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AFRRI TECHNICAL HOTE

SERUM CHEMISTRY VALUES
FOR THE BEAGLE



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#### SERUM CHEMISTRY VALUES FOR THE BEAGLE

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# FOREWORD (Nontechnical summary)

In the investigation of prominent sites of radiation injury in mammals, chemical analyses of the components in biological fluids and tissues are being performed. The research reported herein was necessary to establish the normal values and variations for a large number of the chemical components present in the fluid portion of blood (serum) from normal untreated beagles. These values will serve as base lines from which changes in the serum of irradiated beagles can be detected.

Base line information on dog serum components can be found in the scientific literature; however, only meager information is available specifically for the beagle, and factors which may influence the levels of the components such as age or sex have not been considered.

In our study, microanalytical procedures were applied to make possible a battery of measurements on a single specimen of serum. Sodium, potassium, and calcium determinations were made using a flame photometer which records the intensity of colored light given off by these ions when they are excited by the heat of the flame. Chloride and lipase were measured using wet chemistry titrations. All other measurements were made in a spectrophotometer which analyzes, at a specific wavelength, the optical density of a substance in solution. (Optical density is a function of concentration of the chemical component.)

This report supplies the normal values obtained for 25 different chemical components of beagle serum, measurement of which is expected to assist in evaluating the sites of injury in irradiated dogs. The values for these constituents were determined from 10 males and 10 females, 2 to 3 years of age. Sex-related differences were evaluated. Four of the 25 chemical components studied were found to be significantly higher in males than in females. For the 21 other components, no significant sex-related differences were found.

#### ABSTRACT

Normal values for 25 serum chemical components were determined for AKC registrable beagles. Analyses were performed on serum from 10 males and 10 females, and sex-related differences in the levels of the constituents were evaluated. The serum levels of albumin, glutamic-oxalacetic transaminase, glutamic-pyruvic transaminase, and acid phosphatase were significantly higher (p > .05) in males than in females. For 21 other serum components, including electrolytes, glucose, cholesterol, nonprotein nitrogenous substances, and enzymes, no significant sex-related differences were found.

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#### I. INTRODUCTION

The analysis of whole blood, plasma, or serum for chemical components has been performed with increasing frequency in the diagnosis and treatment of disease. In the research laboratory, blood chemistry techniques are often used to monitor the condition of animals or to indicate changes in various tissues resulting from an applied experimental procedure. A the Armed Forces Radiobiology Research Institute (AFRRI) serum analyses are being performed as one of the parameters measured in a program to characterize injury produced by whole or partial body irradiation. The animal used in many of these studies is the AKC registrable beagle. While several sources are available listing normal values for many serum constituents of the dog<sup>1,4,6,8,14</sup> there are little data specifically for the beagle. Data published<sup>12,13</sup> do not provide the information required for the AFRRI studies since the number of serum constituents considered is meager and variations associated with sex are not reported. This investigation was undertaken to establish base line values for a wide spectrum of serum components in beagles and to evaluate sex-related influences on those components.

# II. MATERIALS AND METHODS

Animals used in this study were AKC registrable beagles, 2 to 3 years of age. Blood was withdrawn from the jugular vein through a 21 gauge 1-inch needle into a 10 ml syringe, and immediately transferred to a clean glass test tube. All blood samples were obtained between 0800 and 0900 hours from animals fasted for 16 hours.

The serum was separated from the clot approximately 45 minutes after blood withdrawal. Any sample exhibiting hemolysis was discarded. That portion of each sample not analyzed immediately was stored at -25°C up to 7 days. Serum stored in the frozen state was used only to analyze constituents which are stable under such conditions as reported by Henry, <sup>10</sup> McKelvie et al., <sup>13</sup> and Frankel and Reitman. <sup>9</sup>

Serum chemical analyses were performed on samples from 10 male and 10 female beagles. All flame photometric procedures were carried out using a Beckman\* flame photometer, Model 105. All spectrophotometric measurements were made with a Beckman\* spectrophotometer, Model DU. The following techniques were employed:

Sodium and Potassium. Flame photometric method described by Annino, susing a 1:200 dilution.

Calcium. (a) Flame photometric method described by Annino, 3 using a 1:25 dilution; and (b) method of Ferro and Ham as described by Damm. 7

Chloride. Mercuric nitrate titration as described by Annino.<sup>3</sup>

<u>Inorganic Phosphorus</u>. Fiske-SubbaRow method as described by Frankel and Reitman.<sup>9</sup>

Glucose. Hycel P-M-S procedure. 11

<u>Cholesterol</u>. Ferro-Ham direct method as described by Frankel and Reitman.<sup>9</sup>

Bilirubin. Modified Malloy-Evelyn technique as described by Annino.<sup>3</sup>

<sup>\*</sup> Beckman Instruments, Inc., Fullerton, California.

 $\underline{\text{Urea Nitrogen}}. \hspace{0.2cm} \text{Gentzko-Masen method as describ 1 by Frankel and}$  Reitman.  $^9$ 

Creatinine and Creatine. Methods as described by Frankel and Reitman.9

Uric Acid. Uricase method as described by Henry. 10

Allantoin. Method of Christman et al.5

Total Protein, Albumin and Globulin. Reinhold method as described by Damm.<sup>7</sup>

 $\underline{\hbox{Glutamic-Oxalacetic (GOT) and Glutamic-Pyruvic (GPT) Transaminases}.}$  Methods described by Amador and Wacker.  $^2$ 

Total Lactic Dehydrogenase (LDH). Berger-Broida method as described by Frankel and Reitman.<sup>9</sup>

Aldolase. Method described by Amador and Wacker.<sup>2</sup>

Creatine Phosphokinase (CPK). Method described by Amador and

 $\underline{\text{Amylase}}$ . Caraway method as described by Annino.<sup>3</sup>

Wacker.2

Lipase. Crandall and Cherry method as described by Annino.3

Phosphatases. Acid and alkaline phosphatases according to the p-nitro-phenylphosphate procedure described by Frankel and Reitman.<sup>9</sup>

#### III. RESULTS

The beagle serum chemistry values, arranged according to sex, are summarized in Table I.

Table I. Serum Chemistry Values for the Beagle\*

	11-24	Males	t	Female			
Serum constituent	Units	Mean ± S. E.₹	Range	Mean ± S. E.	Range	ρ\$	
Sodium	meq/liter	154.6 ± .6	153 - 158	154.7 ± .6	152 - 157		
Potassium	meq/liter	$5.30 \pm .16$	4.5 - 5.9	5.14 ± .13	4.5 - 5.9		
Calcium**	meq/liter	5,30 ± .06	5.0 - 5.5	5.29 ± .07	5.0 - 5.8		
Calcium††	meq/liter	5.20 ± .09	4.8 - 5.8	5.40 ± .08	5,0 - 5,9		
Chloride	meq/liter	115.0 ± 1.0	110 - 120	113.9 ± .8	110 - 117		
Inorganic					ĺ		
Phosphorus	mg/100 ml	3.30 ± .21	2.5 - 4.5	3.80 ± .15	2.8 - 4.3		
Glucos e	mg/100 ml	107.5 ± 4.4	90 - 135	120.0 ± 18.5	80 - 380		
Cholesterol	mg/100 ml	180 ± 11	125 - 240	206 <u>+</u> 24	125 - 380		
Bilirubin	mg/100 ml	.19 ± .03	.1040	.20 ± .04	.1050		
Urea Nitrogen	mg/100 ml	12,3 ± .5	9 - 14	11.5 ± .6	7 - 14		
Creatinine	mg/100 ml	1.04 ± .06	.8 - 1.5	.88 ± .03	.8 - 1.0		
Creatine	mg/100 ml	.15 ± .05	0 ~ .50	. J8 ± . 04	n40		
Uric Acid	mg/100 ml	all values <0.1		all values <0.1			
Allantoin	mg/100 ml	1.38 ± .09	.95 - 1.8	1.69 ± .14	1.3 - 2.3	ł	
Total Protein	g/100 ml	6.14 ± .13	5.4 - 6.9	5.98 ± .13	5.3 - 6.8	1	
Albumin	g/100 ml	3.16 ± .06	2.9 - 3.5	2.86 ± .12	2.3 - 3.5	p < .05	
Globulin	g/100 ml	2.98 ± .14	2.2 - 3.5	3.12 ± .11	2.4 - 3.7	Į.	
Glutamic-Oxal-		Į				İ	
acetic Trans-	Sigma-Frankel	1					
aminase	units/ml	24.8 ± 1.9	18 - 34	18.9 ± .8	15 - 24	p < .01	
Glutamic-Pyruvic	Sigma-Frankel	1		-		Ì	
Transam:nase	units/ml	28.0 ± 2.0	20 - 40	20.8 ± 1.5	14 - 32	p < .02	
Total Lactic	Berger-Broida						
Dehydrogenase	units/ml	306 ± 28	120 - 450	344 <u>+</u> 24	280 - 510		
Aldolase	Sibley-Lehninger						
	units/ml	4.4 ± .4	3 - 6	4.5 ± 1.0	2 - 12		
Creatinine	<u> </u>						
Phosphokinase	Sigma units/ml	$2.7 \pm 1.1$	0 - 9	4.2 ± 2.0	0 - 18		
Amylase	Caraway units/					<u> </u>	
	100 ml	377 ± 36	240 - 584	365 ± 23	252 - 498	1	
Lipase	Sigma-Tietz						
	units/ml	.20 ± .03	04	.25 ± .05	05		
Alkaline						]	
Phosphatase	Sigma units/ml	1.39 ± .15	.9 - 2.2	1.41 ± .13	.8 - 2.2		
Acid	"	İ					
Phosphatase	Sigma units/ml	.48 ± .07	.1575	.25 ± .04	.1045	p < .05	

<sup>\*</sup> All animals were AKC registrable beagles, 2-3 years of age, and were fasted 16 hours prior to blood withdrawal.

<sup>†</sup> Each mean represents 10 animals.

<sup>\$</sup> S. E. Standard Error of the Mean.

<sup>•</sup> p - Probability that the two means are members of the same population, as determined by Student's t-test. 15 No value is listed for comparisons in which p > .05.

<sup>\*\*</sup> Measured by spectrophotometry.

tt Measured by flame photometry.

Of the 25 serum components evaluated, 4 showed sex-related differences which were significant at the .05 level or lower. These were: albumin, GOT, GPT, and acid phosphatase. In each case, males had higher levels than females.

#### IV. DISCUSSION

The serum chemistry values reported in this study agree substantially with those in standard reference sources.<sup>1,4</sup> However, most sources report the values for "dogs" only and are not concerned with the breed of dog or the genealogy of the individuals. A well-known example of the difference the breed of a dog can make in its serum chemistry is the high pric acid level found in the Dalmatian.<sup>4</sup>

The one serum component for which results from two methods are reported is calcium. The simplicity of direct serum calcium measurement by flame photometry is, in part, nullified by several drawbacks, notably interference from other substances (e.g., sodium, protein, phosphate).<sup>3,10</sup> In this study, measurement of calcium by spectrophotometry produced essentially the same mean values as measurement by flame photometry. However, variation was more limited in the former procedure, as indicated by the smaller standard errors and ranges.

In selecting a spectrum of serum chemical components for evaluation in the beagle, several factors were considered. These included: (a) availability of a rapid, acceptably accurate analytical method adaptable to small sample sizes; (b) applicability to the detection of general tissue destruction, specific organ damage, altered metabolic processes, or other deviations from normal physiology in irradiation studies.

Before definitive research could be initiated in the use of serum chemistry to aid in characterizing radiation injury, base lines had to be compiled and possible sex-related differences ascertained. The larger mean values of albumin, GOT, GPT, and acid phosphatase in males must be taken into consideration when designing an experiment or interpreting results involving these components. Similarly, when using base line values reported in the literature, it is important that the sex of the animals be known and the existence or nonexistence of any sex-related differences be noted.

#### V. SUMMARY

Base line serum chemistry values for AKC registrable beagles are presented. Males had significantly (p < .05) higher levels of albumin, GOT, GPT, and acid phosphatase than did females. No significant sex-related differences were found for 21 other serum components.

#### REFERENCES

- 1. Altman, P. L., comp. Blood and Other Body Fluids. Washington, D. C., Federation of American Societies for Experimental Biology, 1961.
- 2. Amador, E. and Wacker, W. E. C. Enzymatic methods used for diagnosis. Meth. Biochem. Anal. 13:265-356, 1965.
- 3. Annino, J. S. Clinical Chemistry: Principles and Procedures, 3rd ed. Boston, Little, Brown and Company, 1964.
- 4. Bloom, F. The Blood Chemistry of the Dog and Cat. New York, Gamma Publications, Inc., 1960.
- 5. Christman, A. A., Foster, P. W. and Esterer, M. B. The allantoin content of blood. J. Biol. Chem. 155:161-171, 1944.
- Coffin, D. L. Manual of Veterinary Clinical Pathology, 3rd ed. Ithaca, N. Y., Comstock Publishing Associates, 1953.
- 7. Damm, H. C., ed. Practical Manual for Clinical Laboratory Procedures. Cleveland, Ohio, The Chemical Rubber Co., 1965.
- 8. Dukes, H. H. The Physiology of Domestic Animals, 7th ed. Ithaca, N. Y., Comstock Publishing Associates, 1955.
- 9. Frankel, S. and Reitman, S., eds. Gradwohl's Clinical Laboratory Methods and Diagnosis, 6th ed., Vol. 1. St. Louis, Missouri, The C. V. Mosby Company, 1963.
- 10. Henry, R. J. Clinical Chemistry: Principles and Technics. New York, Harper and Row Publishers, Incorporated, 1964.
- 11. Hycel P-M-S Sugar Determinations. Houston, Texas, Hycel, Inc., 1964.
- McKelvie, D. H., Powers, S. and McKim, F. Blood Chemistry Values of Beagles. In: The Effects of Sr<sup>90</sup> and Ra<sup>226</sup> on the Beagle, pp. 14-24. School of Veterinary Medicine, University of California, Davis, AEC Report UCD 472-110, July 1964.
- McKelvie, D. H., Powers, S. and McKim, F. Clinical Blood Chemistry Studies in Beagles. In: The Effects of Sr-90 and Ra-226 on the Beagle, pp. 36-43. School of Veterinary Medicine, University of California, Davis, AEC Report UCD 472-112, June 1965.

- 14. The Merck Veterinary Manual. Rahway, New Jersey, Merck & Co., Inc., 1955.
- 15. Steel, R. G. D. and Torrie, J. H. Principles and Procedures of Statistics. New York, McGraw-Hill Book Company, Inc., 1960.

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