by decapitation and the blood was collected for radioimmunoassay (RIA).

PRL and GH were measured by RIA using reagents and proto-

Table 1. B. wt of male rats subjected to hypophysectomy and/or adenopituitary homografts under the kidney capsule (values are expressed in g)

Experimental groups	(n)	Weeks after surgery 0	1	2	3	4	5	6
1 Sham-hypophysectomy + sham-homografts	(8)	125 ± 1.8	160 ± 1.9	181 ± 2.4	210 ± 3.4	232 ± 3.6	241 ± 4.6	262 ± 4.8
2 Sham-hypophysectomy + homografts	(8)	126 ± 1.7	162 ± 2.1	179 ± 2.6	212 ± 3.6	234 ± 3.8	242 ± 4.2	263 ± 4.6
3 Hypophysectomy + sham-homografts	(8)	126 ± 1.5	108 ± 1.2	106 ± 1.3	108 ± 1.4	108 ± 1.6	107 ± 1.7	108 ± 1.6
4 Hypophysectomy + homografts	(8)	126 ± 1.8	107 ± 1.5	118 ± 1.3*	130 ± 2.0*	136 ± 2.3*	140 ± 2.7*	140 ± 2.6*

Values are mean ± SEM. * Significantly different as compared to group 3 ($p < 0.05$, Student's t-test, two-tailed).

Table 2. B.wt of male rats subjected to hypophysectomy and treated s.c. with corticosterone (1 mg/kg, daily) for 6 weeks (values are expressed in g)

Experimental groups	(n)	Weeks after surgery 0	1	2	3	4	5	6
1 Hypophysectomy + saline	(8)	125 ± 1.7	109 ± 1.2	108 ± 1.3	108 ± 1.4	107 ± 1.4	108 ± 1.3	108 ± 1.3
2 Hypophysectomy + corticosterone	(8)	125 ± 1.6	108 ± 1.1	108 ± 1.3	107 ± 1.3	108 ± 1.3	108 ± 1.3	107 ± 1.5

Values are mean ± SEM.

cols provided by the NIADDK Hormone Distribution Program. Interassay and intraassay variation for the PRL assay were 12.7% and 5%, and for the GH assay 11% and 6.2% respectively. RIA of ACTH was performed using materials obtained from CIS (France). The reference preparation was synthetic human $ACTH_{1-39}$ and the tracer was [^{125}I]iodo-porcine ACTH. Bound and free fractions were separated with dextran-coated charcoal. The intra- and interassay coefficients of variation were 9% and 18% respectively. [^{125}I]α-MSH was used as tracer for RIA of α-MSH. Separation of free and bound tracer was achieved by charcoal precipitation. The intra- and interassay coefficients of variation were 9.8% and 6.1% respectively. β-Endorphin immunoreactivity was measured using a New England Nuclear Corporation (USA) kit NEK-003, after extraction by the HPLC procedure. The intra- and interassay coefficients of variation were 10.4% and 12.4% respectively. Serum corticosterone levels were measured using a modification of the corticosterone binding globulin assay[16]. Corticosterone was extracted from serum with ethyl acetate and the bound and free fractions were separated with charcoal. The limit of sensitivity of this assay was approximately 0.5 µg.

Table 1 shows the results concerning the somatic growth of hypophysectomized rats as compared to non-hypophysectomized animals. Hypophysectomy resulted in a total suppression of somatic growth. However, hypophysectomized homografted rats showed a significant increase in b.wt over 6 weeks, although their somatic growth was much lower than that of non-hypophysectomized rats. Furthermore, the daily administration of corticosterone failed to affect the b.wt of hypophysectomized non-homografted rats (table 2). The RIA of GH, ACTH, α-MSH, β-endorphin and PRL revealed that, while the plasma levels of all these hormones were very low in hypophysectomized rats, only the latter showed a sustained increase in homografted rats (table 3). Furthermore, plasma corticosterone levels also appeared to be increased in homografted rats.

Earlier studies on hypophysectomized homografted rats have demonstrated abnormal GH secretion as manifested by diminished basal plasma GH concentrations[13], and abnormal GH responses to stimuli such as thyrotropin releasing hormone and vasopressin[17]. In contrast, Adler et al.[11] have recently shown that non-hypophysectomized rats bearing pituitary homografts have basal serum GH concentrations similar to those of control rats. Consistently, the present results show that hypophysectomized rats, both with and without homografts, exhibit very low plasma concentrations of GH. However, plasma PRL levels in rats which were either hypophysectomized and homografted or non-hypophysectomized and homografted were 3–4 times higher than those of non-hypophysectomized non-homografted animals.

Homografted rats showed an increase in plasma corticosterone levels, which has also been described by others[14,15]. This effect seems to be due to a direct effect of PRL produced by the homografts on the adrenal glands through an inhibition of the formation of reduced steroid metabolites[14]. In our experiments, hypophysectomized non-homografted animals did not show any increase in b.wt after daily treatment with corticosterone, suggesting that this steroid hormone does not affect significantly somatic growth in such animals. Thus, the somatic growth shown by hypophysectomized homografted rats may depend only on the high level of circulating PRL. Indeed, this hormone has been described to stimulate somatic growth in various species, including mammals[6–8].

Collectively, the present findings are consonant with the view that homografted adenopituitaries escape simultaneously the influence of a hypothalamic stimulatory tone on GH release and of an inhibitory tone on PRL secretion. However, other authors have shown a decrease in plasma PRL levels of hypophysectomized homografted rats a week after surgery[13]. A decline after 4 weeks has also been described[18]. In contrast, the present results show that plasma PRL levels in hypophysectomized homografted rats remain constant over a 6-week period.

The exclusive hypersecretion of PRL is a major characteristic of pituitary homografted rats. This model of hyperprolactinemia should be preferred rather than PRL-secreting tumors, which may simultaneously secrete several hormones and affect body and organ weight[19,20].

Table 3. Plasma levels of growth hormone (GH), prolactin (PRL), ACTH, α-MSH, β-endorphin and corticosterone in male rats subjected to hypophysectomy and/or adenopituitary homografts under the kidney capsule

Experimental groups	(n)	GH (ng/ml)	PRL (ng/ml)	ACTH (pg/ml)	α-MSH (pg/ml)	β-Endorphin (pg/ml)	Corticosterone (µg/100 ml)
1 Sham-hypophysectomy + sham-homografts	(8)	175 ± 15.1	18 ± 1.2	52 ± 2.0	154 ± 13.3	211 ± 24.1	1.54 ± 0.21
2 Sham-hypophysectomy + homografts	(8)	162 ± 16.4	74 ± 3.0*	43 ± 2.2	170 ± 15.1	220 ± 21.4	3.72 ± 0.90*
3 Hypophysectomy + sham-homografts	(8)	11 ± 0.1	n.d.	n.d.	n.d.	20 ± 1.0	0.10 ± 0.01
4 Hypophysectomy + homografts	(8)	9 ± 0.2	68 ± 2.7*	n.d.	n.d.	22 ± 1.1	1.11 ± 0.21**

Values are mean ± SEM. * Significantly different as compared to group 1 ($p < 0.05$, Student's t-test, two tailed). ** Significantly different as compared to group 3 ($p < 0.05$, Student's t-test, two tailed). n.d. = not detectable.

1 Zipser, R.D., Licht, P., and Bern, H.A., Gen. comp. Endocr. *13* (1969) 382.
2 Etkin, W., and Lehrer, R., Endocrinology *67* (1960) 457.
3 Licht, P., Gen. comp. Endocr. *9* (1967) 49.
4 Meier, A.H., and Farner, D.S., Gen. comp. Endocr. *4* (1964) 584.
5 Nicoll, C.S., in: Handbook of Physiology, vol. 7: Endocrinology, p. 229. American Physiological Society, Washington 1974.
6 May, R.M., C. r. Soc. exp. Biol. *124* (1937) 920.
7 Goldberg, R.C., and Knobil, E., Endocrinology *61* (1957) 742.
8 Hertz, R., Endocrinology *65* (1959) 926.
9 Meites, J., Nicoll, C.S., and Talwalker, P.K., in: Advances in Neurobiology, p. 238. Ed. A.V. Nalbandov. Un. Illinois Press, Urbana 1963.
10 Welsch, C.W., Negro-Vilar, A., and Meites, J., Neuroendocrinology *3* (1968) 238.
11 Adler, R.A., Herzberg, V.L., and Sokol, H.W., Life Sci. *32* (1983) 2957.
12 Schalch, D.S., and Reichlin, S., Endocrinology *79* (1966) 275.
13 Panerai, A.E., Gil-Ad, I., Cocchi, D., Locatelli, V., Rossi, G.L., and Müller, E.E., J. Endocr. *72* (1977) 301.
14 Witorsch, R.J., and Kitay, J.I., Endocrinology *91* (1972) 764.
15 Marshall, S., Huang, H.H., Kledzik, G.S., Campbell, G.A., and Meites, J., Endocrinology *102* (1978) 869.
16 Murphy, B.E.P., J. clin. Endocr. Metab. *27* (1967) 973.
17 Udeschini, G., Cocchi, D., Panerai, A.E., Gil-Ad, I., Rossi, G.L., Chiodini, P.G., Liuzzi, A., and Müller, E.E., Endocrinology *98* (1976) 807.
18 Chen, C.L., Amenomori, Y., Lu, H.K., Vogt, J.L., and Meites, J., Neuroendocrinology *94* (1974) 1709.
19 MacLeod, R.M., Smith, M.C., and Dewitt, G.W., Endocrinology *79* (1966) 1149.
20 Fang, V.S., Refetoff, S., and Rosenfield, R.S., Endocrinology *95* (1974) 991.

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Antidiuretic effects of oxytocin in the Brattleboro rat

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Summary. The antidiuretic activity of oxytocin (OT) was measured in Brattleboro rats with congenital diabetes insipidus. A dose dependent antidiuretic response was found in animals receiving chronic infusions of 0.1 μg/h, 1.0 μg/h, and 5 μg/h of OT. OT infused at the rate of 5 μg/h over a 7-day period completely reversed the symptoms of diabetes insipidus. The results support the concept that OT serves as a weak agonist of vasopressin at the level of the kidney and at pharmacological levels exhibits antidiuretic activity.
Key words. Oxytocin; antidiuretic activity; Brattleboro rats.

Experiments on the renal effects of oxytocin (OT) have yielded variable results, but most studies indicate that OT serves as both a weak agonist at the renal vasopressin receptor and has important natriuretic effects[1–5]. Elevated levels of OT in the plasma and decreased stores of OT in the pituitary are found in the vasopressin-deficient Brattleboro rat[6,7] indicating that OT is being secreted in response to the severe fluid and electrolyte imbalances resulting from the absence of vasopressin and raising the possibility that OT might correct some of the fluid inbalance. Edwards, LaRochelle, and Gallai [3] found that plasma OT levels increased in the Brattleboro rat concomitant with increased urine osmolalities during a 24-h dehydration study. However, in a second part of their study they suggested that OT had little or no role in the increased concentrating ability of dehydrated DI rats because infusions to produce similar elevated levels of OT in normally hydrated Brattleboro rats led to only a slight rise in urine osmolality.

The present study was undertaken to further clarify the role of OT in fluid regulation in the DI rat. Our research group is investigating the structural and functional development of neural transplants using the Brattleboro rat as a model [8,9]. These studies entail grafting fetal vasopressin neurons into the third ventricle of Brattleboro hosts and measuring the ability of the transplants to ameliorate the host's diabetes insipidus. The proper evaluation of our experiments requires a better understanding of the effects of OT since the transplants may stimulate (or inhibit) OT release. Furthermore, as OT levels may be permanently increased following transplantation, previous results with acute infusions of OT might not be applicable to our studies. Therefore, in the present set of experiments, OT was chronically infused via osmotic minipumps to better mimic the effects of constant elevated levels of OT which may result from transplantation.

Materials and methods. Adult male Brattleboro rats (Blue Spruce Farms, Altamont, NY) homozygous for the diabetes insipidus trait and weighing 225–450 g were used. They had been castrated more than twenty days prior to the beginning of this experiment as part of preliminary study on the effects of sex steroids on water balance; the castration caused only very minor changes, and all animals began the present study from a common baseline. The rats were maintained in stainless steel metabolism cages on a 12/12-h light-dark cycle with food and water provided ad libitum. They were anesthetized with ether and an Alzet osmotic minipump (Alza, Palo Alto, CA), calibrated to deliver 1.0 μl/h of solution, was implanted subcutaneously above the scapula. Pumps were filled either with physiological saline or solutions of synthetic OT (Bachem Inc., Torrance, CA) in saline at titers of 0.1 μg/μl, 1.0 μg/μl, or 5.0 μg/μl. Water consumption and urine osmolality were monitored daily in all animals for a minimum of three days prior to and three days after pump implantation. The animals receiving 5 μg/h OT were monitored for seven days after implantation.

Animals receiving saline, 0.1 μg/h and 1.0 μg/h OT, were used to

Oxytocin measurements

Test Group	Plasma oxytocin μU/ml	Hypothalamic oxytocin mU/total	Pituitary oxytocin mU/total	Urine osmolality mOsm/L
Saline (n = 8)	10.7 ± 2.7	7.51 ± 0.82	749 ± 62	250 ± 20
0.1 μg (n = 6)	71.4 ± 13.4*	8.75 ± 0.42	1653 ± 571	343 ± 47
1.0 μg/h (n = 8)	227.3 ± 21*	8.83 ± 1.4	729 ± 140	853 ± 35*

Data are presented as mean values ± SEM. *Significantly different for the saline controls $p < 0.005$. 1 μU OT = 1.65 ± 0.12 pg synthetic OT (Bachem).