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Authors: GERACI, J. R., and MEDWAY, W.

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SIMULATED FIELD BLOOD STUDIES IN THE BOTTLE-NOSED DOLPHIN *Tursiops truncatus*
3. Changes in Hematology and Chemistry During Blood and Plasma Storage

J. R. GERACI[1] and W. MEDWAY, The Montreal Aquarium, Montreal, Quebec; Dept. of Pathology, Ontario Veterinary College, Guelph; and School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

Abstract: Hemograms were serially performed on dolphin blood stored with EDTA at 4 C and 23 C for 5 days. With the exception of differential counts, the blood remained diagnostically suitable at both temperatures for at least 5 days. Fifteen chemical analyses were serially performed on plasma stored at 25 C and on the plasma of whole blood stored at 4 C and 25 C for 8 days. BUN, uric acid, bilirubin, cholesterol and total protein remained stable under all conditions throughout the 8 day period. Other constituents, including enzymes, were quite stable in stored plasma, and less so in stored whole blood. Many of the changes in stored whole blood were temperature dependent.

INTRODUCTION

At the outset of this study series, we intended to find the reason(s) for the inconsistancies in the literature on marine mammal blood, and to assess the clinical reliability of tests under field conditions. Wide variations have been reported not only between species, which might be expected, but within monotypic colonies, and even within the same individual. The first two reports from this investigation dealt with the influence of peripheral versus systemic sampling sites, and with the effects of exercise and chemical intoxication stress on hematology and blood chemistry.[1,7] The present report concerns the effects of handling blood once it has been drawn from the animal. The techniques which were assessed are consistent with procedures which are commonly used when sampling wild and captive dolphins.

MATERIALS AND METHODS

Three sub-adult and three adult dolphins, *Tursiops truncatus*, were used in this study. All but two sub-adults were females. Their sex and age will not be considered further, since neither appears to have had any bearing on the results. The animals were maintained as a colony either together or in contiguous pools of artificial sea water having a salinity of 30 parts per thousand. When required, they were removed and placed on foam rubber mats where blood could be drawn from the tail flukes, using standard sampling techniques.[1]

Hematology:

Four dolphins were used for this portion of the study. Five ml of blood from each subject was placed into each of four tubes containing disodium EDTA as the anticoagulant. The first set of

[1] Dr. Geraci's present address is Wildlife Disease Section, Department of Pathology, Ontario Veterinary College, University of Guelph, Ontario, Canada N1G 2W1.

hemograms from all of the animals was carried out within 60 min of sampling. Thereafter, the blood tubes from two animals (Nos. 1 & 2) were stored at 4 C, and those from the remaining two (Nos. 3 & 4) were stored at 23 C. Hemograms were then made on days 1, 2 and 5 on all of the stored blood samples. All determinations were performed in duplicate. The packed cell volume (PCV) was determined by the microhematocrit, and hemoglobin by the cyanmethemoglobin methods. Total cell counts were made on A O Spencer hemocytometers, using Hayem's solution and 0.1N HC1 as diluting fluids for the red and white cells, respectively. Blood smears were stained with Wright-Giemsa stain and 500 cells were tallied for the differential counts.

Plasma Chemistry:

Three pairs of dolphins were used in this part of the study. Six tubes of heparinized blood were obtained from each animal. All tubes from the first pair were centrifuged within 10 min of sampling. Plasma analyses from each member of the pair were carried out immediately (0 time), after which the plasma was stored at 25 C, and serially analyzed 4 or 5 times over the next 8 days.

A sample of blood from each member of the two remaining pairs was also centrifuged and analyzed immediately after collection. Thereafter, the *whole blood* samples from the second pair were stored at 4 C, and from the third pair at 25 C. At predetermined intervals over the next 8 days, the stored blood samples from each dolphin were centrifuged and the plasma was analyzed.

The analytical procedures for enzymes were as follows: creatine phosphokinase (CPK), Harleco kit (Hartman-Leddon Co., Phila., Pa.); glutamic pyruvic transaminase (GPT), glutamic oxalacetic transaminase (GOT), and lactic dehydrogenase (LD), Dade kit (Dade reagents, Miami, Fla.. Multi-channel autoanalyzers (Technicon Instruments Corp., Ardsley, N.Y.) were used for the remaining plasma chemical determinations, using the prescribed methodology as outlined in the Technicon bulletins.

RESULTS AND DISCUSSION

The hematologic data is summarized on Table 1. Though there was some variation in results between blood samples, the only consistent change was a shift in white cell distribution as storage time progressed. The shift occurred earlier and was more pronounced on blood stored at 23 C. The change gave the appearance that the lymphocytes were higher and the neutrophils lower than their actual values which were determined on fresh blood. The blood of animal No. 4 showed a remarkable change; the neutrophils decreased from 79 to 52% and the lymphocytes rose from 9% to 33% in 5 days. This is probably a reflection of differences in stability or life span between both cell types; the lymphocytes appear to be more stable. This suggestion requires scientific confirmation, but the literature on other species tends to support it.[10,11] That these changes occurred in the face of rather stable total white cell counts seems to indicate that as time advanced, the increasing numbers of fragmented neutrophils could still be counted on the hemocytometer, but were not morphologically distinct enough to be included in the differential enumeration. This view is upheld by the fact that the smears from 23 C blood contained degenerated white cells and cellular debris beginning on the 2nd day.

With the exception of differential counts, EDTA-treated dolphin blood can be considered to be diagnostically reliable for at least 5 days when stored at either 25 C or 4 C. Human blood is stable for only 1 or 2 days under the same conditions,[6] whereas cooled EDTA-treated harp seal blood has been shown to be suitable for at least 14 days.[4] This study terminated after 5 days, which was felt to be a practical upper limit of delay due to field conditions; dolphin blood may well have been stable for a longer period.

Relying on blood films made from stored blood appears to be rather precarious in the case of dolphins. Since smears so made are generally regarded to be morphologically inferior in any case,[10] their use should be disregarded in favor of films made from fresh blood.

TABLE 1. Hematologic Changes in Dolphin Blood Stored at 4 C and 23 C for 5 Days.

Dolphin No.	Storage	PCV	Hb	RBC	WBC	S	NS	L	M	E	Remarks
					Storage at 4 C						
1	0	38	12.5	3.40	29,100	79	4	9	0	5	
	1	39	12.4	3.56	30,900	75	6	11	1	7	3 nuc. RBC
	2	38	12.8	3.59	31,600	81	0	8	3	8	5 nuc. RBC
	5	38	12.5	3.34	27,000	73	1	17	2	7	7 nuc. RBC
2	0	34	11.1	2.40	31,700	81	4	13	0	10	
	1	34	10.5	2.81	20,700	79	3	10	0	8	1 nuc. RBC
	2	34	10.9	2.80	27,800	78	0	14	0	8	
	5	35	10.9	2.71	30,600	67	0	24	1	8	3 nuc. RBC
					Storage at 23 C						
3	0	50	14.7	3.90	7700	82	0	15	0	3	
	1	50	15.8	3.61	8300	69	2	17	1	11	
	2	50	16.4	3.84	7000	68	0	25	1	6	degen. WBC
	5	49	15.8	3.91	7400	54	0	34	0	12	degen. WBC, some hemo.
4	0	44	14.2	3.32	9300	79	3	9	0	8	
	1	45	12.7	3.57	6200	86	2	6	1	6	1 nuc. RBC
	2	45	14.4	3.30	7300	82	0	12	1	6	1 nuc. RBC, sl. hemo., WBC degen.
	5	46	14.0	3.52	27700	52	0	33	0	5	1 nuc. RBC, sl. hemo., WBC degen.

PCV—Packed cell volume (%)
RBC—Red blood cells (x 10^6/mm^3)
Hb—Hemoglobin (gm/100 ml)
WBC—White blood cells (/mm^3)
S—Segmented neutrophils (%)
NS—Non-Segmented neutrophils (%)
L—Lymphocytes (%)
M—Monocytes (%)
E—Eosinophils (%)

Chemistry:

Of 15 chemical determinations which were performed, BUN, uric acid, total bilirubin, cholesterol and total protein remained stable under all conditions of storage throughout the 8 day period. Table 2 shows the range in values for each parameter. The term "stability" is used with reference to diagnostic usefulness, and does not necessarily have any statistical meaning. Therefore, whereas uric acid levels fluctuated as much as 50%, all the values are well within the normal ranges reported for dolphins.[9] The same applies for total bilirubin which progressively increased in blood stored at 25 C, most likely as a result of hemolysis.

The blood constituents which were notably influenced by storage are shown in Figs. 1, 2, 3, and 4. In the plasma of blood stored at 25 C, sodium decreased and potassium increased after the 2nd day. In blood stored at 4 C, only a less dramatic potassium elevation occurred. These electrolyte changes can be attributed to the intracellular-extracellular cation shifts[8] resulting from red cell membrane permeability or death, which is obviously temperature dependant. Chloride, the major anion, remained unchanged.

Total calcium, inorganic phosphate, and glucose levels were affected by storage conditions (Fig. 2). The temperature dependant increase in phosphate and decrease in glucose in stored whole blood can be explained by hydrolysis and release of intracellular phosphorus esters and by anaerobic glycolysis, respectively. The reason for the calcium decrease in stored plasma is somewhat more complex. The technic used is based on the formation of a colored complex between cresolphthalein complexone and calcium after the release of protein bound calcium. In the original description of the procedure, pooled human serum calcium values were shown to be stable for 8 days.[6] In the present study, plasma was used rather than serum. The fibrin clots, which would appear in the stored plasma, were removed with an applicator stick, prior to analysis, and with it, some protein bound calcium, which is an integral part of the clotting scheme. Very likely, as time progressed, more and more calcium became bound only to be eventually removed—thereby yielding progressively

TABLE 2. Range of Five Stable Constituents in Dolphin Plasma Stored at 25 C, and in Plasma Taken from Blood Stored at 25 C and 4 C for 8 Days.

Determination[1]	Blood 25 C	Blood 4 C	Plasma 25 C
BUN	80 - 85	78 - 81	75 - 77
	71 - 72	75 - 76	75 - 78
uric acid	0.2 - 0.3	0.2 - 0.4	0 - 0.3
	0.3 - 0.6	0.2 - 0.4	0.2 - 0.3
total bilirubin	0.1→ 0.4[2]	0.2	0.2 - 0.4
	0.1→ 0.4	0.2 - 0.3	0.1 - 0.2
cholesterol	190 - 200	150 - 155	240 - 260
	150 - 165	160 - 165	230 - 240
total protein	7.3 - 7.4	7.9 - 8.2	7.6 - 7.8
	8.1 - 8.6	7.3 - 7.5	7.5 - 7.9

[1] All values expressed in mg/100 ml; each set of values represents one animal within the pair grouping.

[2] → progressive increase.

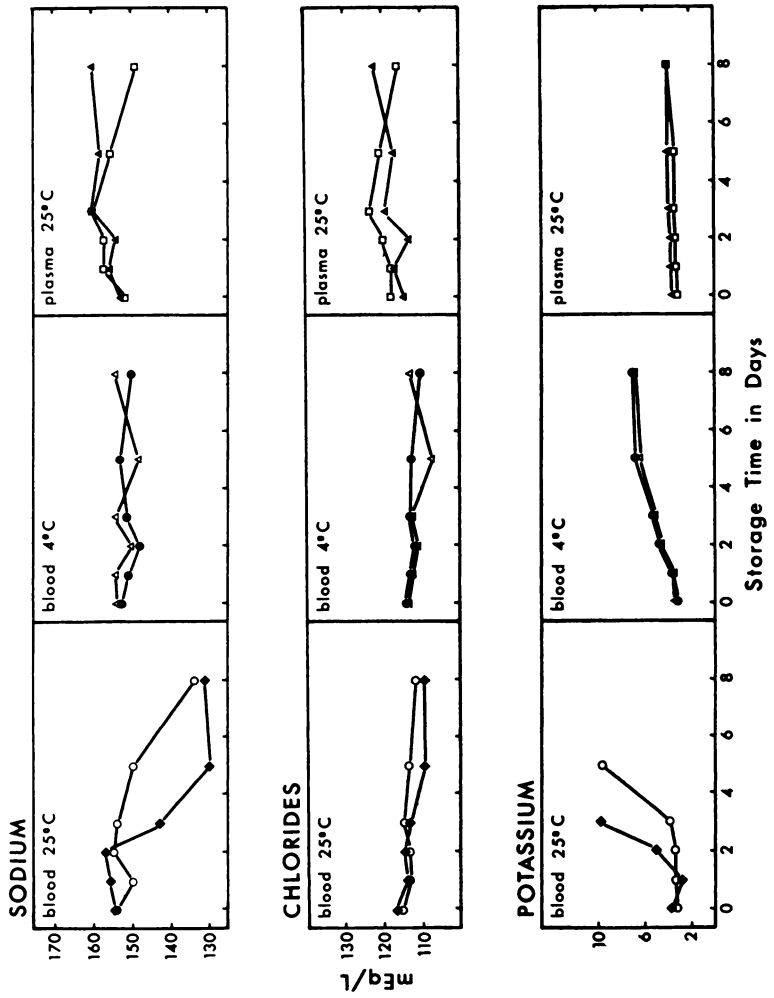

FIGURE 1. Changes in some electrolyte levels in dolphin plasma stored at 25 C, and in plasma from blood stored at 25 C and 4 C.

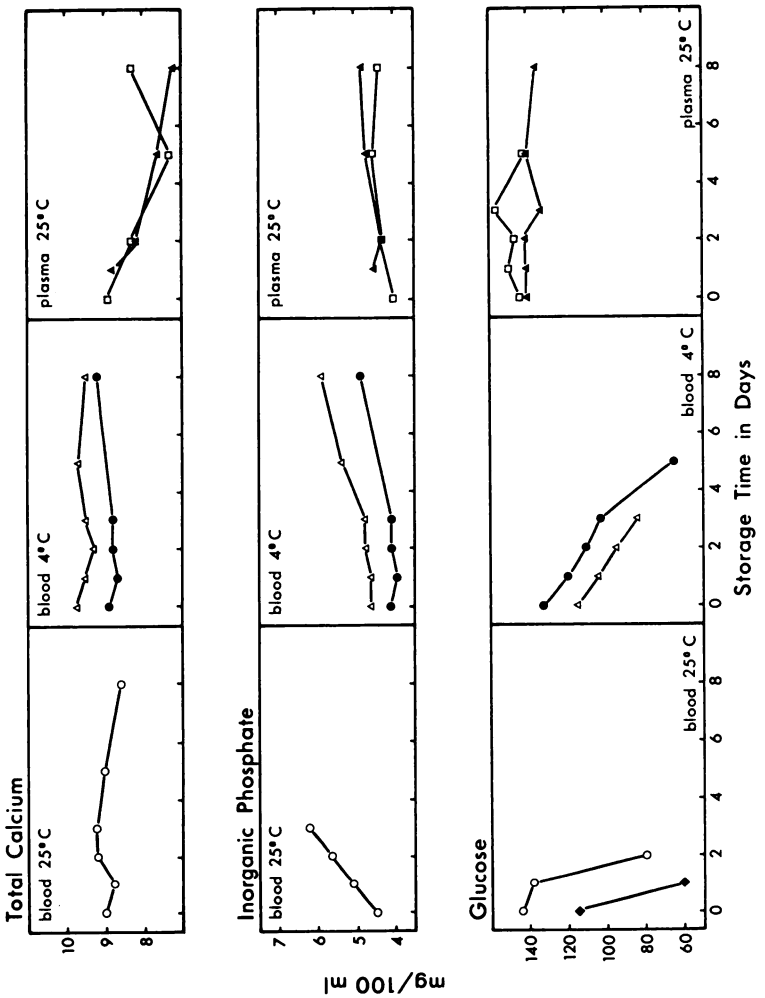

FIGURE 2. Changes in total calcium, inorganic phosphate and glucose levels in dolphin plasma stored at 25 C, and in plasma from blood stored at 25 C and 4 C.

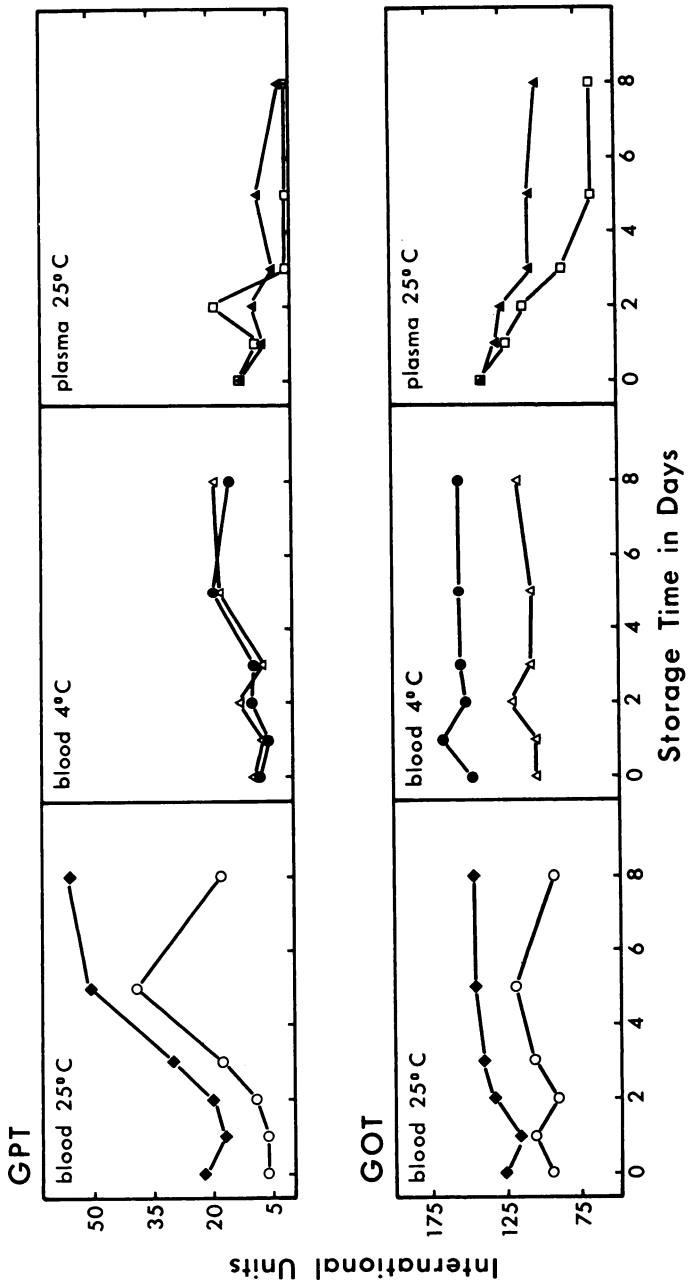

FIGURE 3. Changes in glutamic pyruvic and glutamic oxalacetic transaminase levels in dolphin plasma stored at 25 C, and in plasma from blood stored at 25 C and 4 C.

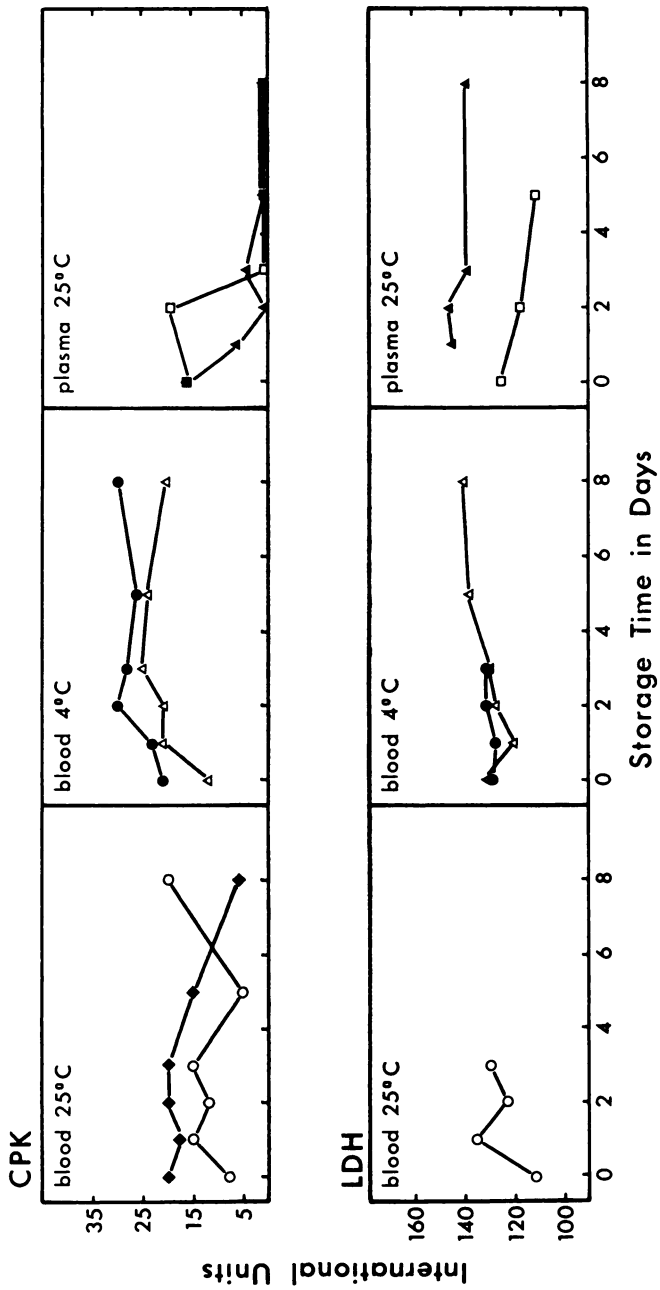

FIGURE 4. Changes in creatine phosphokinase and lactic dehydroginase levels in dolphin plasma stored at 25 C, and in plasma from blood stored at 25 C and 4 C.

lower values. The decreasing calcium values may also be due to entrapment of the ions, or to a gradual increase in pH which would have the effect of decreasing the solubility of calcium phosphate salts. Whatever the reason(s) the calcium shift obviates the value of total calcium determinations on stored dolphin plasma.

The effects of storage on blood enzyme levels are shown in Figs. 3 and 4. Glutamic pyruvic and glutamic oxalacetic transaminase are found in low concentrations in dolphin RBC's (Geraci, J. R., unpublished data). The GPT levels in 25 C whole blood rose sharply after 2 days, indicating either a continual release from red cells or degradation of an inhibiting factor. The more subtle rise in the level of the enzyme in 4 C whole blood suggests that the change is temperature dependant. The levels of both GPT and GOT enzymes progressively decreased in stored plasma. In the case of GPT, it may be due to simple degradation of the enzyme. However, the GOT decrease occurred in the face of relatively stable levels of the same enzyme in stored whole blood, perhaps indicating that whole blood contains a stabilizing or protective component which is absent in stored plasma. The same is true of creatine phosphokinase. Despite the reported inability of this enzyme to withstand storage,[2] it was relatively stable in whole blood stored at 25 C, and 4 C, for 8 days. Yet, CPK activity in 25 C stored plasma was virtually lost in just 2-3 days. There appears to be no precedent for these findings in the literature which we have examined. Perhaps the decreasing levels in plasma are due to selective enzyme degradation which accompanies the clotting mechanism, or the loss of some protective factor which is present in whole blood.

A summary view of the relative stability of all of the plasma chemical constituents in shown on Table 3. As might be expected, plasma is universally superior to whole blood for storage; yet, stored

TABLE 3. Stability (in days) of Constituents in Dolphin Plasma Stored at 25 C and Plasma Taken from Blood Stored at 4 C for 8 Days.

Determination	25 C Blood	4 C Blood	25 C Plasma
sodium	2	+	+
potassium	1	1	+
chloride	+	+	+
total calcium	+	+	1
inorganic phosphate	<1	3	+
glucose	<1	<1	+
blood urea nitrogen	+	+	+
uric acid	+	+	+
total bilirubin	2	+	+
cholesterol	+	+	+
total protein	+	+	+
GPT	2	4	2
GOT	+	+	—
CPK	3	1	<1
LDH	<1(?)[a]	±	±

+ = stable for at least 8 days

± = slight change; not diagnostically significant

[a] = insufficient number of determinations

whole blood need not necessarily be disregarded as useful in diagnosis. A great number of determinations, including some enzymes, can be made with considerable precision and reliability on whole blood stored at refrigerator or room temperature for at least 8 days. The stability of plasma and serum constituents in long term cool and frozen storage conditions is now under investigation.

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