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AIR FORCE MISSILE DEVELOPMENT CENTER

TECHNICAL REPORT

PHYSIOLOGICAL BASE-LINE STUDIES
OF ZOOLOGICAL SPECIMENS

SERUM BIOCHEMICAL VALUES OF CHIMPANZEES

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HOLLOMAN AIR FORCE BASE
NEW MEXICO

August 1961

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Deputy for Development and Test

AIR FORCE MISSILE DEVELOPMENT CENTER
AIR FORCE SYSTEMS COMMAND
UNITED STATES AIR FORCE
Holloman Air Force Base, New Mexico
August 1961

This investigation was conducted in accordance with the principles of laboratory animal care established by the National Society of Medical Research.

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ABSTRACT

Biochemical titres of various components in the sera of 52 chimpanzees are presented. The findings are compared with man and the Macaca mulatta monkey. The method employed for each specific analysis is briefly discussed. The concentrations of the factors in the serum of the chimpanzee herein reported are similar for the most part to those in human serum.

PUBLICATION REVIEW

This Technical Report has been reviewed and is approved for publication.

FOR THE COMMANDER

FELIX H. JONES, JR.
Colonel, USAF
Deputy for Development and Test

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SERUM BIOCHEMICAL VALUES OF CHIMPANZEES

I. INTRODUCTION

There is little information in the literature concerning normal quantitative levels of serum constituents of chimpanzees. One study (Ref. 1) does include some data on liver function in chimpanzees, but only that incidental to the prime objective of producing pathological liver function titres. Serum electrolyte studies were made on the plasma of Rhesus monkeys by White and Knobil (Ref. 2 and 3). The first author cites data obtained from 10 animals and the latter data from 50 animals.

The purpose of this investigation was to determine the base-line serum biochemical values for the colony-stabilized chimpanzee. The findings have assisted in health maintenance and in evaluating the effect of various experimental conditions to which chimpanzees are exposed.

II. MATERIAL AND METHODS

A. Subjects

The 52 animals used for this investigation consisted of 30 males and 22 females ranging from 18 months to 16 years of age and weighing between 11-1/4 and 129 pounds. Only 5 of these were adults, i.e. over 6 years old. Veterinary clinicians performed physical examinations of the whole group of animals throughout the study, and no data are included in the serum ranges from chimpanzees found to be clinically ill. These subjects represent the stabilized chimpanzee colony of the Bioastronautics Research Laboratory, Holloman Air Force Base, New Mexico, and were the source of the serum samples for the data of this report. Although not all biochemical determinations were made on each subject, this report includes information from over 2000 individual determinations and provides significant physiological base-line information.

Released by authors July 1961

B. Procedures

1. Blood Samples.

All blood specimens were taken from unanesthetized animals except for the few older and less manageable individuals. Following a physical examination, the subjects were strapped face down on the examination table (Ref. 4). The venipuncture was made in most instances in a great saphenous vein. Occasionally it was necessary to take the sample of blood from an antecubital vein. In most cases, a 4 to 5 ml. sample of blood was withdrawn, which furnished 2 to 2.5 ml. of serum. The whole blood was immediately centrifuged and the serum transferred into two tubes, one of which was immediately layered over with oil for carbon dioxide content determination. The remaining serum, not under oil, was used for the other determinations. All determinations were made within 48 hours, and usually within 8 hours, after the specimen had been obtained.

2. Analysis.

Whenever possible, the methods used were controlled both by the use of pure standards appropriate to the method, and a commercial reconstituted serum containing the component being measured.* All determinations were done in duplicate except the phosphatases. All techniques used were applicable to human serum.

The flame photometry determinations for sodium, potassium, and calcium were run using a Beckman Model DU spectrophotometer with a photomultiplier and flame attachment. Sodium and potassium together required 0.2 ml. of serum, and the flame photometry method for calcium required another 0.2 ml. sample.

Bilirubin, urea nitrogen, chloride, calcium, uric acid, creatinine, total protein, glucose, and inorganic phosphorus were run on the ultra-micro scale using Beckman-Spinco ultra-micro equipment. Ultra-micro tests were especially appropriate for

*For example: Lab-Trol, Dade Reagents, Miami, Florida

pediatric-aged chimpanzees since only relatively small amounts of blood were available. Several of the ultra-micro techniques were modified for ease of accomplishment and greater reliability. The ultra-micro techniques are generally more difficult than the macro techniques from which they were developed. By concurrently determining serum titres utilizing routine macro techniques on larger serum samples, the accuracy of the micro methods was found comparable to that of the macro methods.

Sodium and potassium: Sodium and potassium concentrations were determined by flame photometry (Ref. 5). The serum samples were run in a 1:50 dilution in a deproteinized solution containing 10 percent isopropanol, 5 percent trichloracetic acid, and water. The precipitated protein was removed by centrifugation. The spectrophotometer was set using standard inorganic salt solutions and commercial reconstituted serum samples as controls (Ref. 5). The wave length for sodium was 588.3 mu., and that for potassium 765 mu.

Calcium: Calcium concentration was determined by flame photometry on a 1:50 serum dilution in 20 percent isopropanol solution containing a small amount of hydrochloric acid. This solution did not cause protein precipitation. The calcium emulsion was determined at 422.3 mu. Calcium titres were also ascertained by a modification of the method of Diehl and Ellingboe (Ref. 6). This method involves titration with EDTA, with calcein as the indicator. The titration was carried out to disappearance of fluorescence in a dark box with fluorescent lamp illumination from above. End points are more easily observed by this modification than by the original method of Diehl and Ellingboe. Titration and flame photometry techniques consistently yielded calcium content values checking within ± 0.1 mg. percent. An inorganic calcium standard solution and a standard commercial reconstituted serum were used as controls. The titration technique was used for a majority of the determinations. It produced results as rapidly and as reliably as did flame photometry and required a smaller sample.

Carbon Dioxide: The serum content was measured by a Natelson microgasometer utilizing the technique of Natelson (Ref. 7 and 8). A standard carbonate solution was used for calibration and control.

Bilirubin: All determinations were accomplished by Powell's method (Ref. 9). Total bilirubin only was determined. In this method, color is developed with the Van Den Bergh reaction in a benzoate-urea solution; the wave length for color measurement is 540 mu. The Malloy-Evelyn method (Ref. 10) which develops color in a methanol solution was found to be unsuitable for chimpanzee serum because variable turbidities developed in the reaction medium. The Powell method proved more suitable for detecting low bilirubin concentrations. Chimpanzee serum invariably exhibited a low titre of bilirubin. A standard curve was established using a fresh standard solution of bilirubin in chloroform, and unknown values were determined from the plotted curve. All bilirubin determinations were made within four hours after the sample was obtained.

Total Protein: A modified biuret method (Ref. 11 and 12) was utilized. Color intensity was measured at 540 mu.

Urea Nitrogen: Fearon and Friedman's method (Ref. 13 and 14) was used with diacetyl monoxime as the color developing agent. The wave length for color measurement was 475 mu.

Uric Acid: A modification of the method of Caraway (Ref. 15) was employed using urea-cyanide solution rather than sodium carbonate as a color intensifier. Color was read at 650 mu.

Creatinine: The Jaffe color reaction with alkaline picrate (Ref. 16 and 17) was used. The wave length for color measurement was 520 mu.

Glucose: These values were obtained on serum using a relatively new method (Ref. 18). It utilizes an enzyme system coupled with a color detector, and reportedly measures only glucose, tending to give lower concentration values than the Folin-Wu or Somogyi methods. No attempt was made to obtain fasting blood samples for the glucose determinations. The wave length for color measurement was 410 mu.

Inorganic Phosphorus: The method of Fiske and Subbarow (Ref. 19) was used. The wave length for color measurement was 650 mu.

Chlorides: The titration method of Schales and Schales (Ref. 20) was used. The titration is accomplished with mercuric nitrate using diphenylcarbazone as indicator.

Total Cholesterol: Two methods were utilized; one was that of Carr and Drekter (Ref 21). It involves the use of the original Lieberman-Burchard color system in a modified manner. The second method was that of Chiamori and Henry (Ref. 22), which employs ferric chloride for color development. The latter technique has the advantage of simplicity and requires less time per sample. Although neither method has proven entirely satisfactory, the results are reliable within 15 to 20 mg. percent. Both methods require 0.2 ml. of serum per sample.

Alkaline Phosphatase: The method (Ref. 23 and 24) utilizes the release of phenolphthalein from a buffered substrate containing sodium phenolphthalein phosphate.* The results are made quantitative rather than semi-quantitative by relating the findings to a curve obtained on a standard phenolphthalein solution. The color measurements were made at 550 mu.

Acid Phosphatase: The method used (Ref. 25) involves measurement of alpha-naphthol released in a buffered substrate.* It was made quantitative by comparison to an alpha-naphthol standard using a spectrophotometer at 520 mu.

Protein Electrophoresis: Beckman-Spinco equipment and the Beckman-Spinco "B" procedure (Ref. 26) was used with a serum sample of 3 ul. The dyed paper strips were scanned with a Spinco Analytrol which gave absorbance curves and automatic integration of the areas under the curves. Data on albumin, alpha, beta, and gamma globulins, and the A/G ratios are presented.

III. RESULTS

In calculating the normal ranges, 5 percent of the data was deleted from the highest extremes of the ranges and 5 percent from the lowest. The use of this 90 percent range should exclude most sub-clinical, non-recognized illness from these studies.

*Phosphatabs, Warner-Chilcott Co., Morris Plains, N.J.

Data obtained by paper electrophoresis on the proteins in chimpanzee sera, are set forth in Table I together with some data for human sera.

Tables II, III, and IV give data on serum electrolytes of chimpanzees, Rhesus monkeys, and man.

Other serum components of chimpanzee serum may be found in Table V; Table VI shows similar human data for purposes of comparison.

IV. DISCUSSION

The findings from this study indicate that there is a high degree of similarity between the biochemical values for chimpanzee and human sera. The differences found cannot be considered significant, especially since the published human sera data do not agree.

The results obtained by protein electrophoresis of chimpanzee serum exhibit some definite qualitative differences as compared with human sera. Chimpanzee serum contains three definite alpha-globulin fractions similar to those found in monkeys (Ref. 31).

The range for total protein concentration found for chimpanzees is somewhat wider than that quoted for man.

The electrolyte ranges observed in chimpanzees were not as wide as those for Rhesus monkeys, but more nearly resemble the narrower ranges found in man.

Total bilirubin values in chimpanzees were uniformly low, as compared to those of humans. No instance has so far been found where the bilirubin content was above 0.4 mg. percent. Somewhat higher levels of bilirubin were found by other investigators (Ref. 1) but they used the Malloy-Evelyn method. This laboratory found the Malloy-Evelyn method unsuitable because variable turbidities developed in the reaction medium. It was also observed to be less sensitive at low concentrations than the Powell technique. Nelson (Ref. 30) states that normal human adult bilurubin values may reach 0.8 mg. percent. Miller (Ref. 32) gives 1.0 mg. percent as a top normal level for bilirubin in humans.

TABLE I

Paper Electrophoresis of Chimpanzee and Human Serum Proteins

Protein Component	Number of Tests	Range* (Percent of Total)	Median (Percent of Total)	Mean (Percent of Total)	Human Ranges** (Percent of Total)
Albumin	39	50.9-69.2	63.1	62.0	51.6-65.6
Alpha Globulins	39	5.2-15.9	10.8	10.7	8.0-14.8
Beta Globulins	39	8.8-14.0	11.0	11.0	9.3-15.7
Gamma Globulins	39	9.7-31.0	14.5	16.0	14.5-20.7
A/G Ratio	39	1.04-2.26	1.70	1.67	

* The range given includes 90 percent of determined figures. Five percent of the data were deleted from both extremes of the ranges.

** Electrophoresis results of ten normal human sera (Ref. 27).

TABLE II

Chimpanzee Serum Electrolyte Values

Electrolyte	Range*	Median	Mean	No. Samples
Sodium mEq/L	139-147	144	145	101
Potassium mEq/L	3.4-4.7	4.0	4.0	83
Chlorides mEq/L	96-111	103	103	128
Calcium mEq/L	4.75-5.75	5.05	5.1	86
Calcium mg. percent	9.5-11.5	10.1	10.2	86
CO_2 mM/L (Content)	17.9-27.9	23.2	23.2	169
CO_2 Vol. percent (Content)	39.7-61.9	51.4	51.4	169

* The range given includes 90 percent of the data. Five percent of the data were excluded from both extremes of the ranges.

TABLE III

Macaca Mulatta Serum Electrolyte Values

Electrolyte	Range*	Mean*	Range**	Mean**
Sodium mEq/L	146.4-161.4	153.0	142.3-177.6	153.8
Potassium mEq/L	2.5-5.3	4.2	3.5-5.5	4.4
Chlorides mEq/L	103.0-114.1	110.2	91.5-124.0	107.7

* These data were for 10 monkeys (Ref. 2).
** These data were for 50 monkeys (Ref. 3).

TABLE IV

Serum Electrolyte Ranges in Man

Electrolyte	Range Spector (Ref. 28)	Range Hawk, Oser, Summerson (Ref. 29)	Range Nelson (Ref. 30)
Sodium mEq/L	132-144	130-144	133-143
Potassium mEq/L	3.6-4.8	4.1-5.6	4.1-5.6
Chlorides mEq/L	97-108	98-106	100-106
Calcium mEq/L	4.8-6.1	4.5-5.75	5-6
CO_2 Vol. percent		55-75 (capacity)	45-70 (content)
CO_2 mM/L (content)	24-31		20.3-31.5

TABLE V

Serum Component Concentrations in the Chimpanzees

Component	Range*	Median	Mean	No. Samples
Inorg. P. mg. percent	3.4-6.0	4.3	4.6	47
Urea N. mg. percent	6.1-15.7	10.0	10.3	41
Uric Acid mg. percent	2.7-5.8	4.2	4.1	50
Creatinine mg. percent	0.8-1.8	1.2	1.2	41
Total Protein mg. percent	6.08-8.85	6.95	7.06	92
Glucose mg. percent	72-137	98	97	54
Bilirubin mg. percent	0.10-0.31	0.20	0.21	53
Alk. Phosphatase BU**	3.0-13.2	9.5	8.6	23
Acid Phosphatase BU**	0.3-1.1	0.7	0.7	10
Total Cholesterol mg. percent	157-311	214	219	42

* The range given includes 90 percent of the data. Five percent were excluded from the extremes of the ranges.

** Bodansky Units.

TABLE VI

Human Serum Component Concentrations

Component	Range Spector (Ref. 28)	Range Hawk, Oser, Summerson (Ref. 29)	Range Nelson (Ref. 30)
Inorganic P. mg. percent	5.1 (mean)	3.7 adult (mean) 5.0 child (mean)	4.5-5.5
Urea N. mg. percent	8.7-12.4	10-15 (whole blood)*	10-17 (plasma)*
Uric Acid mg. percent	4.0-4.8	3-5 (plasma)*	2.5-3.5 (whole blood)*
Creatinine mg. percent	0.7-1.1	1-2	0.6-1.2 (plasma)*
Total Protein mg. percent	5.9-7.2	6.5-8.2	6.5-7.5
Glucose mg. percent	61-130 (97 mean)	65-110	85-120 (fasting whole blood)*
Bilirubin mg. percent	0.1-0.25	0.1-0.25	0.2-0.8
Alk. Phosphatase BU**		1.5-4.0 (adult) 5.0-12.0 (child)	3-13 (child)
Acid Phosphatase BU**			0.0-1.1 (adult)
Total Cholesterol BU**	130-255	110-390	170-259

* Values are for serum unless otherwise noted.

** Bodansky Units

Mature chimpanzees and human adults exhibit lower alkaline phosphatase levels than do immature chimpanzees and children. This affords an explanation of the generally higher values observed in our chimpanzees because most of the animals were immature.

The carbon dioxide combining power of chimpanzee serum exhibits lower values than those quoted in the literature for man. Nelson (Ref. 30) and Albritton (Ref. 33) indicate lower values for children; the over-all lower values reported here are likely because of the immaturity of our subjects.

V. SUMMARY AND CONCLUSIONS

In excess of 2000 determinations were made on various components of the sera of 52 adult and immature chimpanzees. The normal concentration ranges of 20 serum components are presented and discussed. The various techniques for the individual determinations are outlined.

These base-line studies have not revealed significant quantitative differences in the concentrations of these serum components as compared with those of man. Those differences which were found can be ascribed to a proportionately greater density of immature individuals in our subject population. However, there are indications that the protein composition of chimpanzee serum differs qualitatively from that of man.

The base-line data presented in this report have proven useful in following the course of disease in members of our animal colony, including the biochemical responses to therapy. This information has also served for the detection and diagnosis of sub-clinical disease in chimpanzees.

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