

shown that this effect (i) occurs at concentrations similar to those found to be therapeutic in patients, (ii) occurs in primates as well as rodents, and (iii) is transient, lasting less than an hour after a single administration but can be sustained more than 12 hours with long-term treatment similar to that needed for antidepressant response.

These results have both basic and clinical implications. First, three quite different treatments, all capable of altering central adrenergic neurons, alter cerebral fluid dynamics. Second, they have heuristic value, bringing together two discrete hypotheses: the catecholamine theory of affective illness and the central adrenergic vasoregulatory hypothesis proposed by Hartman and colleagues (2). This report indicates that the blood-brain barrier is under the influence of the central adrenergic system and suggests that such homeostatic regulation may be involved in the pathophysiology of affective illness. What exactly is altered (for example, brain water, ions, substrates) of importance to these illnesses remains to be defined. However, the failure of such a neurohumoral system capable of tonically influencing other cerebral tissue and cerebral homeostasis could explain how the global functional impairment—that is, mood alterations, cognitive and psychomotor changes, and disturbances of sleep, appetite, and energy—seen in affective disorders could occur and yet fully remit without residual cerebral tissue pathology.

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## Possible Adaptive Value of Water Exchanges in Flexible-Shelled Eggs of Turtles

**Abstract.** *Use of energy reserves by embryos of common snapping turtles (Chelydra serpentina) is related to the hydric conditions to which eggs are exposed during incubation and to the net exchanges of water through the eggshells. Embryos developing inside eggs with a relatively favorable water balance use more of their energy reserves metabolically and grow larger before hatching than embryos inside eggs with less favorable water exchanges.*

Both laboratory studies (1–4) and field studies (5) indicate that the flexible-shelled eggs of many species of turtles exchange water with surrounding air and soil, but there is no consensus concerning the significance of this phenomenon (6). We report that the use of energy reserves by embryos of common snapping turtles (*Chelydra serpentina*) is related to the net exchange of water in incubating eggs and that hatchlings from eggs incubated in favorable hydric conditions are larger than turtles hatching from eggs incubated in less favorable settings. Because survival of young of other species of turtles is positively correlated with body size (7), the exchanges of water experienced by flexible-shelled eggs of turtles during natural incubation may have adaptive significance.

Eggs collected from two fresh nests (8) were incubated in covered containers at 29°C. Inside the containers, randomly selected eggs from both clutches were either half-buried in substrates, where water potentials were –130, –375, or –610 kilopascals (kPa) (2), or incubated on wire platforms above the substrates (9). Egg mass was measured on day 1 of incubation and then weekly through day 50; hatching began on day 56 (8). Small amounts of water were added to the substrates twice weekly to maintain relatively constant water potentials (2).

Changes in egg mass were similar to those reported for eggs of painted turtles (*Chrysemys picta*) (2). Eggs in the substrates generally increased in mass during the first half of incubation (indicating that eggs experienced net water absorption) and decreased in mass during the second half (indicating that eggs experienced net water loss). Eggs on the plat-

forms generally experienced only small changes in mass during the first half of incubation and large declines in mass during later stages of development. The overall change in egg mass between days 1 and 50, which can be used as an index to hydric balance, was related both to the water potentials in the substrates and to placement of eggs on platforms or in substrates (Table 1).

After hatching, measurements were taken of the live mass, carapace length, carapace width, and head width of young turtles (Table 1). The hatchlings were frozen and their carcasses, including residual yolk withdrawn into the abdominal cavity before hatching, were dried to constant mass at 60°C (Table 1). Because none of these measures of mass or linear dimension is necessarily a reliable index to size (10), we submitted the data to a principal components analysis and generated a size index for each hatchling that summarized the variation in all of the measured variables (10, 11). The original data and the computed size indices were then examined by separate two-way analyses of covariance (12); substrate water potential and placement of eggs in substrates or on platforms were the fixed factors, and egg mass on day 1 was a covariate (13). The covariate was used to reduce variation in the data stemming from differences in the size of the eggs in which the turtles developed (2, 3, 9).

Live mass, linear dimensions, and size indices for hatchlings that emerged from eggs incubated in contact with the substrate were invariably larger than comparable values recorded for turtles hatched from eggs incubated on wire platforms in the same containers ( $P \leq .001$ ) (Table 1

Table 1. Mean values of egg mass on day 1 and net change in egg mass during incubation (days 1 through 50) and of mass, linear dimensions, and size index of hatchlings. Data were adjusted by analysis of covariance (12); egg mass was used as a covariate to compensate for variation in the size of eggs at the outset of the study. The least significant difference (LSD) at  $\alpha = .05$  was computed for each data set from the harmonic mean for sample size (12); LSD = 0.267 g for egg mass on day 1, 0.291 g for net change in egg mass during incubation, 0.209 g for live mass of hatchlings, 0.040 g for dry mass of hatchlings, 0.68 mm for carapace length, 0.85 mm for carapace width, 0.19 mm for head width, and 1.218 for the size index.

Treatment	N	Egg mass (g)		Hatchlings					
		Day 1	Change	Mass (g)		Carapace (mm)		Head width (mm)	Size index
				Live	Dry	Length	Width		
Platform	13	8.967	-1.817	<i>Water potential -610 kPa</i>		24.26	21.81	8.90	-2.513
	12	9.102	-1.371	5.360	1.349	25.14	22.76	9.03	-0.982
Substrate	8	9.253	-1.416	<i>Water potential -375 kPa</i>		25.11	23.12	9.10	-0.543
	10	9.244	-0.534	5.653	1.327	25.26	23.52	9.35	0.686
Platform	11	9.109	-1.045	<i>Water potential -130 kPa</i>		25.40	23.27	9.24	0.351
	13	8.971	1.141	5.957	1.328	26.48	24.34	9.50	2.929
Substrate				6.835	1.262				

and Fig. 1A). Furthermore, all of these variables varied as positive, linear functions of substrate water potential ( $P < .001$ ), whether or not the eggs were on platforms or in the substrates (Table 1 and Fig. 1A).

Variation in the dry mass of hatchlings was the inverse of that for the other variables (Table 1 and Fig. 1B). The mass of dried carcasses was smaller for hatchlings from eggs incubated in contact with substrates than that for turtles hatched from eggs on the platforms in the same containers ( $P < .001$ ), and dry mass also varied among samples as a negative, linear function of substrate water potential ( $P = .002$ ). The interaction between water potential and placement of eggs was not statistically significant.

The size index of hatchling snapping turtles was related to the hydric conditions under which the eggs were incubated (Fig. 1A) and therefore to the net exchange of water experienced by these eggs during incubation (Table 1). Large hatchlings of other species of turtles are more likely to survive the first year of life than are smaller conspecifics (7), indicating that the water exchanges experienced by eggs of snapping turtles incubating in natural nests could be one determinant of the survival of hatchlings. Higher rates of survival of large hatchlings could be related to their superior exploitation of food resources and attendant rapid growth (14). Indeed, this contention is supported by our data on head width of hatchling snapping turtles (Table 1), which indicate that large hatchlings have wider gapes than do small animals. Large hatchlings may therefore be able to exploit a greater variety of prey than is available to smaller conspecifics (15).

The dry mass of the contents of turtle eggs declines during incubation as organic matter is oxidized by developing embryos and used in growth and maintenance metabolism (4). Consequently, the dry mass of hatchlings, including residual yolk, is a measure of how much remains of the energy reserve that was present in eggs at oviposition. Embryos that use relatively large amounts of their

energy reserve have small dry masses at hatching, whereas embryos that use relatively small amounts have large dry masses.

The amount of organic matter consumed by embryonic snapping turtles in this study was related to the hydric conditions to which eggs were exposed during incubation (Fig. 1B) and therefore to the net exchange of water experienced by these eggs (Table 1). Embryos in eggs that experienced a relatively favorable water balance were able to use more of their energy reserves in growth and maintenance than was possible for embryos inside eggs where water exchanges were less favorable. The embryos that used available energy reserves most completely also attained the largest sizes before hatching (Fig. 1).

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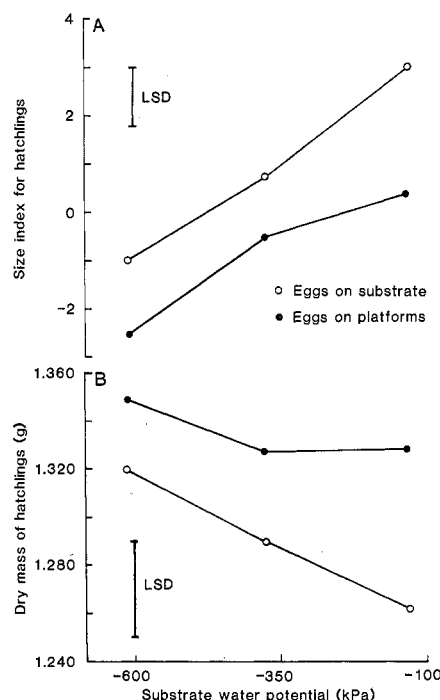


Fig. 1. (A) Mean adjusted values of size indices for hatchling snapping turtles. High (positive) numbers characterize large hatchlings, and low (negative) numbers characterize small hatchlings. (B) Mean adjusted dry mass of carcasses, including residual yolk, of hatchling snapping turtles. Means differing by the least significant difference (LSD) are significantly different at  $\alpha = .05$  (12).

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## Carbon Flow in Plant Microbial Associations

**Abstract.** Measurement of the distribution of the photosynthesis product in the symbiotic association of a legume, a mycorrhizal fungus, and nitrogen-fixing bacteria showed that the fungus incorporated 1 percent of the photosynthesis product and respired 3 percent. The nodules of a 5-week-old plant utilized 7 to 12 percent of the photosynthesis product. The legume compensated in part for the needs of its microbial partners through increased rates of photosynthesis.

Symbiotic associations between plants and microorganisms have a major effect on plant growth and nutrient cycling. Rhizobia associated with legumes can fix 450 kg of nitrogen per hectare per year, and vesicular arbuscular (VA) mycorrhizal fungi enhance the uptake of many elements, notably phosphorus. The carbon flow to the nodules of legumes

grown in sand culture has been measured (1, 2). The dynamics and quantities of the carbon flow to the VA mycorrhiza and the interactions between the two microbial symbionts are unknown.

We used field and growth chamber studies,  $^{14}\text{C}$  and  $^{15}\text{N}$  labeling, and fungal and nodule biomass measurements to determine (i) the quantities of plant car-

bon translocated to the mycorrhizal and rhizobial symbionts of faba beans (*Vicia faba*), (ii) the extent of nitrogen fixation by rhizobia in nodules of mycorrhizal and nonmycorrhizal plants, and (iii) the effect of the carbon utilized by the microorganisms on host growth. The VA fungus *Glomus mosseae*, which we used as inoculum, had significantly increased the growth of *V. faba* and the phosphorus contents in the field at low or moderated levels of soil phosphorus (3).

The cost of the mycorrhizal infection to the plant was studied on 4- to 5-week-old *V. faba* plants growing in a mixture of soil and sand (1:1) with and without mycorrhizal and rhizobial infection. To obtain plants of similar size in the various treatments, nonmycorrhizal plants were supplemented with potassium acid phosphate ( $\text{K}_2\text{HPO}_4$ ), and nitrate nitrogen was added to nonrhizobial treatments. Carbon distribution and flow to symbionts were determined by exposing the above-ground plant parts to  $^{14}\text{CO}_2$  in a Plexiglas chamber designed so that atmosphere beneath the ground could be separated from that above ground. The  $^{14}\text{C}$  contents of plant materials, nodules, and external hyphae were determined by liquid scintillation after dry combustion and absorption of the  $^{14}\text{CO}_2$  in NaOH (4). Carbon dioxide, respired by underground portions during and after the pulse labeling, was absorbed for  $^{14}\text{CO}_2$  determination; fungal biomass was measured by microscopy (5).

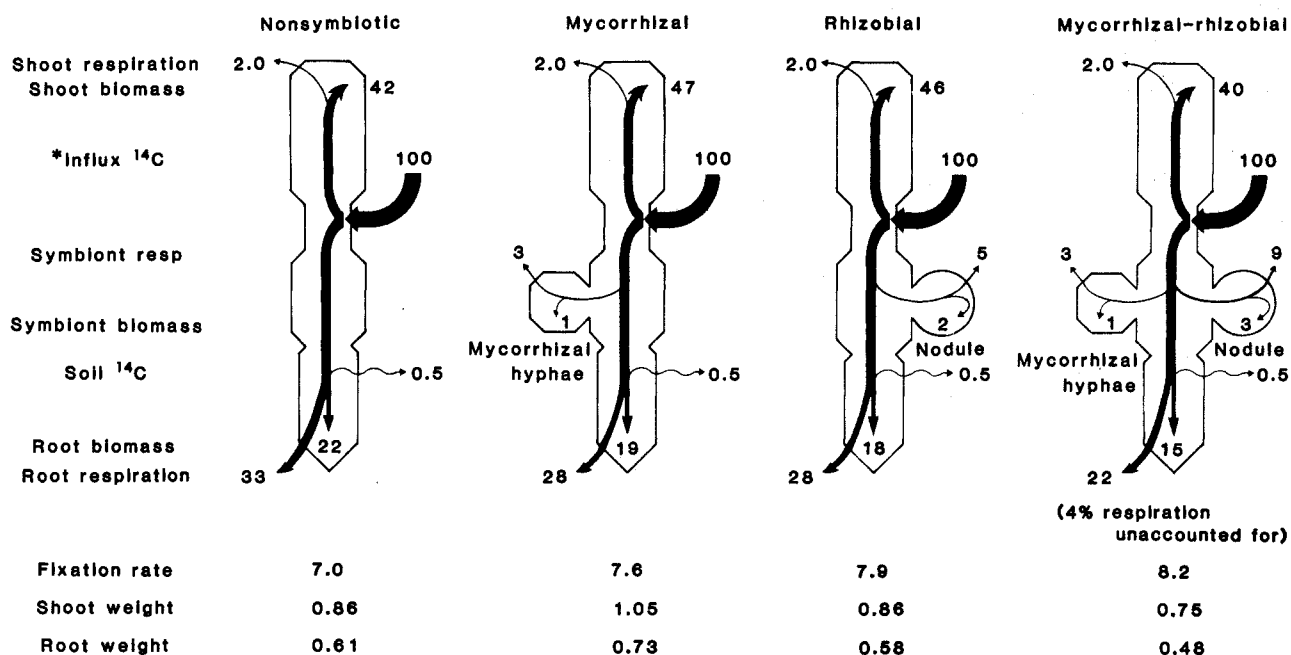


Fig. 1. The  $^{14}\text{C}$  flow to various compartments of symbiotic and nonsymbiotic faba beans (4 to 5 weeks old) after shoots were exposed above ground to  $^{14}\text{CO}_2$  under continuous light. The fixation rate is expressed as milligrams of carbon per gram of shoot per hour. The shoot weight and the root weight are expressed as grams of carbon. The carbon influx has been equalized to 100 units of carbon per gram of shoot carbon.