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Full Length Research Paper

Red blood cell morphology and plasma proteins electrophoresis of the European pond terrapin *Emys orbicularis*

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The European pond terrapin (*Emys orbicularis*) is a turtle found in southern and central Europe, West Asia and North Africa. In this study, we used juvenile *E. orbicularis* (females of 4 - 5 years old) which was captured from the different area of the Mazandaran province in April 2006. Blood was collected from the dorsal sinus into EDTA-coated vacutainer tubes. Plasma proteins were quantified and diluted to different ratios. SDS-PAGE was able to resolve 17 bands at minimum concentration of 51.0 µg/well which contain high and low band with molecular weight of 130.0- 132.0 and 20.0-23.0 kDa.

Key words: *Emys orbicularis,* turtles, erythrocytes, plasma proteins.

INTRODUCTION

The European pond terrapin (also European pond turtle or European pond tortoise), *Emys orbicularis* is one of six species of marine turtles found in southern and central Europe, West Asia and North Africa (Snieshkus, 1995). Various authors have described different circula-ting blood cells of different amphibian and reptile spec-ies (Mateo et al., 1984; Canfield and Shea, 1988; Knotkova et al., 2002; Azevedo and Lunardi, 2003).

Some authors have studied seasonal (Hutton, 1960; Cline and Waldman, 1962; Haggag et al., 1966) or sex-ual (Altland and Thompson, 1958) variations in the number of blood cells of different reptile species. In addition, researchers have studied the number of blood cells of different reptile species (Mateo et al., 1984). Some researchers have studied on a single species (Mateo et al., 1984, Canfield and Shea, 1988; Cannon et al., 1996; Alleman et al., 1992; Sevinç et al., 2000; Sevinç and Ugˇurta, 2001). Plasma protein electropho-resis (Gicking et al., 2004) which has been proposed for use in reptile and amphibian medicine to aid in the diagnosis of disease (Zaias and Cray, 2002) may be useful as a health assessment tool for evaluating injured sea turtles. The effect of anticoagulants on biochemical values in loggerhead sea turtles has been reported (Bolten et al., 1992).

In Iran, hematological studies have generally been conducted on humans and some economically impor-tant animals but there are not any hematological studies of the reptiles living in this country (Sevinç et al., 2000; Sevinç and Ugˇurta, 2001). Studies on Iranian reptiles are usually restricted to morphology and systematics.

Since these turtles are federally listed as threatened in many countries, all turtles brought into rehabilitation facilities are treated with the intent to release them back to the wild. While blood is routinely collected from ill sea turtles and used to determine health status, little inform-ation is available regarding reference intervals. In other hand, to assess its utility, normal reference ranges need to be established. The present paper reports the eryth-rocyte and nucleus measurements and plasma protein pattern of *E. orbicularis* from the Mazandaran province.

MATERIALS AND METHODS

Animals

Eight adult European pond turtles (4 males and 4 females) which were used for the present study was collected from various areas in Mazandaran province of Iran. The study was carried out betw-een September 2005 and August 2006. The females were check-ed by manual examination through the cloaca for eggs in oviducts. All of them were determined as non-pregnant.

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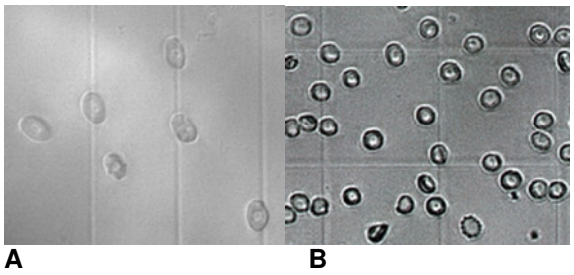

Figure 1. Comparison of erythrocytes and nucleus size: Erythrocytes of *E. orbicularis* (A) and human (B) obtained by isotonic solution.

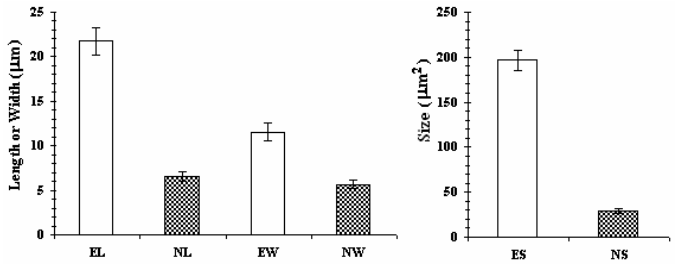

Figure 2. Erythrocyte dimensions of *Emys orbicularis*: Erythro-cytes and nucleus length or width (left) size (right). EL: erythro-cyte length, EW: erythrocyte width, ES: erythrocyte size, NL: nucleus length, NW: nucleus width, NS: nucleus size.

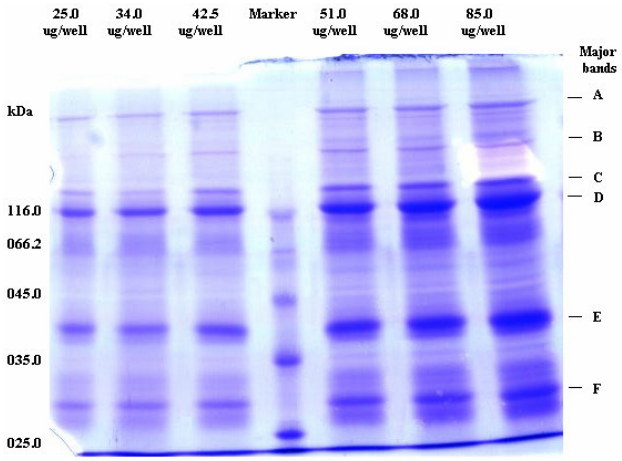

Figure 3. Plasma proteins profile in different concentration per well: Marker is protein marker with 5 bands.

Blood sampling

A blood specimen of each turtle was taken by venepuncture from the postorbital sinuses (Maclean et al., 1973). Blood (1 - 2 ml) was collected using 21 gauge needles and 5 ml syringes. Immediately after blood specimens were transferred into ethylenedia-minetetraacetic acid (EDTA)-coated vacutainer tubes. Blood samples were placed on ice until processing in the laboratory 4 - 6 h after capture. Plasma was separated by centrifugation at 3000 rpm for 10 min. Supernatant plasma proteins quantified by Bard-ford (1976) method and aliquot separated into two or more vials. Sodium azide added to plasma proteins up to 0.02% final concentration.

Blood cell morphology

For red blood cell (RBC) count, fresh blood diluted in isotonic solution (including: 0.25% $FeCl_3$, 2.5% Na_2SO_4 and 0.5% NaCl dissolved in distilled water) was used. RBC counted with chamber method via Thoma's hemocytometer and red blood cell dimension measured Olympus ocular micrometer at a magnification of 400×. Erythrocyte and nuclear sizes were respectively calculated according to the formulas [(EL * EW * π) / 4] and [(NL * NW * π) / 4] described by Ugˇurtas et al. (2003), where EL is the erythrocyte length, EW is the erythrocyte width, NL is the nucleus length and NW is the nucleus width.

SDS-PAGE

Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described by Laemmli (1970), using 1.5 mm x 10 cm polyacrylamide slab gels consisting of 10% resolving gel (30:0.8 acrylamide: N,N'-bis-methylene acrylamide) and a 4% stacking (30:0.8) gel containing 0.1% SDS. Different volumes (1, 2, 3, 4, 5 and 6 µl) of the undiluted plasma proteins were combined with an equal volume of SDS-PAGE reducing buffer and electrophoresed at a constant amperage of 20 mA until the tracking dye was within 1 - 2 mm from the bottom of the resolving gel. Coomassie brilliant blue R250 staining of the gel and des-taining was performed as described by Wilson (1983). The gels were scanned and stored in 10% glacial acetic acid.

RESULTS AND DISCUSSION

The erythrocytes or red blood cells of turtles are nucleated. The cytoplasm of mature erythrocytes appeared light and nuclei observed dark but were homogeneous under isotonic solution (Figure 1). Because there were no significant differences between the erythrocyte sizes of female and male turtles the data from the females and males of individual species were pooled. Turtle's erythrocytes are nuclei cells with nucleus (6.57 ± 0.75) × (5.67 ± 0.47) µm and large size with (21.73 ± 1.51) × (11.53 ± 0.98) in length and width (µm), respectively as shown in Figure 2.

We were able to count the number of erythrocytes of turtles by diluting the blood cells and using Thoma's chamber. Result showed that female turtles have fewer erythrocytes (250,000 ± 20537/mm^3) than male (362 000 ± 60270/mm^3). The erythrocyte measurements results and number of erythrocytes are in agreement with the results of the Ugˇurtas et al. (2003) and Duguy (1970), respectively.

The Bardford (1976) method was used to determine plasma protein concentration of *E. orbicularis* turtles. Our data showed that the mean total protein content of turtle plasma (±SD) was 8.0 ± 0.71 mg/ml. Protein profile of the plasma in 25.5, 34.0, 42.5, 51.0, 68.0 and 85.0 µg/well concentration of plasma were studied by SDS-PAGE (Figure 3). Analysis of molecular weight band measure-

Table 1. Analysis of plasma proteins profile in different concentration.

Line / Band	25.0 µg/well	34.0 µg/well	42.5 µg/well	51.0 µg/well	68.0 µg/well	85.0 µg/well
A	129	129	130.13	130.13	130.13	132.76
a_1	0.000	0.000	0.000	127.51	128.82	128.82
a_2	0.000	0.000	0.000	124.89	124.89	127.51
a_3	0.000	0.000	0.000	118.32	119.64	120.95
B	114.38	115.70	115.70	115.70	115.70	118.32
b_1	0.000	0.000	0.000	111.76	113.07	114.39
b_2	0.000	0.000	0.000	107.83	107.83	109.14
C	102.58	102.58	102.58	102.58	102.58	103.89
D	094.70	094.70	094.70	094.70	094.70	096.01
d_1	0.000	0.000	0.000	090.77	092.08	092.08
d_2	0.000	0.000	0.000	072.39	073.71	076.33
d_3	0.000	0.000	0.000	067.14	068.46	068.46
d_4	0.000	0.000	0.000	063.21	063.21	065.83
E	052.71	051.40	051.40	051.40	052.71	054.02
e	0.000	0.000	0.000	042.21	043.52	044.83
F	025.15	023.84	023.84	025.5	025.5	026.46
F	0.000	0.000	0.000	021.21	022.53	023.84

ments by relation between molecular weight and relative mobility factor, Rf, of protein marker showed that 10% resolving gel of SDS-PAGE are able to resolve 17 bands at minimum concentration of 51.0 µg/well which contain high and low band with molecular weight of 130.0- 132.0 and 20.0-23.0 kDa (Table 1).

In human and domestic animal medicine, electrophoresis of plasma proteins can provide information about chronic or acute inflammatory processes in the patient and may help the clinician determine appropriate treatment (Cray and Tatum, 1998). Recently, plasma protein electrophoresis has been advocated for use in pet bird diagnostics, especially when other tests are nondiagnostic (Cray and Tatum, 1998). In the acute stage of chlamydiosis in birds, there are major changes that occur in the electrophoretogram that may support a diagnosis of this disease (Cray and Tatum, 1998). Electrophoresis also has been utilized for diagnosing other diseases such as aspergillosis, hepatitis, and nephritis (Cray and Tatum, 1998).

Due to the rapid changes that can occur in an animal's plasma, electrophoresis can also be used for serially evaluating the response to treatment (Cray et al., 1995; Cray and Tatum, 1998). Electrophoresis has been proposed for use in reptile and amphibian medicine to aid in the diagnosis of disease (Zaias and Cray, 2002). Plasma protein electrophoresis may be useful as a health assessment tool for evaluating injured sea turtles.

The micronucleated erythrocyte (MNE) count is an indicator of a genetic damage in mature animals. An elevated number of micronucleated cells indicate poor health. However, Zuniga-gonzalez et al. (2000) suggested that in new-born animals, the presence of MNE could be increased, as the reticuloendothelial system might be immature in the young of some species. They also noted that the reticuloendothelial system matures with age. In some species of reptiles such as *Crocodylus acutus, Pituophis depei* and *Macroclemys temminckii*, the MNE counts were found very low, or no MNE was recorded (Zuniga-gonzalez et al., 2000).

We suggest that the biochemical profile described in the present study be used as a standard profile for the healthy *E orbicularis* kept in captivity. Nevertheless, some differences may be expected, especially for young turtles with rapid growth and/or for adult females during the reproductive season.

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