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CLINICAL BLOOD VALUES OF THE NORTHERN FUR SEAL, *Callorhinus ursinus*. II. COMPARISON OF FRESH VERSUS STORED FROZEN SERUM [1]

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Abstract: Analysis of 14 blood components in fresh and stored (107-166 days) frozen serum of the northern fur seal *(Callorhinus ursinus)* revealed significant changes in the values of all but total protein, globulins, phosphorus and creatinine. While most values decreased during storage, cholesterol and bilirubin showed small but significant increases.

INTRODUCTION

In a previous paper,[5] we reported clinical values for 14 blood components in the northern fur seal, *Callorhinus ursinus*. These data were obtained from frozen serum samples, shipped from St. Paul Island, Alaska to the Naval Biosciences Laboratory and analyzed over a 3-6 month period. However, changes that occur in the values of many blood components during storage make the significance of much of the data uncertain, especially since the extent of such changes may vary from species to species. Therefore, during a subsequent field trip to the Pribilof Islands, we decided to analyze freshly collected serum samples and compare the values with duplicate samples frozen for 107-166 days to assess the extent of changes occurring as a result of storage.

It was anticipated that the additional clinical data acquired from the program would not only be of greater scientific interest, but could be more meaningfully applied to the management of the fur seal herd.

MATERIALS AND METHODS

During the fur seal harvest on St. Paul Island, Alaska, in July, 1975 five serum samples per day were analyzed over a 5 day period for 14 components using a Digitek® clinical analyzer [2]. To allow same-day analysis of the samples, clotting times were reduced from overnight to 3-4 h. Subsequently, the matched frozen (-20 to -40 C) samples were analyzed similarly at the Naval Biosciences Laboratory. The resulting data were examined for differences between fresh and frozen samples. The procedures used in the collection and analysis of the fur seal serum samples have been described.[5] Paired comparisons of the data were made using Wilcoxon's Signed Ranks Test.[11] Where differences exceeded chance ($P < 5\%$) the average change was calculated.

In our earlier paper,[5] we could quote only simple averages and the maximum and minimum values for each serum component because of the limited number of data points. With a larger number of samples in the pre-

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[2] Bio-Dynamics, Inc., 9115 Hague Road, Indianapolis, Indiana 46250, USA.

TABLE 1. Fresh serum clinical values for the northern fur seal, *Callorhinus ursinus*.

Serum Component	No. of Animals	Distribution	Mean	Range (±2 S.D.)	Effect of Frozen Storage: Storage Period days	Loss %	Gain %	Normal Human Range[1]	Test C.V.[4] %
Total protein, g/dl	25	log-normal	7.0	6.2-7.9	107	No change		6-8	1.9-3.0
Calcium, mg/dl	25	normal	10.4[2]	9.2-11.6	—	?		9-12	2.7-4.0
BUN, mg/dl	25	normal	26.2	16-36.4	126	5.3	—	7-20	4.4-5.2
Cholesterol, mg/dl	25	normal	260.	160-360	127	—	4.2	150-250	1.5-2.0
Bilirubin, mg/dl	25	log-normal	1.2	0.7-2.1	110	—	8.4	0.2-1.2	6.0-18.0
Globulins, g/dl	25	log-normal	3.8	3.2-4.6	107	No change		2.3-3.5	4.4-7.9
Inorganic phosphorus, mg/dl	21	log-normal	5.7	4.0-8.0	162	No change		2.5-5.0	2.6-3.6
Glucose, mg/dl	25	normal	105.	11-199	125	8.7	—	60-110	3.3-4.5
Uric acid, mg/dl	25	log-normal	3.0	1.5-5.8	163	10.9	—	2.7	6.2-10.0
Creatinine, mg/dl	25	log-normal	1.4	0.8-2.4	166	No change		0.5-1.5	14.9-16.4
Alkaline phosphatase[6], mU/ml	25	normal	150.	72-228	131	7.3	—	30-135	—[5]
SGPT[7], mU/ml	25	log-normal	32.	13-76	161	63.4	—	4.25	2.1-4.0
SGOT[8], mU/ml	15	log-normal	85.	56-129	164	19.5	—	5-20	8.0
LDH[9], mU/ml	25	log-normal[3]	995.	798-1242	133	11.6	—	30-110	6.0

[1]Values taken from compilation by Bio-Dynamics Inc.

[2]This is the mean of the frozen samples. Fresh serum values were slightly higher, but data were invalidated by an analytical procedural error.

[3]Distribution was almost normal.

[4]Coefficients of variation (C.V.) supplied by Bio-Dynamics Inc. and based on analyses of human serum samples.

[5]No value supplied by Bio-Dynamics but probably in 5 - 10% range.

[6]Assay based on enzyme-catalyzed hydrolysis of p-nitrophenyl phosphate.

[7]Serum glutamic pyruvic transaminase. Assay based[12] on coupled reactions: L-alanine + α -ketoglutarate $\xrightarrow{SGPT}$ pyruate and pyruvate + NADH $\xrightarrow{LDH}$ NAD etc.

[8]Serum glutamic oxalacetic transaminase. Assay based[1] on coupled reactions: L-asparatate + α -ketoglutarate $\xrightarrow{SGOT}$ oxalacetate and oxalacetate + NADH $\xrightarrow{MDH}$ NAD etc.

[9]Lactic dehydrogenase. Assay based[10] on reaction: L-lactate + NAD $\xrightarrow{LDH}$ NADH etc.

sent work we were able to establish the type of distribution using a Normal Equivalent Deviate (NED) regression line plot.[9] The appropriate mean, arithmetic or geometric, could then be calculated together with the "normal" range, expressed as ±2 standard deviations. When a log-normal distribution gave the best fit, log standard deviations were obtained from the log NED curve and converted (anti-logs) to actual upper and lower limit values.

RESULTS AND DISCUSSION

Of the 14 blood components analyzed (Table 1) protein, globulins, inorganic phosphorus and creatinine were unaffected by frozen storage. Calcium, also expected to be unchanged, showed an apparent slight decrease, but this may be attributable to an error in the analytical procedure.

Expected reductions in the enzyme levels did occur. Losses ranged from 7% of the alkaline phosphatase to 63% of the serum glutamic pyruvic transaminase (SGPT). The extent of the SGPT reduction is surprising in view of literature references to its excellent storage stability in frozen serum.[6] Glucose levels dropped 9% in 125 days. This may have been due to the absence of the preservative sodium fluoride in the serum; however, our previous results[5] indicated that glucose levels in frozen sera were unchanged by the addition of sodium fluoride.

The most surprising results are the increased values for cholesterol and bilirubin after storage for 127 and 110 days, respectively. The small (4%) but statistically significant ($P < 0.2\%$) increase in cholesterol was confirmed in a second series of identical, separately-stored, frozen samples. A similar increase was found in a series of five rabbit sera (averaging 11% after 8.5 months). The Digitek® procedure for cholesterol is based on the Liebermann-Burchard reaction[3,8] which is reported[4] to give a "total" cholesterol value, including cholesteryl esters. Our results suggest a further slow release of cholesterol during storage from complexes unaffected by the sulfuric acid-acetic anhydride Liebermann-Burchard reagents.

A comparison of the fresh serum values with the frozen serum values in the data previously[5] published shows that all but one of the values from the frozen data are either within the normal range or lower than the fresh serum values. Blood urea nitrogen (BUN) which averaged 40.8 mg/dl in the earlier data, is appreciably above the normal range of fresh serum (16-36.4 mg/dl) in the present report. A likely explanation is that the sera analyzed in the previous report were collected about one month earlier than the sera collected for this study. Since the bachelor bulls arrive at the rookery in order of decreasing age a greater proportion of older animals would be expected in the previous sample. Thus the different BUN values may be reflecting age differences. An additional factor may be seasonal nutritional changes although the known effects of the latter on BUN are thought to be limited to herbivores.[7]

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