Evaporative Water Loss in Box Turtles: Effects of Rostral Brainstem and Other Temperatures

Abstract. *Box turtles were implanted with thermodes astride the preoptic tissue of the brainstem. The rate of evaporative water loss could be transiently increased by heating the rostral brainstem. Heating tissue in the anterior hypothalamus affected evaporative water loss only at high ambient temperatures. The magnitude of the response was proportional both to the change in hypothalamic temperature and to the ambient temperature with which the turtle was in equilibrium. The major function of a high rate of evaporative water loss in turtles is probably to protect the brain from overheating during thermal stress.*

The temperature of the rostral brainstem (hypothalamic area) influences the behavioral regulation of body temperature in ectotherms (*1*) and causes changes in some autonomic functions such as gill ventilation rate in fish (*2*) and arterial pressure in turtles (*3*). The rate of panting in chuckwallas increases during preoptic heating (*4*). Evaporative water loss (EWL) in terrestrial ectotherms by panting or other means is of interest since it is an autonomic function directly concerned with temperature regulation.

Box turtles possess a remarkable capacity for evaporative cooling, maintaining core temperatures as much as 10.5°C below ambient (*5*). Several species of turtles increase their evaporative cooling rate by salivating over their head and forelegs (*6*, *7*). In addition, the box turtle discharges urine from the cloaca over the hind legs (*7*). The relative influence of brain and other body temperatures over the rate of EWL in turtles has not been extensively investigated. Riedesel *et al.* (*7*) attempted to affect salivation in box turtles by heating and cooling the entire head, but their results were equivocal. We report here on a study of the effect of heating the rostral brainstem at different ambient temperatures on the EWL capacity of the box turtle.

Box turtles (*Terrapene ornata* and *Terrapene carolina major*) weighing 200 to 600 g were anesthetized, and thermodes identical to those used by Hammel *et al.* (*1*) were implanted astride the preoptic tissue of the anterior brainstem. The thermode assembly contained a reentrant tube through which brain temperature could be monitored. The thermodes were fixed in place with glue (Eastman 9-10) and would remain in place for 2 to 4 months. After the implanting operation, each animal was fitted with a collar to prevent withdrawal of the head into the shell and was allowed a week to recover.

Temperatures were measured with thermocouples connected to a multipoint recorder (Leeds & Northrup model W. Azar). Evaporative water loss was measured by placing the turtle in a small sealed box (5 liters) through which dried air was pulled at 3.6 liter/min by a small pump. The bottom of the box contained a layer of mineral oil to prevent the evaporation of any salivary or cloacal discharge that fell off the turtle. The air from the box was drawn past the hygrosensor of a relative humidity detector (Hygrodynamics model 15-3050, American Instrument Co.) which was kept at a constant temperature of 40°C. The output of the detector was recorded on the multipoint recorder. The relative humidity, airflow, atmospheric pressure, air temperature, and weight of the animal were used to calculate a scale of evaporative water loss by the animal in milligrams of water per gram per hour. Brain temperature was controlled by adjusting the temperature

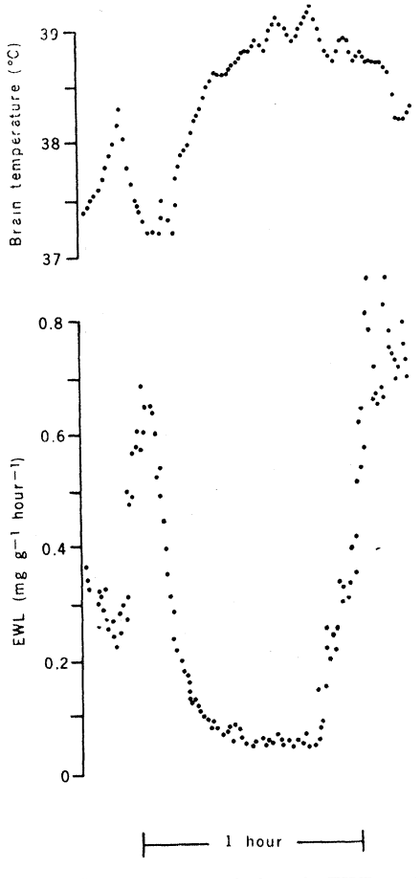

Fig. 1. Spontaneous variations in EWL and brain temperature. Ambient temperature was 40.5°C; the turtle weighed 200 g.

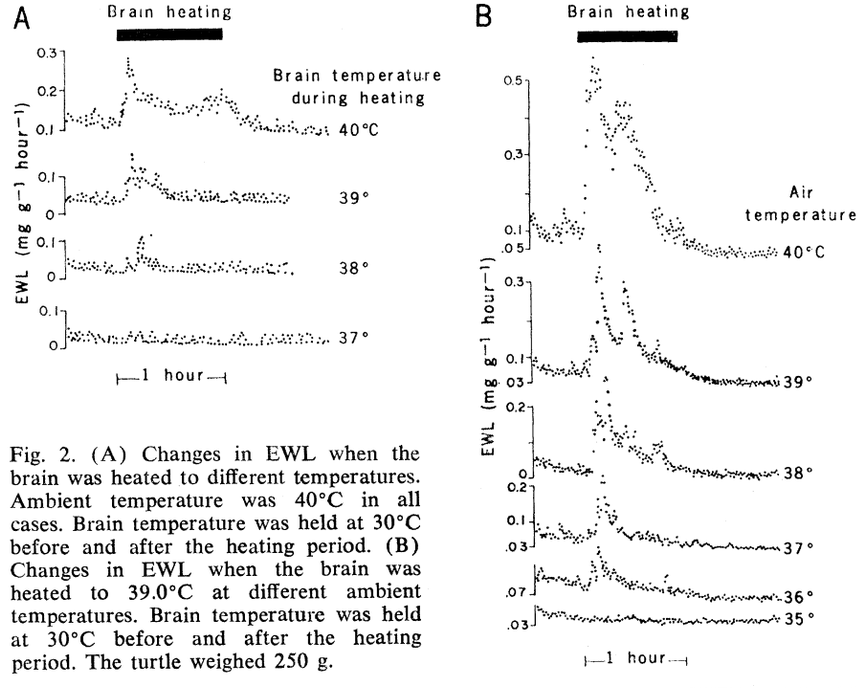

Fig. 2. (A) Changes in EWL when the brain was heated to different temperatures. Ambient temperature was 40°C in all cases. Brain temperature was held at 30°C before and after the heating period. (B) Changes in EWL when the brain was heated to 39.0°C at different ambient temperatures. Brain temperature was held at 30°C before and after the heating period. The turtle weighed 250 g.

and flow rate of the water in the thermode.

The box with the turtle inside was placed in an environmental chamber whose temperature could be controlled within 0.5°C. The box was left open until the animal's cloacal temperature was within 2° to 3°C of the chamber (ambient) temperature. Cloacal temperature is used here as an estimate of body temperature outside the head rather than as a measure of core or heart temperature. The box was sealed and the animal was allowed to reach thermal equilibrium. The temperature of the rostral brainstem was initially lowered from its equilibrated level to 30°C. When EWL had stabilized at a low level the brainstem temperature was raised to 39°C and kept there for 1 hour. Then the brainstem temperature was lowered back to 30°C and kept there for another hour. This was done at ambient temperatures ranging from 30° to 40°C at 1°C intervals. Another series of experiments was done in which the air temperature was kept at 40°C in all cases and the temperature of the brainstem during the heating periods was varied.

In our experiments 40°C was the highest air temperature at which the animals could survive the 4 to 6 hours required for a single run, and higher temperatures were not used. During heating, before the box was sealed, the temperature of the brainstem always increased faster than the cloacal temperature. But brainstem temperature leveled off at 1° to 2°C below air temperature while the cloacal temperature continued to increase. When the turtle was at thermal equilibrium in the sealed box, the cloacal temperature was within less than 1°C of the air temperature. Therefore "ambient" temperature is also an approximation of equilibrated body temperature away from the thermode. In animals whose brain temperature was not being controlled we occasionally noticed spontaneous changes in EWL that appeared to be related to changes in preoptic temperature (Fig. 1). A large increase in EWL would be followed by a decrease in preoptic temperature of up to 1.0°C. Then the EWL would fall and the preoptic temperature would start to rise again. We did not observe any changes in cloacal temperature that could be related to spontaneous changes in EWL.

Figure 2A shows a series of records of EWL during a 1-hour period of preoptic heating to different brain temperatures. In all cases the air temperature was 40°C and cloacal temperature was between 39° and 40°C. The increase in EWL was greatest at a brain temperature of 40°C and lower at lower brain temperatures. There was no response at brain temperatures below 38°C. Figure 2B shows records of EWL during heating of the brainstem to 39°C at each of six different air temperatures. There was no response at air temperatures below 35°C. In all cases the EWL increases caused by brainstem heating were transient, the rate of EWL falling back toward the level that existed before the heating period, even though heating of the brainstem continued and even though the cloacal temperature did not decrease while EWL was increased. This could be the result of input from other parts of the central nervous system or it could represent adaptation by the neurons of the anterior brainstem.

To test whether lowering the brain temperature could cause a decrease in EWL at a very high air temperature, an animal was placed in the EWL box at an ambient temperature of 44°C. Figure 3 shows the EWL record as the preoptic temperature was lowered from an uncontrolled temperature of 40.5° to 32°C. The very high rate of EWL is decreased by about 30 percent during the cooling period. The animal died shortly after this record was taken.

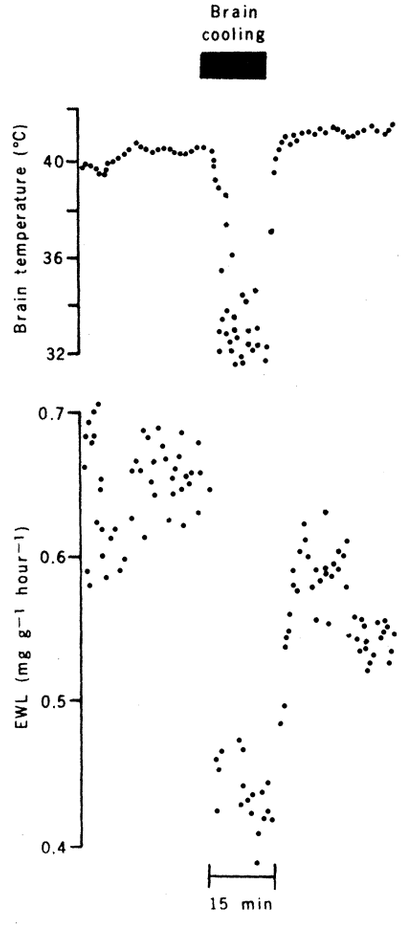

Fig. 3. Evaporative water loss and brain temperature of a box turtle in an ambient temperature of 44°C. Brain temperature was controlled only during the cooling period. The turtle weighed 450 g.

Local heating of the brain of the box turtle results in an increase in EWL only when the body temperature is above about 35°C. The preferred temperature of the box turtle is around 30°C (*8*). The righting reflex is lost at a body temperature near 40°C and the critical thermal maximum (loss of motor coordination) is 41.5° to 43.3°C (*9*). Sturbaum and Riedesel (*5*) reported seeing no salivation or panting by box turtles at air temperatures below 41°C (core temperature = 38.4°C). Thus, the experimental range of 35° to 40°C can be considered as approaching stress temperatures. The large changes in EWL which we observed probably do not play a major role in thermoregulation by the box turtle under normal circumstances, but are induced only by high ambient temperatures at which the animal is in immediate danger of overheating (*6*, *7*).

Brain temperature appears to be a major stimulus to increased EWL at high ambient temperatures, as indicated by the sensitivity of the EWL response to small fluctuations in brain temperature shown in Fig. 1. It is unlikely that core temperatures would be affected by such short-term changes in EWL. While investigating the animals in the EWL box, we observed no changes in cloacal temperatures as a result of induced changes in EWL. It was occasionally possible to induce changes in cloacal temperatures in animals which were only in the environmental chamber but not sealed in the box. Preoptic cooling induced increases in cloacal temperature of up to 1°C, and preoptic heating induced decreases in cloacal temperature of up to 1.5°C. These changes did not occur consistently, and never at ambient temperatures below 41°C.

Even at body temperatures which are fatal, cooling the brain can decrease EWL. This may indicate that the protection of the brain from heat damage is the most powerful influence when the animal is faced with a choice between high body temperatures and ex-

cessive water loss. However, at air temperatures below 35°C no change in EWL was elicited by heating the brain alone. Thus, the magnitude of the EWL response is dependent on a combination of brain temperature and other central and peripheral body temperatures. This is similar to findings in other reptiles (*1, 4*) as well as in mammals (*10*).

Although reptiles do not have the metabolic or evaporative capacity to continually maintain large temperature gradients between themselves and their environment, it is becoming increasingly evident that they do have much if not all the sensory and integrative capacity for temperature control possessed by the so-called higher vertebrates.

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Dopamine β-Hydroxylase Activity in Brains of Chronic Schizophrenic Patients

Abstract. *Postmortem brain specimens from nine chronic schizophrenic patients and nine controls were assayed for activity of dopamine β-hydroxylase, the enzyme responsible for the conversion of dopamine to norepinephrine. Unlike the results of previous reports, there was no statistically significant difference in enzyme activity between the patient and control groups. There were, however, significant negative correlations between dopamine β-hydroxylase activity and the time spent in the morgue before autopsy, and between enzyme activity of schizophrenics and dosage of chlorpromazine or its equivalent.*

Wise and Stein (*1*) reported that dopamine β-hydroxylase (DBH) activity is low in the autopsied brains of schizophrenic patients. This result is exciting because it is consistent with their hypothesis that brain norepinephrine concentrations are low in schizophrenia, as well as with the popular view that schizophrenia is associated with a hyperactive dopaminergic system. Wise and Stein attempted to control the variables that might have produced this abnormality. However, we report data that are in discrepancy with theirs.

Specimens of autopsied brains from five chronic schizophrenic patients were obtained with the aid of the neuropathologist at St. Elizabeths Hospital, Washington, D.C. Brain specimens from four chronic schizophrenics and nine controls were obtained by a similar arrangement with the D.C. Medical Examiner's Office. Information about the subjects was obtained by interviews with family members and from hospital and police records. The control brains were obtained from persons without evidence of a psychiatric history, although one was from an individual with a police record and two were from heavy drinkers. The mean age of the schizophrenics (seven males and two females) was 49.2 ± 6.3 [standard error of mean (S.E.M.)]. Four died suddenly after traumatic suicides; three, from cardiac arrest; one, from pulmonary aspiration; and one, suddenly from pulmonary edema. The mean age of controls (all male) was 42.3 ± 4.5. Six died suddenly of trauma; and three, from cardiac arrest.

The periaqueductal pons-mesencephalon, hypothalamus, and hippocampus were dissected out at autopsy and immediately placed on Dry Ice and subsequently stored at − 80°C for up to 1 year [Wise and Stein (*1*) found that DBH activity was stable for 1 year at − 15°C]. There was no difference in mean storage time for the schizophrenics and controls.

Samples (100 to 600 mg) from the dissected brain parts were homogenized in 40 volumes of 0.005*M* tris(hydroxymethyl)aminomethane acetate buffer (*p*H 7.0) containing 0.1 percent Triton X-100 and assayed by the method of Molinoff *et al.* (*2*). For optimal enzyme activity, the final copper sulfate concentrations for both normal and schizophrenic brain parts were as follows: hippocampus, $1.3 \times 10^{-5}M$; hypothalamus, $2.2 \times 10^{-5}M$; and pons, $1.6 \times 10^{-5}M$. All assays were performed by a person unaware of whether the samples were from schizophrenics or controls.

No significant differences between the two groups were found for DBH activity in any of the brain regions (Student's two-tailed *t*-test) (Table 1), nor was there a significant difference when all three regions were taken into account ($P > .50$, multivariate *t*-test) (*3*). Although the differences were not statistically significant, the regional means for the schizophrenics ranged between 77 and 89 percent of control values. The possible reasons for this were explored.

Wise and Stein indicated that the presence of phenothiazines was probably not a cause of the DBH differences between controls and schizophrenics. They gave rats chlorpromazine (20 mg per kilogram of body weight) daily for 12 weeks and found a small increase in DBH activity. In our study, seven of nine patients were taking phenothiazines at the time of death. Although the patients were also taking a number of nonneuroleptic drugs, there were significant ($P < .05$) negative correlations between the daily dosage of chlorpromazine or chlorpromazine equivalent and the DBH activity in the hypothalamus ($r = -.60$) and pons ($r = -.65$) (*4*). This could mean that the neuroleptics tend to decrease brain DBH activity in schizophrenics, or that there is a negative correlation between severity of clinical disease (as determined by the need for higher drug dosages) and DBH activity.

Wise and Stein attempted to determine the effects of death-to-morgue