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SIMULATED FIELD BLOOD STUDIES IN THE BOTTLE-NOSED DOLPHIN *Tursiops truncatus*

2. Effects of Stress on Some Hematologic and Plasma Chemical Parameters.

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Abstract: Hematologic and plasma chemical constituents were analyzed through a 72 hour period, on a bottle-nosed dolphin that had been subjected to excessive manipulation and carbon tetrachloride intoxication. Of the 19 parameters examined, creatine phosphokinase, glucose, and potassium, seem to have been mildly affected.

INTRODUCTION

With the aid of rapidly advancing technology, the current emphasis on wildlife field investigations is yielding information which was virtually unattainable a decade ago. This is particularly evident in the area of clinical chemistry, where the advent of automated analyzers has resulted in a plethora of data. Textbook interpretation of some of this information may be misleading, as animals sampled under field conditions are subjected to the rigors of restraint superimposed on a number of undeterminable natural environmental pressures, i.e., parasitism, competition, etc. These factors, arbitrarily referred to as stress, can influence blood parameters.[11]

Attempts to investigate the nature of "stress" in aquatic mammals have been limited. Some hematologic and biochemical aspects of transportation stress in the bottle-nosed dolphin, *Tursiops truncatus*, have been described,[8,9] as have the hematologic effects of a steroid and its relationship to stress in the same species.[10] Some partial effect of captive-stress in the harbour porpoise, *Phocaena phocaena*, have also been assessed.[3]

In the present study we have attempted a more detailed evaluation of the effects of a rigorous 72 hour handling and chemical intoxication stress on some hematologic and blood chemical parameters in a dolphin, with the hope that similar information obtained from wild animals can be interpreted more meaningfully.

MATERIALS AND METHODS

One adult female dolphin was the subject of the experiment. The pitfalls in using a single animal are recognized. Nevertheless, practical considerations limit the availability of such costly exhibit animals. The dolphin was maintained in a shallow (1 m) pool of artificial sea water having a salinity of 3%. For the purpose of the study, it was periodically removed and placed on a foam rubber mat where blood could be drawn with ease, using standard sampling techniques.[2] Following the initial sampling, 10 ml of carbon tetrachloride (CCl_4) was administered orally. Thereafter, the animal was returned to the pool where, except for the nine times required to remove it for repeated blood sampling, it was kept continuously moving for 72 hours by splashing and nudging. The CCl_4 was used in order to assess the added stress of liver damage, as reflected by the pouring into the circulation of hepatocellular enzymes

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which had been previously located by tissue distribution studies (Geraci, unpublished data). During the course of the experiment, the animal, maintained without food, became visibly irritated and unusually aggressive; following the study, and to the present time (2 years later) the dolphin has resumed normal behaviour, is in apparent good health, and has been the subject of many additional less stressful studies.

Blood for hemograms was placed in a tube containing the dipotassium salt of ethylenediaminetetra-acetic acid (EDTA) as the anticoagulant, and was maintained at 4 C until analyzed, within 1 hour. The packed cell volume (PCV) was determined by the microhematocrit, and the hemoglobin by the cyanmethemoglobin methods. Total red and white cell counts were made with the aid of Sanborn cell counter (Sanborn Co., Waltham, Mass.).

Blood for chemical studies was collected in tubes containing sodium heparin; it was centrifuged and separated immediately after each sampling. The analytical procedures for enzymes were as follows: creatine phosphokinase (CPK), Harleco kit; glutamic pyruvic transaminase (GPT) and glutamic oxalacetic transaminase (GOT), and lactic dehydrogenase (LD), Dade kit (Dade reagents, Miami, Fla.). Multichannel autoanalyzers (Technicon Instruments Corp., Ardsley, N.Y.) were used for the remaining plasma chemical determinations, using the prescribed methodology in the appropriate Technicon bulletins.

RESULTS AND DISCUSSION

The results of the hematological studies are shown in Table 1. There was no essential difference in PCV, Hb, and RBC counts between the control and trial samples. The WBC count decreased in the interval between the control and first post-stress sampling, from 19,300/mm^3 to 13,000/mm^3. Based on the consistent values which follow, this appears to be a spurious finding; also there is no precedent for such a change. In most species, stress is accompanied by leukocytosis.[11] This appears not to occur in dolphins. The stable WBC values which followed the initial decrease substantiate the findings from a previous study in which white cell counts did not change following 36 hours of quite severe transportation and handling stress in the same species.[8] White cell distributions and total eosinophil counts, which do reflect adrenal stress in mammals including the bottle-nosed dolphin,[5] were not repeated in the present study.

The plasma enzyme results are shown in Table 2. There was a broad range of values for GPT, and some elevation of CPK during the 48 h and 72 h sampling periods. The remaining enzyme values did not change beyond the limits of experimental error, lending some significance to the CPK changes. Creatine phosphokinase is primarily a muscle-cell enzyme in the dolphin (Geraci; unpublished data), as in other mammals.[5] In man, moderate exercise has inconsistently been found to increase circulating levels,[1] whereas it is nearly always elevated during severe exercise.[6] The results of this study suggest that plasma CPK may be an indicator of muscular activity in dolphins as well.

The enzyme GPT is chiefly a hepatocellular enzyme in this species (Geraci; unpublished data). Though there were significant fluctuations in GPT levels, they were not of the order which can be induced by CCl_4 intoxication. The dose rate of 0.07 ml/kg is far less than the usual dosage of 0.25 to 0.5 ml/kg which has been used experimentally to destroy liver cells in domestic species[4]—a probable explanation for this finding.

Table 3 shows the results of 11 additional plasma determinations. The glucose values all fall within the standard range reported for this species,[9] though "experimental" levels were higher than the control. This is likely due to the induced sympathetic and adreno-cortical stimulation leading to glycogen mobilization and gluconeogenesis.[7] The blood urea nitrogen levels were somewhat erratic, though they too fall within a standard range for the species.[9] The two lowest values coincide with the lowest levels of glucose during the experimental period, further suggesting a positive relationship between protein utilization (gluconeogenesis) and the higher glucose levels.

The only remaining noteworthy finding is that of potassium which progressively increased from 3.2 mEq/1 to 3.9 mEq/1 during the first 48 hours, with a return to 3.5 mEq/1 at the termination of the experiment. Potassium is the major intracellular cation, and it may be reasonable to assume that the degree of muscular damage which resulted in leakage of CPK may also account for the slightly elevated plasma potassium concentrations.

These data await confirmation from a more detailed investigation on a large number of animals. Assuming that wild cetacea respond to manipulation as did the animal in this study, then investigations on many of their blood constituents may compare favorably with those performed on the more accessible captive animals.

TABLE 1. Some hematologic parameters measured on blood taken from **Tursiops truncatus** during a 72 hour stress period.

Time	PCV (%)	Hb (g/100 ml)	RBC (millions/mm³)	WBC/mm³
0 hour (control)	38	15.0	3.58	19,300
1½ hours	38	14.2	3.15	13,000
5½ hours	38	14.6	3.43	13,700
24 hours	40	14.4	3.65	13,900
48 hours	38	14.6	3.41	12,000
72 hours	39	14.6	3.47	13,400

PCV = packed cell volume
Hb = hemoglobin
RBC = red blood cells
WBC = white blood cells

TABLE 2. Enzyme Analyses of Serial Plasma Samples taken from **Tursiops truncatus** during a 72 hours stress period.

Time	C.P.K.[1]	G.P.T.[2]	G.O.T.[3]	L.D.H.[4]
0 hour	11	8	95	116
½ hour	12	18	109	138
1½ hours	14	13	110	123
2½ hours	8	18	97	qns*
5½ hours	11	13	97	117
8½ hours	10	9	103	119
24 hours	11	8	100	113
48 hours	18	13	110	qns
72 hours	18	8	100	115

All values expressed in international units.
[1] C.P.K.—creatine phosphokinase
[2] G.P.T.—glutamic pyruvic transaminase
[3] G.O.T.—glutamic oxalecetic transaminase
[4] L.D.H.—lactic dehydrogenase
*qns—quantity insufficient for analysis

TABLE 3. Metabolite and Electrolyte Analyses of Serial Plasma Samples taken from **Tursiops truncatus** during a 72 hour stress period.

	Total Protein[1]	Glucose	Cholesterol	Blood Urea Nitrogen	Total Bilirubin	Ca	Ca (ionized)	P (inorganic)	Na[2]	K[2]	chloride[2]
0 hour	8.6	124	155	41	0.2	9.0	3.5	4.9	156	3.2	117
½ hour	8.7	152	160	40	0.2	8.5	3.2	4.5	157	3.4	117
1½ hours	8.6	165	160	42	0.2	8.9	3.4	4.9	158	3.4	117
2½ hours	8.5	160	158	42	0.2	8.2	3.2	4.9	156	3.5	115
5½ hours	8.5	156	158	41	0.2	8.8	3.4	4.8	157	3.4	116
8½ hours	8.9	147	202	39	0.1	8.4	3.1	4.3	156	3.6	118
24 hours	8.6	135	155	28	0.2	8.7	3.3	4.1	154	3.6	116
36 hours	8.6	135	158	29	0.2	9.1	3.5	4.1	155	3.6	114
48 hours	8.6	170	175	48	0.1	8.8	3.4	4.5	149	3.9	114
72 hours	8.2	145	175	44	0.1	8.6	3.4	4.5	153	3.5	116

1—g/100 ml
2—mEq/1
All other constituents expressed in mg/100 ml

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