

flecting the evolution of mat-forming filaments of increasingly larger diameter.

3) Walter *et al.* (2) have postulated that gliding motility, phototaxis, and interfilament cohesion are the essential characteristics that enable cyanophytes to form the conical laminae of modern *Conophyton*. As evidenced by the prevalence of discarded, empty sheaths (and naked trichomes) in *C. gaubitzia*, it seems probable that oscillatorian gliding and phototaxis (or other tactic response) played a similar role in the formation of fossil conical laminae. Although there is little evidence in *C. gaubitzia* of interfilament cohesion, the preserved filaments are moderately to heavily ensheathed; such cyanophytes secrete copious mucilage, a factor possibly required for formation of the resilient, relatively high amplitude, peaked, and often ridged mats of *Conophyton*. In addition, the apparently differential distribution of filament types in *C. gaubitzia* could be of significance; the broad oscillatoriaceans (and associated mucilage) may have provided structural support on flanks of the growing stromatolites while, in a manner similar to that postulated for modern *Conophyton* (2), relatively narrow, more active, rapidly gliding taxa were dominant in crestal zones.

4) The microorganisms in *C. gaubitzia* are generally quite similar to those known from other microbiotas of comparable age. There is no evidence to suggest that fossil *Conophyton* was produced by an atypical stromatolitic community or by taxa that became extinct at the close of the Precambrian. On the contrary, many of the *C. gaubitzia* filaments seem virtually identical in morphology to living cyanophytes (Fig. 1), a similarity well-illustrating the evolutionary conservatism characteristic of such prokaryotes (15). With regard to the demise of *Conophyton*, and as Awramik (16) was apparently first to suggest, it seems possible that such stromatolites may have been excluded from the Phanerozoic by the advent of grazing metazoans (17), especially if early invertebrates were limited in distribution to (or were particularly abundant within) permanently submerged settings of the type in which *Conophyton* most commonly occurs (18).

5) In recent years there has been a marked increase of interest in the possible use of stromatolites for global biostratigraphic zonation of the Precambrian. Although many aspects of such zonation remain open to question, available data seem to establish that some Proterozoic stromatolites had limited range zones and that assemblages

of stromatolites therefore changed ("evolved") through time (1, 3, 4, 10). However, because few well-preserved microbiotas have previously been reported from relatively complex, stratigraphically useful stromatolites, the presumed biologic bases of these changes have yet to be defined. In light of the results here reported, it seems likely that it will soon prove feasible to determine whether differing types of coeval complex stromatolites were formed by differing microbial communities, whether the microorganisms themselves can provide a reliable basis for biostratigraphic correlation (14), and whether the evolution of such organisms was the causative factor resulting in the evolution of stromatolites.

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19. We thank T. R. Fairchild, C. D. Gebelein, R. J. Horodyski, I. N. Krylov, V. Yu. Shenfil', M. R. Walter, and M. S. Yakshin for helpful discussions, M. R. Walter for providing a preprint of (2), and I. N. Krylov for providing an isotype of *C. gaubitzia*. This investigation was initiated in April 1975, when J.W.S. was an Exchange Scientist in Novosibirsk, sponsored by the Academies of Sciences of the Soviet Union and the United States. Studies at the University of California, Los Angeles, were supported by NSF grant GB 37257 (Systematic Biology Program) and by NASA grant NGR 05-007-407.

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Vasopressin and Oxytocin Are Depleted from Rat Hypothalamic Nuclei After Oral Hypertonic Saline

Abstract. *Vasopressin and oxytocin were measured by radioimmunoassay in rat posterior pituitary and microdissected hypothalamic areas after 3 and 10 days of oral 2 percent sodium chloride in place of drinking water. There was a significant decrease in concentration of both hormones in posterior pituitary and in specific areas of the hypothalamus. Supraoptic, paraventricular, and arcuate hypothalamic nuclei and the retromammillary area had decreased concentration of one or both hormones following hypertonic saline, while hormone concentration in the suprachiasmatic nucleus and median eminence was unaffected.*

Vasopressin and oxytocin have traditionally been thought to originate in neuronal cell bodies in supraoptic and paraventricular nuclei of the hypothalamus and to be transported along axons to the posterior pituitary for storage and release in response to physiologic stimuli (1). Dehydration is a potent stimulus for release of vasopressin and probably also oxytocin, as continued dehydration causes marked depletion of posterior pituitary stores of biologic activity of both hormones (2). Determination of the effect of dehydration on vasopressin and

oxytocin in individual hypothalamic nuclei has been difficult because of the complex structure of the hypothalamus. Using a recently devised technique for microdissection of individual hypothalamic nuclei (3) and radioimmunoassay, we have found vasopressin in six of 32 microdissected hypothalamic areas (4). Here we report the effect of oral hypertonic saline on vasopressin and oxytocin concentration in these six areas.

Twelve male Wistar rats with an average weight of 261 g were divided into two groups of six rats each. One group was

given 2 percent NaCl (saline group) as drinking water, while the control group drank tap water. After 3 days the control group had increased average body weight by 5 percent, while the saline group had lost 7 percent of initial body weight. An additional 12 rats were similarly divided into two groups drinking 2 percent NaCl or tap water, respectively, for 10 days. The initial average weight of these second 12 rats was 281 g; the control group gained 22 percent of initial weight during the 10-day period while the average body weight of the saline group did not change.

At the end of the 3- and 10-day periods the two saline and two control groups of rats were killed by decapitation and their brains and posterior pituitaries were removed and quick-frozen in Dry Ice. Serial alternating 300- μ m and 100- μ m frozen sections were cut in the frontal plane in a cryostat at -10°C . The 100- μ m sections were stained to locate hypothalamic nuclei and areas according to the atlas of Konig and Klippel (5), and tissue samples were removed from the 300- μ m frozen sections with stainless steel cannulas 300 or 400 μ m in diameter with the aid of a stereomicroscope. These diameters are well within the diameter of the hypothalamic nuclear areas, so that only tissue from nuclear areas is obtained. This has been verified by staining of tissue slices after microdissection. Tissue from each microdissected area from each rat was homogenized in 0.1 ml of 0.1N HCl by ultrasound. A sample was taken for protein determination by the micromethod of Lowry *et al.* (6), and the remainder was assayed for vasopressin and oxytocin by separate, specific radioimmunoassays sensitive to 4 pg of hormone and each not interfered with by 1000 pg of the other hormone. The inter-assay coefficient of variation of the assays was less than 10 percent. The methodology for development and performance of the radioimmunoassays has been reported (7). Vasopressin was assayed in all four groups of rats, while oxytocin was assayed in the two groups in the 10-day experiment.

Data were analyzed by mixed model analysis of variance with repeated measures followed by *t*-test with Satterthwaite's approximation to combine error terms (8). The results of vasopressin measurement after 3 days of oral hypertonic saline and after 10 days of oral hypertonic saline, and the results of oxytocin measurement after 10 days of oral hypertonic saline, were each considered as a separate set of data. Because of a systematic inhomogeneity of the data, \log_{10}

Table 1. Hormone concentration (mean picograms per microgram of protein \pm standard error of the mean) after 3 or 10 days, during which control (C) rats drank tap water and the saline (S) groups drank 2 percent NaCl. The *P* values refer to the significance of the difference between certain tissues of the control groups and the same tissues of the corresponding saline groups. Abbreviation: N., nucleus.

Region	Rats	Vasopressin after 3 days	Vasopressin after 10 days	Oxytocin after 10 days
Posterior pituitary	C S	$18,321 \pm 1,220$ $8,406 \pm 1,827$ [*]	$18,077 \pm 2,086$ $4,318 \pm 1,712$ [*]	$2,650 \pm 247$ $1,272 \pm 245$ [*]
N. supraoptic	C S	$7,876 \pm 2,008$ $11,338 \pm 1,293$	$2,709 \pm 457$ $1,465 \pm 245$	72.1 ± 10.0 40.2 ± 6.9 [†]
N. arcuatus	C S	506 ± 83.3 214 ± 28.8 [*]	642 ± 206 165 ± 40 [*]	26.8 ± 4.0 14.0 ± 2.8 [†]
Retrochiasmatic area	C S	17 ± 5.2 61 ± 6.4 [‡]	133 ± 40 40 ± 12 [‡]	9.3 ± 2.0 11.2 ± 2.5
N. paraventricularis	C S	594 ± 260 $1,057 \pm 309$ [†]	$1,990 \pm 1,120$ 959 ± 242	34.6 ± 5.1 27.9 ± 3.2
N. suprachiasmatic	C S	273 ± 44 229 ± 32	210 ± 40 188 ± 39	19.8 ± 3.0 20.7 ± 5.6
Median eminence	C S	$5,460 \pm 368$ $5,965 \pm 855$	$2,838 \pm 283$ $2,256 \pm 247$	100.3 ± 17.1 70.8 ± 11.3

^{*}*P* < .01. [†]*P* < .05. [‡]*P* < .02.

transformation was performed before statistical analysis.

As expected, the posterior pituitary glands were markedly depleted of vasopressin by even 3 days of oral 2 percent NaCl and more so after 10 days (Table 1). There was also significant depletion of oxytocin in the posterior pituitary after 10 days of drinking 2 percent NaCl. In the hypothalamus after 3 days of oral 2 percent NaCl, there was a significantly lower concentration of vasopressin in the arcuate nucleus when compared with controls and significantly higher concentration of vasopressin in the paraventricular nucleus and retrochiasmatic area. After 10 days of oral 2 percent NaCl the arcuate nucleus and retrochiasmatic area had significantly lower concentrations of vasopressin. Also after 10 days of oral 2 percent NaCl, oxytocin concentration in supraoptic and arcuate nuclei was significantly lower. Although there appeared to be some increase in vasopressin concentration in the supraoptic nucleus after 3 days of dehydration and a decrease in vasopressin concentration in supraoptic and paraventricular nuclei after 10 days of dehydration, there were no statistically significant differences in hormone concentration in these areas or in the suprachiasmatic nucleus or median eminence. There is no reason why the two control groups of rats, in the 3-day and 10-day experiments, should have differing vasopressin concentration in posterior pituitary or hypothalamus although the 3-day and 10-day experiments were performed at different times. Indeed, vasopressin concentrations in posterior pituitaries of the two groups are re-

markably similar. However, there were differences between the two control groups in vasopressin concentration in various hypothalamic nuclei, indicating perhaps greater sensitivity of the hypothalamus to minor differences in housing and handling and emphasizing the necessity for having concurrent control and experimental groups.

Thus, oral hypertonic saline not only depletes vasopressin and oxytocin in the posterior pituitary but also produces changes in hormone concentration in specific areas of the hypothalamus. The paraventricular nucleus and the retrochiasmatic area, which contains axons connecting supraoptic and paraventricular nuclei with the posterior pituitary, both had significant increases in vasopressin concentration after 3 days of oral hypertonic saline at a time when posterior pituitary vasopressin content was significantly decreased. As hormone concentration in microdissected brain areas represents the net effect of changes in hormone synthesis and release, this implies that in the first few days of oral 2 percent NaCl there is greater increase in vasopressin synthesis and transport within the hypothalamus than in hormone release. Dehydration has been reported to stimulate incorporation of labeled amino acids into vasopressin in slices of guinea pig hypothalamus (9). In monkeys, water deprivation caused an acceleration of action potential firing of supraoptic neurons (10), and this is presumably related to increased hormone secretion from the terminals of these axons in the posterior pituitary. The signal for increased hormone synthesis in response to hyper-

tonicity is unknown. By 10 days of oral 2 percent NaCl, vasopressin concentration in both the supraoptic nucleus and retrochiasmatic area was less (although only in the latter area was the decrease statistically significant), implying that the rate of transport of hormone away from these areas had exceeded synthesis.

Hypertonic saline ingestion was chosen as the dehydration stimulus because it is as potent as complete water deprivation in causing depletion of vasopressin and oxytocin biologic activity from the rat posterior pituitary (2). Hypertonic saline ingestion is probably less of a general stress to the animal than complete water deprivation and thus should be a more specific hypertonic stimulus. There was no significant difference in protein content of each microdissected area when tissue from dehydrated rats was compared with tissue from control rats. Thus, the differences reported here with hormone expressed as picograms per microgram of protein are due to changes in the content of hormone and not protein. Microdissection was confined to within nuclear areas so as not to include extraneous tissue, and therefore total hormone content of hypothalamic nuclear areas was not measured.

It is of interest that there was no significant change in vasopressin or oxytocin concentration in median eminence at times of significant changes in concentration of both hormones in the posterior pituitary and in various areas of the hypothalamus. There was also no significant change in concentration of either hormone in the supraoptic nucleus in response to oral hypertonic saline. Neurophysins, proteins that bind vasopressin and oxytocin, have been found by immunofluorescence in supraoptic, paraventricular (11), and supraoptic nuclei (12). There is also a recent report of neurophysin in the bovine arcuate nucleus (13).

The depletion of vasopressin from the arcuate nucleus after 3 days of oral hypertonic saline is presumably not due to changes in axons in passage through the nucleus, as vasopressin concentration in the retrochiasmatic area, which we assume to contain axons in passage, was increased after 3 days of oral hypertonic saline. The arcuate nucleus lies just above the median eminence, and it is possible that vasopressin and oxytocin are serving as neurotransmitters in this nuclear area and may be involved in control of anterior pituitary function (14).

The arcuate nucleus has not been thought to be part of the neurosecretion system for vasopressin or oxytocin, but from our findings here it appears to be in-

volved in the dehydration response. This is consistent with an earlier autoradiography study in which we found increased incorporation of [³H]uridine into RNA in response to oral hypertonic saline in the arcuate as well as the supraoptic nucleus but not in supraoptic or paraventricular nuclei (15). Evidently, in both supraoptic and arcuate nuclei, dehydration causes depletion of vasopressin and oxytocin and possibly synthesis of new proteins or polypeptide hormones.

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Heart Rate in Spiders: Influence of Body Size and Foraging Energetics

Abstract. Resting heart rates in 18 species of spiders as determined by a cool laser transillumination technique range from 9 to 125 beats per minute. Cardiac frequencies obtained in this fashion may readily serve as a measure of standard rates of metabolism. A spider's resting heart rate is a function of body size and of foraging energetics.

Spiders are interesting to physiological ecologists because of their highly organized, yet relatively simple body plan and because they constitute a major link

as predators in most terrestrial food webs. Little is known about their energy budgets under natural conditions. Standard metabolic rates can be derived from

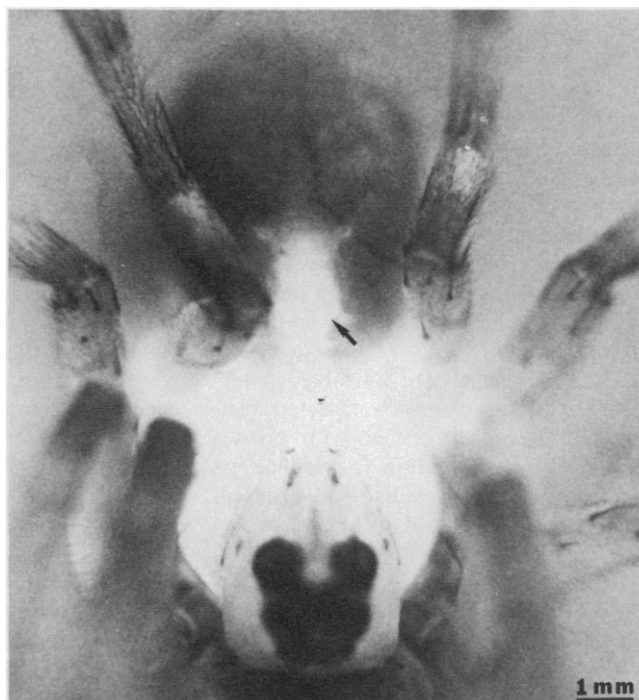


Fig. 1. Laser-illuminated wolf spider (*Lycosa ceratiola*). The tubular heart is visible as a pulsating oval spot on the dorsal surface of the abdomen (arrow) when the spider is irradiated from below by a cool He-Ne laser.