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SHORT COMMUNICATIONS

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Hematological Changes in the Platypus (*Ornithorhynchus anatinus*) Following Capture

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ABSTRACT: Blood samples were collected from the bill sinus of nine free-living platypuses (*Ornithorhynchus anatinus*) within 12 min of capture of each and again after 1 to 12 hr, New South Wales, Australia, 1981 to 1988. In seven animals which were not anesthetized, there was a significant ($P < 0.01$) fall in lymphocyte count between the two samples. The reduction ranged from 10 to 58% of the initial lymphocyte count and caused a significant reduction in the total white cell count ($P < 0.05$). Both the neutrophil and the lymphocyte counts increased in two platypuses which were anesthetized with ether prior to collection of the second blood sample. We propose that the peripheral blood lymphocyte count is a simple means of monitoring the stress response of non-anesthetized, newly-captured platypuses and may be a useful adjunct to behavioral observation.

Key words: Platypus, *Ornithorhynchus anatinus*, hematology, physiology, captive husbandry, clinical pathology, stress.

A high mortality rate may occur within only weeks of capture of the platypus and stress has been proposed as a general mechanism underlying many of the mortalities (Smyth, 1973; Grant et al., 1977; McColl, 1983; Whittington, 1991). Evidence for a stress response in captive platypuses include behavioral changes (Carrick et al., 1982; Whittington, 1991), adrenal hypertrophy at necropsy (McColl, 1983), and an increase in plasma cortisol and catecholamine levels in live newly captured platypuses (McDonald et al., 1992).

Hematology is a routine means of indirect assessment of health status in many species and normal hematological values for platypuses have been determined (Whittington and Grant, 1983, 1984; Canfield and Whittington, 1983). The conscious, free-living, adult platypus has an unusually high total white cell count, with lymphocytes predominating over neutrophils in most individuals (Whittington and Grant, 1984). Hematological values for normal captive platypuses have not been reported.

A decline in the lymphocyte count occurs following release of adrenocorticosteroids in some species and is one outcome of the stress response (Claman, 1972; Lee and McDonald, 1985). Our objective was to determine whether reductions in the lymphocyte count occurred following capture of the platypus. Such a finding would find application in the objective assessment of the degree of adaptation of platypuses to captivity, as lymphocyte counts would be expected to return to normal levels as the stress response waned.

Nine platypuses were caught in unweighted gill nets in the Upper Kangaroo River (150°30′E, 34°45′S) in November 1981 (four animals) or in the Upper Shoalhaven River (149°40′E, 35°30′S), New South Wales, Australia, in March 1988 (five animals) as described by Grant and Carrick (1974). Animals were removed within 5 min of entanglement and placed individually in cloth bags. Age and sex were determined by examination of morphology of the spur (Temple-Smith 1973). Blood was collected from the upper bill sinus (Whittington and Grant, 1983) as soon as possible after capture and this sampling was repeated after at least 1 hr. For blood collection, each animal was restrained in a cloth bag with the bill protruding from

a hole. Chemical restraint was used only in the case of two animals which were lightly anesthetised with ether just prior to collection of the second blood sample, to facilitate insertion of a transponder tag (Grant and Whittington, 1991). Between blood samplings, animals were kept individually in cloth bags in a quiet place.

Hematological parameters were determined using manual methods (Whittington and Grant, 1983). Total plasma protein was determined using a temperature compensated refractometer. Differences between the values of each parameter at the first and second samplings were calculated and the significance of the differences was evaluated using a paired t test (Minitab Statistical Software, State College, Pennsylvania, USA).

Samples of kidney, gut, thymus, spleen, and mesenteric lymphoid nodules from two dead, wild platypuses were placed in 10% buffered neutral formalin, embedded in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin or Warthin-Starry stain (Luna, 1968). Smears of heart blood were stained with Giemsa (Benjamin, 1978).

Seven adult female and two juvenile or subadult male platypuses were netted. The first blood sample was collected 6 to 9 min (seven animals) or 12 to 16 min (two animals) after entanglement in the net while the second sample was collected 1 to 2 hr (four animals), 4 to 7 hr (two animals) or 8 to 12 hr (three animals) after entanglement. Eight platypuses were marked with stainless steel leg bands or transponder implants, but the juvenile was not marked. Six of the eight marked individuals were caught again at the same sites after intervals of several months to several years indicating that the procedure was not deleterious.

For the seven animals not anesthetised, there was a significant fall in the mean total white cell count (TWCC) ($P < 0.05$) and the lymphocyte count ($P < 0.01$) after ≥ 1 hr in captivity (Table 1). Although there was an increase in the mean total neutrophil count at the second sampling this was significant only at the 10% level. The decline in lymphocyte count was dramatic in four of seven animals, ranging from 10.50 to 13.88 $\times$ 10^9/l and in these animals there was a correspondingly large fall in TWCC. In the other three animals the decline in lymphocyte count ranged from 1.37 to 3.43 $\times$ 10^9/l and there were only small changes in TWCC (-0.38 to 1.12 $\times$ 10^9/l). The neutrophil : lymphocyte ratio increased between samplings in each animal. There were no consistent trends in eosinophil, monocyte, or basophil counts between the two samplings. However, there were significant reductions ($P < 0.05$) in both mean packed cell volume and mean total plasma protein which probably represent a redistribution of body water into the intravascular compartment (Table 1).

Hematological changes were quite different in the two animals anesthetised with ether prior to the second sampling. In both animals the TWCC, and total neutrophil and lymphocyte counts increased substantially (Table 1). Statistical analysis was not undertaken due to the small sample size.

McDonald et al. (1992) reported an increase in the plasma cortisol concentration of wild platypuses from less than 50 nmol/l 6 to 15 min after capture in gill nets to approximately 300 nmol/l after 30 min, with maintenance of elevated levels for at least 6 hr. Thus we postulate that the reduction in lymphocyte count observed in the present study was the result of elevated levels of adrenocorticosteroids.

The magnitude of the fall in lymphocyte count varied among conscious platypuses from 10% to 58% of the initial count and did not appear to be related to the initial count or the interval between successive samplings; thus there may be differential responses among animals to the stresses of capture and handling. Correlations between the post-capture behavior and the lymphocyte counts of individual platypuses have not been made due to the lack of an objective behavioral scoring system; however, there is a spectrum of be-

TABLE 1. Leukocyte, erythrocyte, and plasma protein values from platypuses captured in New South Wales, Australia, 1981 to 1988, with reference values. Each animal was sampled soon after capture (T1) and 1 to 12 hr later (T2). Data are mean ± SD.

Blood parameter	Platypuses without anesthesia (n = 7)	Platypuses with anesthesia before T2 (n = 2)	Reference values (n = 9)[a]
Total white cell count ($\times 10^9$/l)			
T1	30.76 ± 8.74	29.21 ± 5.68	28.63 ± 3.15
T2	25.01 ± 5.43[b]	49.75 ± 2.06	
Total neutrophils ($\times 10^9$/l)			
T1	5.97 ± 1.60	5.54 ± 0.94	6.90 ± 1.19
T2	8.01 ± 3.67[c]	20.40 ± 1.07	
Lymphocytes ($\times 10^9$/l)			
T1	23.70 ± 8.42	22.89 ± 4.12	20.32 ± 2.65
T2	15.88 ± 5.40[d]	28.36 ± 1.17	
Neutrophil:lymphocyte ratio			
T1	0.27 ± 0.10	0.24 ± 0	0.43 ± 0.11
T2	0.58 ± 0.40[c]	0.72 ± 0.01	
Eosinophils ($\times 10^9$/l)			
T1	0.48 ± 0.21	0.53 ± 0.50	0.41 ± 0.04
T2	0.44 ± 0.16	0.41 ± 0.33	
Monocytes ($\times 10^9$/l)			
T1	0.63 ± 0.12	0.38 ± 0.06	0.57 ± 0.10
T2	0.56 ± 0.36	0.50 ± 0.25	
Basophils ($\times 10^9$/l)			
T1	0.04 ± 0.06	0	0.03 ± 0.02
T2	0.05 ± 0.05	0	
Packed cell volume (%)			
T1	53 ± 8[e]	58 ± 3	49 ± 2
T2	50 ± 6[b,e]	57 ± 1	
Total plasma protein (g/l)			
T1	75 ± 5	78 ± 4	72 ± 2
T2	70 ± 6[b]	79 ± 7	

[a] Healthy, conscious, adult, wild-caught platypuses (Whittington and Grant, 1984).
[b] T1 and T2 significantly different at $P < 0.05$.
[c] T1 and T2 significantly different at $P < 0.1$.
[d] T1 and T2 significantly different at $P < 0.01$.
[e] Based on six animals.

havior, ranging from quiet recumbency to persistent escape efforts in platypuses within 12 hr of capture (T. R. Grant and R. J. Whittington, unpubl.), and we believe that such an analysis would be worthwhile.

The stress which precipitated or accompanied serious systemic disease also has been associated with a reduction in lymphocyte count in the platypus, although the low levels attained would be termed more appropriately lymphopenia in this species. Whittington and McColl (1983) reported a lymphocyte count of 3.3 × 10^9/l in a wild platypus with terminal aspiration pneumonia following a flood, while a captive animal with septicemic salmonellosis had a lymphocyte count of 2.6 × 10^9/l (Whittington, 1988).

Anesthesia with ether induces leucocytosis due to catecholamine release (Lee, 1959; Soma, 1971) and platypuses anesthetised with ether had significantly greater

TWCC and neutrophil counts than conscious platypuses (Whittington and Grant, 1984). In this study we demonstrated increases in lymphocyte count following anesthesia with ether, changes which probably were due to a catecholamine response and mobilization of lymphocytes from a resting site, as may occur in species such as the domestic cat (Benjamin, 1978).

Two adult, male platypuses became entangled in a fishing net in the Murrumbidgee River near Canberra, Australian Capital Territory (149°10'E, 34°40'S) during a survey of fish populations in 1986. Animal 1 drowned and the carcass was placed on wet ice while the second, live individual was placed in a 45-l container on dry bedding. Animal 2 died 12 hr later and the carcass was placed on wet ice. Both platypuses were necropsied within 24 hr of the estimated time of netting. Both were in good body condition and had the same types and intensities of internal and external parasites. These animals were considered to be age, sex and disease matched individuals that differed in their duration of captivity from 0 hr (animal 1) to 12 hr (animal 2). The thymus of each animal was well developed. There was no evidence of necrosis within lymphoid organs of animal 1. In contrast, there was generalized degeneration and necrosis of individual lymphocytes in the thymus and abdominal lymphoid nodules of animal 2. These results provide indirect evidence that lymphocytolysis occurs following capture of the platypus. Similar changes occur during the stress response in species such as the mouse, rat, hamster and rabbit (Claman, 1972).

The platypus has an unusually high lymphocyte count among the mammalia (Whittington and Grant, 1983, 1984) and we provide evidence that the lymphocyte count is very sensitive to the stress of capture. Due to the range in normal lymphocyte counts, single lymphocyte counts probably would have little predictive value; however, counts below 5×10^9/l have preceded death due to bacterial infection (Whittington and McColl, 1983; Whittington, 1988). Determination of the change in lymphocyte count over time may be a ready means of objective monitoring of the degree of adaptation of platypuses which have been newly captured and introduced to captivity or transferred between institutions.

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