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A Review of Chelonian Hematology

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Abstract Hematologic investigations have been used successfully to diagnose disease and assess the physiological status of chelonians. Here, the microstructure, ultrastructure, development, and function of chelonian blood cells are summarized, and factors that affect hematologic results are reviewed. The limited body of chelonian hematology research is discussed, and recommendations for future work are provided.

Keywords chelonian, blood cell, morphology, hematology

1. Introduction

Many turtle and tortoise species have experienced precipitous population declines. Habitat loss or degradation, human use for food and pets, incidental fisheries capture, and affliction with diseases (e.g., fibropapillomatosis of *Chelonia mydas*) threaten chelonian survival (Work *et al*., 2001). In Southeast Asia, food markets have become the main threat to the survival of chelonians (Altherr and Freyer, 2000). With an increase in turtle consumption, there is also an accelerated development of captive maintenance and breeding of chelonian in China (Zhou and Wang, 2009). In recent years, turtle farming has developed very rapidly, and is important to some regional economies (Zhou and Wang, 2009). To a certain extent, the development of chelonian farming has been hindered by various diseases encountered in captive conditions (Zhou, 2006).

The hematological studies of reptiles can be traced back to the 1940s (DuGuy, 1970; Li, 1997; Li and Lu, 1999; Li, 1999). Since then, a substantial increase in literatures relating to microstructure and ultrastructure of blood cells of chelonian have been reported (Wood and Ebanks,1984; Daimon *et al*., 1987; Fu *et al*., 2004; Li and Zhu, 1990, 1993; Aguirre *et al*., 1995; Li, 1997; Work *et al*., 1998; Wang *et al*., 1999; Cao *et al*., 2001; Gelli *et al*., 2004; Metin *et al*., 2006; Casal and Orós, 2007; Casal *et al*., 2007; Zhang *et al*., 2009; Kassab *et al*., 2009), but the studies of haematopoiesis, cytochemical characterization and development of blood cells are few (Li and Zhu, 1991; Cannon, 1992; Li, 1997; Li *et al*., 2000; Li *et al*., 2001; Azevedo *et al*., 2003; Casal and Orós, 2007). Some reviews related to chelonian hematology and blood chemistry have been reported in China (Li and Zhu, 1997; Li and Lu, 1999; Lu, 1999; Wang, 2001; Fu *et al*., 2003).

Hematologic analysis provides an easy diagnostic and prognostic tool for lower vertebrates (Canfield, 1998; Campbell, 2004; Tavares-Dias *et al*., 2009). Many diseases (e.g., hemoparasites, inflammatory diseases) are associated with changes in hematologic parameters in chelonians. Hematologic parameters have been used to diagnose chelonian diseases and to assess the health status of individuals (Work and Balazs, 1999; Christopher *et al.*, 2003; Joyner *et al*., 2006), and as a prognostic indicator after treatment (Knotkova *et al*., 2005). Normal reference ranges of hematologic and biochemical parameters are considered important for assessing and monitoring the health status of chelonians. Such evaluations are dependent on reliable reference values for healthy animals. In recent years more normal reference ranges of hematologic variables of free-ranging and captive chelonians have been established (Samour *et al*., 1998; Hidalgo-Vila *et al*., 2007; Knotkováet *et al*., 2002; Metin *et al*., 2006). Blood parameters of chelonians are influenced by many factors, including season, age, sex, health status, geographic sites, physiological state and reproductive status (Christopher *et al*., 1999; Dickinson *et al*., 2002; Jacobson, 2007).

Health status has become an increasingly important point of discussion for chelonian conservation and

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mana-gement, as well as for the use of chelonian as biological monitors of the health of local freshwater ecosystems (Diaz-Figueroa, 2005; Chaffin *et al*., 2008). Evaluation of hematologic and biochemical responses to physiologic and environmental factors, and comparative studies of clinically healthy and diseased turtles can provide insightful information for their management and conservation. This is especially important for critically endangered species where such information can be used to plan conservation strategies (Bolten and Bjorndal, 1992; Brenner *et al*., 2002; Diaz-Figueroa, 2005). Hematologic analysis also provides important information for the evaluation of rehabilitated chelonians prior to be released back to the wild.

2. Blood Cell Morphology

Blood cells in the peripheral blood of chelonians consist of erythrocytes, leukocytes, and thrombocytes. Leukocytes can be subcategorized as granulocytes, including heterophils, eosinophils and basophils, and agranulocytes, including lymphocytes and monocytes. Both heterophils and eosinophils display acidophilic granules, while basophils display basophil cytoplasmic granules.

It should be mentioned that the classification criteria of chelonian leukocytes vary among studies. Some cells are not easily identified on the basis of their morphological differences. For example, small lymphocytes may be very similar morphologically to thrombocytes. Most authors agree that reptiles do not have neutrophils, whereas they do have heterophils and eosinophils, which both show acidophilic granules (Canfield, 1998). Some studies classify acidophils (i.e., heterophils and eosinophils) as one type of cell at different stages of maturation (Azevedo and Lunardi, 2003). Neutrophils have been reported only in some reports (Wood and Ebanks, 1984; Pitol *et al.*, 2007). Some literatures (Wang *et al*.,1999; Christopher *et al*.,1999; Knotková *et al*., 2002; Dickinson *et al*., 2002) refer to the presence of azurophils in the peripheral blood of chelonians, and the existence of azurophil is still in dispute (Rosskopf, 2000). Additional cytochemical and ultrastructural studies are recommended to better characterize chelonian blood cells.

2.1 Erythrocytes The mature erythrocytes of chelonian are nucleated, ellipsoidal cells, with oval, centrally positioned nuclei containing dense chromatin clumps. When stained, the nuclei are purple-red, while the cytoplasm is uniform orange-pink or pale pink under Wright's stain (Li, 1997; Wang *et al*., 1999; Cao *et al*., 2001; Fu *et al*., 2004; Metin *et al*., 2006). There are no organelles in mature erythrocytes. Some erythrocytes were observed to have small basophilic inclusions in their cytoplasm, without any signs of illness (Li, 1997; Davis and Holcomb, 2008; Zhang *et al*., 2009). Ultrastructural examination of such inclusions showed pleomorphic densities, which were identified as degenerating oranelles (Li, 1997; Work *et al*., 1998; Casal *et al*., 2007). Light microscopically, immature erythrocytes are sometimes observed in chelonian blood. These cells appear more rounded, and have a rounded, slightly weaker staining nucleus than mature erythrocytes (Li, 1997; Knotková *et al*., 2002; Zhang *et al*., 2009).

2.2 Heterophils Heterophils are large round cells, which range from 12.0 μm to 23.8 μm in diameter. The unlobed nucleus is usually round or oval and located at the periphery of the cytoplasm. The cytoplasm exhibits weak eosinophilia, and contains polymorphic cytoplasmic granules, which are most commonly rod-shaped, but may be rounded or dumbbell-shaped (Li, 1997; Cao *et al*., 2001; Azevedo *et al*., 2003; Fu *et al*., 2004; Casal and Orós, 2007; Zhang *et al.*, 2009). At electron microscopic level, two types of cytoplasmic granules can be classified in terms of size and electron density: one has large size and is electron-dense, while the other has small size and low electron density (Li and Zhu, 1993; Li, 1997; Cao *et al*., 2001). Mitochondria, endoplasmic reticulum, and an inconspicuous Golgi apparatus were also observed ultrastructurally in chelonian heterophils (Li and Zhu, 1993; Li, 1997; Azevedo *et al*., 2003; Casal *et al*., 2007).

2.3 Eosinophils Eosinophils are round, variably sized cells in chelonians, with sea turtles having larger cells which can reach 25 μm, and freshwater turtles having smaller cells. The nucleus is positioned eccentrically and sometimes was bilobate. Large and small eosinophils have been described in *Lepidochelys kempii* (Cannon, 1992) and *C. mydas* (Work *et al*., 1998). The cytoplasm exhibits weak basophilia and is filled with round granules, which appear pink-red under Wright's stain (Li, 1997; Li and Zhu, 1993; Wang *et al*., 1999; Cao *et al.*, 2001; Metin *et al.*, 2006). Well-defined round electron-dense homogeneous granules are observed and mitochondria, endoplasmic reticulum, Golgi complex can be identified in the cytoplasm by electron microscopy (Li and Zhu, 1993; Li, 1997; Cao *et al*., 2001; Azevedo *et al*., 2003; Casal *et al*., 2007).

2.4 Basophils Basophils are round, and are usually the smallest granulocytes ranging between 9.5–16.0 μm . The round to oval nucleus is centrally or eccentrically located.

The cytoplasm is filled with large round granules with a color varying from dark blue to dark purple-black with Wright's stain (Li and Zhu, 1990, 1993; Li, 1997; Wang *et al*., 1999; Metin *et al*., 2006). The basophilic granules often mask the nucleus, which makes nucleus difficult to be observed (Li and Zhu, 1990; Fu *et al*., 2004; Zhang *et al*., 2009). The ultrastructure of the cytoplasmic granules of basophils varies in different turtles. The basophil of *Pelodiscus sinensis* contains electron-dense homogeneous granules, while two types of cytoplasmic granules are observed in *Chinemys reevesii* and *Mauremys mutica* (Li and Zhu, 1990, 1993; Li, 1997). One type has electronopaque contents with structures resembling myelin (*C. reevesii*) and honeycomb bodies (*M. mutica*), while the second type in both species contains numerous electron-dense particles (Li and Zhu, 1990, 1993; Li, 1997).

2.5 Lymphocytes Lymphocytes are small to medium sized (5.0 to 11.0 μm), round cells with a centrally or eccentrically positioned nucleus and scant cytoplasm. The area ratio of nucleus to cytoplasm is large. Lymphocytes have very dense, clumped chromatins that stains dark blue, and the cytoplasm is pale blue, sometimes containing small azurophilic granules under Wright's stain (Wang *et al*., 1999; Cao *et al*., 2001; Fu *et al*., 2004; Zhang *et al*., 2009). Reactive lymphocytes display increased cytoplasmic volume and basophilia, and the nuclei of reactive lymphocytes may show smooth or delicate nuclear chromatin (Campbell, 2004). Two types of lymphocytes were seen in *C. reevesii* by scanning electron microscopy, one type showed a smooth surface, and the other displayed microvilli on the surface of the cells (Cao *et al*., 2000). The scant cytoplasm contained mitochondria, polyribosomes, endoplasmic reticulum and small electron-dense granules under transmission electron microscopic observations (Li and Zhu, 1990, 1993; Li, 1997; Wang *et al*., 1999; Cao *et al*., 2000; Casal *et al*., 2007).

2.6 Monocytes The morphology of monocytes are more variable than other types of leukocytes. They can be round, oval, or rhomboid in shape. The nucleus is eccentric and round, oval, kidney- or rod-shaped. The cytoplasm is basophilic and pale blue under Wright's stain. The nuclear chromatin is slightly less clumped compared with the nuclei of lymphocytes. Monocytes often contain some vacuoles in the cytoplasm (Wang *et al*., 1999; Casal and Orós, 2007; Zhang *et al*., 2009). Ultrastructrally, few projections are seen on the surface of the cells. The abundant cytoplasm contains more organelles: mitochondria, rough faced endoplasmic reticulum, ribosomes and Golgi apparatus (Li and Zhu, 1993; Li, 1997; Wang *et al*., 1999; Casal *et al*., 2007).

2.7 Thrombocytes Thrombocytes are oval-shaped cells. They usually clump together in the blood smear, this characteristic helps in identifying thrombocytes from small lymphocytes. The nucleus is generally oval and contains clumped chromatin. The cytoplasm is scant, with a slim cytoplasmic halo around the nucleus, and is very pale grey to blue in coloration, and may be almost transparent (Casal and Orós, 2007; Zhang *et al*., 2009). Immature thrombocytes are larger than the mature form, and their cytoplasm is light blue (Jacobson, 2007). At the electron microscopic level, the cell edges present some finger-like projections (pseudopodia) and the cytoplasm contains small dense granules. Mitochondria, roughly surfaced endoplasmic reticulum, and golgi complex were observed in *Geoclemys reevesii* (Daimon *et al*., 1987). The prominent and important characteristics are the existence of a surface connected canalicular system (open canalicular system) in thrombocyte in many reptiles (Daimon *et al*., 1987; Work *et al*., 1998; Casal *et al*., 2007). As an open canalicular system, it obviously amplifies the surface area of the plasmalemma, so increasing the efficiency of the organelle in the exchange of metabolites between the cells and extracellular space (Daimon *et al*., 1987). The canaliculi also provide a pathway for the extrusion of endogenous chemicals in the release reaction during aggregation (Daimon *et al*., 1987).

3. Development and Function of Blood Cells

3.1 Haematopoiesis Li (1997) and Lu and Li (1996) described the haemopoiesis of turtles in China, especially in *Pelodiscus sinensis* and *M. mutica*. Guo and Jia (2003) studied the ontogeny of haematopoitic organs in *P. sinensis*, and found that the earliest hemopoietic organ was the yolk sac blood islands, subsequently in the fetal thymus, liver, spleen and bone marrow. After birth, the haematopoiesis is restricted to bone marrow (Guo and Jia, 2003). The spleen is the main organ to produce monocytes and lymphocytes, and also participates in erythropoiesis. Polychromatic normoblast and orthochromatic normoblast were seen in spleen printing slides, which develop into mature erythrocytes in the spleen (Li *et al*., 2000; Li *et al*., 2001; Jiang *et al*., 2003; Jiang, 2004). This was also proven in the snake, *Bothrops jararaca,* in its early postnatal, where sparse islets of erythropoiesis were seen in the spleen, while kidney and liver revealed no discernible haematopoietic activity (Dąbrowski *et al*., 2007).

Li *et al*. (2000) and Jiang *et al*. (2003) divided erythrocyte development into 3 stages in *C. reevesii* and *P. sinensis*, respectively: the primitive stage (pronormoblast), the immature stage (early normoblast, intermediate normoblast and orthochromatic normoblast) and the mature stage. The development of white blood cells also consists of three stages, the primitive, immature (promyelocyte, myelocyte and metamyelocyte) and mature stage (Li *et al*., 2001; Jiang, 2004). Mitotic activity of erythrocyte was occasionally observed in chelonian blood films, and was associated with an erythrocytic regenerative response in peripheral blood in low vertebrates (Zhang *et al*., 2009).

3.2 Function of blood cells The erythrocyte contains little else but hemoglobin, which transports oxygen from the lungs to the various tissues of the body, and transports carbon dioxide from the tissues back to the lungs. Slightly polychromasia (e.g., immature erythrocytes having cytoplasmic basophilia) or reticulocytosis are usually observed in blood films, and greater polychromasia may indicate an erythrocytic regenerative response to anemia (Campbell, 2004).

Heterophils are primarily phagocytic and therefore are associated with inflammatory diseases, especially those associated with infectious diseases or tissue injury (Campbell, 2004). Pan and Zou (2000) designed a trial to study phagocytic and bactericidal function of heterophils, and proved that heterophils of *P. sinensis* could kill more than 60% of *Staphylococcus aureus* and *Escherichia coil* in 180 minutes. Eosinophils are phagocytic and are particularly involved in the destruction of parasites. Increased numbers of eosinophils in blood are associated with parasitism and nonspecific immune stimulation (Mihalca *et al*., 2002). Eosinophils are proven to participate in the immune response of chelonians and are found to phagocytize immune complexes (Mead and Borysenko, 1984).

The turtle basophil has an immune capacity analogous to the mammalian basophil or mast cell. It has 5-hydroxytryptamine and histamine (Li and Zhu, 1991), and contains on surface immunoglobulins that induce histamine release (Mead *et al*., 1983). Degranulation of basophils was sometimes observed (Li and Zhu, 1991; Jacobson, 2007), and is correlated with cell histamine release (Mead *et al*., 1983).

Lymphocytes play several important immune roles in reptile, including producing antibodies and attacking foreign material (Diaz-Figueroa, 2005). The numbers of lymphocytes reflect the body's immune function. For example, low number lymphocytes were found in hibernation due to suppressed immune function (Christopher *et al*., 1999). Lymphopenias may occur with malnutrition and conditions of stress (Campbell, 2004). Lymphocytosis occurs with wound healing, inflammatory disease, parasitic infections (Mead and Borysenko, 1984), and viral diseases (Campbell, 2004).

Monocytes are phagocytic cells. Monocytosis is suggestive of an inflammatory disease, especially a granulomatous. Highly vacuolated monocytes suggest increased phagocytic activity and may indicate a response to a systemic antigen (Campbell, 2004). Monocytes and heterophils often response to phagocytic events together. For example, heterophilia and monocytosis occurred in green sea turtles afflicted with fibropapillomatosis and in *Terrapene carolina carolina* with phaeohyphomycosis (Work and Balazs, 1999; Joyner *et al*., 2006).

Thrombocytes serve the same function as the anucleated mammalian platelet, playing a key role in hemostasis and leading to the formation of blood clotting. The nuclei of thrombocytes may be polymorphic with severe inflammatory disease (Campbell, 2004). Pellizzon *et al*. (2002) described that the aggregation process of thrombocytes of *Phrynopys hilarii* resulted in structural alterations with developing numerous filopodial projections, an increased number of vacuoles and changed from spindle to spherical shape.

4. Hematologic Parameters

Evaluation of hematologic parameters includes packed cell volume (PCV), hemoglobin concentration (Hb), red blood cell count (RBC), white blood cell count (WBC) and differential leukocyte count, as well as examination of blood cell morphology using stained peripheral blood smears. Evaluation of the hematology provides rapid and valuable information for clinical laboratory of reptiles (Jacobson, 2007; Tavares-Dias *et al*., 2009). DuGuy (1970) gave a detailed summary on the numbers of blood cells and their variation in reptiles, containing much useful information for chelonians. Many factors affect hematologic parameters, including species, season, physiological state (e.g., hibernation and reproduction), nutrition, health status, gender and age. Moreover, when interpreting the hematologic data, more consideration should be given to the great influence that external factors have on the normal physiology and health of ectothermic vertebrates compared with endothermic vertebrates.

4.1 Differential leucocyte counts The percentage of each kind of leukocytes varies with species. Heterophils are reported to be the most predominant leukocytes found in most chelonians, and can be up to 50% (Hidalgo-Vila, 2007; Dickinson *et al*., 2002; Wang *et al*., 1999; Casal and Orós, 2007). Eosinophils are often uncommon in the peripheral blood of some chelonians, but can be found in higher percentage in others, such as *L. kempii* (Cannon, 1992), *Mauremys leprosa* (Hidalgo-Vila *et al.*, 2007) and *C. mydas* (Work *et al*.,1998). Zhang *et al*. (2009) postulated that wild turtles usually suffer from parasites, so that they might have higher eosinophils number. The basophil number is highly variable in the peripheral blood, depending on species. Basophils are usually the least common of the leukocytes, but some freshwater turtles have very high percentages. The basophils of *Chelydra serpentina* are up to 63% of the blood leukocytes (Mead *et al*., 1983). Li and Zhu (1993) found that basophils were the most common granulocytes in *Mauremys mutica*, and it also comprises a high percentage (24.9%) in *M. reevessii* (Cao *et al.*, 2001). Basophils are relatively rare in marine turtles, such as *C. mydas* (Work *et al*., 1998) and loggerhead turtle (Casal and Orós, 2007). Deem *et al*. (2006) did not find basophils in the peripheral blood smears from 35 specimens of *Dermochlys coriacea*. Lymphocytes are the most common leukocytes in the peripheral blood of some chelonians, with a high percentage of 80% in wild *C. mydas* (Work *et al*., 1998). Other studies have found that lymphocytes are the second most prevalent of the circulating leukocytes (Diaz-Figueroa, 2005; Casal and Orós, 2007; Deem *et al*., 2006; Cheng *et al*., 1996). Monocytes generally present in low numbers in the peripheral blood and account for 0–10% of the differential leucocyte count (Jacobson, 2007).

4.2 Seasonal differences Seasonal changes have significant effects on hematologic values of chelonians, as in other vertebrates. *P. sinensis* and *Geochelone radiata* have a higher RBC in summer, and a higher WBC in spring (Cheng *et al.*, 1996; Zaias *et al.*, 2008). Higher PCV, Hb and RBC were reported during summer for free-ranging desert tortoises, *Gopherus agassizii* (Christopher *et al*., 1999). Lymphocytes are lowest in winter and highest in summer in reptiles (Campbell, 2004). Muñoz and Fuente (2005) found *Mauremys caspica* had higher proportion of lymphocytes in spring and summer, when these turtles are more active, and the risk of infections is higher. In free-ranging *Gopheerus polyphemus*, tortoises captured in autumn had a lower WBC and heterophil count than those captured in spring (Diaz-Figueroa, 2005). Hb values and lymphocyte counts were higher in autumn compared to those in spring and summer in *Gopherus agassizzi* (Dickinson *et al*., 2002).

Morphology of blood cells is also affected by seasons. Erythrocyte area increases in high temperature season (August), and decreases in low temperature season. Similar changes are found in white blood cells (except heterophils), and the area of leukocytes increases with elevating of temperature (Fu *et al*., 2004).

4.3 Effects of physiologic status Specific physiological states, such as hibernation, can cause hematologic changes in chelonians. Christopher *et al*. (1999) reported that lymphocyte and basophil numbers and RBC mass (PCV, RBC, and Hb concentration) were lower during hibernation, whereas monocyte and azurophil numbers were highest at this time in *G. agassizii*. The number of RBC increased 2 times in *Sacalia quadriocellata* during the breeding season, which may increase immune function (Fu *et al*., 2004).

Other physiological states also affect the hematologic status. The migrating *C. caretta*, had significantly higher red blood cell counts and percent heterophils, and significantly lower percent lymphocyte and absolute eosinophil counts than the residential (Stamper *et al*., 2005). They suggested that the elevated white blood cell counts with increased lymphocyte, and eosinophil levels may indicate antigenic stimulation in the residential, and the results that the migratory turtles had a higher percentage of heterophils and a lower lymphocyte and eosinophil count could be interpreted as a "stress leukogram", supporting a stress hypothesis in migratory animals (Stamper *et al*., 2005). Deem *et al.* (2009) also compared the hematologic values in foraging, nesting, and stranded *C. caretta*, and found that the differences in hematologic values included a lower packed cell volume, higher number of lymphocytes, and lower number of monocytes in stranded turtles; lower white blood cell counts in foraging turtles; and significant differences in total solid values among turtles exhibiting all behaviors with the lowest values in stranded turtles and the highest values in nesting turtles.

4.4 Age, size, gender and geographic effects Hematologic parameters are also influenced by individual factors, such as age, size and gender (DuGuy, 1970). Wood and Ebanks found the PCV and Hb of *C. mydas* were positively correlated with age (Wood and Ebanks, 1984). Red blood cell parameters of *Caretta caretta*, *C. mydas* and *Eretmochelys imbricata* were correlated with carapace lengths, with larger size turtles having larger erythrocytes and lower RBC number (Frair, 1977). Generally, hematologic differences exist between sexes (DuGuy, 1970). The Asian yellow pond turtle, *Ocadia sinensis*, had significant sex differences in the parameters of packed cell volume, eosinophil count, heterophils and monocytes ratio (Chung *et al*., 2009). The males of *Geochelone gigantea* had higher RBC, PCV and Hb than females (Hart *et al.*, 1991), and similar result is seen in *G. agassizii* (Christopher *et al*., 1999), but *C. mydas* has no sexual difference in PCV, Hb, RCC and WCC (Bolten and Karen, 1992; Wood and Ebanks, 1984). Geographic differences likely result in environment differences, such as rainfall and forage availability, and can produce hematologic changes (Christopher *et al*., 1999).

4.5 Health status Most infectious agents for chelonians such as blood parasites, bacteria, fungi and viruses can cause an inflammatory response in affected tissues, which may result in significant changes in the peripheral blood. Blood parasites are commonly found in wild chelonians (Mihalca *et al*., 2008). The presence of parasites within erythrocytes was associated with anaemia, low haemoglobin, basophilia, eosinophilia, heterophilia and azurophilia (Knotkova *et al.*, 2005). Fungal infections (Phaeohyphomycosis) caused severe anemia, leukocytosis (heterophil leucocytosis and monocytosis) (Joyner *et al*., 2006). Eastern box turtle (*Terrapene carolina carolina)* with virus infection (Iridoviral) had a low PCV of 13%, and intracytoplasmic inclusions were observed within leukocytes (Allender *et al.*, 2006). Green Sea turtles afflicted with fibropapillomatosis had low PCV and Hb concentration (Norton, 1990), and heterophil leucocytosis and monocytosis (Work and Balazs, 1999). Pang (1998) found leukocytosis occurred in *P. sinensis* afflicted with liver disease. Anemia also shows an increase in the number of polychromatic erythrocytes in the peripheral blood, which indicates erythrocytic regenerative response (Campbell, 2004). Poor nutrition also can affect the hematologic parameters. The malnourished freshwater turtles, *Podocnemis expansa*, had a significant decrease in red blood cell counts, white blood cell counts, azurophils and heterophils, and malnutrition also caused severe normocytic-hypocromic anemia and marked immune depression (Tavares-Dias *et al*., 2009). In chelonian, red blood cell indices may be interpreted as a comparative index of condition, nutrition, or general health (Campbell, 1998, 2004; Oliveira-Junio *et al.*, 2009) because anemia is the common effect of chronically poor nutrition, particularly with respect to protein intake (Christopher, 1999).

4.6 Other influential factors Environmental pollution, sample collection and research methods can cause hematologic changes as well. The effect of crude oil exposure was studied in the laboratory for juvenile *C. caretta*, and the oiled turtles had up to a four-fold increase in white blood cell count, a 50% reduction in red blood cell count and red blood polychromasia (Lutcavage *et al*., 1995). The proper collection and handling of chelonian blood samples are important in hematologic parameters information and analysis (Stamper *et al*., 2005). The inappropriate site for venipuncture can result in lymph dilution which may dilute blood sample and can skew research results (Gottdenker and Jacobson, 1995). It is necessary to look for better methods of collecting accurate blood samples without getting lymph contamination or harming or sacrificing the animal, especially for some endangered species. It has been previously noted that WBC counts vary greatly in sea turtles based on the method employed (Arnold, 1994). Similar result has been proved by Deem *et al*. (2006), which studied WBC counts by Natt Herrcik's and eosinophil Unopette methods, and WBC counts determined by the eosinophil Unopette method were significantly higher than those estimated from blood slides. This finding should be taken into consideration when one is comparing the results between studies (Deem *et. al,* 2006). The anticoagulants also should be considered to affect hematologic data.

5. Conclusion

To date, relatively few hematologic studies of chelonians have been reported compared to mammals. There are many species for which reference values are unknown or imprecise, and additional studies are warranted.

There is a general lack of hematologic studies of captive chelonians, even though keeping and breeding chelonians in captivity have developed very fast in recent years (Zhou and Wang, 2009). Hematologic investigation of captive chelonians will likely aid in diseasing diagnosis, and make it more easy to monitor the health of these animals. Several studies of chelonian hematology did not describe environmental conditions, making them less meaningful.

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