Although amphibians live successfully in many different environments, their highly water-permeable skins mean that water balance is a constant challenge. Evaporative water loss is a major consideration in terrestrial environments, and only a few species can cope with the hyperosmotic stress of brackish water. On land, evaporative water loss can result in the loss of approximately 6–9% of total body mass per day (Hillman, 1980). Among anurans, the survival limits for dehydration tolerance range from 25 to 60% of total body water lost (Thorson and Sviha, 1943; Hoar, 1986; Shoemaker, 1992), the extent of dehydration tolerance showing a positive correlation with the degree of terrestriality of a species.

Amphibians regulate their water balance at behavioural, physiological and metabolic levels (Duellman and Traub, 1986; Shoemaker, 1992). At the metabolic level, two general principles are evident: (1) amphibians show the highest tolerance among vertebrates for variation in body fluid osmolality and ionic strength (Hillman, 1987, 1988), and (2) water loss, and the high ionic strength conditions this causes, are frequently offset by the synthesis and accumulation of low molecular mass osmolytes. For example, species that aestivate in dry conditions and brackish-water species both accumulate high concentrations of urea in their body fluids to help retard water loss (for reviews, see Shoemaker, 1992; Pinder et al. 1992). Freeze-tolerant amphibians accumulate high levels of glucose or glycerol to limit cellular dehydration during the sequestration of up to 65% of their total body water content as extracellular ice (for reviews, see Storey and Storey, 1986).

### METABOLIC EFFECTS OF DEHYDRATION ON AN AQUATIC FROG, RANA PIPIENS

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Accepted 19 August 1994

**Summary**

Cellular responses to dehydration were analyzed in six organs of leopard frogs *Rana pipiens*. Frogs at 5°C endured the loss of up to 50% of their total body water content but water contents of individual organs were strongly defended. Skeletal muscle water content was strongly affected by dehydration, dropping from 80.7% of wet mass in controls to 67.2% in frogs that had lost 50% of their total body water. However, water contents of internal organs dropped by only 3–8% of their wet masses. Water contents of all organs except skeletal muscle were fully restored by 24 h of rehydration in water at 5°C. Dehydration had no consistent effect on the protein content of five organs but in a sixth, the kidney, protein levels were elevated (by 60–72%) at the higher levels of dehydration and during rehydration. Dehydration led to a rapid increase in glucose concentration in the liver; compared with control values of 13±2 nmol mg⁻¹ protein, levels were doubled by 12.2% dehydration and continued to increase to a maximum of 307±44 nmol mg⁻¹ protein (20 μmol g⁻¹ wet mass) in 50% dehydrated frogs. Glucose accumulation was supported by a decrease in liver glycogen content and a parallel rise in glucose 6-phosphate levels, but not in the levels of other glycolytic intermediates, confirming that glycogenolytic flux was being directed into glucose synthesis. Blood glucose levels also increased as a function of increasing dehydration, reaching values 13.8 times higher than controls, but only the kidney and brain showed a significant accumulation of glucose over the course of dehydration. All organs (except skeletal muscle) had increased lactate levels during dehydration, particularly at 36.6 and 50% dehydration. Severe dehydration also compromised cellular energetics, with ATP levels dropping by 44% in liver of 50% dehydrated frogs. However, frogs readily recovered from dehydration stress and, after rehydration for 24 h, organ lactate levels were sharply reduced relative to 50% dehydrated frogs and liver energy status was largely restored. The hyperglycaemic response elicited by dehydration in this semi-aquatic frog mirrors the extreme hyperglycaemia seen in freeze-tolerant frogs during comparable dehydration exposure at 5°C or during freezing. This suggests that the cryoprotectant response of freeze-tolerant species may have grown out of a pre-existing hyperglycaemic response to dehydration, a common stress experienced by all anurans.

Key words: anuran, desiccation tolerance, leopard frog, *Rana pipiens*, freeze tolerance, glucose metabolism.

### Introduction

Although amphibians live successfully in many different environments, their highly water-permeable skins mean that water balance is a constant challenge. Evaporative water loss is a major consideration in terrestrial environments, and only a few species can cope with the hyperosmotic stress of brackish water. On land, evaporative water loss can result in the loss of approximately 6–9% of total body mass per day (Hillman, 1980). Among anurans, the survival limits for dehydration tolerance range from 25 to 60% of total body water lost (Thorson and Sviha, 1943; Hoar, 1986; Shoemaker, 1992), the extent of dehydration tolerance showing a positive correlation with the degree of terrestriality of a species.

Amphibians regulate their water balance at behavioural, physiological and metabolic levels (Duellman and Traub, 1986; Shoemaker, 1992). At the metabolic level, two general principles are evident: (1) amphibians show the highest tolerance among vertebrates for variation in body fluid osmolality and ionic strength (Hillman, 1987, 1988), and (2) water loss, and the high ionic strength conditions this causes, are frequently offset by the synthesis and accumulation of low molecular mass osmolytes. For example, species that aestivate in dry conditions and brackish-water species both accumulate high concentrations of urea in their body fluids to help retard water loss (for reviews, see Shoemaker, 1992; Pinder et al. 1992). Freeze-tolerant amphibians accumulate high levels of glucose or glycerol to limit cellular dehydration during the sequestration of up to 65% of their total body water content as extracellular ice (for reviews, see Storey and Storey, 1986).
In a recent study, we have shown that glucose biosynthesis in a freeze-tolerant species is also elicited by simple dehydration of the animals (Churchill and Storey, 1993). The wood frog *Rana sylvatica* tolerated high levels of dehydration (50–60% of total body water lost) and dehydration triggered a rapid increase in organ glucose content. For example, in autumn-collected wood frogs dehydrated to 50% of total body water lost, liver glucose rose to 127 μmol g⁻¹ wet mass, a value very similar to the effects of freezing on liver glucose concentrations in this species (Storey and Storey, 1986). These findings indicated that the cryoprotectant response to freezing was primarily a cell volume regulatory response and suggested that various elements of natural freeze-tolerance had arisen from pre-existing metabolic mechanisms of dehydration-tolerance used by amphibians. However, conversely, cryoprotectant synthesis might simply be very sensitive to cell volume reduction, one of the major effects of freezing on cells. To investigate these possibilities, we chose to analyze the metabolic response to dehydration of a freeze-intolerant species. The present study analyzes the effects of experimental dehydration on organ water contents, carbohydrate metabolism and cellular energetics of the leopard frog *Rana pipiens*, a freeze-intolerant semi-aquatic species found throughout the same geographical range as *R. sylvatica*.

**Materials and methods**

**Chemicals and animals**

All chemicals and biochemicals were purchased from Boehringer Mannheim Corp., Montreal, Quebec, or Sigma Chemical Co., St Louis, Missouri. Leopard frogs (*Rana pipiens* L.) were collected near Ottawa, Ontario, in early October 1991. Animals were acclimated to 5°C in a moist environment without food for 4 weeks prior to experimentation.

**Experimental design and protocol**

Control animals were sampled directly from the 5°C incubator. Experimental frogs were dehydrated at 5°C over desiccant (silica gel) at a rate of 0.5–1% of total body water lost per hour, essentially as described by Churchill and Storey (1993). Briefly, the experimental chambers were glass vacuum desiccators containing a layer of silica gel covered by a 1–2 cm thick piece of sponge to prevent the frogs from coming into contact with the desiccant. Four frogs were placed in each desiccator and individuals were identified by labelled bands of tape around a hind leg. During experimental dehydration, each frog was briefly removed from the 5°C incubator approximately every 12 h (to minimize handling) and weighed to determine water loss. When the calculated water loss was within 5% of the targeted sampling point (see below), frogs were weighed every 2 h until the target was reached. The percentage change in body water content was calculated as:

\[
\text{Percentage change} = \left[\frac{(M_i - M_d)}{(M_i \times BWC_i)}\right] \times 100,
\]

where \(M_i\) is initial body mass (g), \(M_d\) is the mass at any given time during dehydration (g) and \(BWC_i\) is the initial body water content of frogs prior to dehydration (0.814±1.9 g H₂O g⁻¹ body mass, mean ± S.E.M., \(N=6\)). The initial body water content of control frogs was obtained from the initial and final masses of frogs that had been killed and then dried to constant mass for 72 h at 80°C.

Initial tests indicated that *R. pipiens* could recover after the loss of 50% of total body water but not after higher losses (55 and 60%) and, therefore, a time course of dehydration was designed using targeted sampling points at 10%, 25%, 35% and 50% of total body water lost. In addition, another group of frogs was dehydrated to approximately 45% of total body water lost and then rehydrated to approximately the initial body water content. To rehydrate frogs at 5°C, animals were placed in a plastic container with about 2 cm of distilled water in the bottom; initial tests showed that individual animals required 12–24 h for complete rehydration (as determined by body mass regain), so all were sampled after 24 h.

**Tissue sampling and extraction**

Frogs were killed by pithing, a blood sample was taken, and then the liver, heart, kidney, brain, hind leg skeletal muscle and gut (stomach + intestine) were quickly excised and frozen in liquid nitrogen. Blood was sampled as described by Churchill and Storey (1993); this involved exposing the heart and snipping one of the atra, then sampling carefully with a capillary micropipette to avoid collection of any blood that had been diluted by extra-organ water. Samples were stored at −80°C until processing.

Perchloric acid extracts of blood and liver samples were prepared as described previously by Storey and Storey (1984); a sample of the well-mixed liver homogenate was removed prior to centrifugation for the determination of glycogen levels by the method of Keppler and Decker (1974). Portions of the neutralized liver extract were used immediately for the measurement of adenylate levels and the remainder was frozen at −80°C for the subsequent assay of glucose, lactate and glycolytic intermediates. All metabolites were measured in coupled enzyme assays by the methods of Lowry and Passonneau (1972) using an Amico-Bowman spectrofluorometer with an excitation wavelength of 340 nm and an emission wavelength of 460 nm.

**Tissue wet mass/dry mass and protein determinations**

Organ water content is expressed as g H₂O g⁻¹ dry mass and was determined from the initial and final masses of portions of each organ after drying to a constant mass at 80°C for 72 h. Total acid-precipitated protein levels (in pellets obtained from the perchloric acid extracts above) were measured spectrophotometrically using the Coomassie Blue dye-binding method and the Bio-Rad assay kit with bovine serum albumin as a standard.

**Data analysis**

All data are reported as means ± S.E.M., \(N=4\) animals in each group. Statistical analysis was performed using one-way
Results

Initial tests showed that leopard frogs could endure and recover from the loss of 50% of their total body water. To examine the effects of dehydration on the metabolism of frogs, an experimental dehydration course was set up that aimed to sample frogs at 10%, 25%, 35% and 50% loss of total body water. The actual mean (± S.E.M.) values calculated from the final body masses in each experimental group were 12.2±0.6%, 25.8±0.1%, 36.6±1.4% and 50.0±1.7% of total body water lost. Another group of frogs was first dehydrated to 45.5% of total body water lost (actual mean 45.5±1.5%) and then allowed to regain water over 24 h to nearly full rehydration (final mean 8.1±2.1% less than their initial water content).

Fig. 1 shows the effects of dehydration on the water and protein contents of six organs of *R. pipiens*. Values are means ± S.E.M., N=4, expressed per gram dry mass of tissue. From left to right the bars represent: control (0% dehydrated), 12.2% dehydrated, 25.8% dehydrated, 36.6% dehydrated, 50% dehydrated, and rehydrated frogs. Rehydrated frogs first lost 45.5% of their total body water and then regained water over 24 h to a final mean of 8.1% less than their initial water content. Muscle is leg skeletal muscle; gut combines stomach and intestine. Asterisks denote values significantly different from the corresponding control value (Dunnett’s test, two-tailed), *P<0.05; **P<0.01.

when the total body water content had dropped by 36.6 and 50%, respectively. Calculated in another way, as percentages of the wet mass, organ water contents for control frogs were 81.3±0.9%, 80.7±0.9%, 77.8±0.9%, 82.8±1.5%, 83.8±0.9%, and 85.8±0.7% for liver, skeletal muscle, gut, kidney, brain and heart, respectively. In 50% dehydrated frogs, these values decreased in all organs to 78.5±0.8%, 67.2±3.1%, 70.5±1.2%, 75.5±0.5%, 81.0±0.8%, and 78.0±1.7%, respectively (all values significantly different from the corresponding control values, P<0.05 after arcsin√y transformation of the percentage data). All organs showed water regain when the frogs were rehydrated (Fig. 1A) and, except for skeletal muscle, which did not fully recover, the water content of rehydrated organs was not significantly different from the corresponding control values.

Fig. 1B shows organ protein content as mg protein g^-1 dry mass during experimental dehydration and rehydration of *R. pipiens*. Dehydration had no significant effect on protein content of the brain, heart and gut (stomach + intestine). There were small decreases in liver protein content at intermediate levels of dehydration (a 30% decrease at 25.8% dehydrated, compared with controls), but liver protein content was not significantly different from controls in 50% dehydrated or in rehydrated frogs. Muscle showed an anomalous increase in protein content only in 36.6% dehydrated animals. However, kidney protein content was significantly elevated in 36.6% and 50% dehydrated frogs, as
well as in rehydrated animals; levels were about 66, 60 and 72% higher than the control values, respectively.

The effects of whole-body dehydration on blood glucose and lactate concentrations are shown in Table 1. Data are expressed as μmol g⁻¹ wet mass and, hence, are not adjusted for the passive increase in metabolite concentrations that would be caused by net water loss from the plasma during experimental dehydration. However, assuming that the maximum loss of plasma water would not exceed 50% (since this was the maximum for total body water loss by any frog), the change in metabolite concentrations that could be due to passive concentration of the plasma could only be twofold. Table 1 shows that increases in blood glucose and lactate concentrations are of a much greater magnitude than this and, hence, a specific elevation of these two compounds is indicated. Levels of both compounds rose progressively as the frogs lost water, with a significant rise in blood lactate levels occurring even in 12.2% dehydrated frogs. In 50% dehydrated frogs, blood glucose values were 13.8 times the control values, whereas lactate levels had risen 22-fold. Blood lactate levels were sharply reduced relative to 50% dehydrated frogs after 24 h of rehydration and glucose levels declined slightly.

Fig. 2 shows the changes in organ glucose and lactate levels over the course of dehydration. Liver showed a rapid and large increase in glucose concentration. Glucose levels doubled from 13 nmol mg⁻¹ protein in controls (equal to 0.86 μmol g⁻¹ wet mass) to 25 nmol mg⁻¹ by 12.2% dehydration, and continued to rise with increasing dehydration reaching a final level of 307 nmol mg⁻¹ at 50% dehydration, a 23.6-fold increase over control values. After 24 h of rehydration, however, liver glucose levels had dropped back to 57 nmol mg⁻¹. Glucose levels also rose significantly in the kidney and brain over the course of dehydration, maximal increases being seven- and 20-fold, respectively, and remained high after rehydration. In the gut and heart, however, glucose levels were significantly elevated in the organs only after rehydration. Muscle glucose showed an increasing trend during

Table 1. Effect of dehydration on blood glucose and lactate levels in autumn-collected Rana pipiens

<table>
<thead>
<tr>
<th>Dehydration (%)</th>
<th>Glucose (μmol g⁻¹ wet mass)</th>
<th>Lactate (μmol g⁻¹ wet mass)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.36±0.06</td>
<td>0.48±0.06</td>
</tr>
<tr>
<td>12.2</td>
<td>0.96±0.30</td>
<td>2.32±0.37*</td>
</tr>
<tr>
<td>25.8</td>
<td>1.67±0.22</td>
<td>2.69±0.60*</td>
</tr>
<tr>
<td>36.6</td>
<td>2.25±0.52*</td>
<td>6.03±0.86**</td>
</tr>
<tr>
<td>50</td>
<td>4.95±0.71**</td>
<td>10.74±0.19***</td>
</tr>
<tr>
<td>Rehydrated</td>
<td>4.03±0.58**</td>
<td>1.93±0.54</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M., N=4.

Values significantly different from the corresponding control value (two-tailed Dunnett’s test) are marked with asterisks, *P<0.05; **P<0.005.

Rehydrated frogs were dehydrated to 45.5% of total body water lost and then rehydrated to a final mean of 8.1% less than initial control levels.

Fig. 2. Effects of dehydration on levels of (A) glucose and (B) lactate in organs of autumn-collected Rana pipiens. Other details as in Fig. 1.
dehydration, but the changes were not significant; maximum values for muscle glucose of 31 nmol g$^{-1}$ protein corresponded to approximately 3 µmol g$^{-1}$ wet mass.

Lactate levels rose significantly in five of the six organs investigated during dehydration, skeletal muscle being the exception (Fig. 2B). Liver lactate concentration rose from 8±1 nmol g$^{-1}$ protein (0.51 µmol g$^{-1}$ wet mass) in control animals to 23 nmol g$^{-1}$ protein at 12.2 % dehydration and a peak of 96 nmol g$^{-1}$ (8.2 µmol g$^{-1}$ wet mass) at 50 % dehydration. After 24 h of rehydration, however, liver lactate had fallen to a mean of 22 nmol g$^{-1}$ protein. The increase in lactate levels was smaller in gut, kidney, brain and heart, but all showed their greatest increase in lactate concentration between 36.6 and 50 % dehydration, suggesting that aerobic metabolism became impaired when dehydration reached extreme levels. The increase in lactate concentration in these organs ranged from two- to eightfold, with peak levels of 22.1, 57.8, 98.8 and 111.3 nmol g$^{-1}$ protein, respectively, corresponding to 2.5, 7.4, 8.0 and 13.4 µmol g$^{-1}$ wet mass.

The possibility that the rise in glucose levels found in all organs arose from liver glycogenolysis and glucose export was investigated. Table 2 shows liver glycogen content over the course of dehydration and rehydration. Liver glycogen levels in control frogs were 10.7±1.5 † µmol mg$^{-1}$ protein (0.85±0.03 µmol g$^{-1}$ wet mass). Mean glycogen content was higher in gut, kidney, brain and heart, but all showed their greatest increase in liver glycogen content between 36.6 and 50 % dehydration, suggesting that aerobic metabolism became impaired when dehydration reached extreme levels. The increase in liver glycogen content in these organs ranged from two- to eightfold, with peak levels of 22.1, 57.8, 98.8 and 111.3 nmol g$^{-1}$ protein, respectively, corresponding to 2.5, 7.4, 8.0 and 13.4 µmol g$^{-1}$ wet mass.

Table 2. Effect of dehydration on liver glycogen content in autumn-collected Rana pipiens

<table>
<thead>
<tr>
<th>Dehydration (%)</th>
<th>Glycogen content (µmol mg$^{-1}$ protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10.7±1.5†</td>
</tr>
<tr>
<td>12.2</td>
<td>18.6±0.6*</td>
</tr>
<tr>
<td>25.8</td>
<td>16.3±1.6*</td>
</tr>
<tr>
<td>36.6</td>
<td>14.0±1.1†</td>
</tr>
<tr>
<td>50</td>
<td>10.4±0.7†</td>
</tr>
<tr>
<td>Rehydrated</td>
<td>9.6±1.0†</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M., N=4.

Values significantly different from the control value (0 % dehydrated) (Student–Newman–Keuls test) are marked with an asterisk, P<0.01. Values significantly different from the 10 % dehydrated value, P<0.01, are marked with a dagger.

Details of rehydration are as in Table 1.

Glucose export from the liver is typically facilitated by inhibitory controls on endogenous routes of glucose 6-phosphate catabolism, primarily glycolysis. Inhibitory controls on glycolytic enzymes can frequently be pinpointed by monitoring changes in the concentrations of the substrates and products of key glycolytic enzymes. Fig. 3 shows changes in the levels of liver glucose 6-phosphate (G6P), fructose 6-phosphate (F6P), fructose 1,6-bisphosphate (F1,6P$2$) and triose phosphates (glyceraldehyde 3-phosphate + dihydroxyacetone phosphate) over the course of dehydration and rehydration. G6P levels rose rapidly, increasing by 4.5-fold by 12.2 % dehydration and indicating an activation of glycogenolysis. G6P concentration continued to rise throughout the dehydration exposure but, despite this, concentrations of the next intermediates in glycolysis, F6P, F1,6P$2$ and the triose phosphates, remained constant to 36.6 % dehydration. However, at 50 % dehydration, levels of F6P, the substrate of phosphofructokinase, rose sharply by fourfold. G6P levels fell during rehydration, but levels of the other compounds did not.

The effects of dehydration stress on the energy status of the liver are shown in Fig. 4. Between 0 and 25.8 % of total body water lost, liver energy status was largely unaffected; indeed, liver ATP levels were slightly higher in 25.8 % dehydrated frogs than in control animals (Fig. 4A). Liver energetics were compromised, however, when dehydration reached 36.6 and 50 %. At 50 % dehydration, ATP levels were only 44 % of control values, ADP levels were elevated, total adenylates ([ATP]+[ADP]+[AMP]) were reduced by 30 % and energy charge ([ATP]+1/2[ADP])/(([ATP]+[ADP]+[AMP])) had fallen from 0.85±0.03 to 0.64±0.05. Upon rehydration, liver energy status showed good recovery; ATP levels recovered to
31 nmol mg\(^{-1}\) protein (equal to 2.3 \(\mu\)mol g\(^{-1}\) wet mass) and energy charge was 0.90 ± 0.01 after 24 h. However, the total adenylate pool was only 80% of the initial control value because of depressed levels of ADP and AMP.

**Discussion**

For a semi-aquatic species, *R. pipiens* showed a very good tolerance of dehydration, recovering after the loss of 50% of total body water (equal to about 40% of total body mass). This is similar to the reports of other authors, who found that *R. pipiens* survived the dehydrational loss of about 37% of total body mass (Thorson and Svhila, 1943; Hillman, 1980). In comparable experiments (using similar rates of body water loss) with *R. sylvatica*, we found that autumn-collected wood frogs could also survive the loss of 50% of total body water, whereas spring-collected animals withstood a 60% loss (Churchill and Storey, 1993). Another terrestrially hibernating freeze-tolerant species, the spring peeper *Pseudacris crucifer*, endured the loss of 55% of total body water (Churchill and Storey, 1994). Thus, *R. sylvatica* and *P. crucifer* appear to have marginally better dehydration tolerance than *R. pipiens*, which is not unexpected, given the more terrestrial lifestyle of these two species, and agrees with previous findings showing strong positive correlations between increasing dehydration tolerance and an increasingly terrestrial lifestyle among amphibians (for a review, see Shoemaker, 1992).

However, despite the loss of 50% of total body water, the water contents of individual organs of leopard frogs showed remarkably little change (Fig. 1A). Clearly, organ water content is defended during desiccation, at the expense of extra-organ water. Indeed, this was supported by visual observations made during dissection; the liquid normally found in the abdominal cavity disappeared at higher dehydration levels and the blood became viscous. The greatest change in organ water content during dehydration was found for skeletal muscle, as might be expected for a peripheral organ. The change in skeletal muscle water content found in the present study, a decrease from 4.23 to 2.15 g H\(_2\)O g\(^{-1}\) dry mass from controls to 50% dehydrated frogs, compares favourably with values that can be calculated from Hillman (1982); at 34% of body mass lost (about 42.5% of total body water), water content of *R. pipiens* gastrocnemius muscle was 1.51 g H\(_2\)O g\(^{-1}\) dry mass, compared with a control value of 3.6 g H\(_2\)O g\(^{-1}\) dry mass. However, even at extreme dehydration levels, the water contents of most internal organs changed very little. Furthermore, there appeared to be a rank order among organs in the defence of organ water content. Significant changes in gut, kidney and heart water content were first noted at 25.8% dehydration, whereas liver water content was affected only at 36.6% dehydration, and the brain only in 50% dehydrated frogs. This may suggest that mechanisms exist to maintain brain water content preferentially during dehydration of the whole organism. Data for *R. pipiens* brain suggest a better dehydration tolerance than that reported for another aquatic ranid, *Rana catesbeiana*; in this species, brain water content decreased by 13% even with relatively low changes (20% decrease) in body mass (Hillman, 1988).

Analysis of the effects of dehydration on organ protein content showed that, in general, it was largely unaffected by hydration state. This was also true of *R. sylvatica* (Churchill and Storey, 1993), so it appears that changes in bulk protein content are not a strategy of organ defence against dehydration. However, it remains to be determined whether dehydration
In contrast, all five extra-hepatic organs analyzed in both (36.6 and/or 50% dehydration) and this is indicative of an elevated lactate concentrations as dehydration became severe. However, most organs showed would be of minimal use in the colligative retention of cell water during dehydration. Nevertheless, most organs showed elevated lactate concentrations as dehydration became severe (36.6 and/or 50% dehydration) and this is indicative of an activation of glycolysis in these organs. The activation of liver glucose output during dehydration may, therefore, support the delivery of this fuel for anaerobic metabolism to other organs. Other studies have also shown sharp increases in body lactate levels in anurans at high levels of dehydration, generally above 20–30% of body mass lost (Gattan, 1987; Hillman, 1978; Churchill and Storey, 1993). When dehydration becomes severe, the decrease in blood volume and increase in blood viscosity greatly increase the work load on the heart, requiring the recruitment of glycolysis to increase ATP production. Not surprisingly, then, the highest organ lactate levels were recorded in the heart of 50% dehydrated R. pipiens. Furthermore, these same factors compromise circulatory oxygen delivery to all organs, and whole-animal oxygen consumption declines whereas lactate levels rise as the consequence of dehydration-related hypoxic stress (Hillman, 1978, 1987).

It is clear from the data in Fig. 4 that dehydration also compromises organ energetics. These data for liver show a sharp decline in organ ATP and total adenylate levels, as well as in energy charge, between 25.8 and 36.6% dehydration and a further decline in all three variables at 50% dehydration. The decrease in the total adenylate pool suggests the involvement in organ energetics of the enzyme AMP deaminase, which converts AMP to IMP. The action of this enzyme, by preventing AMP accumulation, helps to promote the adenylate kinase reaction (2ADP \rightarrow ATP + AMP) and to maintain energy charge at the highest level possible. Notably, however, the energy status of the liver almost completely recovered after 24 h of rehydration. Both energy charge and ATP levels were restored to values not significantly different from the controls and the total adenylate pool was only about 20% less than the control value. Metabolic recovery after rehydration was also evident by the sharp drop in organ lactate levels (Fig. 2B), which returned to control values in four organs, remaining only slightly elevated in the liver and heart, after 24 h.

The present data show that liver glycogenolysis resulting in glucose accumulation in response to dehydration is a direct response to dehydration and this sheds new light on the cryoprotectant-biosynthesis response of freeze-tolerant frogs. If the activation of liver glucose output in response to dehydration is seen in both freeze-intolerant, aquatic-hibernating frogs (e.g. R. pipiens) and freeze-tolerant, terrestrially hibernating frogs (R. sylvatica, P. crucifer), then it can be strongly suggested that cryoprotectant synthesis in response to freezing evolved out of a pre-existing hyperglycaemic response to dehydration in anurans. To use glucose as a cryoprotectant, freeze-tolerant frogs have linked the activation of glucose synthesis to a freezing-induced dehydration event (probably at a peripheral skin site) and enhanced both the magnitude and the speed of the response in several ways. Other comparisons between R. sylvatica and R. pipiens show that the freeze-tolerant species had much greater glycogen reserves and higher glycogenolytic enzyme activities in liver, as well as increased numbers of glucose transporters in both liver and extra-hepatic organs to facilitate the rapid distribution of large quantities of glucose.
Thus, the present study, when coupled with our comparable studies on freeze-tolerant frogs, provides a clear example of biochemical adaptation in which a pre-existing anuran metabolic capacity has been selected and enhanced to serve a novel purpose by a small group of freeze-tolerant species.

Thanks to Dr F. Schueler for collecting leopard frogs. This work was supported by a grant from the US National Institute of General Medical Sciences GM 43796 to K.B.S. and by an NSERC postgraduate scholarship to T.A.C.

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