Metabolism of bears before, during, and after winter sleep

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THE ABILITY OF THE BEAR to survive hibernation marks it as a unique animal model for the study of severe renal failure in human beings. The bear is not a deep hibernator (6). Its period of hibernation has been called "carnivore lethargy," "dormancy," or "heavy sleep" (7, 12) to distinguish it from the deep hibernation of other mammalian species which demonstrate a profound decrease in their body temperature (12). In winter, the bear, although it does not eat for 100 days or more at near normal body temperatures, without urinating or defecating, by not producing catabolic products of protein metabolism requiring urinary excretion.

nitrogen metabolism; urea formation; urine; water balance; thyroxin; blood lipids; respiratory quotients

Our first objective was to determine whether end products of nitrogen metabolism accumulate in bears during winter dormancy. As a secondary goal we evaluated the function of the thyroid gland, because of its central position in regulating intermediary metabolism. The 1st year of study was primarily directed toward monitoring the concentrations of nitrogenous compounds in blood before, during, and after winter sleep and toward measuring thyroid function.

Our next objective was to determine whether a solute load, urea, if given intravenously to bears during their winter sleep, would induce formation of urine.

METHODS

Tests were done on three male bears, 1, 2, and 3 years of age before winter sleep in December 1969, during winter sleep in February and March 1970, and after winter sleep in May 1970. In the 2nd year, confirmatory studies were done in two of the same bears (ages 2 and 3 years) before and during winter sleep; then, while the animals were still in winter sleep, an intravenous urea injection was given. In the 2nd year of study, data were collected before winter sleep in December 1970, during winter sleep in March 1971, and after winter sleep in June 1971. A total of 9 experiments were done, each lasting between 24 and 96 hr. In their waking months bears were fed dog food containing 23% protein and 8% fat.

In the 1st year of study, two American black bears and one Himalayan bear were immobilized with phencyclidine HCl and promazine HCl (150 mg each, well mixed). The initial anesthetic dose lasted 3-4 hr. To administer it, the syringe containing the drugs was placed in a retractable syringe holder mounted on the end of a 4-ft aluminum rod. Usually the bears sat alert, watching the investigators as they entered the enclosure to give the anesthetic. The animals would attempt to push aside the oncoming syringe or retreat to another portion of the cage. Occasionally, one bear immediately after being anesthetized would charge the rapidly retreating investigator as he left the outdoor enclosure. Once the anesthetic had been administered, the bears were fairly quiet. They began to stagger 15-30 min later and usually fell over and were ready to transport to the experimental laboratory. Two of the bears were immobilized a total of 9 times and one bear was immobilized 4 times. The initial anesthetic dose lasted 3-4 hr. To administer it, the syringe containing the drugs was placed in a retractable syringe holder mounted on the end of a 4-ft aluminum rod. Usually the bears sat alert, watching the investigators as they entered the enclosure to give the anesthetic. The animals would attempt to push aside the oncoming syringe or retreat to another portion of the cage. Occasionally, one bear immediately after being anesthetized would charge the rapidly retreating investigator as he left the outdoor enclosure. Once the anesthetic had been administered, the bears were fairly quiet. They began to stagger 15-30 min later and usually fell over and were ready to transport to the experimental laboratory. Two of the bears were immobilized a total of 9 times and one bear was immobilized 4 times. The bears were kept immobilized during the 24-hr experiments by subsequent injections of 75 mg each of the two drugs, which were required at about 3- to 4-hr intervals.

After immobilization, the bears were weighed, base-line blood samples were taken from an antecubital or femoral...
vein, and they were injected with thyroxine-\textsuperscript{131}I (100 \textmu C, 65 \textmu g) and triiodothyronine-\textsuperscript{125}I (100 \textmu C, 1.68 \textmu g). Blood samples were taken for the next 24 hr and once again at 96 hr to follow the disappearance of the radioactive thyroid hormones. Once during the first few hours of the experiment a specially constructed, airtight mask with tubing leading from a one-way air valve to a Douglas bag was positioned over each bear's nose and mouth and for 10 min expired air was collected. When the experiment was over, the bears were moved to their winter quarters, where the temperature was 2°C. The bears remained in their quarters for 97 days with no access to food or water. Each bear had a straw-filled "den" made from half a metal culvert of the type used in road construction with removable bars at each end.

Procedures during the 2nd year of study were the same as those of the 1st year, except thyroxine and expired gas studies were not done; instead, in December 1970, the bears were catheterized and urine was collected for 24 hr. The first study in winter sleep was done on March 11, 1971. Three weeks later, 10 g of urea in 50 ml of saline solution was injected as a bolus into each bear and the disappearance of urea from the blood and its appearance in the urine were determined. In June 1971, an experiment similar to the study in December 1970 was performed. Studies were made of the following factors. Water and electrolytes: sodium, potassium by flame photometer, water in red cells and plasma by dehydration (3). Nitrogen: amino acids (90), urea (13), uric acid (11), ammonia (21), creatinine (25), and total proteins (9). Lipids: cholesterol (27), triglycerides (8), phospholipids (8), ketones (1). Glucose: (16). Thyroid hormones: total thyroxine (T\textsubscript{4}) (17), triiodothyronine (T\textsubscript{3}) (5), free thyroxine (23), thyroid-binding proteins (26), \textsuperscript{131}I-labeled T\textsubscript{3} and T\textsubscript{4} (24) distribution studies.

Samples of expired air were analyzed for O\textsubscript{2} and CO\textsubscript{2} by Haldane analysis; \textsuperscript{131}I- and \textsuperscript{125}I-labeled thyroid hormone samples were counted simultaneously using a dual-channel well counter and double-isotope counting procedures. Urine osmolality was determined by using a Fiske osmometer. Samples were counted simultaneously using a dual-channel well counter and double-isotope counting procedures. Urine osmolality was determined by using a Fiske osmometer.

In the urine excreted over 24 hr, nitrogen, uric acid, ammonia, urea, and creatinine were measured. Also, during winter sleep, the urine was analyzed for total ketone content.

RESULTS

Clinical course. The bears were active during the summer and fall but in winter when placed in their culvert dens they made a small bed of straw, curled up, and went to sleep. Although all bears went to sleep, from time to time they would lift their heads if an observer were in the cellars housing the dens. When the experimenter was preparing to administer drugs to immobilize the bears for withdrawal of blood samples, the bears sat up and occasionally charged toward the experimenter at the culvert opening. No evidence was found of urination or defecation during the 97 days of winter sleep in the first winter or during the 102 days of the second winter. The dens were free of any odor.

In winter, the rectal temperature was 34–35°C. At the end of the winter sleep, body weight had decreased approximately 25%. The body weights for the three bears in the 1st year of study were 39, 81, and 91 kg before and 33, 59, and 55 kg at the end of winter sleep. In the 2nd year, their body weights were 92 and 130 kg before and 74 and 100 kg at the end of winter sleep.

One bear (Himalayan bear 2) died in the 2nd year of the study. Necropsy findings revealed only pneumonitis as a possible cause of death. This bear was the oldest and largest of the three animals and had lost the most weight in the 1st year of study.

Respiratory quotient (RQ), blood lipids, and thyroid hormones. The RQ taken before winter sleep decreased from 0.78 to values approaching 0.60 during winter sleep (Fig. 1). In spring, after the bears were active and awake, the RQ returned to 0.80.

Control values for blood lipids were higher than levels established for normal human beings. During winter sleep, and apparently concomitant with the decrease in RQ, all blood lipids increased, but they returned to prewinter sleep levels in the spring (Fig. 1). Similar changes in blood lipids as depicted in Fig. 1 were recorded during the 2nd year of winter sleep, which involved two of the three bears.

Thyroid hormone studies revealed that bears have lower levels of circulating thyroxine but equal or higher levels of serum triiodothyronine than do humans (Table 1). Before winter sleep, T\textsubscript{3} was cleared from the blood much more rapidly than T\textsubscript{4} (as in humans). The mean half-life of T\textsubscript{3} was 1.8 days, which is similar to the half-life in humans. The disappearance of T\textsubscript{3} and T\textsubscript{4} during winter sleep was not sig-

![Blood lipids and respiratory quotients before, during, and after winter sleep. Mayo Clinic ranges of normal blood values for human beings are (mg/100 ml): cholesterol, 150–300; triglycerides, 150 or less; phospholipids, 180–320.](http://ajplegacy.physiology.org/)

**TABLE 1. Thyroid hormone levels in serum of bears before, during, and after winter sleep**

<table>
<thead>
<tr>
<th>Bear</th>
<th>Total T\textsubscript{4} (\mu g/100 ml)</th>
<th>Free T\textsubscript{4} (\mu g/100 ml)</th>
<th>T\textsubscript{3} (ng/100 ml)</th>
<th>Before</th>
<th>During</th>
<th>After</th>
<th>Before</th>
<th>During</th>
<th>After</th>
<th>During</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.2</td>
<td>0.8</td>
<td>1.6</td>
<td>0.6</td>
<td>0.3</td>
<td>0.6</td>
<td>0.8</td>
<td>0.5</td>
<td>0.5</td>
<td>227</td>
<td>233</td>
</tr>
<tr>
<td>2</td>
<td>0.9</td>
<td>0.8</td>
<td>1.1</td>
<td>0.3</td>
<td>0.5</td>
<td>0.5</td>
<td>0.8</td>
<td>0.5</td>
<td>0.5</td>
<td>164</td>
<td>213</td>
</tr>
<tr>
<td>3</td>
<td>0.9</td>
<td>0.5</td>
<td>1.5</td>
<td>0.5</td>
<td>0.4</td>
<td>0.8</td>
<td>0.8</td>
<td>0.4</td>
<td>0.8</td>
<td>290</td>
<td>332</td>
</tr>
</tbody>
</table>

Mayo Clinic ranges of normal blood values for human beings are (for 100 ml serum): total T\textsubscript{4}, 4–11 \mu g; free T\textsubscript{4}, 0.8–2.8 \mu g; T\textsubscript{3}, 180–320 ng.
significantly different, sufficient data were not obtained for $T_4$ due to the difficulty in acquiring serum samples daily for a protracted time. Serum levels did not change during winter sleep. Bears, like some other mammals, have no binding proteins in the prealbumin region, and interalpha protein (TBG) and albumin (TBA) are the only binding sites for thyroxine and $T_4$ (Fig. 2). The amount of $T_4$ turned over daily was 80 $\mu$g, or 0.8 $\mu$g/kg body wt, equal to results obtained in humans. Examination of the thyroid gland of the bear that died after 2 months of winter sleep showed a normal thyroid gland, that is, a uniform picture of follicles, formed by cuboidal epithelial cells filled with colloid. The measurement of thyroid stimulating hormone (TSH) by immunoassay was attempted, but there was no cross reaction with the human TSH antibody used in the assay and thus zero values were obtained.

**Nitrogen metabolism.** Creatinine concentration more than doubled during the bears' winter sleep, but total blood amino acids (determined by adding the values obtained on ion-exchange chromatography), urea, uric acid, and total protein remained practically unchanged throughout the 2 years of sampling. As indicated, the concentrations of individual amino acid showed no consistent change in winter sleep (Table 2).

Urine volume was between 1 and 2 liters/24 hr before winter sleep, but during sleep the urine volume decreased to only 100 ml/24 hr (Table 3). The appearance of urea, uric acid, and ammonia in urine and the total nitrogen content were all greatly reduced during winter sleep; the creatinine, however, remained unchanged.

The excretion of ketones over a 24-hr period was low during winter sleep. Bear 1 excreted 8 mg and bear 3, 13 mg/24 hr (bear 2 had died before the test was done).

**Other blood studies.** No significant changes were noted during winter sleep in levels of plasma sodium and potassium or in water content of plasma and red cells when these values were compared with samples taken before and after winter sleep. The decreases in mean value of blood glucose and hematocrit in winter sleep were not significant statistically; the changes were considered to represent normal variations (Table 4).

The March 11, 1971, experiment was done to establish a base line for evaluation of the effect of injection of urea on formation of urine. The results of this experiment have been discussed in the context of nitrogen levels of urine during winter sleep (Table 3). Before establishing these values, however, all residual urine collected when the bears were
TABLE 4. Blood levels in bears before, during, and after winter sleep

<table>
<thead>
<tr>
<th>Substance</th>
<th>Before</th>
<th>During</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium, mEq/liter</td>
<td>137±3</td>
<td>144±1</td>
<td>141±0.2</td>
</tr>
<tr>
<td>Potassium, mEq/liter</td>
<td>4.2±0.3</td>
<td>3.8±0.2</td>
<td>4.4±0.1</td>
</tr>
<tr>
<td>Glucose, mg/100 ml</td>
<td>79±11</td>
<td>61±8</td>
<td>97±5</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>44±0.7</td>
<td>50±2</td>
<td>46±2</td>
</tr>
<tr>
<td>Water In plasma, ml/ml</td>
<td>0.9±0.01</td>
<td>0.9±0.001</td>
<td>0.92±0.0001</td>
</tr>
<tr>
<td>Water In red cells, ml/ml</td>
<td>0.70±0.01</td>
<td>0.69±0.01</td>
<td>0.69±0.001</td>
</tr>
</tbody>
</table>

Values are means ± se.

FIG. 3. Urine volume and urea represent accumulative data collected for 24 hr. Experiment performed on March 11, 1971 was control study, and experiment performed on March 31, 1971 represents data collected after intravenous injection of 10 g of urea. On March 11, total urea collected was determined on pooled collections of 24-hr experiment (-----).

first catheterized was measured and analyzed. Residual volumes were 33 and 67 ml, containing 0.8 and 1.6 g of urea, respectively. The rate of formation of urine for the next 24 hr is shown in Fig. 3, with the total urea content of each collection amounting to approximately 4 g (Table 3 and Fig. 3). Osmolality did not vary appreciably during the 24-hr collection period (Fig. 3).

Three weeks later, 10 g of urea in 50 ml of saline was injected intravenously in the two bears (Fig. 4). Just prior to the injection, blood urea measured 8 and 9 mg/100 ml; as a result of the injection it increased to 40 mg/100 ml. Within an hour, blood values had returned to more normal levels (se, ± 2; Table 5) and remained near the mean value for winter sleep (23 mg/100 ml) for the next 23 hr.

After the intravenous injection of 10 g of urea, the osmolality of urine promptly decreased, remained low for 3 hr, then increased steadily for the next 21 hr (Fig. 3). Associated with the changes in osmolality were a prompt increase in volume of urine in bear 1 and a more delayed increase in bear 3 when the rates of urine formation were compared with the rates on March 11, 1971 (Fig. 3).

Urine volume was an exponential function of urea excretion (Fig. 5). The two points at either end of the graph were representative of data before and during winter sleep; the middle two points on the line were generated from data collected after the injection of urea.

No change in plasma water content was noted as a result of the increased urine volume produced by injection of blood urea, but red cell water decreased in bear 1 from 0.69 to 0.61 and in bear 3 from 0.70 to 0.64 ml water/ml red cells.

Fig. 4. Change in blood urea concentration in bears during winter sleep after intravenous injection of 10 g of urea.

Feces. Four fecal samples were obtained on the 1st day after winter sleep. They contained between 0.13 and 3.9 g of nitrogen. There was no evidence for intestinal nitrogen storage during winter sleep.

DISCUSSION

The behavior of the bears in winter sleep was similar to that reported earlier (4, 14, 15). They assumed a "hibernation" position of head between forepaws (4) and would raise their heads and occasionally make movements. Morbid drowsiness or profound sleep was not noted in our confined animals and easy arousal has been described in wild bears during winter sleep (14, 15, 22).

We concluded that changes in circulating thyroid hormone levels, presumably thyroid hormone secretion, did not take part in induction or maintenance of winter sleep in bears. The disappearance rates of T₃ and T₄ indicated that peripheral utilization of thyroid hormone was unchanged. Thyroid hormone may have a permissive but not a causative role in winter sleep of the bear. Furthermore, the morphology of the thyroid gland was normal in winter sleep; no evidence of atrophy was found. Despite the absence of food intake during winter sleep, iodine deficiency will not develop, since all the iodide secreted from the gland as thyroid hormones will be available for reabsorption by the gland after the metabolism and liberation of the bound iodide. This is in
TABLE 5. Blood nitrogen values in bears before, during, and after winter sleep

<table>
<thead>
<tr>
<th>Substance</th>
<th>Before</th>
<th>During</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acids, μmoles/100 ml</td>
<td>280±31</td>
<td>266±32</td>
<td>277±7</td>
</tr>
<tr>
<td>Urea, mg/100 ml</td>
<td>30±3</td>
<td>23±6</td>
<td>21±2</td>
</tr>
<tr>
<td>Uric acid, mg/100 ml</td>
<td>0.85±0.12</td>
<td>1.11±0.18</td>
<td>1.24±0.07</td>
</tr>
<tr>
<td>Ammonia, μg/100 ml</td>
<td>9.0±4</td>
<td>10.3±4</td>
<td>5.4±4</td>
</tr>
<tr>
<td>Creatinine, mg/100 ml</td>
<td>1.06±0.07</td>
<td>3.18±0.48</td>
<td>1.38±0.02</td>
</tr>
<tr>
<td>Total proteins, g/100 ml</td>
<td>7.55±0.11</td>
<td>8.03±0.27</td>
<td>7.10±0.02</td>
</tr>
</tbody>
</table>

Values are means ± se.

FIG. 5. Urine volume as a function of urea excretion.

contrast to the human being without food intake, who continues to lose iodide through urinary excretion.

The length of winter sleep for our animals was about 100 days and during this period they received no food or water. At the end of winter sleep, however, the bears were in almost perfect water balance with normal water content of plasma and red cells, and hematocrits were only slightly increased from presleep values.

The data indicate that bears achieve this independence of food and water intake by using fat as the main source of calories and by not accumulating products of protein catabolism requiring urinary excretion. Not only the concentration of blood urea, usually the main end product of protein catabolism, but also the concentrations of uric acid and of ammonia were unchanged and only a minimal change was observed in serum creatinine over the 100 day interval. These findings confirm and extend those of Brown et al. (2).

The normal end products of nitrogen metabolism do not accumulate in the bear during winter sleep. These data, when coupled with evidence that the water content of blood was unchanged, that the hematocrit is only slightly increased, that the blood volume remains constant (2), and that excretion of feces or urine is lacking, indicate that there was no net formation of the common metabolic end products of nitrogen metabolism. Since the nitrogenous end products did not accumulate or were not excreted, either they were not formed or (1) the nitrogen in them was salvaged by some as yet undetermined manner or (2) the enterohepatic cycle of urea metabolism was functioning at a level adequate to balance the production of the end products and the recycling of the nitrogen into the body “pool.” In either event it appeared that nitrogen metabolism, protein catabolism in particular, was reduced when the bear stopped eating and went into winter sleep. To test this hypothesis, urea was injected intravenously during winter sleep to determine if the bear would catabolize it or if the urea would stimulate formation of urine. The data showed that the injection of urea induced diuresis and, although blood urea was elevated only for the first hr after injection, 60–90% of the injected urea appeared in the urine at a time when blood levels were normal. The diuresis was most likely caused by osmotic equilibration of plasma urea by intracellular water. The briefly expanded blood volume was caused by a much more rapid diffusion of water than of urea in the equilibration process. The added water appeared in the urine.

The source of water was most likely intracellular, since red cell water decreased by 9–11% as a result of the injection of urea, while plasma water content was unchanged. The twofold increase in volume of urine and the appearance of 60–90% of the injected load of urea in urine clearly showed that the injected urea overcame the bear’s ability to conserve water and that, under the conditions of the test, the urea load was not disposed of metabolically but was excreted mainly in the urine.

The experiment on March 11, 1971, however, demonstrated that water and urea entered the bladder during winter sleep even when the only experimental stimulus was the administration of anesthesia. Brown and co-workers (2) showed that glomerular filtration in the bear in winter sleep decreased from approximately 122 ml/min to 37 ml/min. If our findings were not due to an anesthetic effect on kidney function, then not all of the filtered fluid is reabsorbed by the kidney. The bladder also must reabsorb water, urea, and other substances not reabsorbed by the kidney. Leaf and Hays (10) have shown that urea, water, and sodium are transported across the wall of the toad bladder. It is entirely possible, therefore, that in the bear, substances entering the bladder during winter sleep will and do move across the bladder epithelium into blood. In our experience, residual urine is obtainable from bears in winter sleep although they have had no water for several months. Urine, 50–150 ml, can be collected within half an hour after the bears have received anesthesia and while their body temperatures are 34–35 C (observations on three separate occasions during winter sleep).

Further support for this conclusion was the finding that the blood levels of urea in the two bears just prior to the urea-load injection were only 8 and 9 mg/100 ml. Three weeks earlier, during the control experiment, the bears were catheterized for 24 hr. Four grams of urea were collected, which normally, if our hypothesis is correct, would have been reabsorbed by the bladder. The removal of 4 g depleted the body pool of urea and decreased the concentration...
of blood urea. No indication of urea repletion occurred during the next 3 weeks.

The data suggest that in bears reabsorption of urine by the bladder proceeds during winter sleep at a rate sufficient to prevent accumulation of enough urine in the bladder to stimulate urination. Further experiments of bladder transport are needed to determine the role of the urinary bladder in the bear in winter sleep.

The decrease in urea synthesis, however it was accomplished, appeared to be the most important means by which the state of winter sleep was maintained. Since obese human beings also reduce urea formation to low levels after 5–6 weeks of starvation (19), the decrease in urea production in the bear need not involve special mechanisms peculiar to hibernation. It may be that when the bear stops eating, the inhibition of urea formation occurs sooner and is more complete than in human beings. When no urea is synthesized, no urine need be excreted.

An attempt to produce a condition like “winter sleep” in bears was tried in anephric human beings. The purpose was to develop a program to save time and money for anephric humans waiting kidney transplantation (18).

REFERENCES