Hematologic and plasma biochemical findings in cold-stunned Kemp's ridley turtles: 176 cases (2001–2005)

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Objective—To document hematologic and plasma biochemical values for a large number of cold-stunned Kemp's ridley turtles at the beginning of rehabilitation, to investigate differences in hematologic and plasma biochemical values of turtles that ultimately survived versus those that died, and to compare values of survivors during convalescence with initial values obtained at the time of admission.

Design—Retrospective case series.

Animals—176 stranded, cold-stunned Kemp's ridley turtles hospitalized between 2001 and 2005.

Procedures—Hematologic and plasma biochemical values obtained at the time of admission were compared retrospectively for turtles that died versus turtles that survived. Initial results for survivors were compared with convalescent results obtained later in rehabilitation.

Results—Turtles that died had significantly greater plasma concentrations of sodium, chloride, potassium, calcium, phosphorus, and uric acid than did turtles that survived. For survivors, values obtained during convalescence for BUN concentration and plasma calcium concentration were significantly greater than initial values obtained at the time of admission, whereas values obtained during convalescence for glucose, sodium, and uric acid concentrations were significantly lower than initial values.

Conclusions and Clinical Relevance—Cold-stunned Kemp's ridley turtles may be affected by electrolyte derangements, dehydration, and decreased renal function. Hematologic and plasma biochemical evaluation of such turtles provided useful clinical and prognostic information during the rehabilitation process. (*J Am Vet Med Assoc* 2009;235:426–432)

Kemp's ridley turtle (*Lepidochelys kempii*) is the smallest and rarest species of sea turtle. It is listed as critically endangered by the World Conservation Union and is listed as endangered by the US Endangered Species Act. Recent population estimates report approximately 2,000 to 3,000 nesting females, but the total population of adult males and juveniles is unknown.[1] Although adult Kemp's ridley turtles are most commonly found in the Gulf of Mexico, juveniles frequent the northeastern coast of the United States during the summer.[2] In autumn, juveniles leave these summer foraging grounds for warmer waters. In some instances, however, turtles fail to migrate to warmer waters and may become cold-stunned as water temperatures fall below 50°F (10°C). Cold-stunned juvenile Kemp's ridley turtles are commonly found stranded on beaches of New York and Massachusetts from October to December.[3–5] Cold-stunned sea turtles have also been found in the southern United States and Europe.[6,7] Reasons for these cold-stunning events are not completely understood, but it appears that a combination of oceanographic, geographic, and meteorologic conditions are involved.[5]

Abbreviations

ALKP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
CK	Creatine kinase
GGT	γ-Glutamyltransferase
LDH	Lactate dehydrogenase

In Massachusetts, a coordinated rescue and rehabilitation program for cold-stunned sea turtles has been in operation for approximately 15 years. Volunteers and staff of the Massachusetts Audubon Society patrol beaches of Cape Cod Bay to recover stranded turtles, which are then transported to the New England Aquarium for medical care. Unfortunately, 35% to 85% of cold-stunned turtles are already dead when found on the beach.[3,7,8] Details of the triage and medical management of cold-stunned turtles have recently been published.[9] Briefly, turtles are gradually warmed over several days and treated for dehydration, metabolic derangements, cardiorespiratory depression, and concurrent pathologic conditions (eg, pneumonia).

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Assessment of health is obtained via physical examination, radiography, echocardiography, Doppler blood flow evaluation, serial blood gas tension and electrolyte evaluations, CBCs, and plasma biochemical analyses. In some instances, additional diagnostic testing including endoscopy, computed tomography, nuclear scintigraphy, and magnetic resonance imaging is used. Once rehabilitated, turtles are kept in captivity until the following summer and are then released. In some instances, turtles are transferred to secondary care facilities to complete rehabilitation.

Hematologic and plasma biochemical analyses are recognized as an important part of reptile health evaluation.[10] Several studies[8,11–13] have reported hematologic and plasma biochemical values for cold-stunned Kemp's ridley turtles. However, most of these reports were published in non–peer-reviewed literature, included small numbers of turtles, and, in 1 instance, used blood obtained from dead turtles. The purposes of the study reported here were to document hematologic and plasma biochemical values for a large number of cold-stunned Kemp's ridley turtles at the beginning of rehabilitation and to investigate differences in hematologic and plasma biochemical values of turtles that ultimately survived versus those that died. In addition, hematologic and plasma biochemical values for surviving cold-stunned turtles were compiled later in the course of rehabilitation to compare values of these turtles during convalescence with values at the time of admission.

Materials and Methods

Sea turtle rehabilitation at the New England Aquarium was conducted with authorization of the US Department of the Interior Fish and Wildlife Service and the US Department of Commerce National Marine Fisheries Service, in compliance with guidelines of the New England Aquarium Animal Care and Use Committee.

Criteria for selection of cases—Medical records for all live, cold-stunned Kemp's ridley turtles admitted to the New England Aquarium between 2001 and 2005 were reviewed. Data collected included date of stranding, weight, straight carapace length, cloacal temperature at the time of blood sample collection, success or failure of rehabilitation (ie, survived vs died), and values from multiple hematologic and plasma biochemical panels spanning the rehabilitation period. Dates of blood sample collection were compared with dates of admission or death. From the medical records, it was determined whether turtles were receiving parenteral administration of fluids, were eating voluntarily, or had any concurrent illness.

Procedures—A standardized method of blood sample collection and analysis had been used during the 5-year period. Two to 3 mL of blood was collected from the dorsal cervical sinus and transferred to 2 heparinized blood collection tubes.[14,a] One tube was centrifuged within 10 minutes of collection at 1,500 × *g* for 5 minutes, and the plasma was harvested and refrigerated at 39°F (4°C). Heparinized blood from the second tube was used to make 2 blood smears, and the remainder was then refrigerated at 39°F (4°C). Hematologic and plasma biochemical assays were performed within 18 hours at a commercial veterinary diagnostic laboratory.[b] The Hct was measured after centrifugation of blood at 13,000 × *g* for 5 minutes in heparinized capillary tubes. Total WBC count was performed manually by use of a disposable pipette system.[15,c] Differential WBC count was performed by a board-certified veterinary clinical pathologist. One hundred WBCs were identified on fixed blood smears stained with a modified Wright-Giemsa stain[d] by use of an automated stainer.[e] Plasma biochemical values, including ALKP, ALT, AST, CK, LDH, GGT, albumin, total protein, globulin, total bilirubin, BUN, creatinine, cholesterol, glucose, calcium, phosphorus, chloride, potassium, sodium, and uric acid, were measured by use of an automated clinical chemistry analyzer.[f] The analyzer was used to calculate the plasma sodium-to-potassium and albumin-to-globulin concentration ratios.

Hematologic and plasma biochemical values were categorized as initial values, clinically convalescent values, or calculated convalescent values on the basis of the time of blood sample collection, final outcome of the turtle (survived vs died), and clinical status of each turtle. Initial values were measured from blood samples collected during the first 5 days of hospitalization and were defined as coming from a turtle that died if the turtle died within 1 week of the date of blood sample collection. Convalescent values were defined by 2 methods. For the first method, clinically convalescent values were measured from blood samples obtained from turtles defined as a convalescent turtle on the basis of the following criteria: had no concurrent illness on the basis of findings on physical examination and radiography, had completed treatments of parenteral administration of fluids and antimicrobials at least 14 days prior to blood sample collection, had been in room temperatures of 70° to 80°F (21° to 26°C) for at least 14 days prior to blood sample collection, and had eaten daily for at least 14 days prior to blood sample collection. For the second method, statistical analysis was used to determine the calculated convalescent value for each hematologic and plasma biochemical variable. The calculated convalescent value was the value at which the variable stabilized during the course of rehabilitation. For example, a variable that was initially deranged in ill turtles may have declined or increased over time to an eventually stable value. This final stable value and the time required to reach that value were mathematically derived independent of clinical observations. This was determined by calculating a coefficient of variation for each variable (coefficient of variation = SD/mean) by use of all available hematologic and biochemical panels for each turtle ($panel_1$ through $panel_n$, where $panel_1$ was the first panel for an individual turtle and n is the total number of panels performed for that individual). Coefficients of variation were then calculated for $panel_2$ through $panel_n$, $panel_3$ through $panel_n$, and so on, with a final coefficient of variation calculated for the last 3 panels for each turtle ($panel_{n-2}$ through $panel_n$). By use of the calculated coefficients of variation for all turtles, the change in the coefficient of variation of each analyte over time was fitted to the simple net rate equilibrium concentration equation $y = b + (a - b)^{(-cx)}$, where a is the maximum coefficient of variation, b is the equilib-

Table 1—Mean ± SD (range) values for physical characteristics, day of blood sample collection, and day of death (if applicable), within the first 5 days of hospitalization for 176 cold-stunned Kemp's ridley turtles.

Variable	Turtles	
	Survived (n = 142)	Died (n = 34)
Body weight (kg)	2.86 ± 1.15 (1.1–8.0)	2.68 ± 0.82 (1.2–4.6)
Body weight (lb)	6.3 ± 2.5 (2.3–17.6)	5.9 ± 1.8 (2.6–10.1)
SCL (cm)	27.2 ± 4.0 (19.5–41.3)	26.9 ± 3.5 (20.3–36.1)
Hosp (d)	2.4 ± 1.2 (1–5)	2.2 ± 0.9 (1–5)
Dead (d)	NA	2.5 ± 1.7 (0–6)
Temp (°F)	60 ± 7.3 (38–75)	58.4 ± 7.2 (46–74)
Temp (°C)	15.5 ± 4.1 (3.3–23.8)	14.6 ± 4 (7.7–23.3)

SCL = Straight carapace length. Hosp = Number of days turtle had been hospitalized at the time of blood sample collection. Dead = Number of days after blood sample collection that the turtle died. NA = Not applicable. Temp = Cloacal temperature at time of blood sample collection.

rium value, and c is a net rate constant.[g] The number of days to reach the equilibrium value b was defined as the calculated convalescent value time, and all hematologic and plasma biochemical data obtained after this time were used to determine the calculated convalescent mean, SD, and range values for each variable. If there was not a pattern to the change in a variable over time, a calculated convalescent time and value could not be calculated.

Statistical analysis—Comparisons of hematologic and plasma biochemical values were conducted to determine which initial values differed between turtles that survived and turtles that died and which initial values for survivors differed from clinically convalescent values. The initial values of turtles that survived were compared with those that died by use of a *t* test.[h] Because of the large number of comparisons, the *P* value was protected and significance was set at a level of $P = 0.0014$ (0.05/35).

Table 2—Mean ± SD (range) initial and convalescent hematologic and plasma biochemical values for cold-stunned Kemp's ridley turtles.

Variable	Turtles				
	Initial		Convalescent		
	Survived (n = 142)	Died (n = 34)	Clinically convalesecent (n = 15)	Calculated convalescent	Day*
ALKP (U/L)	426 ± 714 (20–4,503)	538 ± 833 (2–3,661)	262 ± 120 (99–463)	270 ± 140 (83–462)	204
ALT (U/L)	16 ± 57 (0–673)	29 ± 37 (2–162)	17 ± 17 (5–68)	ND	ND
AST (U/L)	408 ± 362 (110–2,801)	568 ± 373 (6–1,693)	356 ± 156 (88–669)	464 ± 204 (206–1,070)	29
CK (U/L)	18,433 ± 20,575 (212–114,660)	26,275 ± 26,360 (2–81,880)	3,403 ± 2,136 (1,210–8,367)	ND	ND
LDH (U/L)	4,311 ± 8,080 (294–78,729)	5,022 ± 4,970 (143–25,200)	3,878 ± 2,079 (1,093–7,728)	4,014 ± 1,777 (1,252–5,979)	200
GGT (U/L)	3 ± 3 (0–17)	3 ± 3 (0–15)	3 ± 2 (1–6)	ND	ND
Albumin (g/dL)	1.0 ± 0.2 (0.4–1.5)	1.0 ± 0.2 (0.5–1.5)	1.3 ± 0.2 (1–1.7)	1.4 ± 0.2 (1.1–1.7)	84
Total protein (g/dL)	2.9 ± 0.5 (1.9–4.3)	2.7 ± 0.5 (1.4–4.0)	3.3 ± 0.4 (2.5–4.1)	3.9 ± 0.5 (2.5–5)	86
Globulin (g/dL)	1.8 ± 0.3 (1.1–2.8)	1.7 ± 0.4 (0.9–2.5)	2.1 ± 0.3 (1.5–2.7)	2.5 ± 0.4 (1.5–3.3)	62
Bilirubin (mg/dL)	0.0 ± 0.1 (0–0.3)	0.1 ± 0.1 (0–0.5)	0 ± 0 (0–0.1)	ND	
BUN (mg/dL)	22 ± 12 (3–73)	18 ± 12 (5–49)	136 ± 21 (87–164)‡	133 ± 22 (86–178)	31
Creatinine (mg/dL)	0.1 ± 0.1 (0–0.4)	0.0 ± 0.1 (0–0.4)	0.2 ± 0.1 (0–0.3)	0.2 ± 0.2 (0–0.4)	172
Cholesterol (mg/dL)	333 ± 116 (94–666)	359 ± 124 (161–599)	185 ± 58 (138–356)	202 ± 58 (139–336)	176
Glucose (mg/dL)	153 ± 78 (28–523)	158 ± 90 (26–344)	116 ± 16 (99–146)‡	118 ± 11 (90–134)	37
Calcium (mg/dL)	6.3 ± 1.4 (4.3–11.0)†	8.4 ± 2.5 (5–17.4)	7.5 ± 1.3 (6.1–10.8)‡	7.6 ± 0.6 (6.8–9.8)	8
Phosphorus (mg/dL)	8.1 ± 2.6 (3.6–19.3)†	11.2 ± 4.4 (5.2–19.9)	9.0 ± 1.7 (4.9–11.7)	9.4 ± 2.7 (6.7–14)	196
Chloride (mEq/L)	118 ± 12 (96–139)†	127 ± 16 (77–176)	116 ± 4 (109–122)	ND	ND
Potassium (mEq/L)	3.4 ± 0.9 (1.6–7.6)†	4.0 ± 1.2 (1.8–8.0)	3.9 ± 0.6 (3.0–5.1)	3.8 ± 0.5 (3.0–5.1)	110
Sodium (mEq/L)	160 ± 6 (145–179)†	169 ± 14 (114–198)	154 ± 5 (145–161)‡	154 ± 2 (150–159)	30
A:G ratio	0.6 ± 0.1 (0.2–0.8)	0.6 ± 0.1 (0.5–0.8)	0.6 ± 0.1 (0.5–0.7)	0.6 ± 0.1 (0.3–0.7)	80
Uric acid (mg/dL)	5.3 ± 2.6 (0.9–15.2)†	7.5 ± 3.5 (3.6–16.9)	0.5 ± 0.2 (0.2–0.9)‡	0.5 ± 0.2 (0.1–1.2)	28
Na:K ratio	50 ± 11 (22–107)	45 ± 12 (21–76)	40 ± 5 (32–50)	41 ± 5 (32–52)	104
WBC (cells/µL)	10,446 ± 7,619 (1,000–43,000)	8,194 ± 6,175 (600–33,700)	4,263 ± 2,448 (1,100–10,167)	3,829 ± 1,080 (1,600–6,050)	69
Hct (%)	39 ± 8.9 (12–73)	41 ± 12 (18–62)	30 ± 3 (26–37)	31 ± 4 (24–41)	9
Heterophil (%)	70 ± 13 (15–92)	65 ± 18 (12–91)	55 ± 11 (35–80)	52 ± 10 (31–67)	13
Lymphocyte (%)	23 ± 12 (4–62)	28 ± 17 (7–86)	35 ± 11 (15–60)	43 ± 16 (22–87)	73
Monocyte (%)	6 ± 5 (0–31)	7 ± 4 (0–17)	7 ± 5 (1–16)	ND	ND
Eosinophil (%)	0 ± 0 (0–1)	0 ± 1 (0–3)	2 ± 2 (0–6)	6 ± 2 (1–8)	194
Basophil (%)	0.4 ± 1.1 (0–8)	0.3 ± 0.6 (0–2)	0.8 ± 1.4 (0–5)	ND	ND
Heterophil (cells/µL)	7,399 ± 5,704 (624–27,360)	5,545 ± 4,524 (72–21,905)	2,201 ± 1,006 (556–4,119)	1,921 ± 1,036 (72–5,865)	73
Lymphocyte (cells/µL)	2,076 ± 1,496 (154–8,118)	1,927 ± 1,850 (343–9,099)	1,719 ± 1,502 (255–5,579)	1,720 ± 1,258 (84–9,180)	6
Monocyte (cells/µL)	627 ± 853 (0–5,947)	559 ± 577 (0–2,696)	258 ± 223 (85–688)	280 ± 406 (0–3,224)	24
Eosinophil (cells/µL)	4 ± 19 (0–156)	9 ± 48 (0–285)	98 ± 155 (0–562)	193 ± 199 (12–986)	15
Basophil (cells/µL)	25 ± 81 (0–512)	28 ± 62 (0–272)	29 ± 61 (0–205)	60 ± 56 (0–280)	17

*Day that calculated convalescent value was reached. †Significantly ($P < 0.0014$) different initial values between turtles that survived and turtles that died. ‡Significantly ($P < 0.0014$) different convalescent values than initial values by use of paired comparisons.

A:G ratio = Plasma albumin-to-globulin concentration ratio. Na:K ratio = Plasma sodium-to-potassium concentration ratio. ND = Not determined.

Initial values for a survivor were compared with that individual's clinically convalescent values by use of a paired *t* test, with a protected *P* value of 0.0014.[h] Finally, clinically convalescent values were compared with calculated convalescent values by use of a meta-analysis, in which each blood variable was considered an observation. Whether the clinically convalescent values were greater or lesser than the calculated convalescent values was determined, and the resultant binomial data were then tested for deviation from equal probability.[i]

Results

Two hundred eight live, juvenile Kemp's ridley turtles were admitted to New England Aquarium during the study period. Turtles were found between late October and late December of their respective years of stranding. Eighteen turtles died prior to collection of blood samples for CBC and plasma biochemical analysis. Initial hematologic and plasma biochemical analysis values (ie, blood samples collected within the first 5 days of hospitalization) were available for 190 turtles. All turtles had been treated with parenteral administration of fluids at the time of initial blood sample collection and were not yet eating. Of those turtles for which initial hematologic and plasma biochemical analysis values were available, 142 turtles survived to be released. Thirty-four turtles for which initial hematologic and plasma biochemical analysis values were available died within 6 days of blood sample collection (mean, 2.5 days). Fourteen turtles died > 1 week after initial blood sample collection and were excluded from the study (range, 8 to 193 days; mean, 28 days; **Tables 1 and 2**). Initial plasma calcium, phosphorus, chloride, potassium, sodium, and uric acid concentrations were significantly greater in turtles that died than in turtles that survived. For these variables, values that were associated with mortality rates of approximately 50% (range, 44% to 77%) and 100% were determined **(Table 3)**.

Hematologic and plasma biochemical values were measured from turtles during convalescence (Table 2). Of the surviving turtles, only 15 convalescent turtles met the criteria for determining clinically convalescent values. Blood samples from these 15 turtles were collected, on average, on day 178 of hospitalization (range, 116 to 304 days). Many surviving turtles were transported to secondary care facilities for completion of rehabilitation prior to meeting the definition of a convalescent turtle, and subsequent hematologic and plasma biochemical values for these turtles were not available. In addition, many surviving turtles that completed rehabilitation at New England Aquarium had minor but persistent physical abnormalities, which prevented them from meeting the definition of a convalescent turtle.

For survivors, clinically convalescent values for BUN concentration and plasma calcium concentration were significantly greater than initial values, whereas clinically convalescent values for plasma glucose, sodium, and uric acid concentrations were significantly lower than initial values (Table 2). In paired comparisons, calculated convalescent values were similar to clinically convalescent values, having a mean difference of 8.2% (95% confidence interval, ± 4.0%). Some plasma biochemical variables reached calculated convalescent values more rapidly than others (eg, BUN, plasma calcium, sodium, and uric acid concentrations and Hct [8 to 30 days] vs plasma cholesterol concentration, LDH activity, and phosphorus concentration [176 to 200 days]).

Table 3—Abnormal initial plasma biochemical values of cold-stunned Kemp's ridley turtles associated with mortality rates of approximately 50% (range, 44% to 77%) and 100%.

	Mortality rate			
	Approximately 50%		100%	
Variable	Value	No. of turtles that died	Value	No. of turtles that died
Sodium (mEq/L)	> 170	17/27	> 180	7/7
Chloride (mEq/L)	> 130	14/25	> 140	10/10
Potassium (mEq/L)	> 5.5	4/9	> 8	2/2
Uric acid (mg/dL)	> 11	7/11	> 16	3/3
Phosphorus (mg/dL)	> 15	10/13	> 18	4/4
Calcium (mg/dL)	> 7.5	23/46	> 11	3/3

Discussion

Although hematologic and plasma biochemical values of cold-stunned Kemp's ridley turtles have been documented in several studies,[8,11–13] most of these studies were confined to the non–peer-reviewed literature, provided data for few turtles, or provided data from postmortem samples. By comparison, the present study provided data for a larger number of turtles and clinical variables and for a longer period. In addition, this study is the first to provide detailed antemortem hematologic and plasma biochemical values for cold-stunned sea turtles that ultimately did not survive and the first to report uric acid values and differential WBC counts for cold-stunned Kemp's ridley turtles. In general, initial values in this study are similar to those previously described for cold-stunned turtles, including a low BUN concentration and high plasma sodium, phosphorus, and glucose concentrations; LDH, AST, ALKP, and CK activities; and Hct.[8,11–13] Clinically convalescent values in this study were similar to those of healthy Kemp's ridley turtles, with the exception of moderately high plasma phosphorus concentration and LDH, AST, ALKP, and CK activities.[8,11–13,16]

Initial values for plasma sodium, potassium, chloride, calcium, phosphorus, and uric acid concentrations were significantly higher in turtles that died than in turtles that survived. These findings suggested that many cold-stunned Kemp's ridley turtles are affected by dehydration and decreased renal function and that severe derangements of these values may indicate a poor prognosis. These data are consistent with previous general descriptions of the physiologic status of cold-stunned turtles.[9,11] Although acid-base and blood gas tension data were not evaluated as part of this study, previously published data indicate that cold-stunned Kemp's ridley turtles may be affected by metabolic and respiratory acidosis.[12] Collectively, previous findings of acidosis, plasma electrolyte derangements (eg, severe hyperkalemia), dehydration, and decreased renal function provide a view of the physiologic status of many cold-stunned Kemp's ridley turtles. Severe derangements of selected critical variables were associated with a high mortality rate (Table 3).

Healthy carnivorous sea turtles may have much higher BUN concentrations than terrestrial vertebrates; however, turtles in this study initially had low BUN concentrations.[8,11–13,16–18] This phenomenon has been observed previously in studies[8,11,12,19] of cold-stunned Kemp's ridley turtles and injured loggerhead sea turtles. Although the reason for low BUN concentrations is unclear, it is possible that it is related to decreased urea production under conditions of anorexia or decreased hepatic function. Interestingly, several turtles had increasing BUN concentrations during rehabilitation even though they had not yet accepted food, which suggests that anorexia alone may not explain the low BUN concentration at the time of admission. Results of this study indicate that BUN concentrations of cold-stunned Kemp's ridley turtles can be expected to be low at the time of admission and increase over the course of rehabilitation.

There was great variability in initial plasma glucose concentrations, and hypoglycemia and hyperglycemia were detected. This phenomenon has been observed previously in cold-stunned sea turtles and likely represents the variable metabolic status of the turtles.[8,12] Possible causes of hypoglycemia in these turtles include exhaustion, prolonged anorexia, and sepsis. Hyperglycemia in reptiles may reflect a stress response, overcompensation of gluconeogenic mechanisms, liver disease, pancreatic disease, or exogenous dextrose administration.[20] Hyperglycemia has been observed by the authors in cold-stunned turtles in the absence of exogenous dextrose supplements, and hyperglycemia of unknown etiology has also been detected in debilitated loggerhead sea turtles.[21,22] In general, plasma glucose concentrations of surviving turtles stabilized over several weeks. Further investigation of the pathophysiologic mechanisms of hyperglycemia in sea turtles is warranted.

It is likely that the initially high plasma calcium and phosphorus concentrations in some cold-stunned turtles were the result of decreased renal function and dehydration. In contrast to the high plasma total calcium concentrations found in nonsurvivors in this study, initial ionized calcium concentrations of surviving cold-stunned Kemp's ridley turtles often indicate hypocalcemia.[12] To our knowledge, no study has yet been conducted to evaluate the relationship between total calcium and ionized calcium in Kemp's ridley turtles; however, the available data would suggest that it is important to evaluate both variables in this species.[12]

In many convalescent turtles, plasma phosphorus concentration was greater than calcium concentration. In contrast to many other vertebrates, healthy Kemp's ridley and loggerhead sea turtles have lower plasma total calcium concentrations and often have an inverse calcium-to-phosphorus ratio.[8,11,13,16–19,23,24] It is possible, therefore, that the inverse calcium-to-phosphorus ratio detected in many of the convalescent turtles in our study is normal for the species. It is also possible that this reflects juvenile bone growth, as detected in other juvenile reptiles.[25] However, disorders such as nutritional secondary hyperparathyroidism should also be considered in captive juvenile turtles. Additional studies of the nutritional status of hospitalized sea turtles are warranted.

Fifty-nine turtles had initial plasma albumin concentrations < 1 mg/dL, whereas no clinically convalescent values were < 1 mg/dL. Low plasma albumin concentrations have also been detected in injured loggerhead sea turtles.[19] Although the cause of hypoalbuminemia in cold-stunned turtles is unknown, it could be the result of prolonged anorexia, decreased hepatic function, protein-losing nephropathy, or enteropathy.

Plasma LDH, AST, ALKP, and CK activities were higher than those reported for healthy Kemp's ridley turtles.[8,11,13,16] High serum or plasma activities of tissue enzymes have been previously detected in cold-stunned turtles and injured loggerhead turtles.[8,11,19] Tissues of origin for these enzymes have not been specifically determined for sea turtles, but in snakes and lizards, these enzymes are found in a variety of tissues; thus, these enzymes may be nonspecific indicators of tissue injury.[26,27] Possible reasons for persistent increases in plasma enzyme activities in these juvenile turtles include rapid tissue growth, undetected pathologic conditions, and exertion as a result of confinement in captivity.

Consistent with observations in other reptiles, values for plasma bilirubin concentration, GGT activity, ALT activity, and creatinine concentration were uniformly low in all turtles. These variables are generally considered to be of little clinical importance in reptiles. Bilirubin and creatinine may not be produced at all in most reptiles, and GGT and ALT are found in low quantities in reptile tissues.[10,26,27]

Although statistical analysis of paired samples from individuals did not reveal a significant difference in initial versus convalescent values of Hct, convalescent Hct values were most often lower than initial Hct values. For example, no convalescent Hct value was > 41%, whereas 19 turtles had initial Hct > 50%. High Hct values were likely attributable to dehydration, as most turtles with high Hct values also had high plasma sodium and chloride concentrations. Several turtles were anemic at the time of admission. Although detailed evaluation of turtles on an individual basis was beyond the scope of this study, we have observed that anemic cold-stunned turtles were often affected by chronic pathologic conditions (eg, bacterial or fungal pneumonia) and that anemia generally resolved with management of the primary disorder.

White blood cell counts in this study were generally similar to values previously reported for a smaller cohort of cold-stunned Kemp's ridley turtles.[8] Although analysis of paired initial and convalescent WBC counts did not reveal a significant difference, clinical observations suggested that initial WBC counts for cold-stunned turtles were often high in comparison to convalescent values. Support for this is found by comparing the range of maximum initial WBC counts to the range of maximum convalescent WBC counts (ie, 30,000 to 40,000 cells/μL vs 6,000 to 10,000 cells/μL). Fifty-one of 176 turtles had initial WBC counts > 11,000 cells/μL, whereas no convalescent turtle had WBC counts > 11,000. Initially high WBC counts in cold-stunned turtles are likely a reflection of inflammation, immune response, physiologic stress, or systemic pathologic conditions.[10] Previous studies[16,18,19,23,28–30] of sea turtle WBC results have reported values ranging from 2,000

to 23,000 cells/μL. Convalescent values of the WBC count in the present study were within the lower end of this range.

The types of leucocytes reported in this study were those typically described for chelonians. Most cells were heterophils followed by moderate numbers of lymphocytes and smaller numbers of monocytes, eosinophils, and basophils. These findings were consistent with those of most previous reports[16,18,23,29,30] of hematologic findings for sea turtles. However, there is some inconsistency and disagreement over the nomenclature of sea turtle leucocytes. For example, in a previous study[31] on hematologic findings for Kemp's ridley turtle, granulocytes are described as large eosinophils and small eosinophils, rather than heterophils and eosinophils. In the present study, granulocytes were reported as heterophils, eosinophils, and basophils. This nomenclature is consistent with that most typically used in the clinical veterinary literature for sea turtles.[8,16,18,23,29,30]

Chelonian hematologic evaluation and plasma biochemistry results have varied greatly with different laboratory methods, venipuncture sites, species, age, sex, and season.[23,28,32–34] Thus, results of the present study were most useful for comparison to results of other captive juvenile Kemp's ridley turtle and were obtained by the same methods described here. The dorsal cervical sinus is the most commonly used venipuncture site in sea turtles.

Although there was maximally an 18-hour delay between sample collection and laboratory analysis, methods of sample preparation and handling used in this study were likely to have minimized artifactual changes. These methods included immediate preparation of blood smears at the time of collection, centrifugation within 10 minutes of collection, and refrigeration of the specimens prior to analysis. In a recent study[35] in loggerhead sea turtles, no significant changes were found in plasma biochemical variables (except for GGT activity) when whole blood samples were stored under refrigeration for 24 hours prior to centrifugation and analysis. It is possible that WBC counts may have been affected by a delay in analysis; however, the maximal delay (18 hours) is typical of current clinical practice in which many hospitals send samples for hematologic evaluation to regional diagnostic laboratories for analysis.

No standardized selection criteria exist for defining ill versus healthy reptiles in retrospective studies. As such, many of the selection criteria that were used to categorize turtles in this study were made on the basis of a desire to be conservative in assigning hematologic and plasma biochemical values to any particular category of turtles, while still maintaining reasonable numbers of individuals for statistical analysis. We feel that our defined selection criteria are reasonable. For example, the hematologic and plasma biochemical values that are reported for turtles that died were from blood samples collected, on average, 2.5 days prior to death. Although it would be ideal for this group to only include data from blood samples collected on the day of death, this would exclude information from many turtles of this study group. To exclude data from turtles that may have had any degree of illness, we were quite conservative in defining turtles as convalescent. This resulted in only 15 turtles meeting this definition, but ensured that data from convalescent turtles were not skewed by recent medical treatment or ongoing physical abnormalities. We acknowledge that data obtained at the beginning of hospitalization may have been affected by any treatment provided to turtles prior to blood sample collection. On average, the hematologic and plasma biochemical values reported as initial data were collected on day 2 or 3 of hospitalization (range, days 1 to 5). Although treatments were generally similar among turtles, each turtle was treated as an individual at the discretion of the attending clinician, and determining the effects of specific variations in fluid administration and other modes of supportive care was beyond the scope of this study.

Our method of defining turtles as convalescent was somewhat unconventional, but resulted in several interesting findings. For example, for many variables (eg, ALKP, LDH, albumin, BUN, glucose, sodium, potassium, calcium, phosphorus, Hct, and WBC count), the calculated convalescent value was similar (and in some instances identical) to the clinically convalescent value. This seems to indicate that these variables may stabilize prior to the turtle meeting our definition of convalescent. Additionally, there was great variability in the time required for individual variables to stabilize to clinically convalescent values. For example, glucose, BUN, and sodium concentrations stabilized after approximately 1 month of rehabilitation, whereas ALKP and LDH values did not stabilize for approximately 6 months. This indicated that physiologic processes of rehabilitating sea turtles stabilize at variable rates and that some critical variables (eg, glucose and sodium) stabilize despite the presence of ongoing physical abnormalities or disease. These data may give clinicians temporal context with which to evaluate hematologic and plasma biochemical values of cold-stunned sea turtles throughout rehabilitation. It is also interesting that calculated convalescent values for some variables could not be obtained (eg, ALT, GGT, and bilirubin) because there was not a significant pattern to the change in variation of the analyte and a curve could not be fitted to the data. Such variables may be less useful for monitoring the progress of rehabilitation.

Results of this study indicated that retrospective analysis of clinicopathologic data for reptiles may be of use in identifying derangements associated with morbidity and mortality and may provide useful prognostic information. Similar studies in other species of reptiles are warranted.

a. Microtainer, Becton, Dickinson & Co, Franklin Lakes, NJ.
b. IDEXX Laboratories, North Grafton, Mass.
c. Eosinophil Unopette, Becton, Dickinson & Co, Franklin Lakes, NJ.
d. Modified Wright-Giemsa stain, Fisher Scientific, Middletown, Va.
e. HemaTek 2000, Bayer Health Care, Tarrytown, NJ.
f. Hitachi 747-200 chemistry analyzer, Hitachi Instruments Inc, San Jose, Calif.
g. TableCurve 2D, Systat Software, San Jose, Calif.
h. SigmaStat, Systat Software, San Jose, Calif.
i. Stat Trek Web site. Binomial distribution calculator. Available at: www.stattrek.com/Tables/Binomial.aspx. Accessed May 3, 2008.

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