THE EFFECT OF TEMPERATURE AND SEASONAL ACCLIMATIZATION ON RENAL FUNCTION OF PAINTED TURTLES, *CHRYSEMYS PICTA*

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Abstract—1. Urine production (V) and glomeruler filtration rate (GFR) was measured in summer- and winter-acclimatized painted turtles at 5, 15 and 25°C.

2. V and GFR increased with increasing temperature and were greater in winter than in summer turtles at each test temperature, however the difference was not significant ($P = 0.12$ and 0.09, respectively).

3. The inulin U/P ratio decreased with decreasing temperature indicating that proportionally less filtrate is reabsorbed as temperature decreased ($P < 0.001$).

4. Experimental hydration led to a marked increase in both V and GFR ($P < 0.001$) in both seasons, but there was no effect of season on the ability to excrete the additional water load ($P = 0.37$). The inulin U/P ratio did not change significantly with hydration ($P = 0.46$) indicating that the diuresis in hydrated turtles is best explained by changes in glomerular rather than tubular function.

5. The results indicate that renal function is temperature dependent and does not undergo significant seasonal acclimatization. The antidiuretic effect of cold temperature and apparent lack of seasonal compensation may reflect an energetic constraint resulting from the need to conserve metabolic energy during hibernation when temperature and oxygen availability are low.

INTRODUCTION

Maintenance of homeostasis is an important task for organisms in any environment. This appears especially so for water and electrolyte balance in overwintering aquatic turtles. Fresh water animals face a hypo-osmotic environment and the persistent problems of gains of water and loss of electrolytes across epithelial surfaces. In painted turtles, *Chrysemys picta*, for example, it has been calculated that uncompensated water influx would dilute internal fluids about 3% per day at 23°C (Trobec and Stanley, 1971). Furthermore, sodium efflux results in a 0.9% per day loss in total exchangeable sodium (Dunson, 1967). Fresh water vertebrates normally compensate for water gain and electrolyte loss by filtering large volumes of plasma (high glomerular filtration rates), and reabsorbing most of the electrolytes from the filtrate, thereby excreting a copious dilute urine. Small renal and dermal electrolyte losses are balanced through the diet and active uptake of salt across the gill, skin or pharyngeal membranes from the dilute environment (Maetz, 1971; Kirscher, 1983; Dunson, 1967). However, during the winter northern amphibians and reptiles are torpid and anorexic, precluding dietary uptake of electrolytes.

The excretion of a large volume of dilute urine depends upon the ultrafiltration and the active transport of ions by the renal tubules, bladder, colon and/or cloaca. Ultrafiltration in turn depends upon the maintenance of an adequate glomerular filtration pressure. Filtration pressure is the difference between the net hydrostatic pressure between the lumen of the capillary and the lumen of Bowman's capsule, which favors filtration, and the colloid osmotic pressure, which opposes it. Net filtration pressures of ectothermic vertebrates tend to be far lower than those of endotherms. For example, the values for man and the salamander *Necturus* are 45 and 5.8 mmHg, respectively (Pitts, 1968). This is due for the most part to the lower blood pressures observed in ectotherms. Systolic blood pressures in the major arteries of chelonians range between 20 and 30 mmHg at 20–25°C (Herbert and Jackson, 1985; Brown *et al.*, 1983; Steggerda and Essex, 1957). Depressed cardiac function associated with the depressed metabolic activity at low body temperatures results in a drop in blood pressure of reptiles (Rodbard and Feldman, 1946; Templeton, 1964). Temperature and anoxia combine to reduce the systolic blood pressure of the painted turtle from 28.9 mmHg at 20°C in normoxic water to 7.7 mmHg at 3°C during submergence in anoxic water (Herbert and Jackson, 1985). The depression of blood pressure in renal arterioles at temperatures such as those occurring during hibernation may therefore limit or preclude glomerular filtration.

The painted turtle, *Chrysemys picta*, is an air breathing ectotherm that hibernates submerged in fresh water at middle latitudes in North America. In southeastern Michigan, it ceases feeding in late September or early October, when water temperatures fall below 10°C (Sexton, 1959). Shortly after this, activity levels decline and *C. picta* become torpid, remaining dormant until mid-March (Sexton, 1959; Gibbons, 1968; Crawford, in press). Feeding resumes when water temperatures reach 15°C. Thus,

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at the northern limit of their range, painted turtles may be anorexic for 6–7 months and submerged in ice covered bodies of water without the benefit of aerial respiration for up to 4 months during the annual cycle.

In summary, hibernating amphibians and reptiles continue to face the same osmoregulatory challenges as active animals during summer, but their problems are complicated by depressions of energy metabolism and general physiological function. Osmoregulatory function in aquatic ectotherms at low body temperatures and, especially, osmoregulatory function under natural conditions such as hibernation, are poorly understood. The purpose of this investigation is to examine the effects of body temperature and any potential seasonal acclimatization on renal function in the painted turtle, *Chrysemys picta*. The rates of urine production and glomerular filtration were determined for summer- and winter-acclimatized turtles at 5, 15 and 25°C. These data enabled me to examine the temperature dependence of renal function and observe whether it undergoes seasonal acclimatization.

MATERIALS AND METHODS

Male and female painted turtles, *Chrysemys picta marginata* were trapped at Hiland Lake, Livingston County, Michigan, using a combination of baited and basking traps. Turtles collected from 27 July to 5 August, 1986 were designated as summer-acclimatized. Turtles used to represent the winter-acclimatized condition were collected 15–29 September, and allocated among three 1 m³ enclosures in Rash Pond at the University of Michigan's Botanical Gardens. This process was necessitated because of difficulties in collecting free living animals in winter. The enclosures were so constructed that turtles could breathe aerially or hibernate at the bottom. These animals were retrieved on 15 January, 1987. Traps were checked daily and turtles were transported to the laboratory for processing immediately after capture. Each individual was assigned to a given experimental group based upon its mass. The assignments were made in a manner producing similar means and ranges of body mass among the various groups. This minimized any potential differences in physiological response that might reflect differences in body size. Prior to experimentation, turtles were fasted for 7 to 10 days in a 1000 l aquarium under conditions of temperature and photoperiod loosely approximating the natural environment at the particular season (i.e., summer, 25°C 15:9 hr photoperiod with a heat lamp and platform available for basking; winter, 5°C, 9:15 hr photoperiod with no opportunity to bask).

Glomerular filtration rate (GFR) was measured using methods adapted from Dantzler and Schmidt-Nielsen (1966). Modifications were necessary because of the smaller size of painted turtles that were used in this study. Turtles ($n = 15$ for each season) were anesthetized with an intraperitoneal (20 mg/kg) injection of sodium pentobarbital. Repeated access to the heart and major vessels was achieved by drilling a 0.8 cm diameter hole in the plastron over the heart and plugging it with a rubber stopper. After recovery, (48 hr at the test temperature) turtles were given an intracardiac injection of 2 μCi/kg ¹⁴C-inulin and the placed in individual 2 l containers to allow distribution and equilibration of the label. After mixing of the label (2 hr at 25°C, 4 hr at 15°C and 6 hr at 5°C) turtles were restrained on a ring stand at a 45°C angle, plastron side down. A No. 8 Foley catheter with 3 cm³ balloon was inserted through the cloaca into the urinary bladder and any urine present emptied. The

balloon was then inflated and a 0.2 ml blood sample drawn from the left aortic arch with a heparinized syringe and 26 gauge needle. This represented the beginning of a urine collection period. Repeated clearance periods were performed on an individual whenever possible (as many as five at 25°C). Urine produced by the kidney travels down the ureter where it enters the cloaca. With the catheter inserted and inflated, urine cannot move anteriorly into the bladder but travels down the outside of the catheter out of the cloaca and is collected in a 10 ml graduated cylinder containing 0.5 ml of mineral oil to retard evaporation of water from the collected urine. In this manner, ureteral urine can be collected from restrained but unanesthetized turtles. The collection of ureteral urine is essential for accurate determinations of GFR, as inulin may be absorbed by the bladder (Braysher and Green, 1972). At the end of a urine collection period, another 0.2 ml blood sample was drawn from the aortic arch. Fifty microlitre aliquots of plasma or urine were added to scintillation vials for determinations of ¹⁴C activity. Sample were counted using a Beckman LS-230 scintillation counter.

GFR was measured as inulin clearance and was calculated as the activity of ¹⁴C in urine in cpm/ml multiplied by the rate of urine prodution in ml/min divided by the activity of ¹⁴C in plasma in cpm/ml ($U \times V/P$). GFRs were later converted to units of ml/(kg × hr) to reduce effects of differences in body size and to facilitate comparison with values in the literature. GFR was measured for turtles at 5, 15 and 25°C ($n = 5$ at each temperature). When more than one clearance period was performed on an individual turtle, the mean GFR was calculated. For statistical purposes sample size represented the number of turtles tested, not the number of clearance periods performed.

Potentially significant effects of temperature and season were tested by performing 2-way ANOVA (BMDP 2v). In all statistical analyses, the 0.05 level was used as the criterion for significance.

RESULTS

Glomerular filtration rate (GFR) increased significantly with temperature in both summer- and winter-acclimatized turtles ($P < 0.0001$) (Fig. 1A). The temperature sensitivity for GFR indicated by Q_{10} (Table 1), was much greater between 5 and 15°C than between 15 and 25°C. GFR did not undergo significant seasonal acclimatization ($P = 0.09$).

Urine flow showed very similar patterns as GFR with respect to temperature and season. An increase in temperature caused a highly significant ($P < 0.0001$) increase in the rate of urine production (Fig. 1b). Urine flow was also more sensitive to temperature between 5 and 15°C than between 15 and 25°C (Table 1). Urine flow did not indicate any significant seasonal acclimatization ($P = 0.12$) even though urine production was greater in winter turtles at all temperatures tested (Fig. 2).

The inulin U/P ratio (the concentration of inulin in urine divided by the concentration in plasma) also increased with increasing temperature (Fig. 2). Inulin U/P is a measure of the proportion of filtered water that is reabsorbed. Significantly less water is reabsorbed as the temperature is decreased ($P < 0.0001$). There was no significant indication of seasonal acclimatization on the inulin U/P ($P = 0.98$).

The effect of experimental hydration (intraperitoneal injection of 100 ml/kg distilled water) is
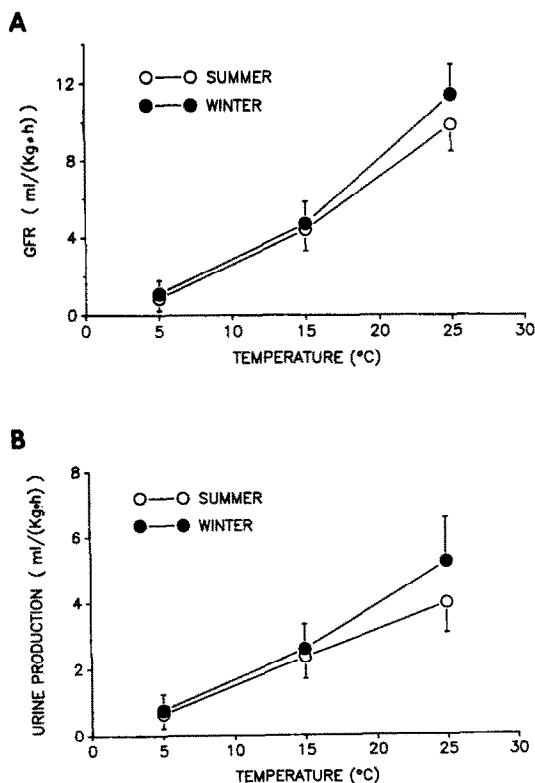
A

B

Fig. 1. Renal function of summer- and winter-acclimatized painted turtles as a function of experimental temperature. (A) Glomerular filtration rate in ml/(kg × hr), measured as inulin clearance. Values are means for five turtles ± 1 standard deviation. (B) Urine flow rates in ml/(kg × hr). Values are means for five turtles ± 1 standard deviation.
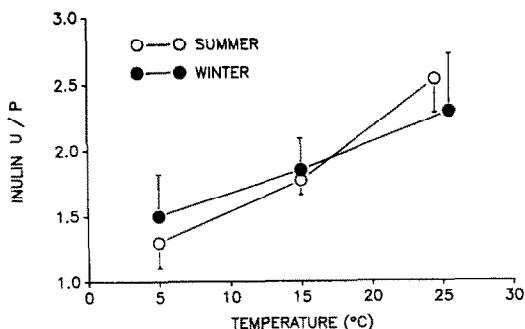

Fig. 2. Inulin concentration in urine divided by concentration in the plasma (inulin U/P) for summer- and winter-acclimatized turtles. Values represent means for five turtles ± 1 standard deviation.

DISCUSSION

Body temperature had a profound effect on renal function of turtles in this study. Both urine flow and glomerular filtration rate (GFR) increased significantly with temperature (Fig. 1). Rate of urine flow depends both on the GFR and the amount of the filtered volume reabsorbed along the kidney's tubules. As temperature increased, GFR rose but so did the inulin U/P ratio, indicating reabsorption of a greater proportion of water in the glomerular filtrate at higher temperatures (Fig. 2). This circumstance, which involves tubular as well as glomerular function, leads to a relatively low sensitivity of urine flow to temperature (cf. Q_{10}'s for GFR and urine flow in Table 1).

The diuretic effect of higher body temperatures seems best explained by their positive effect on heart rate, blood pressure, and, consequently, on net filtration pressure (Rodbard and Feldman, 1946; Templeton, 1964; Herbert and Jackson, 1985). Other studies have also observed increased GFR and urine flow in ectothermic vertebrates at higher body temperature. Urine flow in the white sucker fish *Catostomus commersoni* increases with a Q_{10} of 2.2 from 2 to 18°C (Mackay and Beatty, 1968). In the frog *Rana clamitans*, it increases from 3.16 to 13.2 ml/(kg × hr) and GFR from 4.5 to 34.2 ml/(kg × hr) between 5 and 22°C (Schmidt-Nielsen and Forster, 1954). This frog also increases the creatinine U/P ratio, indicating an improved ability to reabsorb the filtrate as body temperature rises. At 5°C, 29.7% of the filtrate is reabsorbed whereas at 22°C the value if 61.4%. Shoemaker *et al.* (1966) found that the lizard *Tiliqua rugosa* increases both GFR and urine flow with an increase in temperature ($Q_{10} = 4.2$ between 14 and 37°C). From the results of the present study and evidence from three classes of vertebrates it appears that increased body temperature has a diuretic effect on ectothermic vertebrates.

Considering the results here from a different perspective, a decrease in temperature leads to anti-diuresis. This may have important ramifications for aquatic turtles, especially during hibernation. A depression of renal function at low temperatures during the winter should lead to a decrease in the rate of water efflux and to a possible disturbance of water balance. Aquatic ectotherms at low temperature

summarized in Table 2. Hydration elicited a marked increase in GFR and urine flow. However, it did not affect inulin U/P. In hydrated turtles, GFR and urine flow increased significantly with an increase in temperature ($P < 0.0001$) (Fig. 3). Neither GFR nor urine flow underwent significant seasonal acclimatization ($P = 0.37$ and 0.15 respectively). Because of the absence of a significant effect of season, data for winter and summer turtles were combined to facilitate statistical analysis of the effect of hydration. Injection of water caused a significant increase in GFR and urine flow ($P < 0.0001$ for both). In general, hydration led to a doubling of GFR and urine flow at all temperatures tested. However, the effect of hydration on these functions was slightly greater at higher temperatures (Table 2). Inulin U/P did not change significantly with hydration ($P = 0.46$). The stability of inulin U/P ratio indicates that the increase in urine flow and GFR with hydration is due to changes in glomerular function rather than tubular function.

Table 1. The temperature dependence of renal function (Q_{10})

Range	Summer		Winter	
	GFR	Urine flow	GFR	Urine flow
5–15°C	4.91	3.69	4.29	3.42
15–25°C	2.34	1.68	2.52	2.01
5–25°C	3.39	2.49	3.22	2.62

Table 2. The effect of hydration* on renal function

Temperature	Control			Hydrated		
	GFR ml/(kg × hr)	Urine flow ml/(kg × hr)	Inulin U/P	GFR ml/(kg × hr)	Urine flow ml/(kg × hr)	Inulin U/P
5°C	0.98	0.70	1.42	1.81	1.32	1.37
15°C	4.47	2.48	1.83	10.6	6.48	1.77
25°C	10.66	4.59	2.41	23.40	10.55	2.23

*The hydrated state was produced by the intraperitoneal injection of 100 ml/kg distilled water.

should accumulate water and thus become edematous. This in turn would lead to an expansion of body fluid compartments and a decrease in solute concentration. Fluid accumulation and solute dilution have been observed for many aquatic ectotherms at low body temperature (e.g., Jorgensen *et al.*, 1978; Semple *et al.*, 1969). However, the antidiuresis associated with low temperature would result in a conservation of body solutes by reducing renal efflux. At 25°C, painted turtles reabsorb nearly 99% of filtered sodium (Brewer and Ensor, 1980). Therefore, approx. 1% of the filtered sodium is excreted in the urine and lost to the animal. A large reduction in GFR at low temperature in winter turtles would serve to reduce sodium loss and conserve the energy that would have been required for active reabsorption of sodium from the filtrate. Low temperature apparently also

depresses the solute-linked reabsorption of water. The active transport of sodium from the filtrate produces the osmotic gradient which permits water to move osmotically. The decrease in inulin U/P with a decrease in temperature observed in this study suggests that active transport processes are depressed at low temperature. An abrupt decrease in body temperature from 28 to 6°C resulted in an increase in the urine concentration of sodium, chloride as well as total osmolality in the alligator, *Alligator mississippiensis* and the slider turtle, *Pseudemys scripta* (Hernandez and Coulson, 1957). Renal glucose reabsorption was also depressed by low temperature in the sucker fish (Mackay and Beatty, 1968). Therefore, the antidiuretic effect of low body temperature may result in the conservation of plasma solutes which would otherwise be excreted by the animal. The renal portal system possessed by non-mammalian vertebrates may also serve to reduce solute efflux. In this portal system, venous blood enters the kidney via the renal portal vein and supplies actively secreting tubular cells. This supplies additional blood to the kidney and may take on added importance in low pressure situations (when GFR is low). The renal portal system allows for a functional separation of filtration and secretion as renal tubules are provided with a secretory load without the imposition of a large filtrate volume and the necessarily high rates of tubular reabsorption.

Neither GFR nor urine flow underwent significant seasonal acclimatization. Though glomerular filtration rate was higher in winter than in summer turtles at all temperatures tested, these differences are statistically insignificant ($P = 0.09$). Urine flow also was higher at all temperatures in winter turtles but again the differences are statistically insignificant ($P = 0.12$). The lack of seasonal compensation in renal function in the painted turtle may indicate the primacy of the need to conserve energy and suppress metabolism during hibernation. As stated above, compensation of GFR and urine flow during hibernation would necessitate either the expenditure of additional metabolic energy for active reabsorption of the additional solute load from the increased volume of filtrate, or an increased loss of electrolytes. Neither of these alternatives may be appropriate at a time when oxygen and energy stores could be limiting or when water influx and a lack of dietary solute uptake are already causing a dilution of body fluids.

Experimental hydration (the injection of 100 mg/kg distilled water) led to a marked diuresis (Fig. 3B). Turtles in this state approximately doubled GFR and urine flow at all temperatures tested indicating an ability to excrete an acute water load. Actual differences in the rates of water output were more pronounced at 15 and 25°C than at 5°C (Table 2).
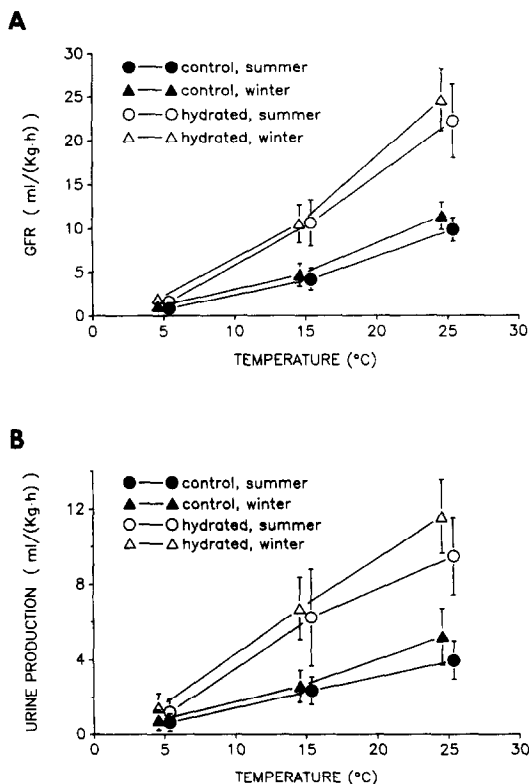
Fig. 3. The effect of hydration (intraperitoneal injection of 100 ml/kg distilled water) on renal function of summer- and winter-acclimatized turtles at 5, 15 and 25°C. Values represent means for five turtles ± 1 standard deviation. (A) Glomerular filtration in ml/(kg × hr), measured as inulin clearance. (B) Urine production in ml/(kg × hr), measured as the volume of urine collected during inulin clearance periods.

Thus, low temperature partially depresses the ability to excrete an additional water load. The increase in GFR and subsequent increase in urine flow after imposition of the water load can best be explained by its effect on physical parameters affecting filtration. An increase in total body water and subsequent expansion of the extracellular space and plasma volume would necessarily cause an increase in blood pressure. In addition, the dilution of plasma non-filterable solutes (e.g. proteins) would cause a decrease in the oncotic pressure at the glomerulus. Both of these circumstances would serve to increase the net filtration pressure and therefore favor glomerular filtration. From this experiment it is impossible to determine whether the expansion of fluid spaces (and subsequent increases in pressure) or the dilution of fluid spaces are responsible for the diuresis. Repeating the experiment with an injection of isotonic saline would help elucidate this problem. Similar effects of experimental hydration were observed in the frog *Rana clamitans* and the turtle *Pseudemys scripta*. The injection of 150 ml/kg distilled water led to a 2.4-fold increase in GFR and a 4-fold increase in urine flow in the bullfrog (Schmidt-Nielsen and Forster, 1954). In the slider turtle, a water load of 100 ml/kg 0.5% dextrose led to a 2.17-fold increase in GFR and a 2.69-fold increase in urine flow (Dantzler and Schmidt-Nielsen, 1966).

At all temperatures tested, the inulin U/P ratio was unaffected by experimental hydration ($P = 0.46$) (Table 2). Therefore, hydration had little effect on the fractional water reabsorption from the filtrate. However, the absolute volume of water reabsorbed per unit time increases as the volume of filtrate produced per unit time increases. This indicates that hydration exerts its primary effect on glomerular function, having an insignificant action on tubular function. Creatinine U/P decreased during hydration of the bullfrog (Schmidt-Nielsen and Forster, 1954), but showed no changes in the slider turtle (Dantzler and Schmidt-Nielsen, 1966). It appears that amphibian and reptilian kidneys respond quite differently to a water load. A decrease in the inulin U/P ratio would have the effect of eliminating the water load more quickly, but could also lead to an increased renal electolyte loss. However, it is important to note that ureteral urine was collected in these studies and post-renal modification by the cloaca, bladder and/or colon may play an imporatant part in ion and water reabsorption.

In summary, renal function in painted turtles is temperature dependent, but it does not appear subject to processes of seasonal acclimatization. The depression of renal function at low temperature may be responsible for the observed water accumulation and electrolyte dilution in these turtles. However, a significant water load can be eliminated and thus prevent a possibly fatal dilution of body fluids. The lack of acclimatizational changes in renal function is of interest. It may reflect a constraint resulting from the need to conserve metabolic energy during hibernation when temperatures and oxygen availability are low.

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