

CERTAIN PHYSIOLOGICAL STUDIES ON INDIAN ELEPHANTS

By

K. P. SREEKUMAR



THESIS

submitted in partial fulfilment of the
requirement for the degree

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

Department of Physiology and Biochemistry
COLLEGE OF VETERINARY AND ANIMAL SCIENCES

Mannuthy - Trichur

1986

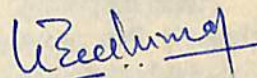
**Dedicated to
Lord Ganapathy**

DECLARATION

I hereby declare that this thesis entitled
"CERTAIN PHYSIOLOGICAL STUDIES ON INDIAN ELEPHANTS"
is a bonafide record of research work done by me
during the course of research and that the thesis has
not previously formed the basis for the award to me
of any degree, diploma, associateship, fellowship, or
other similar title, of any other University or
Society.

Mannuthy,

23.7.1986



R.P. SREEKUMAR

CERTIFICATE

Certified that this thesis, entitled "CERTAIN
PHYSIOLOGICAL STUDIES ON INDIAN ELEPHANTS" is a record
of research work done independently by Sri.K.P. Sreekumar
under my guidance and supervision and that it has not
previously formed the basis for the award of any degree,
fellowship or associateship to him.



Dr. G. NIRMALAN
(Chairman, Advisory Board)
Professor,
Department of Physiology
& Biochemistry.

Mannuthy,

23rd July, 1986

Thanks are due to Dr. P.A. Wahid, Professor and Mr. K. Madhavan Nair, Associate Professor, Kerala Agricultural University, for their valuable assistance in atomic absorption spectroscopy.

Thanks are also due to Dr. B.C. Joshi, S₄ Scientist, I.V.R.I., for his wise counselling.

The study could not have been carried out had not been for the whole hearted co-operation extended to me by the then Forest Veterinary Officer, Dr. Ramakrishna Pillai, Government of Kerala, and the staff of Guruvayoor Devaswom. I record my sincere appreciation to them.

I am thankful to the Indian Council of Agricultural Research, New Delhi, for the financial assistance extended for pursuing part of the study and to the Kerala Agricultural University for the facilities provided.

K.P. SREEKUMAR

CONTENTS

<u>Chapter</u>		<u>Page</u>
I	GENERAL INTRODUCTION	1
II	ESTIMATION OF BODY WEIGHT AND HEIGHT IN INDIAN ELEPHANTS	
	Introduction and Review	4
	Materials and Methods	9
	Results	12
	Discussion	19
III	SURFACE AREA AND BASAL HEAT PRODUCTION IN INDIAN ELEPHANTS	
	Introduction and Review	32
	Materials and Methods	36
	Results	41
	Discussion	60
IV	CERTAIN HAEMATOLOGICAL CHARACTERISTICS OF INDIAN ELEPHANTS	
	Introduction and Review	66
	Materials and Methods	77
	Results	79
	Discussion	83
V	SERUM PROTEINS IN INDIAN ELEPHANTS	
	Introduction and Review	92
	Materials and Methods	101
	Results	102
	Discussion	104

<u>Chapter</u>		<u>Page</u>
VI	ELECTROPHORETIC FRACTIONATION STUDIES IN INDIAN ELEPHANTS	
	Introduction and Review	109
	Materials and Methods	113
	Results	115
	Discussion	118
VII	NORMS FOR CERTAIN SERUM ENZYMES OF CLINICAL VALUE IN INDIAN ELEPHANTS	
	Introduction and Review	120
	Materials and Methods	129
	Results	130
	Discussion	132
VIII	MINERAL STATUS OF INDIAN ELEPHANTS	
	Introduction and Review	139
	Materials and Methods	148
	Results	149
	Discussion	152
	SUMMARY	160
	REFERENCES	164
	ANNEXURES	i-xxviii

LIST OF TABLES

<u>Table</u>	<u>Page</u>
II.1 Prediction equations for estimating body weight and height in Indian elephants	6
II.2 Values for body measurements and body weight of Indian elephants	13
II.3 Correlation matrix	14
II.4 Prediction equations for estimating body weight and height at shoulder in Indian elephants of all age groups	15
II.5 Estimated body weight using different models	22
II.6 Estimated body weight using formulae earlier reported	24
II.7 Estimated height at shoulder using model-4	28
II.8 Body weight of elephants estimated using the three models recommended as compared with actual weight recorded a year back	30
III.1 Measured body surface area and the areas of individual region	42
III.2 Correlation matrix	43
III.3 Prediction equations for computing total body surface area in Indian elephants	44
III.4 Measured body surface area and the body surface area estimated using the different models suggested	45
III.5 Prediction equations for body surface area in relation to body weight alone and to both body weight and height at shoulder	47
III.6 Measured and estimated surface area in Indian elephants	48
III.7 Body weight, measured surface area and the estimated heat production per kg body weight and per square meter body surface using Brody's formula	50

<u>Table</u>		<u>Page</u>
III.8	Computed heat production in Cal/day/kg body weight using the formulae recommended by other workers	55
III.9	Estimated metabolic rate in Cal/day/square metre body surface area using the reported formulae	57
IV.1	Reported values on haematological norms in elephants and other domestic animals	69
IV.2	Certain haematological norms in Indian elephants	80
IV.3	Erythrocyte sedimentation rate in Indian elephants	81
V.1	Reported values on serum/plasma protein levels in the blood of elephants and other domestic animals	94
V.2	Distribution of serum proteins and A/G ratio in Indian elephants	103
VII.1	Reported levels of serum enzymes in animals	123
VII.2	Activities of certain serum enzymes in Indian elephants	131
VIII.1	Reported values of certain major and trace elements in the sera of domesticated animals	142
VIII.2	Major minerals in the serum of Indian elephants	150
VIII.3	Trace elements in the serum of Indian elephants	151

LIST OF FIGURES

<u>Figure</u>		<u>Page</u>
II.1	Growth curve in Indian elephants	16
II.2	Growth rate expressed as percentage in Indian elephants	17
III.1	Relationship of body surface to body weight in Indian elephants	52
III.2	Basal metabolism per day per kg body weight of Indian elephants	53
III.3	Basal metabolism per day sq.m. surface area of Indian elephants	54
VI.1	Serum protein electropherogram (agar-gel)	116
VI.2	Serum lipoprotein electropherogram (agar-agarose gel)	116
VI.3	Electrophoretic variants of serum albumin (agar-gel)	117
VI.4	Haemoglobin electropherogram (agar-gel)	117

CHAPTER I

GENERAL INTRODUCTION

CHAPTER I

GENERAL INTRODUCTION

Elephants, the largest of the present day terrestrial mammals, are the sole living representatives of the order Proboscidea. The prehistoric elephants maintained their ascendancy by virtue of faunal prosperity till the Pleistocene era - period of about two and a half million years immediately preceding the emergence of modern man - at which time they reached the culmination of many millions of years of evolution. Their presence in wild life parks, sanctuaries, zoos, circuses, temple devaswoms and forest ranges often make us forget that they belong to an endangered species facing extinction. The increasing numbers of elephant hunts and incidences of poaching pose severe threat to their existence.

Of all the animals that have ever existed, the elephants rank among the most successful ones. As had been rightly stated by the famous British ecologist, Sir Frank Fraser Darling, "watching the elephants, one is conscious afresh that no other animal can occupy so many habitats and that the elephant is still the most thriving and resurgent species".

The success of elephants in a situation where failure exacts the supreme penalty of extinction is attributable to their high level of intelligence, efficiency in exploiting the habitats, adaptability, and the power of collective action from their well ordered social organisation. A sincere effort to provide a proper ecology for them in their natural habitat is also very essential for this. It is high time that organised efforts to breed the elephants in captivity, to explore the possibility of artificial insemination in elephants, and to rear them in wild life parks in large numbers and the like, are resorted to avoid the possibility of an early extinction of this valuable species. Such an approach necessarily demands availability of adequate baseline data on the physiology of Indian elephants.

The present study was undertaken with the following objectives:

1. Deriving prediction equations for estimating body weight and height from body linear measurements,

2. To find out the metabolic body weight and true total surface area in elephants,
3. To estimate total surface area from areas of parts of the body,
4. To study the correlation between body weight and surface area and to derive prediction equations for estimating surface area from height and/or weight,
5. To compute the basal heat production in elephants,
6. To determine norms for certain haematological parameters like specific gravity of whole blood and plasma, relative and absolute viscosity of whole blood, serum icterus index, pH of whole blood and plasma and coagulation time,
7. To find out the optimum time for determination of erythrocyte sedimentation rate to make it clinically important,
8. To estimate total proteins, albumin, globulin and albumin:globulin ratio in serum,
9. To fractionate serum proteins and lipoproteins by electrophoresis and to identify variants of albumin and haemoglobin,
10. To assay the activities of glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, lactic dehydrogenase and creatine phosphokinase in serum, and
11. To estimate the levels of sodium, potassium, calcium, magnesium, iron, copper, iron:copper ratio and zinc in serum.

CHAPTER II

**ESTIMATION OF BODY WEIGHT AND
HEIGHT IN INDIAN ELEPHANTS**

CHAPTER II

ESTIMATION OF BODY WEIGHT AND HEIGHT IN INDIAN ELEPHANTS

INTRODUCTION AND REVIEW

Knowledge on the body weight of an animal is of paramount importance in determining the metabolic activity in estimating the dietary needs and in calculating the dosage of drugs to be administered. Elephants are not an exception to this general rule. In metabolic studies, the mass and linear dimensions are essential for correlating the heat production and vital activity. Correct dosages of tranquilizers are essential for the successful control of elephants in musth. Since recording the actual weight with sensitive platform balance/ weigh-bridge is not feasible in many places where the elephants are normally housed, the practice is to estimate

the body weight by prediction equations. These equations have been derived using linear measurements as parameters. Benedict (1936) had commented that height and general massiveness, as judged by visual impression, are excellent indices of the size. Another reliable index of the size is the body weight. Nutritional status of the animal, age, sex, work load, physiological status and environment are some of the important factors that influence the body weight. Kurt and Nettasinghe (1968), Laws et al. (1975), Krishnamoorthy and Nair (1979) and Ananthasubramaniam et al. (1982) have presented prediction equations for estimating weight of elephants (Table II.1).

In the present study, prediction equations had been derived for estimating the body weight and height at shoulder of Indian elephants from linear measurements recorded from a sizeable number of elephants. With a view to recommend relatively simpler and safer methods, attempts were also made to find out better measures for recording the length and girth from among the different techniques, which are scientifically and factually acceptable.

Table II.1

Prediction equations for estimating body weight and height in Indian elephants

Number of elephants studied	Sex	Prediction equations		References
1	2	3		4
39	Male and Female	$Y = -60.6 + 28.9x$	$x =$ cube root of body weight in kg $y =$ chest girth in cm	Kurt and Nettasinghe (1968)
39	Male and Female	$y = -22.39 + 18.9x$	$x =$ cube root of body weight in kg $y =$ shoulder height in cm	Kurt and Nettasinghe (1968)
	Male	$w = 0.00507 h^{2.803}$	$w =$ body weight in kg $h =$ shoulder height in cm	Laws <u>et al.</u> (1975)
	Male	$w = 0.000306 h^{2.890}$	$w =$ body weight in kg $h =$ shoulder height in cm	Laws <u>et al.</u> (1975)
	Female	$w = 0.001267 h^{2.631}$	$w =$ body weight in kg $h =$ shoulder height in cm	Laws <u>et al.</u> (1975)
	Female	$w = 0.000258 h^{2.917}$	$w =$ body weight in kg $h =$ shoulder height in cm	Laws <u>et al.</u> (1975)

(continued)

Table II.1 continued

1	2	3	4
		$w = \frac{1.25 LG^2}{300}$	L = length between point of shoulder to point of buttocks (PS-PB) in inches G = chest girth in inches
20	Male and Female	$w = 23 g - 4984.$	g = chest girth in cm Ananthasubramaniam <u>et al.</u> (1982)
20	Male and Female	$w = 6.9 l + 20.7g - 5556.$	g = chest girth in cm l = body length (PS-PB) in cm Ananthasubramaniam <u>et al.</u> (1982)
20	Male and Female	$w = 8.2g + 18.4ng - 3927.$	g = chest girth in cm ng = neck girth in cm Ananthasubramaniam <u>et al.</u> (1982)
20	Male and Female	$w = 12.8(g+ng) - 4281.$	g = chest girth in cm ng = neck girth in cm Ananthasubramaniam <u>et al.</u> (1982)
20	Male and Female	$w = 10^{-4} \times 2.4313 l^{0.2} g^{2.6}$	l = body length between point of shoulder and point of buttocks (PS-PB) in cm g = chest girth in cm Ananthasubramaniam <u>et al.</u> (1982)

(continued)

Table II.1 continued

1	2	3	4
20	Male and Female	$w = 10^{-5} \times 12.0539 l g^2$	<p>l = body length between point of shoulder and point of buttocks (PS-PB) in cm</p> <p>g = chest girth in cm</p> <p>Ananthasubramaniam <u>et al.</u> (1982)</p>
37	Male and Female	HF = 2 FC	<p>HF = height in m</p> <p>FC = right foot circumference in m</p> <p>Benedict (1936)</p>

MATERIALS AND METHODS

The study was conducted on 36 Indian elephants - 8 baby elephants and 28 adults - varying in age from nine to fifty years, maintained by the Forest Department of the Government of Kerala in their elephant camps, by Guruvayoor Devaswom and by private owners. Excepting for two female baby elephants, the rest were all males. For studying growth rate, four more baby elephants varying in age from one year to seven years were employed. All the animals were clinically healthy and maintained under ideal nutritional regime. An elephant was considered as an adult when it attained the age of 15 years (Parkes, 1956).

Based on the height, the adult elephants were classified into three groups: Group I- (8' and above), Group II- (7' to 8') and Group III- (6' to 7').

The leaves of Caryota urens formed the bulk of the diet, supplying the roughage. The ration also included cooked rice, ragi, horse-gram, wheat, coconut, gingelly oil, salt and molasses. The schedule of ration in adult working elephants was as follows:

Group	Rice kg	Ragi kg	Horse-gram kg	Wheat kg	Fodder kg
I	4	8	5	4	400
II	3	6	4	4	350
III	2	5	3	2	300

They were fed daily at 0900 and 1700 hours. The animals were let loose to forage in the forest during night. The adult elephants were subjected to muscular work-hauling and piling of timber- during the day time. All the animals were weighed twice in the morning before feeding in a weigh-bridge (Avery - Sensitivity ± 10 kg) and the mean of the two weights was taken. The body measurements were then recorded, in duplicate, with a good tape measure (in cm), which withstood the rough handling without stretching, shrinking, curling or breaking and the mean was noted. Each elephant was made to stand squarely on all the four legs on a level ground with the head straight in an upright steady position. The height at withers was measured by keeping the tape fixed to the tip of a straight rod, provided with a spirit level, horizontally placed at the top of the shoulder. The distance between the rod and the ground was taken as the height at shoulder of the animal. The chest girth was measured by tightly encircling the tape around the body of the animal just behind the elbows. The neck girth was determined in the same way at the base of the neck in front of the shoulder. The body length was recorded in two ways - by noting the distance between the base of fore-head to the base of tail (BFH-BT) in one and in the other, the distance between the point of shoulder to the point of buttocks (PS-PB). The circumference of the right fore-foot was taken by using a thin thread wound round the foot in such a way as to cover

the nails at the lower portion and then measuring the thread. The correlation between the different parameters studied and the body weight as well as the relationship between the circumference of right fore-foot and height were then worked out in elephants varying in age from 9 to 50 years. The method of least squares (Snedecor and Cochran, 1967) was used to determine the various relations.

For studying the growth rate, the elephants were grouped into batches according to their age: 0-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30-35, 35-40, 40-45 and 45-50. This was necessitated due to the small sample size. The body weights of the animals in an age group were pooled and the mean was plotted against age to get the curve. Percentage of growth as well as body weight were plotted against age.

RESULTS

Mean values for the body measurements as well as the true body weight of the elephants, recorded in the present study, are given in table II.2. The raw data for these are given in annexure 1. The correlation matrix calculated from the data on body weight and body measurements is presented in table II.3. The body parameters in the decreasing order of their correlation with body weight were chest girth, length from base of fore-head to base of tail (body length), neck girth, height at shoulder, right fore-foot circumference, and length between point of shoulder and point of buttocks (body length). The parameters having a correlation of 0.90 and above with the body weight alone were used in deriving the prediction equations. Linear models were tried with single and possible meaningful combinations of the selected parameters. Though the multiple correlation coefficients were very high in most cases, few of the estimated figures in each case showed wide variations from the observed values. Hence, many non-linear models were tried and the better suitable ones were fitted. Table II.4 presents three prediction equations for body weight of the entire group of elephants (elephants varying in age from 9 to 50 years) (models 1,2,3) and another prediction equation for height (model-4) together with multiple correlation coefficients. Fig.II.1 represents the relationship between body weight and age. Fig.II.2 gives

Table II.2

Values for body measurements and body weight of Indian elephants

Parameter	Mean \pm S.E.
1. Height at shoulder (HF) (cm)	252 \pm 5.79
2. Length between base of fore-head to base of tail (BFH-BT) (cm)	326 \pm 7.42
3. Length between point of shoulder to point of buttocks (PB-PS) (cm)	192 \pm 7.35
4. Chest girth (CG) (cm)	367 \pm 8.49
5. Neck girth (NG) (cm)	242 \pm 2.59
6. Right fore-foot circumference (FC) (cm)	130 \pm 3.00
7. Body weight (W) (kg)	3352 \pm 201.61

Table II.3
Correlation matrix

Body weight (BW) (kg)	Length between point of shoulder to point of buttocks (body length) (PS-PB) (cm)	Right fore-foot circumference (FC) (cm)	Neck girth (NG) (cm)	Chest girth (CG) (cm)	Length between base of fore-head to base of tail (body length) (BFH-BT) (cm)	Height at shoulder (HT) (cm)
1	0.7946	0.9001	0.9228	0.9729	0.9288	0.9226
	1	0.8250	0.7086	0.7970	0.7420	0.7111
		1	0.8534	0.8821	0.8688	0.8982
			1	0.8849	0.8688	0.8603
				1	0.8859	0.8852
					1	0.8426
						1

Table II.4

Prediction equations for estimating body weight and height at shoulder in Indian elephants of all age groups (rounded to nearest figure)

Number of animals studied	Model No.	Prediction equations	Multiple correlation coefficient
36	1	$W = -1637.07 + 0.035(\text{BFH-BT}) \times \text{CG} + 3.072\text{HT}$	0.98
36	2	$W = -1442.23 + 0.036(\text{BFH-BT}) \times \text{CG} + 3.013\text{FC}$	0.98
36	3	$W = -1237.21 + 0.038(\text{BFH-BT}) \times \text{CG}$	0.98
36	4	$\text{HT} = 21.04 + 1.77 \text{FC}$	0.90

W = body weight in kg

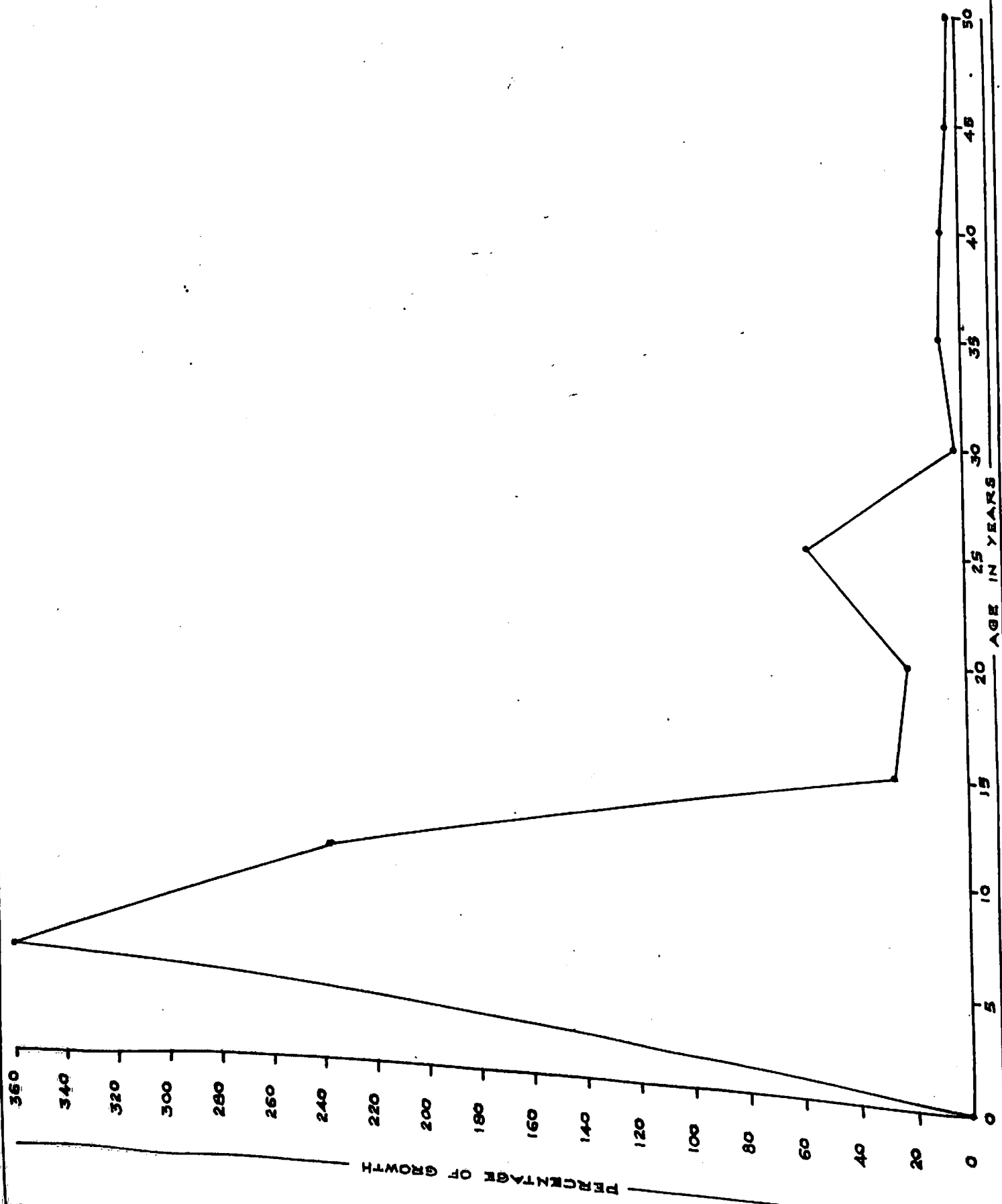
BFH-BT = length between base of fore-head to base of tail (body length) in cm

CG = chest girth in cm

HT = height at shoulder in cm

FC = right fore-foot circumference in cm

Fig. II.1: Growth curve of Indian elephants



1971

The following table shows the growth rate of Indian elephants in different parts of the country. The growth rate is expressed as a percentage of the total population of elephants in each part of the country.

**Fig. II.2: Growth rate expressed as percentage
in Indian elephants**

the relationship between percentage growth rate and age. Annexure-2 presents the raw data on weight and age of the 40 elephants studied.

DISCUSSION

From the correlation matrix (Table II.3), it was inferred that among the different parameters studied influencing the body weight, chest girth (CG) was found to be the best ($r=0.97$) followed by, in descending order of importance, the body length from base of fore-head to base of tail (BFH-BT) ($r=0.93$), neck girth (NG) ($r=0.92$), height at shoulder (HT) ($r=0.92$), right fore-foot circumference (FC) ($r=0.90$) and the body length between point of shoulder and point of buttocks (PS-PB) ($r=0.79$). Ananthasubramaniam et al. (1982) reported the correlation of the different parameters studied with body weight, in the decreasing order of importance, as neck girth ($r=0.98$), chest girth ($r=0.97$), shoulder height ($r=0.96$) and the length between point of shoulder and point of buttocks ($r=0.78$). Ananthasubramaniam et al. (1982) considered the neck girth as a more better measure of body weight compared to that of the chest girth. In the present study, though girth was taken in two forms (chest girth and neck girth), the data gathered revealed that the correlation of chest girth to body weight was higher ($r=0.97$) than that given by neck girth ($r=0.92$). This is suggestive of the better reliability of chest girth over neck girth to predict the body weight of elephants. The findings of the present study, with more number of elephants, failed to substantiate the view expressed by Ananthasubramaniam et al. (1982). They also reported that the relationship

between body length (point of shoulder to point of buttocks) and body weight was good ($r=0.78$). But, in the present study, the body length between the base of fore-head to base of tail was found to have a better relationship ($r=0.93$) with body weight than the length taken between point of shoulder and point of buttocks ($r=0.79$). This is indicative of a greater linear growth between the fore-head and base of tail than the point of shoulder and point of buttocks. Moreover, measurement of length between the point of shoulder and point of buttocks is highly susceptible for errors since a slight movement of the animal or a shift in its stance will move these points. From the point of view of convenience of measuring, reliability and high correlation, it, thus, appears that the length between base of fore-head to base of tail (BFH-BT) can be chosen over the conventional length between point of shoulders and point of buttocks (PS-PB) for predicting weight in elephants. Laws et al. (1975) contended that height is not subjected to rapid seasonal fluctuations and hence, could be used to characterise the body weight of elephants. In the present study, equations were fitted in, both by incorporating height at shoulder and by excluding it. No difference in the coefficients of variations or in the multiple correlation coefficients could be detected, indicating that height at shoulder is not a superb parameter compared to the other ones tested. The inclusion of foot circumference instead of height at shoulder as a parameter also did not

give any change in the coefficient of variation nor in the multiple correlation coefficient. The foot circumference was tried since it is a parameter that could be easily measured from the foot print of the animal. The three models (1,2,3) fitted in the present study for prediction of body weight in elephants aged nine years and above explained 98% of the variation in the original body weight, and the coefficients of variations of the estimated values were very close to the observed values and hence, all the three models could be equally applied for predicting the body weight. Table II.5 gives the observed body weight and the estimated values using the different models.

The measurement of height is easily subject to error, since a slight change in posture greatly affects the measurements. Availability of suitable rods, with spirit level, at remote areas is also a problem. Besides the aim is to predict the body weight with precision, minimum possible effort and body parameters, adopting easy steps in calculation. It appears that the prediction equation given as model-3 could be preferred over the other two (model 1 and 2) in elephants aged 9 years and above. Table II.6 gives the observed body weight of elephants used in this study as well as the body weights calculated by applying the formulae recommended by Kurt and Nettasinghe (1968), Krishnamoorthy and Nair (1979) and Ananthasubramaniam et al. (1982).

Table II.5

Estimated body weights(kg) using different models (rounded to nearest kilogrammes)

Sl. No.	Observed body weight (kg)	Model-1 (kg)	Model-2 (kg)	Model-3 (kg)
1	2	3	4	5
1	2080	2116	2165	2209
2	1615	1582	1587	1607
3	1940	1927	1970	1952
4	1750	1724	1746	1734
5	2000	1829	1854	1869
6	2170	2465	2527	2461
7	2240	2271	2247	2263
8	1780	1749	1732	1742
9	2290	2575	2588	2588
10	1740	1827	1781	1784
11	2060	1959	1949	1930
12	1630	1577	1549	1558
13	2890	2982	3018	3004
14	3445	3291	3296	3278
15	1755	1743	1707	1732
16	3870	3682	3631	3628
17	3625	3629	3621	3585
18	3725	3840	3832	3862
19	4130	4041	4147	4175

(continued)

Table II.5 continued

1	2	3	4	5
20	3500	3219	3234	3229
21	4950	4868	4936	4912
22	3710	3888	3883	3890
23	3300	3261	3280	3261
24	4120	4390	4385	4385
25	4390	4368	4345	4334
26	3430	3503	3418	3424
27	4430	4583	4586	4638
28	3760	3808	3761	3753
29	4660	4523	4505	4485
30	4720	4393	4357	4330
31	4510	4905	4863	4868
32	4960	4988	5002	5009
33	4420	4570	4573	4562
34	4885	4756	4708	4746
35	5250	5068	5086	5100
36	4950	4782	4811	4794
Mean	3352	3352	3352	3352
\pm S.E.	\pm 201.61	\pm 199.78	\pm 199.71	\pm 199.65
Coefficient of variation	36.08	35.76	35.74	35.72

Table II.6

Estimated body weights (kg) using formulae earlier reported (rounded to nearest kilogrammes)

Observed body weight (kg)	Kurt and Nettesinghe (1968) $(11k + 22.39)^3$ 18.9 (kg)	Kurt and Nettesinghe (1968) $(g + 60.6)^3$ 28.9 (kg)	Krishnacorthy and Nair (1979) $\frac{1.25 Lg^2}{300}$ (kg)	Ananthasubramanian et al. (1982) 23 g=4984 (kg)	Ananthasubramanian et al. (1982) $6.9 L + 20.7 g$ -5556 (kg)	Ananthasubramanian et al. (1982) 8.2 g + 18.4 ng -3927 (kg)	Ananthasubramanian et al. (1982) 12.8 (g+ng)-4281 (kg)	Ananthasubramanian et al. (1982) $10^{-4} \times 2.4313 \times 10.2 \times g^{2.6}$ (kg)	Ananthasubramanian et al. (1982) $10^{-5} \times 12.0539 Lg^2$ (kg)
1	2	3	4	5	6	7	8	9	10
2080	1460	2195	1823	2261	2034	2060	2119	2086	1854
1615	1586	1785	1595	1686	1551	1708	1697	1693	1622
1940	1788	2143	1846	2192	2006	2495	2401	2067	1877
1750	1765	1991	1413	1985	1606	2090	2055	1818	1428
2000	1629	2025	1708	2031	1827	1978	1991	1919	1738
2170	2381	2604	3096	2767	3007	2608	2657	2691	3149
2240	2270	2284	2002	2376	2207	2377	2375	2201	2037
1780	1907	1910	1389	1870	1523	1976	1940	1749	1413
2290	2325	2624	2437	2790	2683	2893	2861	2582	2479
1740	2240	1695	1243	1548	1220	1677	1633	1538	1264
2060	2216	2431	1607	2560	1413	2627	2605	2224	1634
1630	1883	1710	1492	1571	1413	1924	1812	1608	1518

(continued)

Table II.6 continued

1	2	3	4	5	6	7	8	9	10
2890	2553	2785	2057	2974	2607	3161	3105	2628	2098
3445	3161	3544	3680	3756	3794	3789	3783	3628	3742
1755	1932	1895	1317	1847	1461	2004	1953	1718	1340
3870	3991	3665	3795	3871	3904	4106	4039	3758	3859
3625	3818	3448	3871	3664	3814	3572	3604	3578	3920
3725	3300	3867	3057	4055	3731	3896	3949	3764	3109
4130	2410	4657	4214	4722	4697	4446	4538	4762	4723
3500	2864	3308	2596	3526	3207	3376	3425	3191	2640
4950	4096	4862	5649	4883	5111	5166	5089	5163	5746
3710	3561	3892	4379	4078	4242	3922	3975	4068	4453
3300	2994	2995	3229	3204	3296	3482	3399	3061	3284
4120	4177	4429	4672	4538	4601	4436	4474	4595	4752
4370	4516	4263	3157	4400	3994	4515	4487	4115	3210
3430	4096	3308	2661	3526	3235	3431	3463	3206	2706
4430	3665	4628	5042	4699	4815	6241	5780	4842	5127
3760	4136	3496	2879	3710	3442	3533	3591	3414	2928
4660	4740	4318	4689	4446	4553	4955	4807	4501	4762
4720	4917	4318	4722	4446	4566	4753	4666	4508	4802
4510	5108	4457	4797	4561	4656	4426	4474	4643	4879
4960	4516	5845	6520	5596	5760	3893	4423	6194	6632
4420	4408	4318	4582	4446	4518	4495	4487	4481	4660

(continued)

Table II.6 continued

	1	2	3	4	5	6	7	8	9	10
	4885	4647	5104	4300	5067	4801	5489	5370	5091	4374
	5250	4472	4599	5227	4676	4863	5037	4935	4850	5316
	4950	4387	4318	5080	4446	4691	4587	4551	4501	5167
Mean	3352	3220	3281	3273	3491	3357	3531	3513	3399	3340
\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm
S.E.	201.61	191.76	191.33	244.98	198.99	222.26	201.80	197.62	212.96	250.78
Coefficient of variation	36.08	35.73	33.95	44.91	34.20	39.73	34.29	33.75	37.59	45.05

Height at shoulder was found to have a linear relationship with circumference of foot and a prediction equation (model-4) had been worked out. Benedict (1936) and Kurt and Nettasinghe (1968) estimated the height at shoulder using right fore-foot circumference and observed it to be approximately twice the circumference of the foot. In the present study also height was found to be approximately twice to circumference of the right fore-foot. Table II.7 gives the observed and estimated height at shoulder in elephants.

In several places due to lack of platform balance/ weigh-bridge, weight of the elephant is not regularly taken. Using the prediction equations arrived at in this study, the body weights of 21 adult elephants were estimated from the body measurements and the same was found to be in reasonable agreement with the weights recorded a year back (Table II.8).

The body weights (kg) recorded in the present study (Annexure 2) were plotted against age (in years) and presented in Fig.II.1. The percentage of growth was plotted against age and presented in Fig.II.2. It can be seen from the graphs that the percentage growth rate was high upto 20 to 25 years in the elephants studied. The data further revealed that even after attaining maturity, the elephants recorded a moderate rise in body weight.

Table II.7

Estimated height at shoulder (cm) using model-4

Sl.No.	Observed (cm)	Estimated (cm)
1	192	198
2	198	198
3	207	228
4	206	220
5	200	207
6	230	269
7	226	216
8	212	207
9	228	234
10	225	212
11	224	228
12	211	204
13	236	251
14	255	260
15	213	198
16	277	260
17	273	278
18	259	246
19	231	255
20	246	251

(continued)

Table II.7 continued

Sl.No.	Observed (cm)	Estimated (cm)
21	280	303
22	266	260
23	250	260
24	282	276
25	290	287
26	280	250
27	264	251
28	281	266
29	295	290
30	299	290
31	305	285
32	290	282
33	287	287
34	293	262
35	289	285
36	287	296
Mean	252	251
\pm S.E.	\pm 5.79	\pm 5.31

Table II.8

Body weight of elephants estimated using the three models recommended as compared with actual weight recorded a year back (rounded to nearest kilogrammes)

Sl. No.	Actual weight recorded a year back (kg)	Model-1	Model-2	Model-3
1	2	3	4	5
1	3330	3288	3294	3323
2	4275	4275	4195	4264
3	2890	2921	2836	2897
4	3600	3531	3478	3543
5	3200	3064	3007	3093
6	2500	2562	2543	2587
7	2475	2498	2376	2482
8	2998	2921	2911	2945
9	2990	2842	2886	2886
10	4670	4564	4560	4605
11	4668	4484	4483	4508
12	3400	3389	3399	3403
13	4460	4491	4443	4483
14	4350	4275	4290	4321
15	2900	2757	2783	2810

(continued)

Table II.8 continued

1	2	3	4	5
16	3200	3112	3138	3152
17	4250	4206	4206	4216
18	3400	3395	3384	3403
19	3880	3905	3909	3950
20	3700	3627	3602	3674
21	2010	2027	1950	2007
Mean	3485.52	3434.95	3413.00	3454.86
\pm S.E.	\pm 165.45	\pm 161.78	\pm 164.06	\pm 162.76

NATIONAL BUREAU OF
 GEOPHYSICAL RESEARCH
 WASHINGTON, D. C.

CHAPTER III

**SURFACE AREA AND BASAL HEAT
PRODUCTION IN INDIAN ELEPHANTS**

CHAPTER III

SURFACE AREA AND BASAL HEAT PRODUCTION IN INDIAN ELEPHANTS

INTRODUCTION AND REVIEW

Basal metabolic rate refers to the heat production during complete physical and mental rest in a thermally neutral environment in the post-absorptive condition. The energy utilized for executing the work involved in circulation, respiration, secretion, excretion, muscle tonus, homeothermy and maintenance of the thermodynamically unstable living state is the causative factor for this heat production. Basal energy metabolism is a convenient baseline for measuring various energy increments, such as heat increments associated with muscular work, feeding, lactation, gestation and regulation of body temperature. Since such a basal state cannot be attained in animals with ease even with training,

measurement of resting energy metabolism is often resorted to.

The basal metabolic rate per unit body weight decreases rapidly with an increase in body weight in homeotherms. It is well known that the basal metabolic rate, per unit body weight, is greater in small animals than that in large animals, obviously due to the greater surface area of small animals in relation to the body weight. The size of the internal organs and tissues tend to vary more nearly with surface than with body weight. As the animal increases in size, the pull of gravity increases directly with body weight, whereas the strength of the supporting structures such as the legs tends to increase with two-third power of body weight. Hence, to retain stability the supporting structures grow more rapidly than the visceral organs, approximately in proportion to surface area. The surface area is estimated in living animals by formulae or by means of surface integrator and in dead animals by removing the skin and determining its area after cutting it into pieces. However, closely agreeable results are seldom possible with these three approaches in the same animal and it is well established that the results vary quite considerably. According to Meeh's (1879) formula, surface area in square metres (S) = $k \times W^{2/3}$, where 'W' is the weight in kg and 'k' a constant which varies from animal to animal (from 0.085 in guinea pig to 0.123 in man). Benedict (1936) suggested that 'k' be assigned a uniform value of 0.10

for all species. Another formula available for use in the human being is the one suggested by DuBois (1936):

$A = 71.84 W^{0.425} H^{0.725}$, where 'A' is the surface area in square centimetres, 'W' is the body weight in kg and 'H' the height in centimetres. Brody (1945) suggested the formula, $A = 0.15 W^{0.56}$, where 'A' is the surface area in square metres and 'W' the body weight in kilogrammes.

It is true that surface area varies with two-third power of body weight in geometrically similar bodies of constant specific gravity. Animals, irrespective of whether they are small or large, young or old, fat or thin, are not all geometrically similar and not of constant specific gravity. Hence, Brody (1945) favoured the determination of the exponent for W, based on actual data, to adopting $W^{2/3}$ as a general rule.

Only very few studies have been conducted on the basal heat production in elephants. It will be academically highly interesting to study the resting heat production in elephants - the largest living land mammal. Benedict (1936) reported a high heat production per square metre of surface area in Indian elephants. Brody (1945) preferred calculation of basal metabolic rate using body weight raised to some power, the true value to be determined from actual metabolism data. He suggested the formula: $Q = 70.5 M^{0.734}$, where 'Q' is the Calories of heat produced per day and 'M' the body weight in

kilogrammes. Kleiber (1932) came out with another formula for calculating the metabolic rate: Calories per day = $70 W^{3/4}$, where 'W' is the body weight in kilogrammes. The formula $Q = 142 M^{0.61}$, where 'Q' is the Calories of heat produced per day and 'M' the body weight in kg was introduced by Rubner (1883). Lee (1939) recommended a similar formula $Q = 57.5 M^{0.82}$, where 'M' is the body weight in kg and 'Q' the Calories of heat produced per day. The DuBois (1936) formula gave the relationship as $Q = 111 M^{0.57}$, where 'Q' is the basal heat production per day in Calories and 'M' the body weight in kilogrammes. Brody (1945) reported that the maintenance of digestible energy (DE) and of total digestible nutrients (TDN) were double the basal energy metabolism.

In the present study the true surface area had been measured in Indian elephants. Prediction equations for estimating surface area from areas of parts of body and from weight and height at shoulder had been made. The basal heat production per day in Indian elephants was also computed.

MATERIALS AND METHODS

The mean body weight and height at shoulder of 22 Indian elephants was recorded, based on two observations, as detailed out in chapter II. Attempts to derive equations for computing surface area of Indian elephants were made using the actually measured surface area, body weight and height at shoulder. The models attempted were $S = a \times W^b$ and $S = a \times W^b \times H^c$, where 'a' is a constant, 'W' is the body weight in kg, 'b' and 'c' the exponents and H, the height at shoulder in metres.

The body of elephant was roughly divided into different geometrical figures and the area in sq.m. was calculated individually for these regions. The summing up of the areas of these individual regions gave the total body surface area in square metres.

All body measurements were taken in duplicate as laid out in chapter II and the mean value was recorded.

The trunk of the elephant was considered as a cylinder. The length from the central point of a transverse line, connecting the base of the two tusks, to the tip of the trunk, was taken as the length of the trunk. The circumference was taken at the base, body and tip of the trunk and the mean value was taken as the circumference of the trunk.

The face of the animal was considered as a rectangle. The straight line connecting the two fronto-lateral angles

of the fore-head was taken as one side of the rectangle. The other sides were the straight lines drawn downwards connecting the fronto-lateral angles of the fore-head to the transverse line connecting the base of the tusk.

The ear of the elephant resembled a triangle. The length extending from the rostral angle of the attached border to the caudal angle of the ear was taken as the base of the triangle. The rostral border (attached border) and caudal border joined at the free end making a distal angle (apex). The altitude of the triangle was taken as the straight line length between the rostral angle and distal angle of the ear.

The neck was divided into three regions namely, rostral, middle and caudal. The circumference of the rostral part was taken at the level around the neck just at the angle of the mandible. The circumference of the middle part was taken about the level of the fourth cervical vertebra. The caudal part was measured at the base of the neck in front of the shoulder. The mean of these three values was taken as the circumference of the neck. The length of the neck was taken as the length between the rostral transverse line (just behind the mandible) and the caudal transverse line (just in front of the shoulder). Body girth was measured at three levels - anterior, middle and posterior regions. The anterior body girth was taken at the anterior part of the chest behind a transverse line (anterior transverse line) connecting the external angle of scapula to the olecranon process. The middle

body girth was taken at the level of the last rib and the posterior body girth was taken at the region in front of the line (posterior transverse line) connecting the rostral end of the external angle of the ilium to the flap of the skin ventrally (connecting the stifle to the belly). Body girth was taken as the mean of these three values. The length of the body was taken as the distance between the anterior and posterior transverse lines.

The tail gave the geometrical appearance of a cylinder. The circumference of the tail was taken in three regions viz., base, body and tip of the tail. The mean of these gave the circumference of the tail. The straight line length from the base of the tail to the tip constituted the length of the tail.

The limbs were classified as cylindrical figures. The height of the fore-limb was the distance from the dorsal vertebral border of the scapula down to the ground level. The height of the hind-limb was the distance from the middle of the line connecting the external angle of the ilium and tuber ischii to the ground level. The circumference of the fore-limb was taken at the level of the shoulder joint, below the elbow joint, below the carpal joint and at the base of foot. The mean values was taken as the circumference. In the case of the hind-limb, the circumference was recorded at the level of the hip joint, below the stifle joint, below

the hock and at the base of foot. The circumference noted was the average of these three readings.

The perineal area was taken as the triangle extending from the lower part of the anal opening down to the loose flap of skin lying between the thighs. The upper border is the transverse line connecting the tuber ischii. Two lines drawn from the two tuber ischii were connected at the apex so as to include the loose flap of skin in the perineal area. The vertical length from the central point of the transverse line to the apex of the loose skin was taken as the altitude of the triangle.

The areas of the individual geometrical regions were calculated using the following formulae:

1. Area of a cylinder = $2 \pi r (h+r)$,

where 'r' is the radius and 'h' the height.

2. Area of a triangle = $1/2 ab$, where 'a' is the altitude and 'b' the base.

3. Area of a rectangle = $l \times b$, where 'l' is the length and 'b' the breadth.

The areas of fore-limb, hind-limb and ear were recorded on only one side and so the values were doubled while computing the total surface area.

The relationships between the body weight and surface area, and body weight and height at shoulder and surface area were worked out using least squares method of analysis

(Snedecor and Cochran, 1967). Plotted the relationship between the body weight and the measured surface area. The line of best fit was drawn through the points for estimated surface area using the prediction equation employing body weight. The possibility of predicting total surface area from the areas for individual geometric regions was worked out using method of least squares (Snedecor and Cochran, 1967). The individual areas having a correlation more than $r=0.80$ were alone considered for the purpose.

The total basal heat production per day was calculated by using Brody's formula ($Q=70.5 M^{0.734}$). The heat production in Calories per day per kg body weight and Calories per day per square metre surface area were then calculated. The estimated values were then compared with the results obtained by using the formulae of other workers. The relationship between heat production per day per sq.m. of surface area and surface area was plotted out. The relationship between Calories of heat production per day per kg body weight and body weight was also plotted.

RESULTS

The mean values for the measured total body surface area and the areas of individual regions are given in table III.1.

The correlation between the measured total body surface area and the measured surface area of individual regions is given in table III.2. The highest correlation ($r=0.90$) was with the area of the fore-limb. All possible combinations were tried, using the highly correlated areas, to establish prediction equations. Table III.3 gives the best possible prediction equations to determine the total body surface area from the areas of regions. The measured surface area and the surface area estimated by the different models suggested are presented in table III.4.

Table III.5 gives prediction equations for estimating surface area from body weight, and surface area from both body weight and the height at shoulder. Between the two prediction equations suggested, the one incorporating the body weight and height at shoulder was considered more reliable ($R^2=0.87$) and reasonable. Table III.6 presents the data on the measured surface area, surface area estimated with the two prediction equations suggested and the calculated surface area using the conventional Benedict's modification of Meeh's formula.

Table III.1

Measured body surface area and the areas of individual regions (m²)

Parameters	Mean \pm S.E.
1. Measured surface area (m ²)	20.09 \pm 1.01
2. Body area (BA) (m ²)	6.28 \pm 0.32
3. Neck area (NA) (m ²)	1.41 \pm 0.15
4. Hind-limb area (HLA) (m ²)	4.70 \pm 0.25
5. Fore-limb area (FLA) (m ²)	5.06 \pm 0.32
6. Trunk area (TA) (m ²)	1.03 \pm 0.07
7. Tail area (TLA) (m ²)	0.46 \pm 0.07
8. Fore-head area (FHA) (m ²)	0.61 \pm 0.05
9. Ear area (EA) (m ²)	0.42 \pm 0.03
10. Perineal area (PA) (m ²)	0.13 \pm 0.02

Table III.2
Correlation matrix

Total body surface area m^2	Body area (BA) m^2	Neck area (NA) m^2	Hind-limb area (HLA) m^2	Fore-limb area (FLA) m^2	Trunk area (TA) m^2	Tail area (TLA) m^2	Fore-head area (FH) m^2	Ear area (EA) m^2	Perineal area (PA) m^2
1	0.8394	0.8366	0.8790	0.9018	0.7166	0.01249	0.5611	0.6702	0.3077
	1	0.8118	0.5779	0.5950	0.5430	-0.0338	0.4113	0.6039	0.1077
		1	0.5667	0.6968	0.5238	0.1078	0.3761	0.4260	0.1388
			1	0.8498	0.6255	-0.1028	0.4973	0.6273	0.4087
				1	0.5880	-0.0917	0.4564	0.4574	0.4744
					1	0.0365	0.4804	0.6709	0.06605
						1	-0.1250	0.0244	-0.1442
							1	0.5888	-0.00038
								1	-0.1187
									1

Table III.3

Prediction equations for computing total body surface area in Indian elephants

Model No.	Prediction equations	Multiple correlation coefficient
1	$S = (1.3712 BA + 1.772 HLA + 1.2147 FLA) - 0.2304$	0.98
2	$S = (1.4742 BA + 1.9236 FLA) + 1.0472$	0.96
3	$S = (1.5643 BA + 2.3773 HLA) - 0.9043$	0.94
4	$S = (3.3995 NA + 2.3976 HLA) + 4.0187$	0.94

S = total body surface area in m^2
 BA = body area in m^2
 NA = neck area in m^2
 FLA = fore-limb area in m^2
 HLA = hind-limb area in m^2

Table III.4

Measured body surface area and the body surface area (m^2) estimated using the different models suggested

	Measured body surface area (m^2)	Model-1 (m^2)	Model-2 (m^2)	Model-3 (m^2)	Model-4 (m^2)
1	2	3	4	5	6
1	20.48	20.41	21.16	19.73	19.18
2	17.97	19.04	17.94	21.47	16.06
3	15.31	15.74	15.60	15.88	16.90
4	15.32	15.93	16.01	16.02	16.15
5	18.40	18.50	18.92	18.27	17.34
6	21.11	20.95	21.14	20.50	21.56
7	20.83	20.11	18.32	21.89	22.74
8	20.30	20.33	20.55	19.66	20.48
9	16.47	17.04	17.43	16.41	16.93
10	28.82	28.05	27.91	26.41	27.36
11	25.39	26.02	26.74	24.57	23.15
12	31.47	31.64	31.47	30.91	31.68
13	12.2	13.07	13.97	12.68	12.07
14	13.02	11.45	12.52	11.11	12.34

(continued)

Table III.4 continued

	1	2	3	4	5	6
15	18.25	17.35	17.36	17.75	18.48	
16	21.76	21.64	21.06	21.82	22.49	
17	26.44	26.11	24.89	27.59	26.58	
18	20.90	21.89	21.62	21.61	20.92	
19	18.64	18.94	18.30	19.35	20.60	
20	17.84	17.95	17.95	18.15	18.60	
21	22.08	20.89	20.98	21.38	21.08	
22	18.95	18.91	19.10	18.81	19.25	
Mean	20.09	20.09	20.04	20.09	20.09	
\pm S.E.	\pm 1.01	\pm 1.00	\pm 0.96	\pm 0.98	\pm 0.98	\pm 0.98
Coefficient of variation	23.64	23.38	22.53	22.89	22.94	

Table III.5

Prediction equations for body surface area in relation to body weight alone and to both body weight and height at shoulder

Number of animals	Model No.	Formulae	Multiple correlation coefficient
22	5	$S = 0.1025 W^{0.6688}$	0.78
22	6	$S = 0.2533 W^{0.5153} H^{0.3920}$	0.87

where, S = total body surface area in square metres

W = body weight in kg

H = height at shoulder in metres

Table III.6

Measured and estimated surface area in Indian elephants

Sl. No.	Measured surface area (m ²)	Model-5	Model-6	Benedict's modification of Meeh's formula $S = 0.1 W^{2/3}$ (m ²)
1	2	3	4	5
1	20.48	21.81	22.38	20.92
2	17.97	18.63	16.35	17.88
3	15.31	15.92	16.22	15.29
4	15.32	13.76	14.35	13.22
5	18.40	17.08	18.06	16.40
6	21.11	21.28	21.42	20.41
7	20.83	20.46	21.64	19.68
8	20.30	21.01	21.91	20.15
9	16.47	16.20	17.06	15.55
10	28.82	25.45	27.28	24.30
11	25.39	20.90	27.52	25.78
12	31.47	22.17	23.54	21.26
13	12.20	12.40	12.69	11.91
14	13.02	13.98	12.75	13.42

(continued)

Table III.6 continued

1	2	3	4	5
15	18.25	20.41	18.43	19.58
16	21.76	19.71	20.70	18.91
17	26.44	26.07	26.98	24.99
18	20.90	20.91	21.83	20.05
19	18.64	18.58	19.29	17.83
20	17.84	20.67	18.78	19.85
21	22.08	23.26	20.61	22.29
22	18.95	22.36	20.21	21.44
Mean	20.99	19.96	20.00	19.15
\pm S.E.	\pm 1.01	\pm 0.83	\pm 0.90	\pm 1.25
Coefficient of variation	23.64	19.53	21.05	19.46

Table III.7

Body weight, measured surface area and the estimated heat production per kg body weight and per square metre body surface using Brody's formula

Sl. No.	Body weight (kg)	Measured body surface area (m ²)	Estimated heat production Calories/day/kg body weight	Estimated heat production Calories/day/square metre surface area
1	2	3	4	5
1	3025	20.48	8.36	1235.13
2	2390	17.97	8.90	1184.09
3	1890	15.31	9.48	1169.87
4	1520	15.32	10.04	996.28
5	2100	18.40	9.21	1051.67
6	2915	21.11	8.44	1166.13
7	2750	20.83	8.58	1132.32
8	2860	20.30	8.49	1195.82
9	1940	16.47	9.41	1108.52
10	3810	28.82	7.86	1039.67
11	4140	25.39	7.69	1254.31
12	3100	31.47	8.31	818.37
13	1300	12.20	10.46	1115.49
14	1555	13.02	9.98	1192.09

(continued)

Table III.7 continued

1	2	3	4	5
15	2740	18.25	8.56	1288.95
16	2600	21.76	8.71	1040.21
17	3950	26.44	7.79	1163.67
18	2840	20.90	8.50	1155.52
19	2380	18.64	8.91	1138.02
20	2790	17.84	8.54	1336.10
21	3330	22.08	8.15	1229.28
22	3140	19.95	8.28	1372.04
Mean	2683.86	20.09	8.76	1153.80
\pm	\pm	\pm	\pm	\pm
S.E.	163.08	1.01	0.16	25.66
Coefficient of variation	28.50	23.64	6.42	10.43

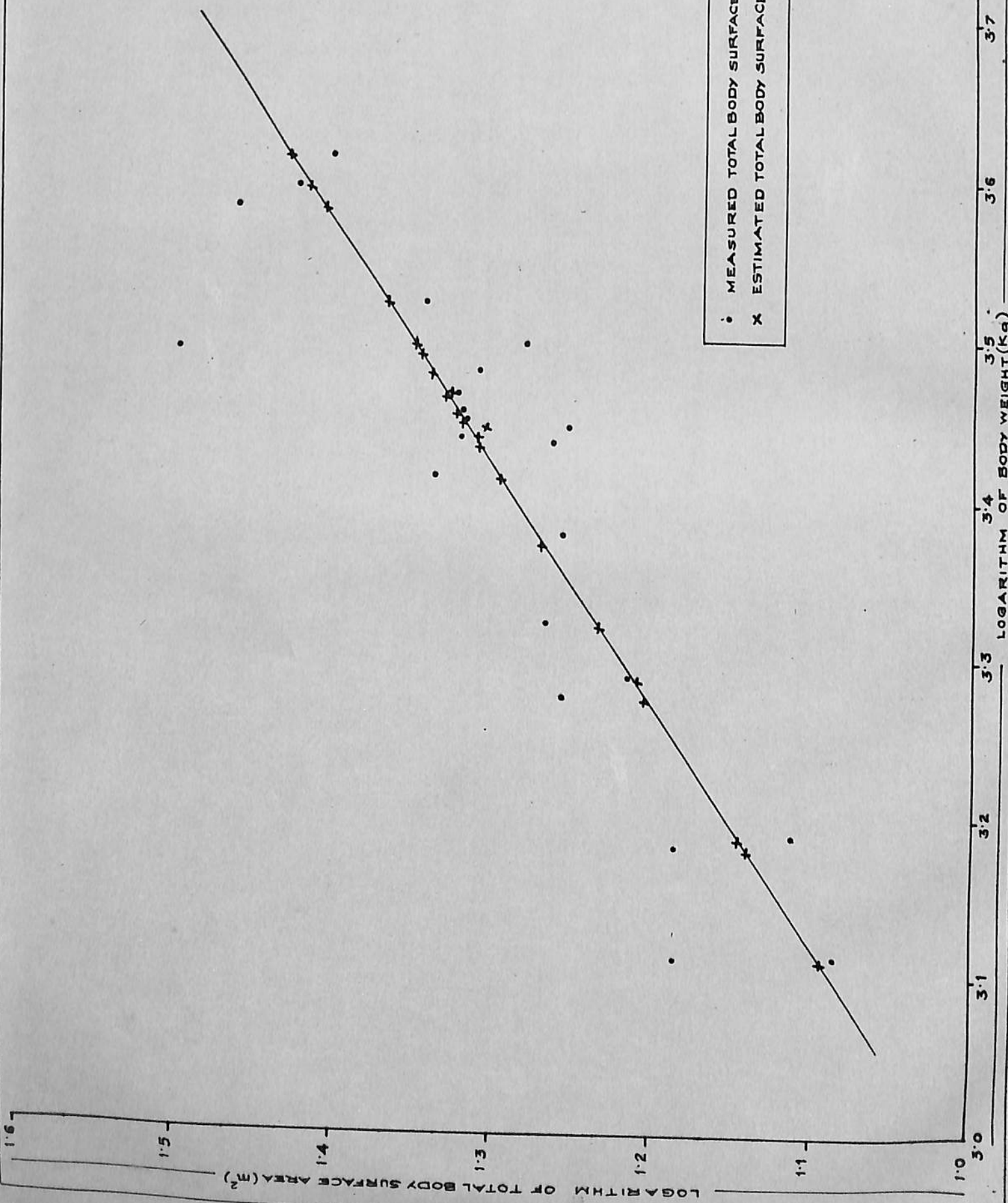


Fig. III.1: Relationship of body surface to body weight in Indian elephants

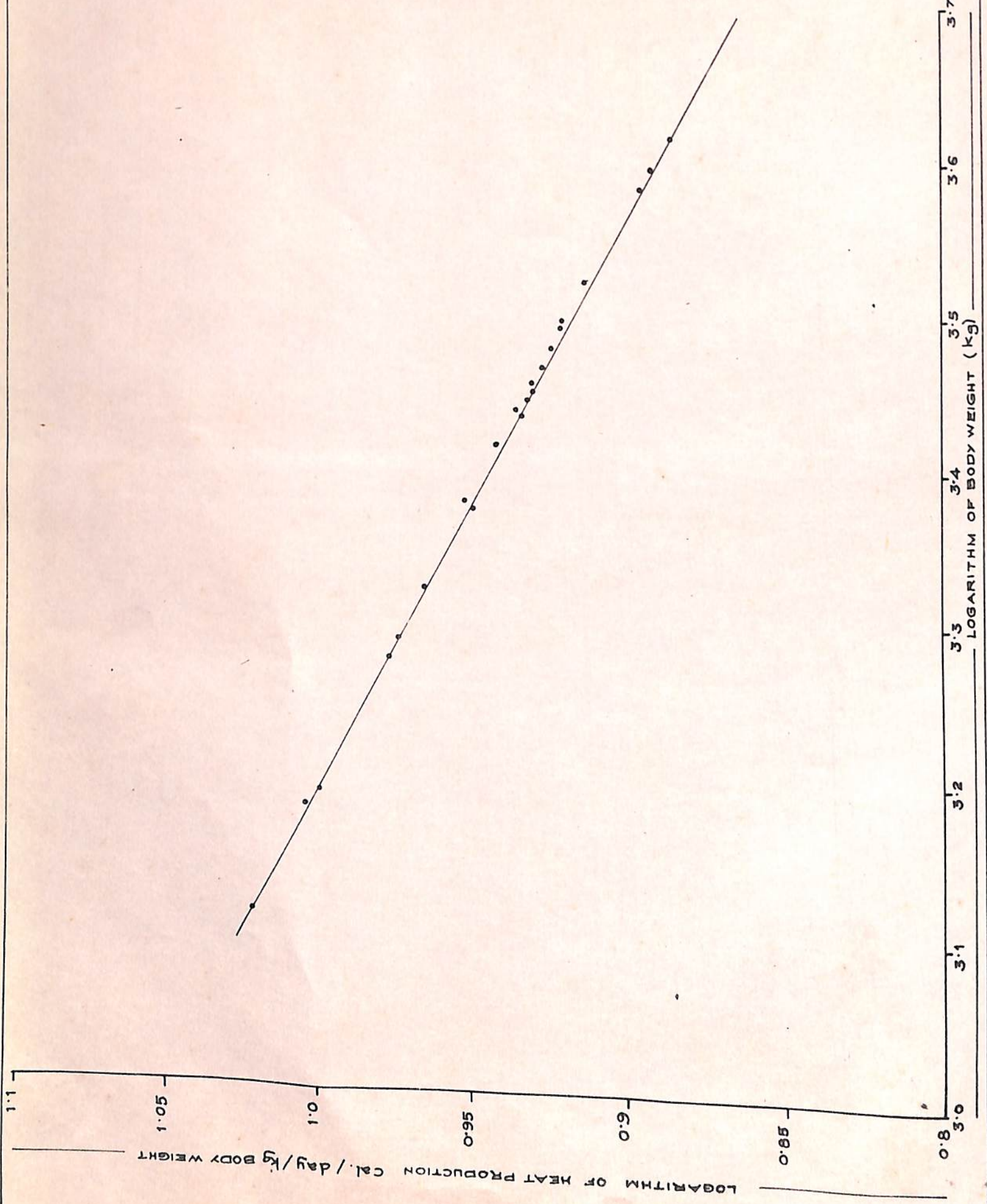


Fig. III.2: Basal metabolism per day per kg body weight of Indian elephants

3.2

3.1

3.0

2.9

2.8

LOGARITHM OF HEAT PRODUCTION Cal./day/m²

1.0

1.1

1.2

1.3

1.4

1.5

1.6

LOGARITHM OF TOTAL BODY SURFACE AREA (m²)

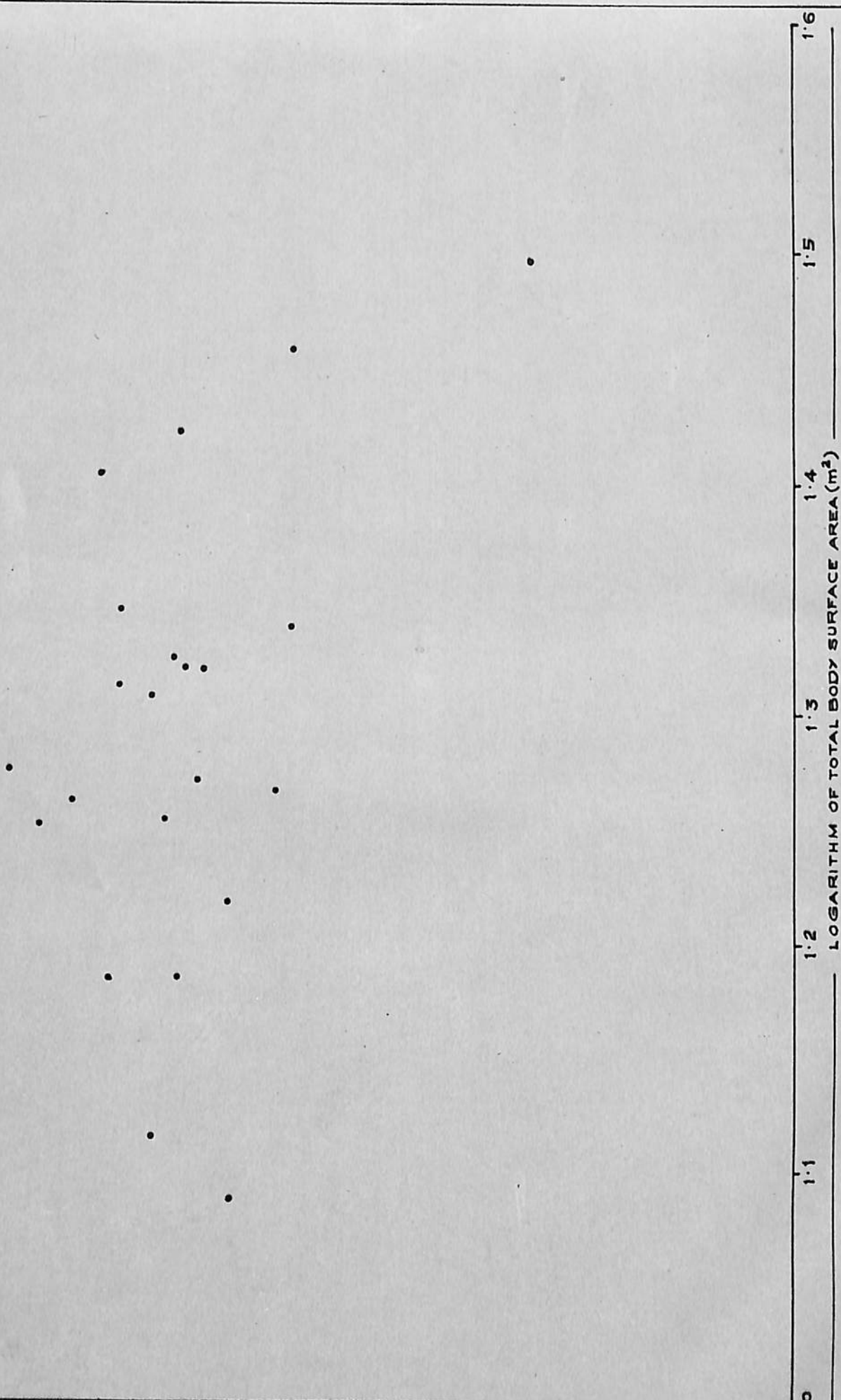


Fig. III.3: Basal metabolism per day per sq.m.
surface area of Indian elephants

Table III.8

Computed heat production in Cal/day/kg body weight using the formulae recommended by other workers

Sl. No.	Body weight (kg)	Brody's formula 70.5 M ^{0.734}	Kleiber's formula 70 W ^{3/4}	Rubner's formula 142 M ^{0.61}	Lee's formula 57.5 M ^{0.82}	DuBois's formula 111 M ^{0.57}
1	2	3	4	5	6	7
1	3025	8.36	9.44	6.23	13.59	3.54
2	2390	8.90	10.01	6.83	14.18	3.91
3	1890	9.48	10.62	7.49	14.79	4.33
4	1520	10.04	11.21	8.15	15.38	4.75
5	2100	9.21	10.34	7.19	14.51	4.13
6	2915	8.44	9.53	6.33	13.68	3.59
7	2750	8.58	9.67	6.47	13.82	3.68
8	2860	8.49	9.57	6.37	13.73	3.62
9	1940	9.41	10.54	7.41	14.72	4.28
10	3910	7.86	8.91	5.70	13.03	3.20
11	4140	7.69	8.73	5.52	12.84	3.09
12	3100	8.31	9.38	6.18	13.54	3.50
13	1300	10.46	11.66	8.67	15.82	5.09
14	1555	9.98	11.15	8.08	15.32	4.71

(continued)

Table III.8 continued

	1	2	3	4	5	6	7
15	2740	8.56	9.68	6.48	13.83	3.69	3.69
16	2600	8.71	9.80	6.61	13.96	3.77	3.77
17	3950	7.79	8.83	5.62	12.95	3.75	3.75
18	2840	8.50	9.59	6.39	13.74	3.63	3.63
19	2380	8.91	10.02	6.85	14.19	3.92	3.92
20	2790	8.54	9.68	6.48	13.83	3.69	3.69
21	3330	8.15	9.21	6.00	13.28	3.39	3.39
22	3140	8.28	9.35	6.14	13.50	3.48	3.48
Mean	2683.86	8.76	9.86	6.69	14.01	3.92	3.92
\pm		\pm	\pm	\pm	\pm	\pm	\pm
S.E.	163.08	0.16	0.17	0.18	0.17	0.11	0.11
Coefficient of variation	28.50	8.42	7.90	12.48	5.68	13.84	13.84

Table III.9

Estimated metabolic rate in Cal/day/square metre body surface area using the reported formulae

Sl. No.	Measured body surface area (m ²)	Kleiber's formula 70 W ^{3/4}	Rubner's formula 142 M ^{0.61}	Lee's formula 57.5 M ^{0.82}	Dupois's formula 111 M ^{0.57}	Brody's formula 70.5 M ^{0.734}
1	2	3	4	5	6	7
1	20.48	1394.16	920.88	2006.93	522.40	1235.13
2	17.97	1331.52	909.00	1885.41	520.55	1184.09
3	15.31	1310.60	924.60	1825.54	534.48	1169.87
4	15.32	1112.30	809.01	1525.88	471.75	996.28
5	18.40	1180.17	820.40	1656.04	472.25	1051.67
6	21.11	1315.49	873.43	1888.79	496.23	1166.13
7	20.83	1276.17	854.26	1824.87	486.47	1132.32
8	20.30	1348.58	897.79	1933.71	510.45	1195.82
9	16.47	1242.38	873.28	1733.70	504.29	1108.52
10	28.82	1177.87	753.28	1723.19	423.41	1039.67
11	25.39	1422.94	899.49	2054.46	503.91	1254.31
12	31.47	924.11	608.31	1332.56	344.75	818.37
13	12.20	1242.21	923.49	1685.55	541.89	1115.49
14	13.02	1331.33	965.24	1829.26	562.34	1192.09

(continued)

Table III.9 continued

1	2	3	4	5	6	7
15	18.25	1452.61	972.87	2076.64	554.09	1288.95
16	21.76	1171.30	790.25	1668.35	451.02	1040.21
17	26.44	1319.12	839.37	1934.71	471.11	1163.67
18	20.90	1302.99	868.29	1867.42	493.82	1155.52
19	18.64	1279.63	874.09	1811.40	500.64	1138.02
20	17.74	1485.99	995.23	2124.36	566.82	1336.10
21	22.08	1389.74	905.69	2003.55	511.68	1229.28
22	18.95	1549.48	1018.14	2236.36	576.72	1372.04
Mean	20.99	1298.21	877.34	1846.76	496.41	1153.80
\pm	\pm	\pm	\pm	\pm	\pm	\pm
S.E.	1.01	28.95	18.89	43.79	11.88	25.66
Coefficient of variation	23.64	10.46	10.10	11.12	11.23	10.43

Table III.7 gives body weight, measured surface area and the estimated heat production per day per kg body weight and the computed heat production per day per sq.m. body surface area.

The relationship between the measured surface area and the body weight is presented in Fig.III.1.

Figure III.2 presents the relationship between the body weight and the estimated heat production per day per kg body weight.

The relationship between measured surface area and heat production per day per sq.m. surface area is shown in Fig.III.3.

Table III.8 presents data on estimated total heat production/day/kg body weight using the different formulae available in literature. The estimated heat production per day per sq.m. of surface area using the different formulae is presented in table III.9.

Raw data for areas of individual regions and the total body surface area have been presented in annexure 3.

DISCUSSION

Surface area assumes importance for proper interpretation of basal metabolic rate. The heat production is always dependent on the surface area. The greater the surface area, the greater is the loss of heat and the greater is the heat production. In spite of this relationship, controversy still exists as to whether body weight or surface area should be given more importance with reference to heat production. Brody (1945) considered use of surface area as a reference base to be a vague approach, since the surface area of living animals is not a constant. Besides, the surface area, as it relates to heat dissipation and conservation changes, is highly variable in hot and cold environments. Effective surface area can be altered by several mechanisms like development of fur, changes in the calibre of blood vessels and the like. Further, metabolic rate is more dependent on neuro-endocrine mechanisms than on external surface area.

According to "Lambert's law", heat radiation and heat loss are proportional to the profile of the projected area and not to simple surface area. Hence, it was recommended that two-third power of body weight be used as a more definitive reference base. Still, there is no justification for assuming that the metabolic body weight should be the same for all animals, since they are not geometrically similar. So far, there are no reports on the actual measurement of surface area

in a large-sized sample of elephant population. In the present study, the body of elephant was divided into different geometrical regions, the area of these individual regions were determined and the values were consolidated to get the true surface area. The body weights and heights of these animals were also recorded whereby prediction equations were fitted in to estimate the total body surface area.

Studies on the relationship between the area of individual regions and the total surface area revealed that the fore-limb area is highly correlated ($r=0.90$). As per model-1 recommended, $S = (1.3712BA + 1.1772 HLA + 1.2147 FLA) - 0.2304$, where S is the total body surface area in square metre, 'BA' represents the body area in sq.m. and 'HLA' and 'FLA' the area in square metres of hind-limb and fore-limb respectively. Here, the mean $20.09 \pm 1.00 \text{ m}^2$ and the coefficient of variation 23.38 were similar to that of the mean 20.09 ± 1.01 and coefficient of variation 23.64 of the measured total body surface area. Hence, model-1 is the most suitable for estimating. This, however, involved measuring the areas of three individual regions. Yet, this approach is recommended since its $R^2=0.98$. Next in preference, model-2 can be employed ($R^2=0.96$) since it has the advantage that measurements are restricted to only two regions. Thus, it is possible to estimate the total body surface area from areas of parts of the body. No surface integrator was used in this study. A beginning has been made to measure the surface area by dividing the body of elephant

into geometrical regions; with refinements, this approach could be made into a reliable method, since the values for measured surface area by this method were in agreement with the estimated values using the conventional formulae.

Relationship between body weight and surface area and body weight and height at shoulder and body surface area were worked out and prediction equations were derived (Models 5 and 6). The correlation coefficient obtained when both weight and height were incorporated in the prediction equation for estimating surface area was better ($R^2=0.87$), than the one in the formula with weight alone ($R^2=0.78$) and hence, the formula incorporating both weight and height is being recommended to get a more accurate estimate of the surface area in elephants. The measured surface area in the present study was 20.09 ± 1.01 sq.m. while the area computed from model-1 (Table III.4) was 20.09 ± 1.00 sq.m. and from model-6 (Table III.6) was 20.00 ± 0.90 sq.m. The surface area estimated by using the conventional Benedict's modification of Meeh's formula was 19.15 ± 1.25 sq.m. It thus appears that the prediction formulae now being recommended for elephants are superior and more reliable compared to Benedict's modification of Meeh's formula. Benedict (1936) reported the surface area of a female Asian elephant weighing 3672 kg, using Benedict's modification of Meeh's formula as 23.8 square metres.

The total heat production per day in elephants was calculated from their body weight by using Brody's formula and expressed in relation to kilogramme body weight and also to square metre surface area. The mean values obtained were 8.76 ± 0.16 Calories/day/kg body weight and 1153.90 ± 25.66 Calories/day/sq.m. surface area.

In his study on the elephant 'Jap', Benedict (1936) reported the heat production as 13 calories per day per kg body weight. This elephant weighed 3672 kg and had an estimated surface area of 23.8 square metres. This information has also been cited by Eltringham (1982). Apparently an error had crept in. The value should have been 13 Cal/day/kg body weight. This assumption is justified since in his monograph Benedict has given the Caloric value of a litre of oxygen at a respiratory quotient of 0.82 as 4.825 cal. (p 263). Benedict (1936) reported the heat production in elephants as 2060 calories per day per sq.m. surface area, after deducting 25% for posture and digestive activity. As stated, the figure should have been 2060 Cal/day/kg body weight. This again was based on observations made during a study with open circuit respiration chamber and a refined trunk breathing apparatus on a single elephant Jap. The higher value reported might probably be due to the unfamiliar environment exposed to and excitation of Jap. The trunk of the elephant is highly sensitive and any attempt to restrict its freedom of movement is sure to result in excitation of the animal.

Brody (1945) reported the range of heat production in three elephants as 8.06 to 11.78 Calories per day per kg body weight. The heat production per day per kg body weight obtained in the present study was found to be in agreement with the values of Brody (1945).

Ananthasubramaniam (1979), working on two elephants with a mean body weight of 3605 kg and surface area of 23.43 sq.m. had observed the digestible energy as 30.6 Cal/day/kg body weight and 4668.9 Cal/day/sq. metre body surface. Brody (1945) had stated that the maintenance of digestible energy is double the basal energy metabolism. Hence, the basal energy in the work of Ananthasubramaniam (1979) should be 15.3 Cal/day/kg body weight and 2334 Cal/day/sq. metre, without 25% deduction recommended by Benedict (1936) towards posture and digestive activity. After deductions, the values should be 11.48 Cal/day/kg body weight and 1750.8 Cal/day/sq. metre surface. The results obtained in the present study agree with the findings of Ananthasubramaniam (1979), the very slight variations being attributed to the small sample size.

Rubner (1883) had concluded in general terms that the total heat production per square metre of body surface area is approximately 1000 Calories in all warm blooded animals. However, further detailed studies by Brody (1945) on cattle and horse gave higher values (cattle - 1094 Calories per day per sq.m.; horse - 1147 Calories per day per sq.m.). The

heat production per unit surface area in elephant is nearly twice the value of 600 Calories per day per sq.m. surface area accepted as the probable basal metabolism of small warm blooded animals at thermic neutrality by Rubner (1883).

According to Benedict (1936), the heat production per kg body weight is, in general, high in small animals, and that the heat production per square metre of body surface is by no means so constant as it was formerly thought to be. In the present study also it was observed that the heat production per square metre of body surface area was not following any specific relationship (Fig. III.3). A variation of 400 per cent in the heat production/sq.m. surface area has been reported in animals varying in size from mouse to elephant (Benedict, 1936).

Figure III.2 revealed a straight line relationship between body weight and heat production/day/kg body weight, thus establishing the fact that the heat production/day/kg body weight records a decrease with an increase in body weight.

CHAPTER IV

**CERTAIN HAEMATOLOGICAL
CHARACTERISTICS OF
INDIAN ELEPHANTS**

CHAPTER IV

CERTAIN HAEMATOLOGICAL CHARACTERISTICS OF INDIAN ELEPHANTS

INTRODUCTION AND REVIEW

Clinical biochemistry has now been recognized as an indispensable adjunct to the study of the functions of the body in both health and disease. The understanding of normal values of the blood constituents characteristic for a species is a prerequisite for correct diagnosis, interpretation and treatment of diseases. Detailed studies on the haematology of Indian elephants are scanty. Normal values for several constituents in the blood of elephants are yet to be established. Hence, the present study was undertaken to characterise the norms for some of the important haematological constituents in clinically healthy elephants.

The specific gravity of whole blood is influenced by the ratio between plasma and cells. The specific gravity of plasma is dependent on the concentration of plasma proteins. A diurnal variation in the specific gravity has been reported in some species. Exercise is observed to increase the specific gravity. The higher specific gravity of the cellular elements results in the settling down of corpuscles. No value is available in the literature for the specific gravity of either the whole blood or the plasma of Indian elephants. Reported values on specific gravity of whole blood or plasma of different species have been tabulated in table IV.1.

viscosity confers on the blood a resistance to flow. With increased viscosity, an increase in resistance and a decrease in flow rate have been observed. Whole blood viscosity is directly related to the packed cell volume (PCV). A high PCV favours enhanced friction between successive layers and when above 50 per cent, the work required of the heart to pump the blood around the body is reported to be twice normal. In solution, weight for weight, fibrinogen has a greater viscosity than globulin, which in turn has a higher viscosity than albumin. Measurement of the plasma viscosity is a very sensitive index and the change in plasma proteins, in response to inflammation and tissue damage, causes variation in plasma viscosity. Several

factors like temperature, aggregation of erythrocytes and mean corpuscular volume (MCV) influence the viscosity. A mucopolysaccharide secreted by the capillary endothelium is believed to lower the viscosity. Literature reports on the viscosity of blood of elephants are not available. However, some of the relevant reports for other species are presented in table IV.1.

Icterus index is an estimate of serum bilirubin. There are several important factors which markedly influence the serum icterus index. The type of ration and the state of water balance affects the index. Dry feed and bone marrow depression causes a decrease in the index while, green pastures, reduced feed intake, fasting, pregnancy, haemolysis, hepatocellular damage and biliary obstruction cause a rise. Lipaemia results in a false elevation in icterus index. The index is a good estimate in carnivores, since the yellow colour of the serum is primarily due to bilirubin while, in horse and cow its value is lessened due to the colour contributed by carotene, carotenoids and xanthophyll of plants and vegetables. Table IV.1 gives the icterus index in Indian elephants and in some other species.

The maintenance of normal concentration of hydrogen ion in body fluids is essential to life, since the properties and net charge of proteins are highly dependent on the hydrogen ion concentration. The level must be narrowly

Table IV.1

Reported values on haematological norms in elephants and other domestic animals

Species	Number of animals	Sex	Age	Specific gravity	Viscosity (centi-poise)	Icterus index	pH	ESR	Coagulation time (sec.)	References	
	1	2	3	4	5	6	7	8	9	10	11
Indian elephants	15	-	Adult	-	-	-	-	36.60±1.69 mm/30 min (25-49 mm/30 min)	-	Simon (1961)	
Indian elephants	11	-	Baby	-	-	-	-	61.3±2.97 mm/h (54-66 mm/h)	-	Nirmalan <u>et al.</u> (1967)	
Indian elephants	14	Male	Adult	-	-	-	-	63.4±5.5 mm/h (50-72 mm/h)	-	Nirmalan <u>et al.</u> (1967)	
Indian elephants	11	Female	Adult	-	-	-	-	61.3±5.5 mm/h (52-70 mm/h)	-	Nirmalan <u>et al.</u> (1967)	
Indian elephants	5	Female	Pregnant	-	-	-	-	67.4±2.7 mm/h (63-70 mm/h)	-	Nirmalan <u>et al.</u> (1967)	
Indian elephants	2	Female	Lactating	-	-	-	-	64.5±0.5 mm/h (64-65 mm/h)	-	Nirmalan (1967)	
Indian elephants	8	-	-	-	-	-	-	54.88 mm/15 min (13-64 mm/15 min)	-	Pillai and Nair (1974)	
Indian elephants	8	-	-	-	-	-	-	56.5 mm/30 min (20.68 mm/30 min)	-	Pillai and Nair (1974)	

(continued)

Table IV.1 continued

1	2	3	4	5	6	7	8	9	10	11
Indian elephants	8	-	-	-	-	-	-	58.3±22.59/h (24-69 mm/h)	-	Pillai and Neir (1974)
Indian elephants	-	Female	Baby	-	-	2.0	-	-	-	Schalm et al. (1975)
Indian elephants	-	-	-	-	-	-	-	49.7 mm/h (36-63.3 mm/h)	-	Brown and White (1980)
African elephants	11	Male	Adult	-	-	-	7.30 (7.10-7.50)	29 mm/h (19-40 mm/h)	-	Young and Lombard (1967)
African elephants	-	-	-	-	-	-	-	34.6 mm/h (21-61.5 mm/h)	-	Brown and White (1980)
Horse (whole blood)	-	-	-	1.053 (1.046-1.059)	3.7*	-	-	-	-	Spector (1956)
Horse (plasma)	-	-	-	1.027 (1.025-1.028)	-	-	-	-	-	Spector (1956)
Horse	-	-	-	-	-	17-21	-	35-40 mm/h	600-660	Purushotham and Mahender (1963)
Horse	38	-	0-6 m	-	-	-	-	32±18.5 mm/h (1-61 mm/h)	-	Archer and Allen (1970)
Horse	38	-	2 y	-	-	-	-	45±13.1 mm/h (22.57 mm/h)	-	Archer and Allen (1970)

(continued)

Table IV.1 continued

	1	2	3	4	5	6	7	8	9	10	11
Horse	34	Gelding	>2 y	-	-	-	-	-	50.8±10.4 mm/h (19-66 mm/h)	-	Archer and Allen (1970)
Horse	24	Mares	>2 y	-	-	-	-	-	34.5±20.9 mm/h (0-59 mm/h)	-	Archer and Allen (1970)
Horse	-	-	-	-	-	-	7.5-20	-	-	-	Kaneko and Cornelius (1970)
Horse	18	Adult	-	-	-	-	-	-	51 mm/30 min (24-60 mm/min)	-	Osbaldiston (1971)
Horse	18	Adult	-	-	-	-	-	-	59 mm/h (51-63 mm/h)	-	Osbaldiston (1971)
Horse	-	-	-	-	-	-	-	-	-	240-900	Schalm <u>et al.</u> (1975)
Horse	-	-	-	-	-	-	-	7.20- 7.55	15-38 mm/20 min	-	Swenson (1977)
Horse	-	-	-	-	-	-	-	-	-	180-900	Benjamin (1978)
Pig	-	-	-	-	-	-	-	-	0-6 mm/30 min	-	Coffin (1953)
Pig	-	-	-	-	-	-	-	-	1-14 mm/h	-	Coffin (1953)
Pig	20	-	1-2 y	-	-	-	-	-	27 mm/30 min (12-36 mm/30 min)	-	Pillel and Nair (1974)
Pig	3	-	-	-	-	-	-	-	-	120-240	Owen <u>et al.</u> (1974)
Pig	-	-	-	-	-	-	2-5	-	-	-	Schalm <u>et al.</u> (1975)

(continued)

Table IV.1 continued

1	2	3	4	5	6	7	8	9	10	11
Pig (whole blood)	-	-	-	1.045 (1.035-1.055)	-	-	-	-	210	Swenson (1977)
Pig	-	-	-	-	-	< 5	-	-	60-300	Benjamin (1978)
Dog	-	-	-	-	-	-	-	1-6 mm/20 min	-	Coffin (1953)
Dog	-	-	-	-	-	-	-	5-25 mm/h	-	Coffin (1953)
Dog	-	-	-	-	-	-	-	0-15 mm/h	-	Knowles (1955)
Dog	-	-	-	-	-	-	-	-	516	Disdisheim <u>et al.</u> (1959)
Dog	6	-	-	1.050-1.052	-	3.2-5.0	-	4-5 mm/h	150-180	Purushotham and Mahendar (1963)
Dog	-	-	-	-	-	-	-	18 mm/h (7.5-26 mm/h)	-	Osbaldiston (1971)
Dog	-	-	-	-	-	2-5	-	-	180-780	Schalm <u>et al.</u> (1975)
Dog (whole blood)	-	-	-	1.046-1.059	-	-	7.35 (7.31-7.42)	5-25 mm/h	-	Swenson (1977)
Dog	-	-	-	-	-	< 5	7.36 (7.31-7.42)	-	60-300	Benjamin (1978)
Cat	-	-	-	-	-	2-5	-	-	-	Schalm <u>et al.</u> (1975)
Cat (whole blood)	-	-	-	1.050 (1.045-1.057)	-	-	7.35 (7.24-7.40)	-	-	Swenson (1977)

(continued)

Table IV.1 continued

1	2	3	4	5	6	7	8	9	10	11
Cat	-	-	-	-	-	<5	-	-	-	Benjamin (1978)
Goat (whole blood)	-	-	-	1.042 (1.035- 1.049)	3.7*	-	-	-	-	Spector (1956)
Goat (plasma)	-	-	-	1.022 (1.019- 1.025)	-	-	-	-	-	Spector (1956)
Goat	-	-	-	-	-	2-5	-	-	-	Schalm <u>et al.</u> (1975)
Sheep	25	-	Adult	-	3.6	-	-	-	-	Gajewski and Pover (1971)
Sheep (whole blood)	-	-	-	-	-	5	7.44 (7.32-7.54)	-	180	Schalm <u>et al.</u> (1975)
Buffalo (whole blood)	-	-	-	1.052- 1.064	-	-	-	-	-	Raghavan and Gaffar (1961)
Buffalo (plasma)	-	-	-	1.024- 1.032	-	-	-	-	-	Raghavan and Gaffar (1961)
Buffalo	45	-	-	-	-	-	-	21-64 mm/h	-	Pillai and Nair (1974)
Buffalo	-	-	-	-	-	-	-	86.4 mm/h	-	Nigham and Pandey (1973)
Buffalo	-	-	-	-	2.0+1.25 (2-5)	-	-	53+12.3 mm/h (17-59 mm/h)	-	Jain <u>et al.</u> (1982)
Buffalo	6	-	-	1.058- 1.060	-	-	-	55-60 mm/h	330-360	Purushotham and Mahendar (1963)

(continued)

Table IV.1 continued

1	2	3	4	5	6	7	8	9	10	11
Cattle (whole blood)	-	-	-	1.052 (1.046- 1.058)	-	-	-	-	-	Spector (1956)
Cattle (plasma)	-	-	-	1.029 (1.026- 1.033)	-	-	-	-	-	Spector (1956)
Cattle	-	-	-	-	-	5-15	-	-	-	240-900 Schalm et al. (1975)
Cattle	-	-	-	-	-	-	7.38 (7.27-7.49)	-	390	Swenson (1977)

* Calculated value

controlled within limits to ensure optimal activity of enzymes and proper functioning of the central nervous system. The absolute status of hydrogen ion concentration in blood at any time is controlled by buffering systems in blood and the elimination of carbon dioxide by the lungs, and acid and base by the kidneys. Table IV.1 gives relevant data collected from literature on pH of blood for various species.

Values for the coagulation time vary with the method employed. Biochemical techniques of isolation and purification, immunological methods of identification and specific clinical disorders of blood coagulation have together produced evidence for the existence of thirteen coagulation factors. The fluidity of the blood in the body depends on the special properties of the intact vascular endothelium, on the rate of blood flow and on the presence of natural anticoagulants. Alterations in the coagulation time may be due to acquired or inherited abnormalities in the complex physiochemical systems concerned in clotting or alterations in the vessel wall. Literature is not available on the coagulation time of elephant blood. The values for coagulation time in different species are furnished in table IV.1.

Rate of sedimentation of corpuscles is influenced by haematocrit, viscosity and concentrations of lipids and proteins in plasma. Abnormalities in plasma composition, particularly an increase in the concentration of fibrinogen,

alpha-globulin, gamma-globulin and hyperlipidaemic state are known to increase the erythrocyte sedimentation rate (ESR) whereas high levels of albumin decrease the rate. Erythrocyte sedimentation rate is dependent on rouleaux formation, attributed to changed zeta potentials under the influence of fibrinogen and globulin. The rate of sedimentation is reported to be elevated during pregnancy and high ambient temperature. Erythrocyte sedimentation rate is used as a diagnostic aid in acute infections, chronic localized infections, and traumatic injuries. Table IV.1 presents data on the ESR in elephants and other species.

In the present study, specific gravity of whole blood and plasma, relative and absolute viscosity of blood, serum icterus index, pH of whole blood and plasma, coagulation time and sedimentation rate of erythrocytes have been determined and norms established.

MATERIALS AND METHODS

Blood samples for the study were collected from Indian elephants maintained by the Forest Department of the Government of Kerala in their elephant camps, and by private agencies in estates (vide chapter II).

Blood samples for analysis were drawn in the morning, before feeding, from the ear vein of 6 baby elephants, 19 adult male and 16 adult females into tubes containing EDTA as anticoagulant, taking care that the free flowing blood through the cannula is collected under a layer of liquid paraffin. Plasma samples were separated by centrifuging the whole blood at $2500 \times g$ for 15 min and collected under a layer of liquid paraffin. All the determinations except icterus index were done in duplicate, in the elephant camps soon after the collection of blood. For determination of coagulation time, fresh blood was drawn into two capillary tubes direct from the ear vein. Icterus index was determined in the laboratory.

The copper sulphate method of Phillips et al. (1950) was employed to determine the specific gravity of whole blood and plasma.

The determination of relative viscosity was done on 25 ml sample using Ostwald's U-tube viscosimeter with water as the standard. The values obtained were then used to calculate absolute viscosity.

Icterus index was found out using the spectrophotometric method of Henry et al. (1953).

The pH of whole blood and plasma was determined with a 'Digital pH meter' (ECIL pH 5651).

The method of Wintrobe and Landsberg (1935) was followed in determining ESR. The readings were taken at 5 min intervals upto 20 min and then at 10 min intervals upto 60 minutes. The 'Capillary Tube' method was adopted to find out the coagulation time of blood.

The mean values obtained were statistically tested by applying Student's t test (Snedecor and Cochran, 1967). Comparisons were made between baby elephants and adult males, baby elephants and adult females and adult males and adult females.

RESULTS

Table IV.2 presents the data gathered for specific gravity of whole blood and plasma, relative and absolute viscosity of whole blood, serum icterus index, pH of whole blood and plasma and whole blood coagulation time in baby, adult male and adult female elephants.

No significant difference could be recorded in the values for specific gravity of whole blood or plasma between baby elephants, adult male and adult female elephants.

Statistically no significant difference could be observed between baby elephants, adult male and adult female elephants with respect to values for relative viscosity and absolute viscosity of whole blood. The mean value for relative viscosity ranged from 6.10 ± 0.18 in baby elephants to 6.37 ± 0.33 in adult male elephants.

Table IV.2 gives the serum icterus index. No difference attributable to age or sex could be detected in the values for icterus index. The mean values were 2.29 ± 0.44 , 2.24 ± 0.22 and 2.35 ± 0.34 units respectively in baby elephants, adult male and adult female elephants.

No significant difference in the pH values of whole blood or plasma could be observed between the different groups. The pH of whole blood varied from 7.13 ± 0.03 to 7.22 ± 0.02 , while, that of plasma from 7.10 ± 0.03 to 7.18 ± 0.04 .

Table IV.2
 Certain haematological norms in the Indian elephants[†]

Constituents	Baby elephants	Adult male elephants	Adult female elephants
Specific gravity -			
whole blood	1.052 ± 0.001(6)	1.054 ± 0.001(19)	1.054 ± 0.001(16)
plasma	1.026 ± 0.001(6)	1.027 ± 0.001(13)	1.028 ± 0.001(14)
Relative viscosity -			
whole blood	6.37 ± 0.33(5)	6.10 ± 0.18(16)	6.36 ± 0.19(15)
Absolute viscosity (centipoise)	5.70 ± 0.28(5)	5.54 ± 0.14(16)	5.68 ± 0.16(15)
Serum icterus index (units)	2.29 ± 0.44(3)	2.24 ± 0.22(12)	2.25 ± 0.34(5)
pH - whole blood	7.21 ± 0.04(6)	7.22 ± 0.02(19)	7.13 ± 0.03(13)
plasma	7.18 ± 0.04(6)	7.15 ± 0.02(19)	7.10 ± 0.03(13)
Whole blood coagulation time (sec.)	323.5 ± 26.0(6) ^a	402.7 ± 15.0(18) ^b	317.8 ± 17.0(13) ^a

Figures in parenthesis indicate the number of animals

[†] Mean ± S.E.

Different superscripts in the same horizontal line indicate significant difference

Table IV.3

Erythrocyte sedimentation rate in Indian elephants⁺

Time (min)	Baby elephants (mm)	Adult male elephants (mm)	Adult female elephants (mm)
5	3.83 ± 1.0(6)	3.89 ± 0.58(18)	3.83 ± 0.70(12)
10	8.67 ± 2.8(6)	11.73 ± 2.0(15)	13.92 ± 1.9(12)
15	20.67 ± 4.5(6)	30.33 ± 2.9(15)	33.92 ± 3.2(12)
20	31.17 ± 4.8(6)	40.25 ± 3.0(16)	44.50 ± 3.4(12)
30	40.17 ± 3.2(6) ^a	51.06 ± 1.9(16) ^b	54.90 ± 2.4(10) ^b
40	47.33 ± 2.2(6) ^a	55.00 ± 1.3(17) ^b	56.7 ± 1.7(10) ^b
50	50.00 ± 2.4(6) ^a	56.31 ± 1.3(16) ^b	57.60 ± 1.6(10) ^b
60	51.67 ± 2.0(6) ^a	57.47 ± 1.1(19) ^b	57.82 ± 1.4(12) ^b

Figures in parenthesis indicate the number of animals

⁺ Mean ± S.E.

Different superscripts in the same horizontal line indicate significant difference

The whole blood coagulation time was significantly higher ($p < 0.01$) in adult males (402.7 ± 15.0 sec) than in baby elephants (323.5 ± 26.0 sec) and adult female elephants (317.8 ± 13.0 sec).

Values for erythrocyte sedimentation rates at 5, 10, 15, 20, 30, 40, 50 and 60 min have been presented in table IV.3. No influence of age or sex could be detected in the rates of erythrocyte sedimentation at 5, 10, 15 and 20 minutes. The ESR at 15 min was 20.67 ± 4.5 , 30.33 ± 2.9 and 33.92 ± 3.2 mm in the case of baby, adult male and adult female elephants respectively. ESR at 30 min and 40 min were significantly higher ($p < 0.01$) in adult males and adult females compared to those in baby elephants. Baby elephants had a lower sedimentation rate ($p < 0.05$) than those in adult males and adult female elephants at 50 and 60 minutes. No influence of sex could be discerned in the values for ESR at different time periods.

Raw data for all the above estimations are presented in annexures 4 to 15.

DISCUSSION

The specific gravity of whole blood was found to vary from 1.052 ± 0.001 to 1.054 ± 0.001 in Indian elephants in this study while that of the plasma ranged between 1.026 ± 0.001 and 1.028 ± 0.001 . The values were within the range reported in literature for other species (Spector, 1956; Raghavan and Gaffar, 1961; Purushotham and Mahendar, 1963; Schalm et al., 1975; Swenson, 1977). As anticipated, the specific gravity of whole blood was found to be higher than that of plasma. No influence of sex or age could be detected in this study. The technique suggested by Raghavan and Gaffar (1961) for calculating specific gravity of serum, when specific gravity of whole blood is known (half of the last two digits of the specific gravity of blood expressed correct to three decimal places plus 1.000 is the serum specific gravity) can be safely applied for calculating the specific gravity of plasma in the case of elephants. The specific gravity of plasma is dependent on the concentration of proteins. In the present study no appreciable difference was noted among the groups in the serum protein concentration (6.17 ± 0.26 to 6.92 ± 0.43 g/dl; Chapter V). Simon (1961) observed the plasma protein concentration in Indian elephants as 10.72 g/dl. Nirmalan and Nair (1971) reported the mean total plasma protein content as 8.25 ± 0.50 g/dl in baby elephants and 9.25 ± 1.21 g/dl in adult non-lactating female elephants. Brown et al. (1978) failed to observe any variation in total serum

proteins due to age, sex, location or season in African elephants. The relative ratio between cells and plasma is established to influence the specific gravity. Simon (1961) reported low erythrocyte counts ($2.81 \pm 0.43 \times 10^6/\text{mm}^3$) in Indian elephants. Bartles et al. (1963) gave the mean RBC count in African elephants, as $3.20 \times 10^6/\text{mm}^3$ of blood. Schmitt (1964) gave the mean RBC count, from his study on one African and three Indian elephants, as $4.64 \pm 0.51 \times 10^6/\text{mm}^3$ of blood. According to Nirmalan et al. (1967) the erythrocyte count is very low in Indian elephants ($2.40 \pm 0.51 \times 10^6/\text{mm}^3$ in non-pregnant non-lactating female elephants to $2.42 \pm 0.44 \times 10^6/\text{mm}^3$ in baby elephants). Further, they could not record any influence of age or sex. Young and Lombard (1967) gave the red cell count in African elephants as $5.02 \times 10^6/\text{mm}^3$ of blood. A low erythrocyte count of $3.19 \pm 0.29 \times 10^6/\text{mm}^3$ of blood was reported in elephants (species not specified) by Usami et al. (1969). A low erythrocyte count ($3.09 \times 10^6/\text{mm}^3$) was also reported by Andrewbutter (1971) in Asian elephants. The values for PCV in elephant blood reported in literature by earlier workers included $38.20 \pm 1.33\%$ (Simon, 1961); 38.2% (Bartles et al., 1963); from $34.7 \pm 3.49\%$ in baby elephants to $34.8 \pm 4.84\%$ in tuskers (Nirmalan et al., 1967); 48% (Young and Lombard, 1967); $35.9 \pm 4.2\%$ (Usami et al., 1969) and 54.6% (Andrewbutter, 1971). The absence of significant differences between the different groups in the specific gravities of whole blood and plasma observed in the present

study is, thus, attributed to similarities in ratio of cell to plasma and in protein concentration.

The absolute viscosity of whole blood ranged between 5.54 ± 0.14 and 5.70 ± 0.16 centipoise. The value is found to be nearly two times higher than those reported in literature for sheep, goat, horse and buffalo (Spector, 1956; Gajewski and Povar, 1971; and Jain et al., 1982). Degree of agglutination has been reported to influence the viscosity (Usami et al., 1969). In elephants, the rouleaux formation is rapid as is revealed by the high ESR, and this could contribute for the elevated viscosity observed. Similarly the globulin content in elephant serum is high (from 4.08 ± 0.31 g/dl in adult males to 4.86 ± 0.52 g/dl in baby elephants in this study; Chapter V). Similar high values for globulin had been reported by Andrewbutter (1971) (6.2 g/dl) and Nirmalan and Nair (1971) (5.41 ± 0.73 to 6.50 ± 1.32 g/dl). Usami et al. (1969) reported that elephants have the highest values for beta-globulin and fibrinogen among man, dog, sheep and goat. They also observed that the shear dependence of erythrocyte suspensions from elephant, man and dog is strikingly increased by the presence of serum proteins and further with the inclusion of fibrinogen. Gel-electropherograms revealed a high beta-globulin fraction in elephant serum in this study (Fig.VI.1, Chapter VI). This concurred with the earlier findings of Giri et al. (1958) and Usami et al. (1969). The relatively high viscosity observed can be explained as due to

the tendency for rapid formation of rouleaux and to the higher total protein and globulin contents and to the low A/G ratio in the blood of elephants (Chapter V). No significant difference in the total protein content or in the level of globulin could be detected among the three groups studied in the present work (Chapter V). Similarly, Nirmalan et al. (1967) did not observe any influence of age or sex on the MCV in elephants (from 142.0 ± 12.89 cubic microns in tuskers to 146.9 ± 13.06 cubic microns in non-pregnant non-lactating female elephants). These observations substantiate the absence of significant differences in viscosity between baby elephants, adult male and adult female elephants. According to West et al. (1966), the heart muscle functions best when working against a certain resistance. It could be safely presumed that the heart in elephants is functioning effectively and efficiently as a pump to ensure adequate circulation around the massive body. Probably in elephants the work carried out by the heart is much more than in other species. A decrease in flow rate could be anticipated with an increase in viscosity. Whether this high viscosity is an advantage or disadvantage can't be predicted unless detailed studies on haemodynamics are carried out.

The value for serum icterus index obtained in this study agreed with the only available value of 2 units in Indian elephants cited by Schalm et al. (1975). Andrewbutter (1971) reported the bilirubin level as $2 \mu \text{mol/l}$ in an Asian elephant.

Brown and White (1980) also reported the mean bilirubin contents in μ mol/l, in Indian elephants as 2, in man as 0 to 20 and in African elephants as 5. The uniformly low value for serum icterus index observed in elephants in the present study is suggestive of the non-contribution of carotenoid pigments from the bulk of the diet of elephant viz., leaves of Caryota urens. This could also possibly mean that efficient excretion of bilirubin by liver is occurring in elephants. The values in units for icterus index are reported to vary: in horse, from 7.2 to 21 (Purushotham and Mahendar, 1963; Kaneko and Cornelius, 1970); in pig, from 2 to 5 (Schalm et al., 1975; Benjamin, 1978); in dog, from 2 to 5.0 (Purushotham and Mahendar, 1963; Schalm et al., 1975; Benjamin, 1978) and in cat, from 2 to 5, in cattle, from 5 to 15 and in goat, from 2 to 15 (Schalm et al., 1975).

The reaction of elephant blood was found to be at the alkaline side of neutrality. The pH of whole blood in the case of Indian elephants appeared to be within compatible limits for life reported in other species. The mean plasma pH value observed in this study is lower than that reported (mean 7.3; range 7.10 to 7.40) by Young and Lombard (1967) in African elephants.

The whole blood coagulation time varied from 317.8 ± 17.0 to 402.7 ± 15.0 seconds. The value falls within the range reported in cattle, buffalo, dog, horse and cat. The time

appeared to be slightly higher than those reported for pig and sheep (Disdisheim et al., 1959; Purushotham and Mahendar, 1963; Swenson, 1977; Schalm et al., 1975 and Benjamin, 1978). As compared to man, the concentration of coagulation factors were reported to be high in elephants (Lewis, 1974). However, the coagulation time obtained in the present study did not vary much from the reported values for man. Seiverd (1973) reported the whole blood coagulation time in man, using capillary tube method, to vary from 120 to 360 seconds. Partial thromboplastin time was reported to be low in elephants. Lewis (1974) has reported the mean platelet count in blood of Indian elephants as $637 \times 10^9/l$ and Brown and White (1980) in 96 African elephants as $294 \times 10^9/l$. Moreover antiprothrombin III also was reported to be absent (Lewis, 1974). According to Boiti and Grosso (1972), an elevated plasma globulin content was found to increase the prothrombin level while albumin exerted an opposite effect. The levels of globulin and fibrinogen were high (3.71 g/dl and 0.61 ± 0.09 g/dl) than those in man (2.46 g/dl and 0.27 ± 0.04 g/dl), dog (3.34 g/dl and 0.28 ± 0.11 g/dl), sheep (3.15 g/dl and 0.28 ± 0.10 g/dl), goat (2.99 g/dl and 0.44 ± 0.10 g/dl) and horse 2.74 g/dl and 0.26 g/dl) (Usami et al., 1969; Jeffcott, 1974 and Schalm et al., 1975). Nirmalan and Nair (1971) had reported the levels of globulin (from 5.41 ± 0.73 g % in baby elephants to 6.50 ± 1.32 g % in adult non-lactating female elephants) and fibrinogen (from 0.57 ± 0.13 g % in tuskers to 0.65 ± 0.14 g % in adult non-lactating females in Indian elephants).

Brown and White (1980) also reported the level of fibrinogen in African elephants (mean 0.64 g/dl and range 0.63 to 0.66 g/dl) and in Indian elephants (mean 0.61 g/dl and range 0.40 to 0.88 g/dl) to be high. Fibrinolysis is generally mediated by a proteolytic enzyme plasmin, which exists as inactive plasminogen in blood. Plasminogen activators are the euglobulin fractions of plasma. According to Buckell (1958) elephant has apparently low levels of plasminogen activators. Lewis (1974) noted that the clot retraction was poor in Indian elephants. Brown and White (1980) reported that they could obtain unhaemolysed serum samples only from about 50% of the total attempted. But, in the present study no such difficulty was encountered, the clot retraction was good, the serum separation was quick and the serum obtained was clear and not haemolysed. Matschiner and Willingham (1974) observed that the plasma prothrombin levels, in castrated male and female rats fed vitamin K deficient diet, dropped after the injection of testosterone and rose or remained high after the injection of oestradiol. In cattle and sheep, Nockles et al. (1978) recorded higher coagulation time in males compared to the young ones and adult females. In the present study too, the coagulation time was high in adult males (402.7 ± 15.0 sec.) than in young ones (323.5 ± 26.0 sec.) and female elephants (317.8 ± 13.0 sec). The higher coagulation time observed in male elephants in this study could be possibly due to the tendency of testosterone

in inducing vitamin K-deficiency and hence lowering the prothrombin synthesis, as already reported in other species.

The rate of erythrocyte sedimentation in elephants was high suggestive of an increased tendency for rouleaux formation. In addition, the high globulin content (4.08 ± 0.31 to 4.86 ± 0.52 g/dl) and low albumin content (2.07 ± 0.09 to 2.17 ± 0.18 g/dl) in the serum observed in the present study (Chapter V) might have also contributed. High levels of fibrinogen and globulin are known to enhance ESR, while increased levels of albumin inhibit. In elephants, fibrinogen and globulin levels in blood were reported to be high, while that of albumin was low (Nirmalan and Nair, 1971). Giri *et al.* (1958) and Usami *et al.* (1969) reported a high beta-globulin content in elephant serum. In the present study also beta-globulin fraction was high in serum protein electrophoresis (Chapter VI). Simon (1961), Bartles *et al.* (1963), Schmitt (1964), Nirmalan *et al.* (1967), Young and Lombard (1967), Usami *et al.* (1969) and Andrewbutter (1971) had reported low erythrocyte counts ($10^6/\text{mm}^3$) in elephants (2.81 ± 0.43 ; 3.20 ; 4.64 ± 0.51 ; from 2.40 ± 0.51 to 2.41 ± 0.44 ; 5.02 ; 3.19 ± 0.29 and 3.09 respectively). In ESR there are 3 phases - initial one of aggregation followed by a period of fast settling and a final phase of packing. ESR could be used as a non-specific diagnostic test to detect ailments, to note the progress of the disease and the effectiveness of therapy. For meaningful interpretation, the rates determined during

the phase of fast settling alone should be used. According to Pillai and Nair (1974) the relationship between ESR values and the time of determination was seen only during the first 10 to 15 minutes. They also observed in the eight elephant blood samples they studied, that 50% sedimentation occurred between 7 and 8 minutes. Hence, they recommended the optimal time for determination of ESR in elephants as 5 minutes. They reported a mean value of 14.5 mm in 5 min and 58.25 ± 22.59 mm in one hour. But in the present study on 36 Indian elephants the ESR attained 50% sedimentation only at 15 minutes. This suggests that ESR could be determined at 15 min time interval to make it a tool for clinical diagnosis. The ESR at 30 min, 40 min, 50 min and 60 min were high in adults than in baby elephants. Raghavan and Gaffar (1960) also reported a rise in ESR with age in the case of buffaloes. The rates of sedimentation observed from 30 min onwards suggest a similar influence of age.

CHAPTER V

SERUM PROTEINS

IN INDIAN ELEPHANTS

CHAPTER V

SERUM PROTEINS IN INDIAN ELEPHANTS

INTRODUCTION AND REVIEW

The level of plasma/serum proteins can be considered as an index of the physiological health status of an animal. They are involved with a wide variety of functions like the maintenance of viscosity, suspension stability and oncotic pressure of blood, the transportation of lipids, vitamins, hormones, in immuno-defensive mechanisms, in the coagulation of blood and in tissue protein synthesis. Dietary amino acids in excess of the requirements, are normally converted to carbohydrates and fats before they are utilized for energy.

Qualitative and quantitative variations in the distribution of serum proteins are often studied for diagnosis and tracing the course of diseases. Yet, their value is still inconclusive. However, abrupt and major differences in any of the components could be used as a diagnostic tool, if analysis had been done on serial samples. The concentration of proteins at any given time is a function of the hormonal balance, nutritional status, water balance, and other factors affecting the state of health. The level of proteins in the serum shows considerable interspecies variations. There is a direct correlation between half-life of protein and body size, the turnover being much faster in small animals. In addition to normal catabolism by the liver, kidney and other tissues, there is a constant loss of plasma proteins into the gut. The literature values on serum proteins in elephants and other species are given in table V.1.

Albumin is an easily available pool of amino acids ready to supply the needs of tissues. Relative to other plasma protein molecules, the albumin molecule is smaller and more compact, with a lower isoelectric pH (4.7) and more prototropic groups, and hence, binds more cations at the pH of plasma. The albumin fraction of plasma normally contributes about 80% of the colloidal osmotic pressure.

Globulins contribute significantly towards the viscosity

Table V.1

Reported values on serum/plasma protein levels in the blood of elephant and other domestic animals

Species	Number of animals	Sex	Age	Total proteins (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Albumin: globulin ratio	References
1	2	3	4	5	6	7	8	9
Indian elephants	7	-	Adult	8.20±0.70	-	-	-	Giri <i>et al.</i> (1958)
Indian elephants (plasma)	15	-	Adult	10.72±0.96	-	-	-	Simon (1961)
Indian elephants and an African elephant	4	-	Adult	6.20±0.3	-	-	-	Schmitt (1964)
Indian elephant	1	-	Adult	8.40	2.20	6.20	0.35	Andrewbutter (1971)
Indian elephant (plasma)	11	Male and Female	Baby	8.25±0.50 (7.48-8.97)	2.20±0.44 (1.56-2.94)	5.41±0.73 (4.38-6.90)	0.45±0.18 (0.23-0.86)	Nirmalan and Nair (1971)
Indian elephant (plasma)	13	Male	Adult	8.49±0.77 (7.86-10.01)	2.36±0.62 (1.69-3.62)	5.56±1.02 (4.25-7.65)	0.46±0.20 (0.22-0.79)	Nirmalan and Nair (1971)
Indian elephant (plasma)	10	Female	Adult	9.25±1.21 (7.37-11.18)	2.10±0.54 (1.62-3.34)	6.50±1.32 (5.51-8.64)	0.34±0.16 (0.21-0.76)	Nirmalan and Nair (1971)

(continued)

Table V.1 continued

1	2	3	4	5	6	7	8	9
Indian elephants and African elephants	7	-	-	7.0	-	-	-	Gabrisch (1978)
Indian elephants	8	Male and Female	12-25y	7.95 (7.3-8.82)	2.03 (1.50-3.19)	5.92 (4.75-6.95)	0.34 (0.26-0.57)	Moses and Gopalakrishnan (1979)
Elephants (species not specified)	15	-	-	7.4±0.59	3.82±0.43	3.71	1.04±0.16	Usami <i>et al.</i> (1969)
African elephants	138	Male and Female	0-60y	8.72	3.79*	4.93*	0.77*	Brown <i>et al.</i> (1978)
Horse	70	-	-	7.3	-	-	-	Irfan (1967)
Horse (plasma)	30	-	-	7.1±0.38	3.1±0.34	-	-	Kaneko and Cornelius (1970)
Horse	-	-	-	6.72	2.6	4.12*	0.63*	Kaneko and Cornelius (1970)
Horse (plasma)	2	-	-	7.81	2.99	-	-	Kaneko and Cornelius (1970)
Horse (plasma)	10	Male and Adult		7.29	2.87	4.15*	0.69*	Kaneko and Cornelius (1970)
Horse	8	-	3 M	5.81	2.74	3.05	0.99*	Jeffcott (1974)
Horse	32	-	6-9 m	5.88	2.81	2.97	2.95*	Jeffcott (1974)

(continued)

Table V.1 continued

1	2	3	4	5	6	7	8	9
Horse	23	-	1 y	5.82	2.96	2.84	1.04*	Jeffcott (1974)
Horse	27	-	3 y	6.07	3.45	2.64	1.31*	Jeffcott (1974)
Horse	10	-	4-6 y	6.46	3.74	2.74	1.36	Jeffcott (1974)
Horse	147	-	-	6-8.5	-	-	-	Schalm <u>et al.</u> (1975)
Horse	-	-	-	6.50	3.25	3.25	1.00*	Swenson (1977)
Horse	-	-	-	5.7-7.8	2.33-3.85	2.62-4.09	0.62-1.46	Benjamin (1978)
Pig	-	-	Adult	6.8±0.5	-	-	-	Squibb <u>et al.</u> (1953)
Pig	-	-	3 m	7.16±0.91	-	-	-	Haaranen (1960)
Pig	-	-	6 m	7.19±0.04	-	-	-	Haaranen (1960)
Pig	-	-	Adult	7.68±1.11	-	-	-	Haaranen (1960)
Pig	-	-	2-3 m	5.5	-	-	-	Miller <u>et al.</u> (1961)
Pig	-	-	4-6 m	6.8	-	-	-	Miller <u>et al.</u> (1961)
Pig	-	-	1 y	7.51	-	-	-	Miller <u>et al.</u> (1961)
Pig	-	-	2 y	7.97	-	-	-	Miller <u>et al.</u> (1961)

(continued)

Table V.1 continued

1	2	3	4	5	6	7	8	9
Pig	10	-	3-4 m	7.77	-	-	-	Irfan (1967)
Pig	79	-	5 $\frac{1}{2}$ - 6 $\frac{1}{2}$ m	7.4	3.4	4.0	0.85*	Kaneko and Cornelius (1970)
Pig	-	-	Adult	7.3	3.3	4.0	0.83*	Baetz and Mengeling (1971)
Pig	-	-	2 m	5.67	2.49	3.18	0.78*	Tumbleson and Kalish (1971)
Pig (plasma)	-	-	2-6 m	6.0-7.0	-	-	-	Schalm <i>et al.</i> (1975)
Pig (plasma)	-	-	1 y	7.0-8.0	-	-	-	Schalm <i>et al.</i> (1975)
Pig	-	-	-	6.30	2.03	3.27	0.62*	Swenson (1977)
Pig	-	-	-	7.90-8.90	1.80-3.30	5.29-6.43	0.37-0.51	Benjamin (1978)
Dog (plasma)	15	-	2-7 y	6.64	-	-	-	Irfan (1967)
Dog (plasma)	20	-	-	6.30	3.36	2.16*	1.56*	Kaneko and Cornelius (1970)
Dog (plasma)	16	-	-	5.94 \pm 0.09	-	-	-	Kaneko and Cornelius (1970)
Dog	-	-	-	6.34 \pm 0.24	-	-	-	Kaneko and Cornelius (1970)
Dog	-	-	2 $\frac{1}{2}$ -3m	5.87 \pm 0.46	-	-	-	Schalm <i>et al.</i> (1975)
Dog (plasma)	-	-	1-2 y	7.03 \pm 0.33	-	-	-	Schalm <i>et al.</i> (1975)

(continued)

Table V.1 continued

1	2	3	4	5	6	7	8	9
Dog (plasma)	-	-	2 y	7.5±0.24	-	-	-	Schalm <i>et al.</i> (1975)
Dog	-	-	-	6.20	3.57	2.63	1.36*	Swenson (1977)
Dog	-	-	-	5.40-7.10	2.30-3.20	2.70-4.40	0.59-1.11	Benjamin (1978)
Cat	-	-	-	7.58	4.01	3.57	1.12	Swenson (1977)
Cat	-	-	-	5.40-7.30	2.10-3.30	2.60-5.10	0.45-1.19	Benjamin (1978)
Goat	-	-	-	6.70-7.85	3.25-4.90	2.30-4.60	-	Millson <i>et al.</i> (1960)
Goat	15	Male and Female	7-9 m	6.25	3.95	2.30*	1.72	Kaneko and Cornelius (1970)
Goat	-	Female	Adult	7.23	-	-	-	Verma and Tyagi (1974)
Goat (plasma)	10	-	Adult	7.1±0.5 (6.6-7.5)	-	-	-	Schalm <i>et al.</i> (1975)
Goat	-	-	-	6.0-6.7	3.96	2.71	1.46*	Swenson (1977)
Goat	-	-	-	6.40-7.90	2.70-3.90	2.70-4.10	0.63-1.26	Benjamin (1978)
Sheep	27	-	4 m	5.81	2.96	2.85	1.04*	Kaneko and Cornelius (1970)
Sheep	-	-	-	6.69±0.23	-	-	-	Schalm <i>et al.</i> (1975)
Sheep	-	-	-	5.38	3.07	2.31	1.33*	Swenson (1977)
Sheep	-	-	-	6.00-7.90	2.70-3.90	2.50-5.70	0.42-0.76	Benjamin (1978)

(continued)

Table V.1 continued

1	2	3	4	5	6	7	8	9
Cattle	-	-	-	6.42-8.92	3.0-3.5	5.0-5.5	0.6-0.75	Labouche (1964)
Cattle	-	-	Adults	7.16	3.08	4.08	0.75	Irfan (1967)
Cattle	30	Male	18-30 m	6.97	3.20	3.77	0.85*	Kaneko and Cornelius (1970)
Cattle	-	-	Adults	7.70	3.4	4.30	0.79*	Payne <u>et al.</u> (1973)
Cattle	-	-	<6 m	7.64	4.08	3.55	1.15*	Tumbleson <u>et al.</u> (1973)
Cattle	-	-	3 y	8.90	4.05	4.84	0.84*	Tumbleson <u>et al.</u> (1973)
Cattle	-	-	6 y	9.42	4.27	5.15	0.83*	Tumbleson <u>et al.</u> (1973)
Cattle	-	-	>10 y	10.2	3.99	6.21	0.64*	Tumbleson <u>et al.</u> (1973)
Cattle	20	Male	Adults	7 ± 0.3	4 ± 0.4	3 ± 0.04	1.33*	Bogin <u>et al.</u> (1977)
Cattle	-	-	-	7.60	3.63	3.97	0.91*	Swenson (1977)
Cattle	-	-	-	6.74-7.46	3.03-3.55	3.00-3.48	0.84-0.94	Benjamin (1978)

* Indicates the calculated values

of plasma. They are also involved in transport of majority of constituents in blood and also in immune mechanisms. Destruction of tissues by direct injury or through surgery upsets the dynamic equilibrium in the distribution of proteins between tissue and plasma and invariably an increase in plasma globulin - particularly, alpha-globulin - could be observed. Alterations in the distribution of the different fractions of globulins can be induced by season, age, nutritional status, a wide variety of clinical abnormalities and the like.

Albumin:globulin ratio (A/G ratio) serves as an important diagnostic aid. The albumin:globulin ratio varies with the species. Albumin predominates over globulins in dog, sheep, goat and man, while globulin either predominates over or equals the level of albumin in horse, cattle and pig.

In the present study, the total proteins as well as the relative distributions of albumin and globulin in the serum of Indian elephants had been studied. The A/G ratio had been calculated.



MATERIALS AND METHODS

The study was conducted on 39 clinically healthy Indian elephants - 6 baby elephants, 16 adult male elephants and 17 adult female elephants - maintained under identical nutritional regime (vide chapter II). The blood samples were drawn into clean glass tubes from the ear vein early in the morning before feeding. The tubes were kept in a slanting position for 25 minutes and then kept at 4°C for 10 hours. It was then centrifuged (2500 x g; 15 min) at ambient temperature. The separated serum was removed by a pasteur pipette to clean tubes. The total serum proteins were estimated by the biuret method (Gornall et al., 1949) using the reagent kit of Miles (India) Limited, Baroda. The serum albumin was determined by BCG method (cited by Sonnenwirth and Jaret, 1980) using the reagent kit supplied by Miles (India) Limited. The difference between the levels of total serum proteins and serum albumin was taken as the globulin content. Albumin:globulin ratio was calculated.

Student's t test (Snedecor and Cochran, 1967) was employed to test the significance of mean values.

RESULTS

The total protein, albumin and globulin levels in the serum and the albumin:globulin ratio obtained in the present study are presented in table V.2. Age or sex did not exert any influence on the total protein, albumin and globulin levels in serum. The A/G ratios obtained were identical among the three groups of elephants.

Raw data have been presented in annexures 16 to 19.

Table V.2

Distribution of serum proteins and A/G ratio in Indian elephants⁺

Constituents	Baby elephants(6)	Adult male elephants (16)	Adult female elephants (17)
Total proteins (g/dl)	6.92 \pm 0.43	6.17 \pm 0.26	6.78 \pm 0.26
Albumin (g/dl)	2.08 \pm 0.14	2.07 \pm 0.09	2.17 \pm 0.18
Globulin (g/dl)	4.86 \pm 0.52	4.08 \pm 0.31	4.61 \pm 0.31
Albumin:globulin ratio	0.46 \pm 0.08	0.55 \pm 0.05	0.53 \pm 0.05

Figures in parenthesis gives the number of animals studied

⁺ Mean \pm S.E.

DISCUSSION

The total serum protein levels among the three groups of elephants viz., baby elephants, adult males and adult female elephants recorded in the present study varied from 6.17 ± 0.26 g/dl in adult males to 6.92 ± 0.43 g/dl in baby elephants. The values for total proteins reported in literature by earlier workers ranged from 6.20 to 8.40 g/dl (Giri et al., 1958; Simon, 1961; Schmitt, 1964; Usami et al., 1969; Andrewbutter 1971; Nirmalan and Nair, 1971; Gabrisch, 1978 and Moses and Gopalakrishnan, 1979). In African elephants, Brown et al. (1978) reported the total protein content in serum as between 6.80 to 10.70 g/dl. Differences in the nutritional status, age, nature of work and the methodology adopted might account for the negligible discrepancies observed between the values obtained in the present study and majority of those already published.

The serum total protein level in the present study on elephants was within the range reported for other species like horse (5.81 to 8.5 g/dl; Irfan, 1967; Kaneko and Cornelius, 1970; Jeffcott, 1974; Schalm et al., 1975; Swenson, 1977 and Benjamin, 1978); pig (5.5 to 8.9 g/dl; Squibb et al., 1953; Haaranen, 1960; Miller et al., 1961; Irfan, 1967; Kaneko and Cornelius, 1970; Baetz and Mengeling, 1971; Tumbleson and Kalish, 1971; Schalm et al., 1975; Swenson, 1977 and Benjamin, 1978); dog (5.4 to 7.5 g/dl;

Irfan, 1967; Kaneko and Cornelius, 1970; Schalm et al., 1975; Swenson, 1977 and Benjamin, 1978); cat (5.4 to 7.58 g/dl; Swenson, 1977; and Benjamin, 1978); goat (6.0 to 7.9 g/dl; Millson et al., 1960; Kaneko and Cornelius, 1970; Verma and Tyagi, 1974; Schalm et al., 1975; Swenson, 1977 and Benjamin, 1978); sheep (5.38 to 7.9 g/dl; Kaneko and Cornelius, 1970; Schalm et al., 1975; Swenson, 1977 and Benjamin, 1978); cattle (6.42 to 10.2 g/dl; Labouche, 1964; Irfan, 1967; Kaneko and Cornelius, 1970; Payne et al., 1973; Tumbleson et al., 1973; Bogin et al., 1977; Swenson, 1977 and Benjamin, 1978).

No influence of sex or age could be detected in this study. This is contrary to the findings of Nirmalan and Nair (1971) who observed a significant difference in the level of total plasma protein between the baby elephants and adults. Brown et al. (1978) failed to observe any difference in the total protein content attributable to the influence of age, sex, location or season in African elephants.

The serum albumin content in the present work ranged from 2.07 ± 0.09 g/dl in adult male elephants to 2.17 ± 0.18 g/dl in adult female elephants. Albumin level in Indian elephants had been reported to range from 1.50 to 3.62 g/dl (Andrewbutter, 1971; Nirmalan and Nair, 1971 and Moses and Gopalakrishnan, 1979). Brown et al. (1978) reported the albumin level in African elephants to vary from 3.42 to 4.10 g/dl. The level of serum albumin had been reported

to vary between 1.80 to 3.40 g/dl in pig (Kaneko and Cornelius, 1970; Baetz and Mengeling, 1971; Tumbleson and Kalish, 1971; Swenson, 1977 and Benjamin, 1978); between 2.33 to 3.85 g/dl in horse (Kaneko and Cornelius, 1970; Jeffcott, 1974; Swenson, 1977 and Benjamin, 1978); between 2.30 to 3.57 g/dl in dog (Kaneko and Cornelius, 1970; Swenson, 1977 and Benjamin, 1978); between 2.10 to 4.01 g/dl in cat (Swenson, 1977 and Benjamin, 1978); between 2.70 to 4.90 g/dl in goat (Millson et al., 1960; Kaneko and Cornelius, 1970; Swenson, 1977 and Benjamin, 1978); between 2.70 to 3.90 g/dl in sheep (Kaneko and Cornelius, 1970; Swenson, 1977 and Benjamin, 1978) and between 3.0 to 4.27 g/dl in cattle (Labouche, 1964; Irfan, 1967; Kaneko and Cornelius, 1970; Payne et al., 1973; Tumbleson et al., 1973; Bogin et al., 1977; Swenson, 1977 and Benjamin, 1978).

The level of serum albumin in Indian elephants, thus, appeared to be lower than in other domestic animals. No influence of age or sex could be detected in albumin level.

Globulin content in the present study varied from 4.08 ± 0.31 g/dl in adult male elephants to 4.86 ± 0.52 g/dl in baby elephants. Information already available indicate the globulin content to vary from 4.26 to 8.64 g/dl (Andrewbutter, 1971; Nirmalan and Nair, 1971; Brown et al., 1978 and Moses and Gopalakrishnan, 1979). Usami et al. (1969) reported the mean globulin content in elephants as

3.82 \pm 0.43 g/dl. No influence of age or sex could be detected in the distribution of globulin among the three groups of elephants studied. A higher globulin level in the serum of baby elephants due to their exposure to sub-clinical infections, though anticipated, was not apparent in the present study ($p > 0.05$). Serum globulin content reported in other species ranged from 2.64 to 4.15 g/dl in horse (Kaneko and Cornelius, 1970; Jeffcott, 1974; Swenson, 1977 and Benjamin, 1978), from 3.18 to 6.43 g/dl in pig (Kaneko and Cornelius, 1970; Baetz and Mengeling, 1971; Tumbleson and Kalish, 1971; Swenson, 1977 and Benjamin, 1978), from 2.16 to 4.40 g/dl in dog (Kaneko and Cornelius, 1970; Swenson, 1977 and Benjamin, 1978), from 2.60 to 4.60 g/dl in goat (Millson et al., 1960; Kaneko and Cornelius, 1970; Swenson, 1977 and Benjamin, 1978), from 2.31 to 5.70 g/dl in sheep (Kaneko and Cornelius, 1970; Swenson, 1977 and Benjamin, 1978), and from 3 to 6.21 g/dl in cattle (Labouche, 1964; Irfan, 1967; Kaneko and Cornelius, 1970; Payne et al., 1973; Tumbleson et al., 1973; Bogin et al., 1977; Swenson, 1977 and Benjamin, 1978).

The albumin:globulin ratio (A/G ratio) in the present study varied from 0.46 \pm 0.08 in baby elephants to 0.55 \pm 0.05 in adult male elephants. In earlier findings, albumin:globulin ratio had been reported to vary from 0.21 to 0.86 (Andrewbutter, 1971; Nirmalan and Nair, 1971 and Moses and

Gopalakrishnan, 1979). However, Usami et al. (1969) reported a value of 1.04 ± 0.16 in elephants (species not specified). In the African elephant, Brown et al. (1978) reported the A/G ratio as 0.77. Age or sex had no influence on the A/G ratio. The results obtained revealed that elephants can be grouped along with species like cattle and pig having a A/G ratio lower than one. The significance of the low A/G ratio vis a vis the haemodynamics of circulation cannot be emphasized until further detailed studies are carried out.

CHAPTER VI

ELECTROPHORETIC FRACTIONATION STUDIES IN INDIAN ELEPHANTS

CHAPTER VI

**ELECTROPHORETIC FRACTIONATION
STUDIES IN INDIAN ELEPHANTS**

CHAPTER VI

ELECTROPHORETIC FRACTIONATION STUDIES IN INDIAN ELEPHANTS

INTRODUCTION AND REVIEW

Genetically controlled protein polymorphism has been associated with many economic traits like milk production and egg production, and in many fitness traits like disease survivability and viability. With the introduction of modern technology for separation and fractionation, it has become possible to study polymorphism in proteins. The association between these polymorphic protein variants with economic and fitness traits had led to the use of these polymorphic protein variants as genetic markers of selective breeding. The widespread differences in frequencies of different alleles controlling polymorphic protein variants might have selective advantages as in the case of haemoglobin

variant like HbS. Human beings with heterozygous HbS phenotype are resistant to malaria. Similarly, certain B antigens in blood group system confer resistance against Rous' sarcoma, Marek's disease and avian leucosis in birds (Briles et al., 1982 and Brown et al., 1982). In poultry heterozygosity at B locus is also associated with faster growth.

Giri et al. (1958) separated five fractions from the serum of Indian elephants viz., albumin and the α_1 -, α_2 - beta- and gamma-globulins by one dimensional and seven fractions namely, albumin and the α_1 -, α_2 -, β_1 -, β_2 -, β_3 - and gamma-globulins by two-dimensional agar-gel electrophoresis. Schmitt (1964) using Michaelis buffer (ionic strength 0.1; pH 8.6) also identified seven components in elephant serum. Nirmalan (1967) studied the electrophoretic pattern of the serum of Indian elephants by both paper and agar-gel techniques (barbitone buffer of ionic strength 0.05, pH 8.6). Seven components namely, albumin and the α_1 -, α_2 -, β_1 -, β_2 -, γ_1 - and γ_2 -globulins were fractionated in paper electrophoresis while by agar-gel technique only five fractions namely, albumin and the α_1 -, α_2 -, beta- and gamma-globulins were separated. Brown et al. (1978) reported five fractions namely, albumin, alpha-globulin, β_1 -globulin, β_2 -globulin and gamma-globulin in the serum of African elephants

(cellulose acetate strips; pH 8.8; tris-barbitone-sodium barbital buffer). Albumin, α_1 -globulin, α_2 -globulin, β_1 -globulin, β_2 -globulin, γ_1 -globulin and γ_2 -globulin were the seven fractions isolated from serum of Indian elephants using Michaelis buffer (sodium barbitone-sodium acetate buffer) by agar-gel electrophoresis (Moses and Gopalakrishnan, 1979).

Literature reports on albumin polymorphism in elephants are scanty. Giri *et al.* (1958) demonstrated a fast moving component in the albumin fraction. The occurrence of two plasma albumin fractions in domestic fowls was first demonstrated by McIndoe (1962). Ashton (1964) reported six albumin alleles in cattle. The results of mating in a beef-cattle herd showed that albumin polymorphism in cattle is controlled by the autosomal co-dominant alleles Alb^A and Alb^B . Alb^B was more frequent in Zebu cattle while it was apparently absent in European cattle. Spooner and Oliver (1969) studied albumin polymorphism in cattle in British Isles and reported five albumin variants. Hans and Lee (1982a) revealed two albumin variants in Korean cattle. Wantanabe and Suzuki (1967) studied the serum albumin types in 1,628 goats of several breeds from Japan and other countries and revealed that there were three albumin phenotypes AA, BB and AB controlled by two autosomal alleles Alb^A and Alb^B . Osterhoff and Cox (1972) reported two albumin variants in three African breeds of goats. Three albumin types had been demonstrated in horses (Gurhev, 1983).

The study on haemoglobin polymorphism dates back to Pouling et al. (1949) who investigated haemoglobin by means of paper electrophoresis and reported the fraction HbS which was different from the normal haemoglobin in humans. Earlier studies on haemoglobin polymorphism in elephants revealed only a single band (Janusch and Janusch, 1964) by vertical starch-gel electrophoresis. Sen et al. (1966) observed the distribution of two haemoglobin types in several breeds of cattle in India using paper electrophoresis. Hans and Lee (1982b) reported that Korean cattle had three types of haemoglobin while Holstein Freisian had only one. Beraide and Enyenihi (1969) demonstrated three haemoglobin types in Nigerian goats. The Hungarian native female goats were reported to have two phenotypes based on the type of haemoglobin (Fesus et al., 1983).

In the present study, electrophoretic techniques have been adopted to fractionate the serum protein and lipoproteins and to identify variants of albumin and haemoglobin in Indian elephants.

MATERIALS AND METHODS

Samples of blood and sera obtained from 36 Indian elephants of all ages and both sexes, were utilized for this study (vide Chapter IV and V).

For fractionating total serum protein, tris-barbiturate buffer (pH 8.6) was used as the vessel buffer. Agar solution, prepared with the vessel buffer (1.5%), was used for the preparation of agar coated slides. The run was made for 3.5 h with a current intensity of 2.5 mA per slide. Fixing was done in two stages, with acetic acid-methanol mixture (Fixative I) for 30 min and then in acetone solution (Fixative II) for 4 hours. It was then covered with a filter paper and dried in an incubator at 40°C. Amido Black 10 B was used as stain (10 min) and later it was destained with methanol-acetic acid mixture (15 min).

For lipoprotein electrophoresis, vermol buffer (pH 8.6) was used as the vessel buffer. The 1.5% agar-agarose gel was prepared using the vessel buffer for preparing the slides. The apparatus was then kept in a refrigerator and a current of 5 mA per slide was supplied for five hours. The slides were then fixed in methanol-acetic acid mixture and then stained with oil red-O overnight.

Tris-citric acid buffer (pH 5.8) was used for the preparation of 1.5% agar solution in electrophoretic studies on albumin. Boric acid-sodium hydroxide buffer (pH 7.8) was

employed as the vessel buffer. The strength of the current supplied was 2.5 mA per slide and the run was continued for 2 hours. The procedures for fixing, drying, staining and destaining have already been detailed out in the case of total serum protein electrophoresis.

For studying haemoglobin polymorphism, about 5 ml of blood was mixed with 5 ml of isotonic saline solution and then centrifuged. The supernatant saline was removed. This was repeated twice. A 2.5% aqueous suspension of washed erythrocytes was used for haemoglobin electrophoresis. The current was run for 10 h at the rate of 2 mA per slide. The pH of vessel buffer was 8.9. Fixing and drying operations were done as in the case of total serum protein electrophoresis. Benzidine was used to stain the band (10 min) and it was destained using the destainers as in the case of total protein electrophoresis. Annexure 20 gives the reagents used in agar-gel electrophoresis.

RESULTS

Agar-gel electrophoresis revealed the fractionation of five fragments in the serum of Indian elephants, viz., albumin, α_1 -globulin, α_2 -globulin, beta-globulin and gamma-globulin (Fig.VI.1).

Fractionation of lipoproteins revealed four fractions (Fig. VI.2).

Albumin polymorphism could be detected in the serum of Indian elephants. Two fractions were observed - one slow moving and the other fast moving. Fast moving fraction was stained more darker than the slow moving one. The fast moving fraction is designated as Alb^A and the slow moving fraction as Alb^B (Fig. VI.3).

Electrophoretically, variants in haemoglobin could not be detected. All samples revealed only one band (Fig. VI.4).

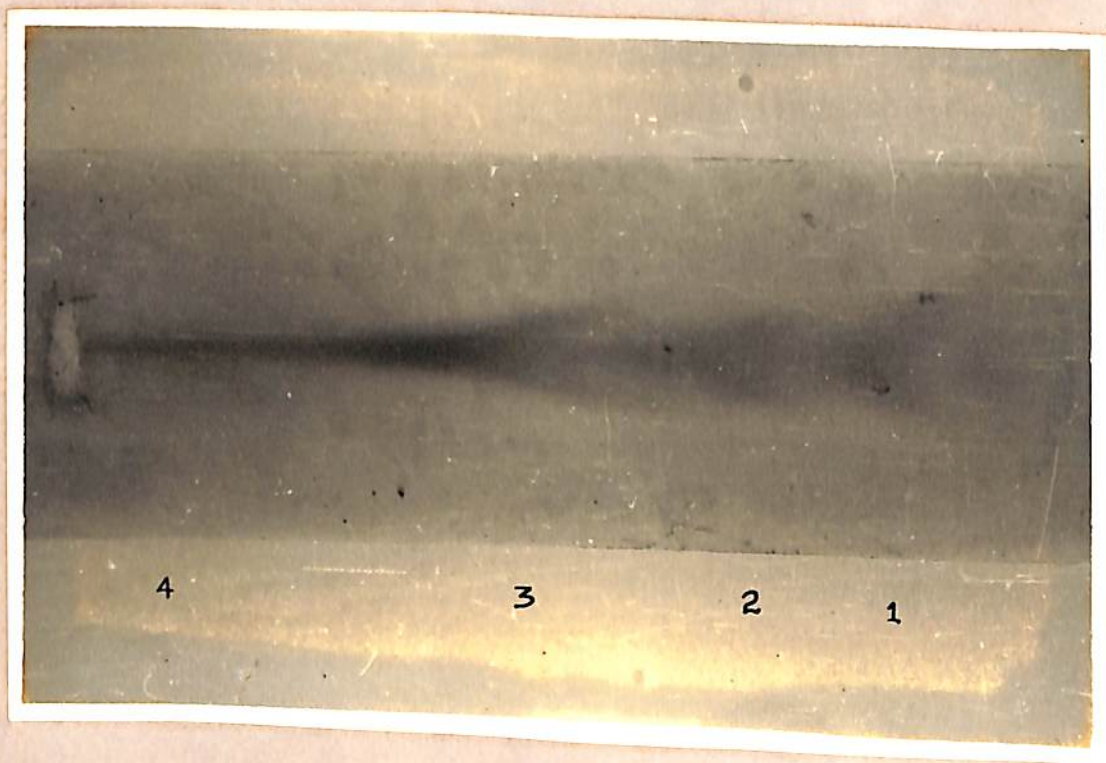
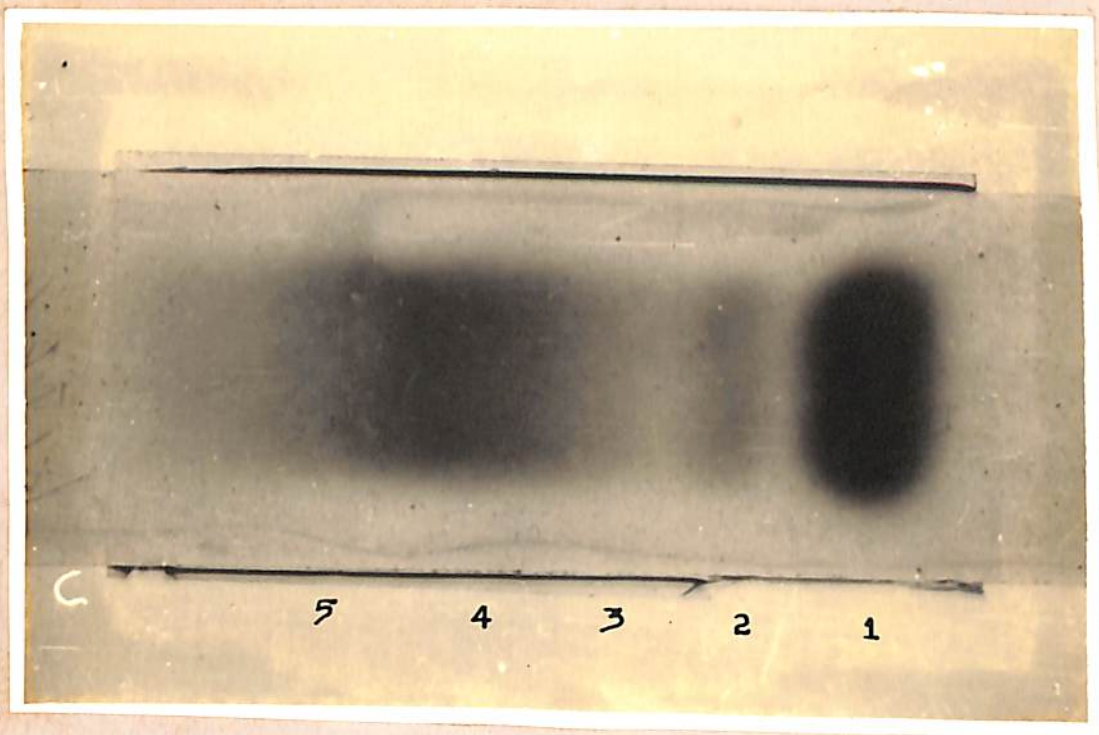


Fig. VI.1: Serum protein electropherogram (agar-gel)

1. Albumin
2. Alpha₁-globulin
3. Alpha₂-globulin
4. Beta-globulin
5. Gamma-globulin

Fig. VI.2: Serum lipoprotein electropherogram (agar-agarose gel)

1. High density lipoprotein
2. Very low density lipoprotein
3. Low density lipoprotein
4. Chylomicrons

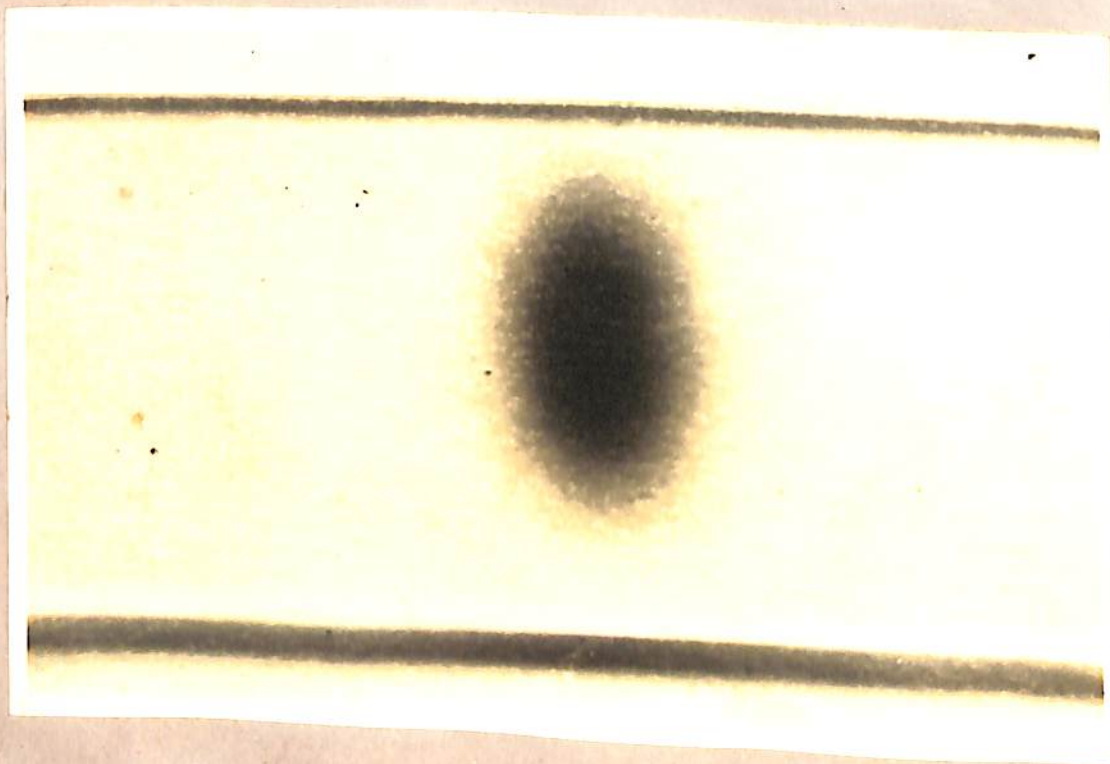
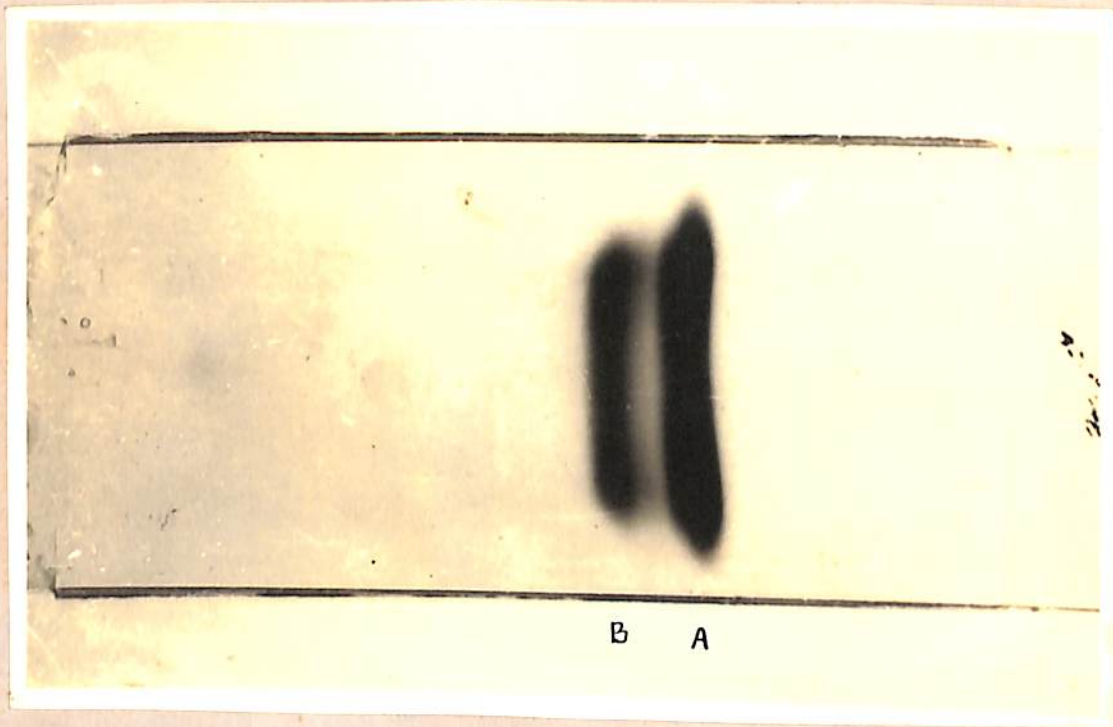


Fig. VI.3: Electrophoretic variants of serum albumin (agar-gel)

Fig. VI.4: Haemoglobin electropherogram (agar-gel)

DISCUSSION

The results of the qualitative study on electropherograms of serum proteins revealed the presence of 5 fractions - albumin and α_1 -, α_2 -, beta- and gamma-globulins. This finding agreed with the earlier reports of Giri *et al.* (1958) and Nirmalan (1967). However, Nirmalan (1967) detected seven fragments by paper electrophoresis. Similarly, Moses and Gopalakrishnan (1979) identified seven fractions in agar-gel electrophoresis using Michaelis buffer. The observation in the present study might probably indicate a poor resolution and incomplete separation under the conditions employed.

The lipoprotein fractionation revealed four fractions *viz.*, chylomicrons, low-density lipoprotein, very low-density lipoprotein and high-density lipoprotein. The chylomicrons are seen very close to the point of origin, followed by the low-density lipoprotein, very low-density lipoprotein, and the fast moving high-density lipoprotein.

Polymorphism of serum albumin could be detected in elephants. Two electrophoretic - a fast moving and a slow moving-fractions namely (Alb^A and Alb^B) were found in all the sera tested. In the present study, single band fractions of serum albumin could not be observed. Albumin polymorphism has been reported in cattle (Ashton, 1964; Spooner and Oliver, 1969), in goats (Wantanbe and Suzuki, 1967 and Osterhoff and Cox, 1972) and in horse (Gurbev, 1983). A

Mendelian co-dominant mode of inheritance in albumin variants has been established beyond doubt. In the present study, elephants homozygous for fast moving (Alb^A) and slow moving (Alb^B) alleles could not be observed as evinced by absence of single bands in the electrophoretic study. The present work had been carried out on 36 elephants only. Studies on a larger number of elephants might reveal more polymorphic variants, if they exist.

The present study revealed only one haemoglobin type in the case of Indian elephants. This is in agreement with the findings of Janusch and Janusch (1964) in African elephants. Based on these limited observations it could be postulated that haemoglobin polymorphism does not exist in Indian elephants.

No conclusion can be drawn based on this limited work regarding the correlation between the polymorphic protein variants and economic and fitness traits in elephants. Further detailed work is planned.

CHAPTER VII

**NORMS FOR CERTAIN SERUM ENZYMES OF
CLINICAL VALUE IN INDIAN ELEPHANTS**

CHAPTER VII

NORMS FOR CERTAIN SERUM ENZYMES OF CLINICAL VALUE IN INDIAN ELEPHANTS

INTRODUCTION AND REVIEW

The serum enzyme levels might serve as effective biochemical tools in studies on zoologic relationships. Distribution patterns of serum enzymes may supplement other biologic characteristics in taxonomy and even aid in recognition of phylogenic relationships. It is believed that serum enzymes represent either enzymes in transit from the site of synthesis to the site of action or spill-over enzymes due to damage to cells. The serum levels of a particular enzyme may be increased by diseases that enhance either the rate of release or the quantity of enzyme available for release.

Among the transaminases, serum glutamic oxaloacetic transaminase (SGOT; aspartate aminotransferase) and serum glutamic pyruvic transaminase (SGPT; alanine aminotransferase) are of prime importance. The highest concentration of GOT is in muscle cells, with slightly lesser amounts in liver and heart muscles. Glutamic oxaloacetic transaminase is not organ-specific. Lesser amounts are found in many other tissues like kidney, pancreas, brain and erythrocytes. Analgesics, antibiotics, corticosteroids and oestrogens are known to increase the level of GOT in serum. Lipaemia and haemolysis interfere with accurate determination of SGOT. The activity of SGOT decreases with an increase in temperature. Muscular damage increases the level in serum by ten-fold. In acute liver damage, myocardial infarction, intestinal complication and septicaemia, an increase in GOT level in serum had been reported. Cornelius et al. (1959), Wroblewski (1959), Buck et al. (1961), Zurgilgen and Ruther (1962), Dotta et al. (1964), Cardinet et al. (1967), Coodley (1970), Kaneko and Cornelius (1970) and Benjamin (1978) have reported on the clinical significance of transaminases. Since all the major tissues contain high concentrations of GOT, a significant elevation in SGOT cannot be ascribed to any specific organ damage.

The high levels of GPT found in the hepatocytes in liver of dogs, cats and primates makes it specific for

hepatocellular damage in these species (Cornelius et al., 1959; Buck et al., 1961; Dotta et al., 1964; Coodley, 1970; Kaneko and Cornelius, 1970 and Benjamin, 1978).

Very little GPT is present in the hepatic tissues of horse, cattle, sheep and pig and so its clinical importance in these species is not high. Very low concentrations of GPT are found in other tissues.

An increase in the value of SGPT is indicative of hepatic cell damage but hepatic necrosis is not necessary for the elevation of SGPT, since alterations in cell membrane permeability can result in leakage of this cytoplasmic enzyme into the blood. Normal to moderate elevation is seen in hypothyroidism, diabetes mellitus and neoplasms of the liver. Table VII.1 gives the SGOT and SGPT levels in elephants and some other species.

Lactic dehydrogenase (LDH) is located in body tissues that utilize glucose for energy. Because of its high concentration in many tissues, it is not organ-specific, and so isoenzyme analysis is essential. The blood LDH-1 and LDH-2 levels increase in cardiac and renal damages while LDH-3 increases in lung disorders. In skeletal muscle involvement there is an increase in the blood level of LDH-4 and LDH-5 fractions. Serum LDH is elevated in many processes in which there is cell necrosis but, because of the ubiquitous distribution of the enzyme, it cannot be considered specific

Table VII.1

Reported levels of serum enzymes in animals (i.u./l)

Species	Number of animals	Sex	SGOT	SGPT	LDH	CPK	References
1	2	3	4	5	6	7	8
Indian elephants	12	-	6.53±1.51 (4.22-8.98)	2.27±0.36 (1.73-2.78)	-	-	Nirmalan and Nair (1969)
Indian elephants	-	-	17-23	8.0-11.0	525	114	Brown and White (1980)
African elephants	-	-	20	3.0	-	153	Brown and White (1980)
Horse	-	-	79.2±6.22	5.28±1.82	-	-	Cornelius <u>et al.</u> (1959)
Horse	17	-	97±21	6±4	148±58	-	Zimmerman <u>et al.</u> (1965)
Horse	43	-	-	-	-	1.3±0.9	Cardinet <u>et al.</u> (1967)
Horse	-	-	109.44±31.68	-	-	-	Kaneko and Cornelius (1970)
Horse	-	-	61.4±16.9	2.13	-	-	Kaneko and Cornelius (1970)
Horse	-	-	101-154	3-7	-	1.0	Doxey (1971)
Horse	-	-	28.5-115.2	-	-	-	Dunavant <u>et al.</u> (1974)

(continued)

Table VII.1 continued

1	2	3	4	5	6	7	8
Horse	-	-	58-94	1-6.7	41-104	-	Benjamin (1978)
					48-242	-	Benjamin (1978)
					29-172	8-58	Benjamin (1978)
Pig	-	-	14.93±6.77	13.10±3.74	-	-	Cornelius <i>et al.</i> (1959)
Pig	24	-	46±18	25±7.0	199±46	-	Zimmerman <i>et al.</i> (1965)
Pig	12	-	19.76±4.16	8.99±1.67	-	-	Nirmalan and Nair (1969)
Pig	-	-	13.92	-	-	< 3	Kaneko and Cornelius (1970)
Pig	-	-	8.2-21.6	9-17	96-160	-	Benjamin (1978)
Dog	-	-	10.90±2.6	10.46±2.98	-	-	Cornelius <i>et al.</i> (1959)
Dog	24	-	15±3	10±2	38±8	-	Zimmerman <i>et al.</i> (1965)
Dog	-	-	1.44-38.4	2.4-24	-	-	VanVleet and Alberts (1968)
Dog	12	-	8.27±2.51	5.86±1.84	-	-	Nirmalan and Nair (1969)
Dog	-	-	<19.2	<14.4	-	-	Kaneko and Cornelius (1970)
Dog	-	-	9.6-23.04	2.4-11.04	-	-	Kaneko and Cornelius (1970)
Dog	-	-	12.96±2.11	8.64±1.1	-	-	Kaneko and Cornelius (1970)

(continued)

Table VII.1 continued

1	2	3	4	5	6	7	8
Dog	-	-	15±3	4.8-16	-	-	Doxey (1971)
Dog	-	-	4.8-19.2	-	-	-	Dunavant <u>et al.</u> (1974)
Dog	-	-	6.2±1.3	4.8-24	10-35	-	Benjamin (1978)
Cat	10	-	23±1	18±6	95±45	-	Zimmerman <u>et al.</u> (1965)
Cat	-	-	9.12±2.30	7.49±4.75	-	-	Kaneko and Cornelius (1970)
Cat	-	-	6.7-11	1.7-14	16-69	0.4-3.4	Benjamin (1978)
Goat	4	-	19.0 (17-20)	5.0 (4-6)	176 (133.198)	-	Zimmerman <u>et al.</u> (1965)
Goat	-	-	43-132	7-24	31-99	-	Benjamin (1978)
Goat	-	-	-	-	-	14-62	Garnier <u>et al.</u> (1984)
Sheep	102	-	40.8 (25.92-61.44)	4.03 (0.24-9.12)	-	-	Buck <u>et al.</u> (1961)
Sheep	45	-	62 ± 28	9 ± 2	302 ± 58	-	Zimmerman <u>et al.</u> (1965)
Sheep	-	-	35.86±6.53	8.35±2.45	-	-	Kaneko and Cornelius (1970)
Sheep	-	-	45.6±2.93	-	-	-	Kaneko and Cornelius (1970)
Sheep	-	-	58.70±2.45	-	-	-	Kaneko and Cornelius (1970)

(continued)

Table VII.1 continued

1	2	3	4	5	6	7	8
Sheep	-	-	78.72 \pm 11.04	11.14 \pm 0.86	-	-	Kaneko and Cornelius (1970)
Sheep	-	-	39-47	2.9 \pm 0.77	-	6.3-1.7	Doxey (1971)
Sheep	-	-	79 \pm 11	11 \pm 1	60-111	-	Benjamin (1978)
Buffalo	-	Female	34.08 \pm 2.16	7.68 \pm 1.14	-	-	Singh <i>et al.</i> (1972)
Buffalo	-	Male	34.32 \pm 3.82	7.2 \pm 1.03	-	-	Singh <i>et al.</i> (1972)
Buffalo	-	-	35.13 \pm 2.21	8.81 \pm 1.10	-	-	Pyne and Maitra (1982)
Cattle	-	-	21.02 \pm 2.74	9.46 \pm 6.05	-	-	Cornelius <i>et al.</i> (1959)
Cattle	10	-	34.08 (24-43.68)	12.19 (7.68-14.88)	-	-	Buck <i>et al.</i> (1961)
Cattle	-	Male	23.66	14.39	-	-	Roussel and Stallcup (1965)
Cattle	100	Female	55 \pm 12	15 \pm 6	473 \pm 97	-	Zimmerman <i>et al.</i> (1965)
Cattle	12	-	22.23 \pm 3.49	7.56 \pm 1.07	-	-	Nirmalan and Nair (1969)
Cattle	-	-	-	-	778.08	-	Prasse (1969)
Cattle	-	-	81.41 \pm 1.54	20.74 \pm 0.34	-	-	Kaneko and Cornelius (1970)
Cattle	-	-	11.52 \pm 8.16	11.57 \pm 2.4	-	-	Kaneko and Cornelius (1970)

(continued)

Table VII.1 continued

1	2	3	4	5	6	7	8	8
Cattle	-	-	11-34	3.9-29	-	0.87	Doxey (1971)	
Cattle	-	Female	21.12 \pm 1.38	8.58 \pm 0.79	-	-	Singh <u>et al.</u> (1972)	
Cattle	-	Male	21.6 \pm 3.58	8.88 \pm 1.08	-	-	Singh <u>et al.</u> (1972)	
Cattle	-	Female	22.32 \pm 3.53	8.04 \pm 0.92	-	-	Singh <u>et al.</u> (1972)	
Cattle	-	Female	20.4 \pm 3.0	8.52 \pm 1.69	-	-	Singh <u>et al.</u> (1972)	
Cattle	-	Male	21.12 \pm 1.41	9.12 \pm 1.09	-	-	Singh <u>et al.</u> (1972)	
Cattle	-	-	17.28-45.6	8.76 \pm 1.23	-	-	Dunavant <u>et al.</u> (1974)	
Cattle	-	-	20-34	4-11	176-365	-	Benjamin (1978)	
Cattle	-	-	-	-	217-420	-	Benjamin (1978)	
Cattle	-	-	-	-	326-517	3-50	Benjamin (1978)	

Results expressed in other units have been converted to i.u./l as per the method suggested by Benjamin (1978)

for any tissue or pathognomonic for any specific condition. Tissue-specific isoenzymes are helpful in identifying the tissue of origin. Malignant neoplasms, leukaemia and haemolysis causes a rise in LDH levels in blood. Excitement and anxiety can contribute to an elevated serum LDH level. It has been shown that oxalates inhibit LDH. The normal levels reported in some species are tabulated in table VII.1.

Creatine phosphokinase (CPK) is found primarily in the skeletal muscle, cardiac muscle and brain. It is absent in liver. Sex or age is not known to exert any influence. However, higher levels are reported in males than in females. The CPK activity was found to rise in blood within a few hours after the onset of myocardial infarction symptoms, reaching the peak in 30 hours and subsiding to normal in two to four days. This suggests that serum CPK is a sensitive indicator of myocardial ischaemia and sub-endocardial infarction. A negligible increase has been reported in pulmonary embolism. In hypothyroidism, the level of CPK rises in blood. It is also seen that in cerebrovascular diseases serum CPK levels are abnormally high. Anxiety and excitement are capable of increasing the CPK levels in blood. The CPK levels reported in literature are presented in table VII.1.

In the present study, normal activities of glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, lactic dehydrogenase and creatine phosphokinase have been assayed in Indian elephants.

MATERIALS AND METHODS

Blood samples were collected from five baby elephants, 10 adult male elephants and 13 adult female elephants (vide chapter IV). The animals were all clinically healthy and maintained under identical nutritional regime. Blood samples were collected in clean glass tubes and the separated serum was kept at 4°C till the assay.

The methods of Reitman and Frankel, as given by King (1965), were followed for estimating SGOT and SGPT, using 'Transaminase Test Kits' of Decrus Corporation (Bombay, India).

'LDH Test Kit' of Span Diagnostics (Private) Limited (Surat, India) was employed in the estimation of LDH, using the method of King (1965).

The serum CPK level was assayed adopting the method of Hughes (1962), 'CPK Test Kit' of Span Diagnostics (Private) Limited (Surat, India) was used for this assay.

The statistical significance of the mean values was tested by Student's t test (Snedecor and Cochran, 1967).

RESULTS

Table VII.2 presents data for GOT, GPT, LDH and CPK levels in sera of baby elephants, adult male and adult female elephants. No influence of sex or age could be detected in the values for any of the parameters studied. Mean values for LDH and CPK in adult males, though higher than in baby elephants, were not significantly different statistically ($p > 0.05$).

The raw data have been presented in annexures 21 to 24.

Table VII.2

Activities of certain serum enzymes in Indian elephants[†]

Constituent	Baby elephants	Adult male elephants	Adult female elephants
Serum glutamic oxaloacetic transaminase (i.u./l)	10.18 _± 3.8(5)	15.65 _± 2.7(10)	18.46 _± 2.4(13)
Serum glutamic pyruvic transaminase (i.u./l)	5.64 _± 0.85(4)	4.75 _± 0.54(10)	4.98 _± 0.49(12)
Lactic dehydrogenase (i.u./l)	366.67 _± 90.04(3)	468.75 _± 55.14(8)	398.75 _± 77.98(4)
Creatine phosphokinase (i.u./l)	30.28 _± 12.0(5)	51.21 _± 7.4(8)	43.80 _± 12.0(3)

Figures in parenthesis indicate the number of animals

[†] Mean _± S.E.

DISCUSSION

Considerable difficulty is experienced in making meaningful comparisons between the plasma/serum enzyme activities obtained in different studies due to differences in the methodology and units employed. In table VII.1 the activities of serum enzymes in elephants and other species of animals reported by earlier workers have been presented. Values have been expressed in International Units (i.u./l) and where needed, results of studies in other units have been converted to i.u./l as detailed out by Benjamin (1978). This was done to ensure effective reasonable comparison.

The SGOT levels in elephants were found to vary from 10.18 ± 3.8 i.u./l in the case of baby elephants to 18.46 ± 2.4 i.u./l in adult females. The SGOT activity in adults seemed to be higher compared to baby elephants but the difference was not statistically significant. No influence of age or sex could be detected in the SGOT levels. The SGOT levels in Indian elephants had been reported to vary from 4.22 to 23 i.u./l (Nirmalan and Nair, 1969 and Brown and White, 1980). Brown and White (1980) reported the mean SGOT level in African elephants as 20 i.u./l which was higher than the observed value in the present study. The serum SGOT levels in Indian elephants were similar to those reported in literature in pigs (8.2 to 46 i.u./l; Cornelius *et al.*, 1959; Zimmerman *et al.*, 1965; Nirmalan and Nair, 1969; Kaneko and Cornelius, 1970 and Benjamin, 1978), in

dogs (1.44 to 38.4 i.u./l; Cornelius et al., 1959; Zimmerman et al., 1965; VanVleet and Alberts, 1968; Nirmalan and Nair, 1969; Kaneko and Cornelius, 1970; Doney, 1971; Dunavant et al., 1974; Benjamin, 1978), in cats (6.7 to 23 i.u./l; Zimmerman et al., 1965; Kaneko and Cornelius, 1970; Benjamin, 1978), in goats (17 to 32 i.u./l; Zimmerman et al., 1965; Benjamin, 1978) and in cattle (11.0 to 45.6 i.u./l; Cornelius et al., 1959; Buck et al., 1961; Roussel and Stallcup, 1965; Zimmerman et al., 1965; Nirmalan and Nair, 1969; Kaneko and Cornelius, 1970; Doney, 1971; Singh et al., 1972; Dunavant et al., 1974; and Benjamin, 1978). However, the SGOT activity in the present work was found to be lower than those reported in horses (28.8 to 115.2 i.u./l; Cornelius et al., 1959; Zimmerman et al., 1965; Kaneko and Cornelius, 1970; Doney, 1971; Dunavant et al., 1974; and Benjamin, 1978), in sheep (25.92 to 79 i.u./l; Buck et al., 1961; Zimmerman et al., 1965; Kaneko and Cornelius, 1970; Doney, 1971 and Benjamin, 1978) and in buffaloes (34.08 to 35.13 i.u./l; Singh et al., 1972 and Pyne and Maitra, 1982). Sato and Vallenias (1962) reported higher SGOT levels in young Thoroughbred horses as compared to adult ones. According to Singh et al. (1972) cattle failed to exhibit any significant influence of age in SGOT while Dotta et al. (1964) reported breed differences. In the present study, however, the adults had a higher value than the baby elephants, but it was not statistically significant. According to Coodley (1970) physical stress can

result in elevated SGPT levels. The higher value in adult elephants, though non-significant, could probably be due to the routine physical stress they are subjected to during the work of hauling and piling of timber.

The SGPT level in Indian elephants ranged from 4.75 ± 0.54 i.u./l in adult males to 5.64 ± 0.85 i.u./l in baby elephants. The serum levels in baby elephants, adult male elephants and adult female elephants were similar revealing the absence of any influence of sex or age. The values obtained in the present work were in agreement with the reported values for SGPT in Indian elephants (1.73 to 11.0 i.u./l; Nirmalan and Nair, 1969; Brown and White, 1980). African elephants had a lower SGPT level (3.0 i.u./l; Brown and White, 1980) than that observed in this study. The SGPT levels in elephants were in close agreement with the reported values in horses (1 to 6.7 i.u./l; Cornelius et al., 1959; Zimmerman et al., 1965; Kaneko and Cornelius, 1970; Doney, 1971 and Benjamin, 1978), in dogs (2.4 to 14.4 i.u./l; Cornelius et al., 1959; Zimmerman et al., 1965; VanVleet and Alberts, 1968; Nirmalan and Nair, 1969; Kaneko and Cornelius, 1970; Doney, 1971 and Benjamin, 1978), in cats (1.7 to 18 i.u./l; Zimmerman et al., 1965; Kaneko and Cornelius, 1970 and Benjamin, 1978), in goats (4 to 24 i.u./l; Zimmerman et al., 1965; and Benjamin, 1978), in sheep (0.24 to 11.14 i.u./l; Buck et al., 1961; Zimmerman et al., 1965; Kaneko and Cornelius, 1970; Doney, 1971 and Benjamin, 1978) and in

cattle (3.9 to 29 i.u./l; Cornelius et al., 1959; Buck et al., 1961; Roussel and Stallcup, 1965; Zimmerman et al., 1965; Nirmalan and Nair, 1969; Kaneko and Cornelius, 1970; Doxey, 1971; Singh et al., 1972; Dunavant et al., 1974 and Benjamin, 1978). The SGPT levels in pigs (9 to 25 i.u./l; Cornelius et al., 1959; Zimmerman et al., 1965; Nirmalan and Nair, 1969 and Benjamin, 1978) and in buffaloes (7.2 to 8.81 i.u./l; Singh et al., 1972 and Pyne and Maitra, 1982) were found to be higher than the values observed in the present study.

The serum LDH level in Indian elephants ranged from 366.67 ± 90.64 i.u./l in baby elephants to 468.75 ± 55.14 i.u./l in adult female elephants. No significant difference could be observed between the baby elephants and adult elephants and between the adult males and adult females. Brown and White (1980) reported the LDH level in serum of Indian elephants (525 i.u./l). The serum level of LDH is known to increase during muscle trauma and during lysis of erythrocytes. If the blood samples had been collected from animals shot dead, the level of the enzyme is, thus, bound to be high. According to Codazzo et al. (1974), anxiety and agitation elevated the serum LDH level in horses. The higher values reported by Brown and White (1980) could be probably due to these reasons. Moreover, the presence of inhibitors or potentiators of the activity of an enzyme or the differences in the kinetic properties of isoenzymes from different sources

might modify the measured level (Boyd, 1962). The levels obtained in the present work were in agreement with the serum LDH levels reported in cattle (176 to 778 i.u./l; Zimmerman et al., 1965; Prasse, 1969; and Benjamin, 1978). The levels in other species like horse (29 to 242 i.u./l), pig (96 to 199 i.u./l), dog (10-38 i.u./l), cat (16 to 95 i.u./l), goat (31 to 198 i.u./l) and sheep (60 to 302 i.u./l) (Zimmerman et al., 1965 and Benjamin, 1978) were lower than the mean values obtained in this study. The serum LDH level was found to be higher in young lambs than in adults (Persechino et al., 1973; and Beatty and Doney, 1984).

The data gathered during the present study revealed a lower level, though non-significant, in baby elephants than adults. Since LDH is present in many tissues, analysis of isoenzyme levels are needed for reliable diagnosis as to the specific tissue of damage.

The serum CPK level in Indian elephants in the present study varied from 30.28 ± 12.0 i.u./l in baby elephants to 51.27 ± 7.4 i.u./l in adult male elephants. Sex or age had no effect on the CPK level. Brown and White (1980) reported the values for CPK level in Indian elephants (153 i.u./l) and in African elephants (114 i.u./l). The value obtained in the present study is lower than those reported. It may be mentioned that muscle trauma could lead to CPK release (Goodley, 1970; Benjamin, 1978). Samples collected from

elephants shot dead are, thus, prone to contain elevated CPK levels. Besides, anxiety and excitement is established to increase the CPK level (Codazzo et al., 1974). The values obtained in the present study were similar to the values reported in species like horse (1.3 to 58 i.u./l; Cardinet et al., 1967; Doney, 1971 and Benjamin, 1978), goat (14 to 62 i.u./l; Garnier et al., 1984) and cattle (0.87-50 i.u./l; Doney, 1971 and Benjamin, 1978). The serum CPK levels in dogs (0.2 to 3.6 i.u./l; Benjamin, 1978), in sheep (0.3 to 1.7 i.u./l; Doney, 1971), and in cats (0.4 to 3.4 i.u./l; Benjamin, 1978) were far below the level reported in the present study. Coodley (1970) reported a lower level of serum CPK in females than males. In the present study too the adult male elephants showed a higher CPK level, even though the difference was not significant statistically. Cardinet et al. (1967) reported significant age and sex variations in CPK levels in dogs with higher values in young ones and males.

In general, the source and physiologic significance of the enzymes found in the blood plasma/serum of normal animals are even now uncertain. These enzymes have been considered to represent the products of disintegration of cells undergoing normal "wear and tear". Had that been the case, the levels of individual enzymes in each species would tend to parallel each other to a much greater degree than what is observed. The observations that intracellular enzymes may

be released from intact cells suggests the alternate possibility that the source of the serum enzymes in normal animals may be normal non-disrupted cells. It would seem more reasonable to infer that the serum levels of enzymes in normal animals relate to other factors in addition to the rate of "turnover" of cells. These may include the molecular nature of the respective enzyme protein, the characteristics of the cell membranes and perhaps other factors. Each of these factors may be presumed to be species dependent. The species differences in the concentration of an enzyme in the tissue of origin may determine at least in part, differences in the serum levels of that enzyme in normal animals. The relative levels of various enzymes in the sera of different animals can be inferred only approximately from the measured activities, since they reflect only in part the concentration of enzyme protein.

The available evidence suggests that most enzymes originated at an early stage in evolution and that adaptive variations of animals and formation of new species are dependent on the evolution of the control mechanism. At the same time, the potential which an organism possesses for multiplication of its genes and diversification of the distribution of its enzymes is a measure of its fitness to survive in an environment which may undergo gradual or sudden and radical change as a result of human interference as well as natural calamities.

CHAPTER VIII

**MINERAL STATUS
OF INDIAN ELEPHANTS**

CHAPTER VIII

MINERAL STATUS OF INDIAN ELEPHANTS

INTRODUCTION AND REVIEW

Serum or plasma is normally preferred over whole blood for analysis of minerals since variations in the number or the size of erythrocytes are known to greatly alter the levels.

Maintenance of optimal level of sodium, the chief cation of plasma, is of paramount importance because of its role in osmotic pressure, acid-base balance, neuromuscular irritability, cellular permeability and enzyme activation. In the tropical working animal, the level is bound to be low, due to sweating and the poor content of sodium in herbage. Values for plasma/serum sodium levels, collected from literature, are given in table VIII.1.

Potassium is the intracellular cation and it plays a very important part, along with sodium, in the maintenance of acid-base equilibrium, osmotic pressure, nerve transmission and enzyme kinetics. Maintenance of proper potassium concentration in the extracellular fluid is essential, particularly for proper functioning of heart. High concentration of potassium causes widespread intracardiac block, while low concentration impairs the contractility of the heart muscles. Literature values for serum potassium are furnished in table VIII.1.

About 99 per cent of the body calcium is seen in the skeleton and teeth and the remaining 1 per cent is widely distributed in cells and tissue fluids. Calcium is found in plasma as free ion, as protein-bound and as a complex. The diffusible ionized form is the physiologically active form. Calcium present in bone is not static but is involved in continuous and very rapid interchange between blood and tissue fluids. Parathyroid hormone and calcitonin play vital roles in maintaining the blood calcium level within normal limits. Optimal ratio of calcium and phosphorus as well as the level of vitamin D influences calcium absorption. Acidic medium and high protein content of the diet favour the absorption of calcium, while the same is inhibited by fat, phytates and oxalates. Calcium is well known to influence several important processes like osteogenesis, neuromuscular irritability, cellular

permeability and acid-base balance. In heavy lactation, growth and pregnancy, there is depletion of calcium reserve. Table VIII.1 presents the serum calcium levels in some species.

Magnesium is an indispensable constituent of all living cells. About 60 per cent of the magnesium of the body is found in the bones. In blood, the magnesium content is very constant. Magnesium is distributed unequally between the red cells and the plasma, the former having a considerably higher concentration. Diffusible form accounts for 80 per cent of the serum magnesium, while the non-diffusible fraction is in combination with serum protein. Magnesium is of importance in osteogenesis, in activation of enzymes and for neuromuscular irritability. The data collected from literature for serum magnesium levels in some species are tabulated in table VIII.1.

The total iron content of the animal body varies with species, age, sex, nutrition and the state of health. In the body, iron is a constituent of several important compounds like haemoglobin, myoglobin, haem enzymes, transferrin and ferritin. In normal individuals of many species, only 30 to 40 per cent of the transferrin carries iron, the remainder being known as the latent iron-binding capacity. Serum iron, and both total and latent iron-binding capacity vary greatly among individuals of the same species under physiologic and

Table VIII.1

Reported values of certain major and trace elements in the sera of domesticated animals

Species	Number of animals	Sex	Age	Sodium (mmol/l)	Potassium (mmol/l)	Calcium (mmol/l)	Magnesium (mmol/l)	Iron (μ mol/l)	Copper (μ mol/l)	Zinc (μ mol/l)	References
1	2	3	4	5	6	7	8	9	10	11	12
Indian elephants	15	-	Adult	-	-	1.72 \pm 0.17 (1.25-2.74)	0.90 \pm 0.53	-	-	-	Simon (1951)
Indian elephants	11	-	Baby	-	-	3.04 \pm 0.24	0.99 \pm 0.24	-	-	-	Nirmalan and Nair (1969)
Indian elephants	14	Male	Adult	-	-	2.94 \pm 0.28	1.07 \pm 0.25	-	-	-	Nirmalan and Nair (1969)
Indian elephants	11	Female	Adult	-	-	3.12 \pm 0.32	0.96 \pm 0.24	-	-	-	Nirmalan and Nair (1969)
Indian elephants	2	Female	Pregnant	-	-	2.77 \pm 0.40	1.10 \pm 0.20	-	-	-	Nirmalan (1967)
Indian elephants	5	Female	Lactating	-	-	2.77 \pm 0.69	1.02 \pm 0.32	-	-	-	Nirmalan et al. (1967)
Indian elephants	-	-	-	132	5.1	2.32	-	-	-	-	Andrewbutter (1971)
Indian elephants	8	-	-	-	-	3.11 \pm 0.06 (2.89-3.39)	1.17 \pm 0.06 (0.57-1.97)	-	-	-	Pillai (1972)
Indian elephants	4	-	-	-	-	2.80	-	-	-	-	Ananthasubramanian (1979)
Indian elephants	-	-	-	126	4.7	1.73-3.13	0.92-1.08	-	-	-	Brown and White (1980)
African elephants (plasma)	-	-	-	150.5	5.6	-	10	-	-	-	Bartles et al. (1963)
African elephants	30	Male and female	0-59 y	125.2	6	2.19	1.51	-	-	-	Dillman and Carr (1970)
African elephants	87	Male and female	0-60 y	-	-	2.80 (2.35-3.27)	1.81 (1.07-2.55)	-	-	-	Brown and White (1977)
African elephants	105	Male and female	2-60 y	136.5 \pm 5.6	6.24 \pm 0.86	-	-	-	-	-	Brown and White (1979)
African elephants	-	-	-	125-137	5.3-6.4	2.19-2.91	1.53-1.84	20	17	-	Brown and White (1980)
Horse	-	-	-	149 (146-152)	3.3 (2.7-3.5)	3.05 (2.8-3.35)	0.75-1.05	-	-	-	Spector (1956)
Horse	30	-	-	-	-	3.09 \pm 0.14	1.03 \pm 0.13	-	-	-	Long (1961)

(continued)

Table VIII.1 continued

1	2	3	4	5	6	7	8	9	10	11	12
Horse	-	-	-	-	-	-	-	27.57	-	-	Seckington <i>et al.</i> (1967)
Horse	-	-	-	-	-	-	-	25.78	-	-	Seckington <i>et al.</i> (1967)
Horse	-	-	-	-	-	-	-	27.9±6.6	-	-	Steward and Clarkson (1968)
Horse	-	-	-	-	-	-	-	29.9±7.2	-	-	Steward and Clarkson (1968)
Horse	30	-	-	-	-	3.09±0.14	1.03±0.14	19.8±1.97 (13.07-25.07)	-	-	Kaneko and Cornelius (1970)
Horse	8	-	-	-	-	2.54±0.25	0.63±0.06	-	-	-	Kaneko and Cornelius (1970)
Horse	-	-	-	147 (147-150)	4.96 (3.94-5.86)	2.62-3.49	0.53-1.11	-	-	-	Doxey (1971)
Horse	-	-	-	-	-	-	1.95	-	14.42-28.96	-	Breazile (1971)
Horse	-	-	3 y	-	-	2.97±0.08	-	-	-	-	Archer and Jeffcott (1977)
Horse	-	-	4 y	-	-	2.94±0.08	-	-	-	-	Archer and Jeffcott (1977)
Horse	-	-	4 y	-	-	2.95±0.08	-	-	-	-	Archer and Jeffcott (1977)
Horse	-	-	-	144±5	4.2±0.6	-	0.79±0.08	-	-	-	Archer and Jeffcott (1977)
Horse	-	-	1 y	-	-	3.19±0.1	-	-	-	-	Archer and Jeffcott (1977)
Horse	-	-	-	-	4.26	-	0.87	-	-	-	Gaola <i>et al.</i> (1983)
Pig	-	-	-	-	-	-	1.1 (0.85-1.65)	-	-	-	Albritton (1952)
Pig	-	-	3 m	-	-	-	-	-	29.2±2.4	-	Lahey <i>et al.</i> (1952)
Pig	-	-	-	155 (140-160)	5.9 (4.9-7.1)	2.25-2.85	-	-	-	-	Spector (1956)
Pig	-	-	Adult	140±4.7	6.0±0.6	-	-	-	-	-	Widdowson and McCance (1956)
Pig	-	-	4 m	-	-	-	-	-	-	8.8±0.5	Hoekstra <i>et al.</i> (1956)
Pig (plasma)	10	-	-	-	-	-	1.3±2.0	31.3±12.1	17.31±6.6	11.47±1.5	Long (1961)
Pig	13	-	-	143.98±4.7	5.98±0.2	-	-	-	-	-	Long (1961)
Pig	11	-	New born	141.5±4.8	8.57±0.2	-	-	-	-	-	Long (1961)
Pig	-	-	-	-	-	3.09	-	-	-	-	Tegeris <i>et al.</i> (1966)
Pig	-	-	6 m	-	-	2.41±0.2	-	-	-	-	Kaneko and Cornelius (1970)

(continued)

Table VIII.1 continued

1	2	3	4	5	6	7	8	9	10	11	12
Pig	-	-	Pregnant	-	-	2.52±0.27	-	-	-	-	Kaneko and Cornelius (1970)
Pig	-	-	Adult	131.0	4.5	2.75	0.85	-	-	-	Baetz and Mengeling (1971)
Pig	-	-	-	-	-	-	-	22.0	-	-	Furugouri (1971)
Pig (plasma)	-	-	-	-	-	-	-	-	-	9.18	Underwood (1971)
Pig	-	-	-	-	-	-	-	-	29.98	13.46±0.3	Doxey (1971)
Pig (plasma)	20	-	-	-	-	2.87 (2.25-3.84)	1.08 (0.37-1.86)	-	-	-	Pillai (1972)
Pig	-	-	3 m	148.9±5.0	5.5±0.5	2.77±0.15	0.91±0.08	-	-	-	Archer and Jeffcott (1977)
Pig	26	-	-	136±9.5	4.91±0.8	2.51±2.29	0.86±0.13	-	-	-	Kupski <i>et al.</i> (1984)
Dog	-	-	-	143 (137-149)	-	2.45 (2.1-2.8)	0.95 (0.7-1.2)	-	-	-	Albritton (1952)
Dog (plasma)	-	-	-	147 (140-154)	4.4 (3.7-5.8)	2.65 (2.85-3.05)	0.9 (0.75-1.0)	-	-	-	Albritton (1952)
Dog (plasma)	-	-	-	147±4.0	4.09±0.28	2.53±0.5	0.86±0.12	29.9±5.8	-	-	Long (1961)
Dog	-	-	-	-	-	2.55±0.5	-	19.34 (16.35-21.85)	-	-	Kaneko and Cornelius (1970)
Dog	-	-	-	146 (139-153)	4.4 (3.7-5.8)	2.62	0.94 (0.69-1.18)	-	-	-	Doxey (1971)
Dog (plasma)	-	-	-	-	-	2.71±0.16 (1.95-3.79)	1.00±0.10 (0.57-1.97)	-	-	-	Pillai (1972)
Dog	-	-	-	141-155	3.7-5.8	2.3-3.04	-	-	-	-	Ledet (1980)
Cat	-	-	-	151 (147-156)	4.3 (4.0-4.5)	-	2.2	-	-	-	Albritton (1952)
Cat (plasma)	-	-	-	153 (150-156)	-	-	-	-	-	-	Albritton (1952)
Goat	-	-	-	-	-	-	1.0	-	-	-	Spector (1956)
Goat	12	Female	-	-	-	-	-	32.59 (18.26-54.43)	-	-	Underwood (1971)
Goat	12	Male	-	-	-	-	-	27.22 (20.41-34.2)	-	-	Underwood (1971)

(continued)

Table VIII.1 continued

1	2	3	4	5	6	7	8	9	10	11	12
Sheep	-	-	-	160	4.8	2.85	0.95 (0.85-1.05)	-	-	-	Spector (1956)
Sheep	-	-	-	146±4.9	4.85±0.39	2.53 (2.17-2.87)	-	-	-	-	Doxey (1971)
Cattle (plasma)	-	-	-	-	4.85	2.71	-	-	-	-	Mcsherry and Grinyer (1954)
Cattle	-	-	-	142 (132-152)	4.8 (3.9-5.8)	2.75 (2.35-3.05)	1.65 (0.5-1.8)	-	23.61	-	Spector (1956)
Cattle	10	-	-	-	-	2.61±0.05	-	-	-	-	Mithulji <i>et al.</i> (1966)
Cattle	-	-	-	-	-	-	-	-	13.77	-	Mills <i>et al.</i> (1967)
Cattle	-	-	-	143±5.2	4.7±0.48	2.54±0.14	0.95±0.15	-	-	-	Mylrea and Bayfield (1968)
Cattle	-	Female	-	-	-	2.67±0.05	1.28±0.03	-	18.89	-	Patel <i>et al.</i> (1969)
Cattle	-	Female	-	-	-	-	-	26.14 (15.94-45.30)	-	-	Underwood (1971)
Cattle	-	Male	-	-	-	-	-	25.96 (16.47-48.35)	-	-	Underwood (1971)
Cattle	-	-	-	139 (135-145)	4.75 (3.9-5.6)	2.62-3.5	0.86 (0.74-1.12)	-	11.0	-	Doxey (1971)
Cattle	30	-	-	-	3.14±0.06	-	-	-	-	-	Desai <i>et al.</i> (1979)
Cattle	-	-	-	-	-	-	-	24.35-35.6	6.9-10.2	16.52-26.26	Ormian <i>et al.</i> (1982)
Cattle	30	Female	-	139.17±0.93	5.4±0.09	2.38±0.4	-	-	-	-	Borvonsin and Sirikhajornbhandu (1983)

Note: The necessary values had been converted to the international (SI) system of units as per Collins and Kelly (1977).

Figures in parenthesis indicate the range

pathologic conditions. Iron deficiency seldom occurs in grazing stock under natural conditions. Deficiency is possible with high cereal diet and low animal protein diet or inadequate absorption or excessive loss of blood or a disturbance in iron metabolism due to parasitic infestations or diseases. The serum iron level in different species are given in table VIII.1.

In the blood, copper is distributed approximately equally between erythrocytes and plasma, except in late pregnancy, when the concentration rises in the plasma. Living cells contain several copper containing enzymes with oxidative functions. Copper acts as a catalyst in the utilisation of iron in haemoglobin formation. The copper in plasma occurs in two main forms - one firmly attached and the other loosely bound. The former consists of ceruloplasmin which is an α_2 -globulin accounting for 96 per cent of the plasma copper. The remaining plasma copper is loosely bound to serum albumin. The normal range of concentration of copper in the blood of healthy animals is similar in all higher animals. Table VIII.1 gives the serum copper level in elephants and some other species. Iron:copper ratio is of clinical importance in diagnosis of certain ailments.

About one-third of the plasma zinc is loosely bound with serum albumin and the remainder is attached firmly

with globulin. Most of the zinc in blood is present in erythrocytes as it forms the integral part of carbonic anhydrase. It is involved in enzyme systems, hormones, protein synthesis and carbohydrate metabolism. The level of serum zinc is influenced by dietary intake, acute stress and pregnancy. The available data on levels of serum zinc in other species are given in table VIII.1.

The present study encompasses an investigation into the levels of sodium, potassium, calcium, magnesium, iron, copper, iron:copper ratio and zinc in Indian elephants.

MATERIALS AND METHODS

Blood samples were collected from eight baby elephants, nineteen adult male elephants and seventeen adult females. All the animals were clinically healthy and maintained under identical nutritional regime (vide Chapter II). Blood samples were collected in clean glass tubes, kept in slanting position for 25 min and then kept at 4°C for 10 hours. They were then centrifuged (2500 x g for 15 min) at ambient temperature. The separated serum was used for the determination of major elements (Na, K, Ca and Mg). The trichloroacetic acid protein-free serum filtrate (annexure 25) was used for the estimation of trace elements (Fe, Cu and Zn).

Serum levels of sodium and potassium were estimated by adopting the method of Osler (1965) using a flame photometer.

The procedures recommended in the manual (1976) supplied by M/s. Perkin-Elmer were followed for the estimation of calcium, magnesium, iron, copper and zinc in serum using atomic absorption spectrophotometer (Perkin-Elmer, Model 2380). Iron:copper ratio was then worked out.

The significance of the mean values obtained was tested by applying Student's t test (Snedecor and Cochran, 1967).

RESULTS

Table VIII.2 presents the values for sodium, potassium, calcium and magnesium in the serum of the animals studied (see annexures 26 to 29 for raw data). No significant difference could be detected among these groups in the values for sodium or potassium. Baby elephants had a significantly higher level of calcium than adult female elephants ($p < 0.05$). Apart from this, age or sex had no influence on serum calcium level. No significant difference could be observed between the three groups in the values for serum magnesium.

Values obtained for iron, copper, iron:copper ratio and zinc are presented in table VIII.3 (annexures 30 to 33 furnish the raw data). Age or sex had no influence on the distribution of serum iron. The baby elephants showed significantly lower level ($p < 0.05$) of serum copper than the adult females. There were no significant differences in the concentrations between adult males and adult females or between adult males and baby elephants. Iron:copper ratio had been calculated. Baby elephants had a higher ratio than adult males. The adult female elephants showed a significantly higher level ($p < 0.05$) of serum zinc than the baby and adult male elephants. There was no significant difference between the levels in the baby elephants and adult males.

Table VIII.2

Major minerals in the serum of Indian elephants⁺

Constituents	Baby elephants	Adult male elephants	Adult female elephants
Sodium (mmol/l)	125.36 \pm 6.7(8)	118.24 \pm 4.3(19)	126.99 \pm 4.6(17)
Potassium (mmol/l)	4.97 \pm 0.17(8)	4.77 \pm 0.11(19)	4.85 \pm 0.12(17)
Calcium (mmol/l)	2.36 \pm 0.058(8) ^a	2.24 \pm 0.038(19) ^a	2.17 \pm 0.046(17) ^b
Magnesium (mmol/l)	1.05 \pm 0.046(8)	1.03 \pm 0.031(17)	0.97 \pm 0.032(17)

Figures in parenthesis indicate the number of animals

⁺ Mean \pm S.E.

Different superscripts in the same horizontal line reveal statistical significance

Table VIII.3

Trace elements in the serum of Indian elephants⁺

Constituents	Baby elephants	Adult male elephants	Adult female elephants
Iron (μ mol/l)	34.78 \pm 14.0(6)	44.48 \pm 8.3(17)	43.26 \pm 8.9(15)
Copper (μ mol/l)	23.84 \pm 3.4(6) ^a	28.74 \pm 2.0(17) ^a	33.69 \pm 2.1(16) ^b
Iron:copper ratio	1.50 \pm 0.17 ^a	1.14 \pm 0.06 ^b	1.33 \pm 0.11 ^a
Zinc (μ mol/l)	31.13 \pm 3.4(6) ^a	33.73 \pm 2.5(11) ^a	42.61 \pm 2.4 (12) ^b

Figures in parenthesis indicate the number of animals

⁺ Mean \pm S.E.

Some superscripts in the horizontal line indicate absence of significant difference

DISCUSSION

The serum sodium level in the present study was found to vary from 118.24 ± 4.3 to 126.99 ± 4.6 mmol/l in the case of baby, adult male and adult female elephants. Bartles *et al.* (1963) reported a higher sodium level in an African elephant (150.5 mmol/l). But, Dillman and Carr (1970) observed a mean level of 125.2 mmol/l in 80 African elephants. Andrewbutter (1971) noted the serum sodium level in Indian elephants as 132 mmol/l. A similar value of 136.5 ± 5.6 mmol/l was reported by Brown and White (1977) from the studies on 97 African elephants. Brown and White (1980) reported the serum sodium level in Indian elephants as 126 mmol/l and in African elephants as from 125 to 137 mmol/l. The values obtained in the present study seem to be in agreement with those reported by Dillman and Carr (1970) and Brown and White (1980). Comparison of the values obtained in the present study with those reported in literature for African elephants did not reveal any major variation. The serum sodium level in elephants was found to be lower than the reported values in horses (140 to 160 mmol/l), in pigs (131 to 160 mmol/l), in dogs (137 to 155 mmol/l), in cats (147 to 156 mmol/l), in sheep (146 to 160 mmol/l) and in cattle (132 to 152 mmol/l) (Albritton, 1952; Spector, 1956; Widdowson and McCance, 1956; Long, 1961; Mylrea and Bayfield, 1969; Baetz and Mengeling, 1971; Doney, 1971; Archer and

Jeffcott, 1977; Ledet, 1980; Borvonsin and Siritthajornbhandu, 1983 and Kupski *et al.*, 1984). The low level noted in elephants may be attributed to the poor content of sodium in herbage, possibly due to the high rainfall in the regions wherein the elephants were stationed. The type of work the elephants were engaged in - hauling and piling of timber - also might have contributed for this. In a survey conducted on 132 African elephants in Uganda game parks, 95 per cent of the animals had a range between 124 to 147 mmol/l (Brown and White, 1979). But, mean level in 80 African elephants much farther south in the Luangwa village in Zambia was 125 mmol/l (Dillman and Carr, 1970). This is suggestive of the existence of a variation due to geographical location. Age or sex was found to have no influence on serum sodium level (Dillman and Carr, 1970; Brown and White, 1979). Brown and White (1979) observed a seasonal variation, the value being always lower in the wet season.

Serum potassium level was found to range from 4.77 ± 0.11 mmol/l to 4.97 ± 0.17 mmol/l in the present study. African elephants appeared to have higher potassium level than that observed in Indian elephants. The potassium level in serum of African elephants had been reported to be 5.6 mmol/l (Bartles *et al.*, 1963); 6 mmol/l (Dillman and Carr, 1970); 6.24 ± 0.86 mmol/l (Brown and White, 1979) and 5.3 to 6.4 mmol/l (Brown and White, 1980). The values obtained in the present work were in agreement with those

reported by Andrewbutter (1971) and Brown and White (1980). The serum potassium level in Indian elephants appeared to be similar to that in horse (2.7 to 5.86 mmol/l), in pig (4.5 to 8.57 mmol/l), in dog (3.7 to 5.8 mmol/l), in cat (4.0 to 4.5 mmol/l), in sheep (4.8 to 4.85 mmol/l), and in cattle (3.14 to 5.18 mmol/l) (Albritton, 1952; McSherry and Grinyer, 1954; Spector, 1956; Widdowson and McCance, 1956; Baetz and Mengeling, 1971; Doxey, 1971; Archer and Jeffcott, 1977; Desai et al., 1979; Ledet, 1980; Gaola et al., 1983; Borvonsin and Sirikajornbhandu, 1983 and Kupski et al., 1984). Values available in literature on the chemistry of blood of African elephants were invariably based on studies on elephants that have been shot. Naturally, the involved delay in the collection of blood might have affected the values as atleast a part of the cells would have disintegrated by then releasing the intracellular constituents to the outside. Dillman and Carr (1970) found that the serum potassium level in African elephants varied with age. Brown and White (1979) observed a high potassium level during the wet season and a drop during the dry season. They could not detect any influence of age, sex or geographical location. In the present study too, no significant change, due to age or sex could be detected in the serum potassium level.

The level of calcium ranged from 2.17 ± 0.040 to 2.36 ± 0.058 mmol/l. Earlier reports on serum calcium level of Indian elephants include those of Simon (1961)

(1.25 to 2.74 mmol/l); Nirmalan and Nair (1969) (from 2.94 \pm 0.28 mmol/l in adult male to 3.12 \pm 0.32 mmol/l in adult non-pregnant non-lactating females); Andrewbutter (1971) (2.32 mmol/l); Pillai (1972) (3.11 \pm 0.06 mmol/l); Ananthasubramanian (1979) (2.80 mmol/l) and Brown and White (1980) (1.73 to 3.13 mmol/l). The calcium level in the present study was within the range reported in other species like dog (1.95 to 3.79 mmol/l), sheep (2.17 to 3.05 mmol/l), horse (2.54 to 3.49 mmol/l), pig (2.25 to 3.84 mmol/l) and cattle (2.38 to 3.5 mmol/l) (Albritton, 1952; McSherry and Grinyer, 1954; Spector, 1956; Long, 1961; Mithulji et al., 1966; Tegeris et al., 1966; Mylrea and Bayfield, 1968; Patel et al., 1969; Kaneko and Cornelius, 1970; Pillai, 1972; Archer and Jeffcott, 1977; Ledet, 1980; Borvonsin and Sirikhajornbhandu, 1983 and Kupski et al., 1984). Dillman and Carr (1970) detected no influence of age or sex on serum calcium level in African elephants. Brown and White (1977) failed to observe any difference in the levels between sexes or different age groups. However, significant differences, due to season and geographical location, were observed in African elephants. Nirmalan and Nair (1969) also reported the absence of any influence attributable to sex or age on serum calcium level in Indian elephants. The present study revealed a significantly higher calcium level ($p < 0.05$) in baby elephants than in adult females. No difference could be observed between the

adult male and baby elephants. Urbanyi (1958) reported that calcium level varied with age, breed and to a lesser extent with sex in the case of cattle, horse and pigs. McSherry and Grinyer (1954), Payne and Leach (1964) and Tumbleson et al. (1973) reported a decreasing trend in calcium levels in dairy cattle with increasing age. Kaneko and Cornelius (1970) reported that oestrogen lowers the level of serum calcium. The low level observed in adult females in this study may be attributed, in part, to the influence of oestrogens.

The present study on Indian elephants revealed that the serum magnesium level varied from 0.97 ± 0.032 to 1.05 ± 0.046 mmol/l. The values were in agreement with the reported serum magnesium levels in Indian elephants (Simon, 1961 - 0.90 ± 0.53 mmol/l; Nirmalan and Nair, 1969 - from 0.96 ± 0.24 mmol/l in adult non-pregnant non-lactating female to 1.07 ± 0.25 mmol/l in adult female; Pillai, 1972 - 0.57 to 1.97 mmol/l; Brown and White, 1980 - 0.92 to 1.082 mmol/l). The values also agreed with those of Bartles et al. (1963) in African elephants (1.0 mmol/l) but, were lower than those reported by Dillman and Carr (1970) (1.51 mmol/l); Brown and White (1977) (1.07 to 2.55 mmol/l) and Brown and White (1980) (1.53 to 1.84 mmol/l). Magnesium is normally found intracellularly and the delay in the collection of blood after the elephant had been shot or the occurrence of

haemolysis might have contributed substantially to the elevated values reported in African elephants. The values in the present study were in agreement with the serum magnesium levels in horse (0.53 to 1.95 mmol/l), in pig (0.37 to 1.86 mmol/l), in dog (0.57 to 1.97 mmol/l), in goat (1.0 mmol/l), in sheep (0.85 to 1.05 mmol/l) and in cattle (0.5 to 1.8 mmol/l) (Albritton, 1952; Spector, 1956; Long, 1961; Mylrea and Bayfield, 1968; Patel *et al.*, 1969; Kaneko and Cornelius, 1970; Baetz and Mengeling, 1971; Breazile, 1971; Doxey, 1971; Pillai, 1972; Archer and Jeffcott, 1977; Gaola *et al.*, 1983 and Kupski *et al.*, 1984). No influence of age or sex could be detected. Similar findings had been reported by Nirmalan and Nair (1969) and Dillman and Carr (1970).

The level of serum iron in Indian elephants in the present study ranged from 34.78 ± 14.0 to $44.48 \pm 8.3 \mu\text{mol/l}$. Brown and White (1980) had reported a mean serum iron content of $20 \mu\text{mol/l}$ in African elephants. The value obtained in the present study seemed to be high. The values reported in goats (18.26 to $54.43 \mu\text{mol/l}$) and in cattle (15.94 to $48.85 \mu\text{mol/l}$) (Underwood, 1971) seemed to agree with that recorded in present study. The serum iron concentration was found to be higher than those in horse (13.07 to $29.9 \mu\text{mol/l}$), in pig (22 to $31.3 \mu\text{mol/l}$) and in dog (16.85 to $29.9 \mu\text{mol/l}$) (Long, 1961; Seckington *et al.*, 1967; Steward and Clarkson, 1968; Kaneko and Cornelius, 1970;

Furugouri, 1971; Underwood, 1971 and Ormian et al., 1982). Nirmalan et al. (1967) reported no significant difference in the haemoglobin content due to sex or age in Indian elephants. The present study also revealed absence of any significant effect attributable to sex or age in the serum iron level in Indian elephants.

The serum copper level in Indian elephants was found to range from 23.84 ± 3.4 to $33.69 \pm 2.1 \mu\text{mol/l}$ in the present study. The value was found to be higher than those reported in African elephant ($17 \mu\text{mol/l}$) (Brown and White, 1980). The values compared favourably with the values reported in literature for horse (14.42 to $28.96 \mu\text{mol/l}$) and pig (17.31 to $29.98 \mu\text{mol/l}$) but, were higher than in cattle (6.9 to $23.61 \mu\text{mol/l}$) (Lahey et al., 1952; Spector, 1956; Long, 1961; Mills, et al., 1967; Patel et al., 1969; Breazile, 1971; Doxey, 1971 and Ormian et al., 1982). Mithulji et al. (1966) reported a low copper level in cows compared to those in calves or bullocks and attributed it to the feeding regime. Patel et al. (1969) also reported a high serum copper level in young calves than in old animals. In the present study, no such effect of age was observed. Underwood (1971) could not observe any influence of sex on serum copper in many species excepting in human beings. It was reported that oestrogens increased the plasma copper level in human beings. In the present study also, significantly higher levels of copper were observed in adult females

compared to adult males; yet, the difference was not of statistical significance. The apparently high level of copper noticed in females might be due to the effect of oestrogens probably through the synthesis of ceruloplasmin.

Iron:copper ratio had been found to vary from 1.14 ± 0.06 to 1.50 ± 0.17 in the elephants studied. In human beings the normal ratio is reported to vary from 0.8 to 1.0. Apparently, the ratio is high in elephants compared to human beings.

The serum zinc level in the present investigation varied from $31.13 \pm 3.4 \mu\text{mol/l}$ to $42.61 \pm 2.4 \mu\text{mol/l}$. No value was available in literature on serum zinc level in either Indian or African elephants. The values obtained in elephants in this study were high uniformly compared with other domesticated animals like pig (8.8 to $13.46 \mu\text{mol/l}$) and cattle (16.52 to $26.26 \mu\text{mol/l}$) (Hoekstra *et al.*, 1956; Long, 1961; Doxey, 1971; Underwood, 1971 and Ormian *et al.*, 1982). In the case of rabbits, the level was reported to be $41.35 \mu\text{mol/l}$ (Underwood, 1971). According to Underwood (1971) there is high individual variability, a small or non-existent difference due to age, race or sex and no seasonal or diurnal variation in zinc level. Halstead *et al.*, (1968) observed an elevated plasma zinc level in females due to the effect of oestrogens. In the present study a significantly higher serum zinc level ($p < 0.05$) was observed in adult female elephants than the baby or adult males and this could probably be due to the influence of oestrogens.

SUMMARY

SUMMARY

Information available on the physiologic norms of Indian elephants are scarce and scanty and so, an attempt had been made to establish norms for some of the physiologic profiles in 44 Indian elephants, of varying ages and both sexes, maintained under ideal conditions of management by the Forest Department, Devaswams and private owners.

Equations to predict body weight and height at shoulder from body measurements had been derived. Several models were tried and the one with the highest multiple correlation coefficient was chosen as the recommended one. Formulae recommended were:

1. $W = -1237.21 + 0.038 (BFH-BT) \times CG$, where

'W' is the body weight in kg, 'BFH-BT', the body length from the base of fore-head to the base of tail in cm and 'CG', the chest girth in cm, and

2. $HT = 21.04 + 1.77 FC$, where 'HT' is the height at shoulder in cm and 'FC', the right fore-foot circumference in centimetres.

The body of the elephant was divided into several geometrical figures (regions) and the areas of these individual regions were measured. These were added up to give the total surface area in square metres. A prediction equation incorporating height along with body weight was derived to estimate the total surface area of elephants. The equation recommended was:

$$s = 0.2533 w^{0.5153} H^{0.3920},$$

where 's' is the total body surface area in m^2 , and 'w' and 'H' are the body weight (in kg) and height at shoulder (in m) respectively.

Attempts were made to predict total body surface area from surface area of individual regions. The formula:

$$s = (1.3712BA + 1.772HLA + 1.2147 FLA) - 0.2304,$$

where 's' is the total body surface area in m^2 , 'BA', the body area and 'HLA' and 'FLA' the hind-limb and fore-limb surface areas in m^2 , had the highest multiple correlation coefficient and is being recommended. The basal heat production in elephant was estimated as 8.76 ± 0.16 Cal/day/kg body weight or 1153.80 ± 25.66 Cal/day/sq.m. surface area.

The specific gravity of whole blood and plasma, relative and absolute viscosity of blood, serum icterus index, pH of

whole blood and plasma, whole blood coagulation time and erythrocyte sedimentation rate were determined in the blood of baby, adult male and adult female elephants. Excepting for erythrocyte sedimentation rate wherein significant differences were noted in the rates of settling at 30, 40, 50 and 60 min between baby elephants and adult elephants, other constituents did not reveal any influence of age or sex. For application of erythrocyte sedimentation rate as a clinical diagnostic aid, the time of 15 min was recommended as optimal.

No influence of age or sex could be detected in the levels of serum total proteins, albumin, globulin and in the A/G ratio. The A/G ratio was found to be less than one.

Electrophoretic studies with agar-gel revealed the presence of 5 fractions - albumin, and α_1 -, α_2 -, beta- and gammaglobulins in the serum. Lipoproteins separated into four fractions - chylomicrons, low-density, very low-density and high-density lipoproteins. Existence of polymorphism in albumin was detected. No variants of haemoglobin could be identified.

Glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, lactic dehydrogenase and creatine phosphokinase levels in the serum were assayed to establish the norms. The results showed absence of any influence of age or sex.

The normal serum levels of the major minerals - sodium, potassium, calcium and magnesium and trace elements - iron, copper and zinc - were determined. The results obtained compared favourably with the reported values in other domestic animals. Iron:copper ratio was found to be above 1.0.

REFERENCES

REFERENCES

- Beatty, E.M. and Doxey, D.L. (1984). The effect of physiological parameters on serum lactate isoenzymes in lambs. Vet. Res. Commun., 54: 33-40.
- Benedict, F.G. (1936). The Physiology of the Elephant. Carnegie Institute, Washington, pp. 58, 93-101, 263-286.
- Benjamin, M.M. (1978). Outline of Veterinary Clinical Pathology. The Iowa State University Press, Ames, Iowa, USA, 3rd Ed. pp. 67, 169, 225-268.
- Beraide, V. and Enyeniki, U.K. (1969). Haemoglobin types in some Nigerian goat breeds. Res. vet. Sci., 10: 309-310.
- *Bogin, E., Avidar, Y., Eyal, J. and Sturam, H. (1977). Blood levels of enzymes, proteins, metabolites and minerals in healthy bulls. Refuch. Veterinarith., 34: 19-20. Abstr. Vet. Bull. (1978), 48: 3312.
- *Boiti, C. and Grosso, G. (1972). Cited by Archer, R.K. and Jeffcott, L.B. (1977).
- Borvonsin, S. and Sirikajornbhandu (1983). Preliminary study on haematology of native heifers. Thai. J. Vet. Med., 13: 1-9.
- Boyd, J.W. (1962). The comparative activity of some enzymes in sheep, cattle and rats - Normal serum and tissue levels and changes during experimental liver necrosis. Res. vet. Sci., 3: 256-268.
- Breazile, J.E. (1971). Textbook of Veterinary Physiology. Lea & Febiger, Philadelphia, pp. 456, 463.
- Briles, W.E., Briles, R.N., Pullock, D.L. and Pattism, M. (1982). Marek's disease resistance of B (MHC) heterozygote in a cross of purebred leghorn lines. Poult. Sci., 6: 205-211.

- Brody, S. (1945). Bioenergetics and Growth. Hafner Publishing Co., New York, pp. 352-390, 475.
- Brown, D.W., Collins, W.M., Ward, P.H. and Briles, W.E. (1982). Complementation of major histocompatibility haplotypes in regression of Rous sarcoma virus induced tumours in non-bred chicken. Poult. Sci., 61: 409-413.
- Brown, I.R.F., and White, P.T. (1977). Serum calcium, magnesium, phosphorus and alkaline phosphatase in the African elephant (Loxodonta africana). Comp. Biochem. Physiol., 56B: 159-162.
- Brown, I.R.F. and White, P.T. (1979). Serum electrolytes, lipids and cortisol in the African Elephant (Loxodonta africana). Comp. Biochem. Physiol., 899-901.
- Brown, I.R.F., and White, P.T. (1980). Elephant blood haematology and chemistry. Comp. Biochem. Physiol., 65B: 1-12.
- Brown, I.R.F., White, P.T. and Malpas, R.C. (1978). Proteins and other nitrogenous constituents in the blood serum of the African elephant (Loxodonta africana). Comp. Biochem. Physiol., 59A: 267-270.
- Buck, W.B., James, L.F. and Binus, W. (1961). Changes in serum transaminase activities associated with plant and mineral toxicity in sheep and cattle. Cornell Vet., 51: 568-585.
- Buckell, M.B. (1958). The effect of citrate on euglobulin methods of estimating fibrinolytic activity. J. clin. Path., 11: 403-410.
- Cardinet, G.H., Litterell, J.F. and Freeland, R.A. (1967). Comparative investigation of serum creatine phosphokinase and glutamic-oxaloacetic transaminase activities in equine paralytic myoglobinuria. Res. vet. Sci., 9: 219-226.

- *Codazzo, D., Genetic, C., Agne, F. and Maffeo, G. (1974). Serum enzyme activities and blood chemical values in race horses after transport and training. Archivio Veterinario Italiano., 25: 145-154. Abstr. Vet. Bull. (1975), 43: 903.
- Coffin, D.L. (1953). Manual of Veterinary Pathology. Comstock, Ithaca, New York, 3rd Ed. pp. 178-179.
- Collins, J.D. and Kelly, W.R. (1977). The haematology and clinical biochemistry of domestic animals: the use of international (SI) systems of units. Ir. vet. J., 31: 127-133.
- Coodley, E.L. (1970). Diagnostic enzymology. Lea & Febiger, Philadelphia, pp. 16-28, 54-63, 259-263.
- Cornelius, C.E., Bishop, J., Switzer, J. and Rhode, E.A. (1959). Serum and tissue transaminase activities in domestic animals. Cornell Vet., 49: 116-125.
- Desai, H.B., Pande, M.B., Desai, M.C. and Shukla, P.C. (1979). Haematological and clinical studies of blood of Dangri cattle. Indian J. Anim. Res., 13: 43-46.
- Dillman, J.S. and Carr, W.R. (1970). Observations on arteriosclerosis, serum cholesterol and serum electrolytes in the wild African elephant (Loxodonta africana). J. comp. Path., 80: 81-87.
- Disdisheim, P., Hattori, K. and Lewis, J.H. (1959). Haematological and coagulation studies on various animal species. J. Lab. clin. Med., 53: 866-875.
- *Dotta, U., Balbo, T. and Gazzola, A. (1964). Serum levels of glutamic-oxaloacetic and glutamic-pyruvic transaminase in healthy calves and in calves with myodystrophy. Zooprofilassi., 19: 553. Abstr. Vet. Bull. (1965), 35: 1526.

- Doxey, D.L. (1971). Veterinary Clinical Pathology. Bailliere Tindall, London, pp. 69, 187.
- *DuBois, E.F. (1936). Cited by Brody, S. (1945).
- Dunavant, Michael, L. and Rich, L.J. (1974). Clinical applications of serum enzyme determination. Am. Soc. Vet. Clin. Pathol., 3: 3-26.
- Eltringham, S.K. (1982). Elephants. Blandford Press, Link House, England, pp. 129-130.
- Faulkner, W.R. and King, J.W. (1970). Manual of Clinical Laboratory Procedure. The Chemical Rubber Co., Ohio, pp. 78.
- Fesus, L., Varkonyi, J. and Ats, A. (1983). Biochemical polymorphism in goats with special reference to the Hungarian native breeds. Anim. Blood Grps. biochem. Genet., 14: 1-6.
- Furugouri, K. (1971). Normal values and physiological variations of plasma iron and total iron binding capacity of pigs. J. Anim. Sci., 32: 667-672.
- Gabrisch, K. (1978). Proteins and free amino acid in serum of African and Indian elephants. Praktische Tierarzt, 2: 112-115. Abstr. Vet. Bull. (1978), 48: 5744.
- Gajewski, J. and Povar, M.L. (1971). Blood coagulation values of sheep. Am. J. vet. Res., 32: 405-409.
- *Gaola, G., Ferlazzo, A.G. and Omer, A. (1983). Serum electrolytes in the Sandratellauo horse after physical exercise. Archivio. Veterinario Italeano, 34: 122-126. Abstr. Vet. Bull. (1984), 54: 5414.

- Garnier, F., Benoit, E., Jacquet, J.P. and Delatour, P. (1984). Serum enzymology of goats normal values of CPK, LDH, ICDH and SDH. E Cole. Nat. Vet., 15: 55-58. Abstr. Vet. Bull. (1984), 54: 6841.
- Giri, K.V., Pillai, N.C. and Ravindra Nath (1958). An electrophoretic study of Indian elephant serum proteins. Arch. Biochem. Biophys., 73: 320-330.
- *Gornall, A.G., Bardawill, C.J. and David, M.M. (1949). Determination of serum protein by means of biuret reaction. J. Biol. Chem., 177: 1751-1752. Cited by Faulkner, W.R. and King, J.W. (1970).
- *Gurbev, I.P. (1983). The origin of Yakut horses. Refrativnyi Zhurnal., 7: 50-57. Abstr. Anim. Breed. Abstr. (1984), 52: 6284.
- Haaranen, S. (1960). Some blood components of growing pigs. Nord. Vet. Med., 12: 239-244.
- Halsted, J.A., Hackley, B.M. and Smith, J.C. (1968). Plasma-zinc and copper in pregnancy and after oral contraceptive. Lancet, 21: 278-279.
- *Hans, S.K. and Lee, K.M. (1982a). Studies on albumin and post-albumin polymorphism in Korean cattle. Korean J. Anim. Sci., 24: 522-526. Abstr. Anim. Breed. Abstr. (1984), 52: 256.
- *Hans, S.K. and Lee, K.M. (1982b). Studies on polymorphism of haemoglobin of Korean and Holstein Friesian cattle. Korean J. Anim. Sci., 24: 517-521. Abstr. Anim. Breed. Abstr. (1984), 52: 255.
- *Henry et al. (1952). Cited by Levison, S.A. and MacFate, R.P. (1969).

- Hoekstra, W.G., Lewis, P.K., Phillips, P.H. and Grummer, R.H. (1956). The relationship of parakeratosis supplemental calcium and zinc to the zinc content of certain body components of swine. J. Anim. Sci., 15: 752-764.
- Hughes, B.P. (1962). A method for the estimation of serum creatine kinase and its use in comparing creatine kinase and aldolase activity in normal and pathological sera. Clin. Chim. Acta., 7: 597-598.
- Irfan, M. (1967). The electrophoretic pattern of serum proteins in the normal animals. Res. vet. Sci., 8: 137-142.
- Jain, N.C., Vengad, J.L., Jain, N.K. and Shrivastava, A.B. (1982). Haematological studies on normal lactating Indian water buffalo. Res. vet. Sci., 32: 52-56.
- Janusch, J.B. and Janusch, V.B. (1964). Plasma proteins and haemoglobins of the African elephants and the hyrax. Nature, 201: 510-511.
- Jeffcott, L.B. (1974). Haematology in relation to performance and potential. 2. Some specific aspects. J. S. Afr. Vet. med. Ass., 45: 278-286.
- Kaneko, J.J. and Cornelius, C.E. (1970). Clinical biochemistry of domestic animals. Academic Press, New York, 2nd Ed. pp. 97-125, 161-207, 313-365.
- King, J. (1965). Practical Clinical Enzymology. D. Van Nostrand Company Ltd., London, pp. 87, 130.
- *Kleiber, M. (1932). Cited by Brody, S. (1945).
- Knowles, R.P. (1955). Interpretation of white cell count, sedimentation rate and packed cell volume. Vet. Med., 50: 127-130.

- Krishnamoorthy, V. and Nair, P.G. (1979). State level workshop on elephants. Kerala Agricultural University, pp. 7-8.
- Kupski, A., Kolb, E., Grundel, G. and Nestler, K. (1984). Contents of electrolytes (calcium, magnesium, inorganic phosphates, sodium, potassium) in the blood of sows at farrowing and in new-born piglets of the whole litter before intake of colostrum and during the first day of life. Archiv fur Experimentalla Veterinar medicin., 38: 704-721. Abstr. Vet. Bull. (1985), 55: 2439.
- Kurt, F. and Nettasinghe, A.P.W. (1968). Estimation of body weight of the Ceylon elephant (Elephas maximus). Ceylon vet. J., 16: 24-26.
- *Labouche, C. (1964). Blood proteins in cows. Revue Elev. Med. Vet. Pays. trop., 721-745. Abstr. Vet. Bull. (1965), 35: 4762.
- Lahey, M.E., Gubler, C.J., Chase, M.S., Cartwright, G.E. and Wintrobe, M.M. (1952). Studies on copper metabolism. II. Haematologic manifestations of copper deficiency in swine. Blood, 7: 1053-1054.
- Laws, R.M., Parker, I.S.C. and Johnstone, R.C.B. (1975). Elephants and their habitats. Clarendon Press, Oxford, pp. 188.
- Ledet, A.E. (1980). Hepatic disease in a dog with cholestasis and normal enzyme activity. Clin. Path. Rev., 3: 121-124.
- *Lee, R.C. (1939). Cited by Brody, S. (1945).
- Levison, S.A. and MacFate, R.P. (1969). Clinical Laboratory Diagnosis. Lea & Febiger, Philadelphia, pp. 437.

- Lewis, J.H. (1974). Comparative haematology: Studies on elephants (Elephas maximus). Comp. Biochem. Physiol., 49: 175-181.
- Long, C. (1961). Biochemists' Hand Book. E & FN Span Ltd., 22, Henrietta Street, London, pp. 841, 873-879, 881-884.
- Matschiner, J.T. and Willingham, A.K. (1974). Influence of sex hormones on vitamin K deficiency and epoxidation of vitamin K in the rat. J. Nutr., 104: 660-665.
- McIndoe, L.M. (1962). Occurrence of two plasma albumins in domestic fowls. Nature, 195: 253-255.
- McSherry, B.J. and Grinyer, I. (1954). The pH values, carbondioxide content and the levels of sodium, potassium, calcium, chloride and inorganic phosphorus in the blood serum of normal cattle. Am. J. vet. Res., 15: 509-510.
- *Meeh, K. (1879). Cited by Brody, S. (1945).
- Miller, E.R., Ullery, D.E., Ackermann, I., Schmidt, D.A., Luecke, R.W. and Hoefner, J.A. (1961). Swine haematology from birth to maturity. II. Erythrocyte population, size and haemoglobin concentration. J. Anim. Sci., 20: 890-897.
- Mills, C.F., Dalgarno, A.C., William, R.B. and Quarterman, J. (1967). Zinc deficiency and the zinc requirement of calves and lambs. Br. J. Nutr., 21: 751-767.
- Millson, G.C., West, L.C. and Dew, S.M. (1960). Biochemical and haematological observations on the blood and cerebrospinal fluid of clinically healthy and scrapie affected goats. J. comp. Path. Ther., 70: 194-198.
- Mithulji, G.F., Shukla, P.C. and Patel, B.M. (1966). Haematological studies in Kankr&j cattle. Indian vet. J., 43: 605-612.

- Moses, J.S. and Gopalakrishnan, A.V. (1979). A note on agar gel electrophoresis of sera of Indian elephants. Cheiron, 8: 145-148.
- Mylrea, P.J. and Bayfield, R.F. (1968). Concentrations of some components in the blood and serum of apparently healthy dairy cattle. Aust. vet. J., 46: 861-864.
- Nigham, I.S. and Pandey, M.D. (1973). Age changes in erythrocyte sedimentation rate in buffalo. Indian vet. J., 50: 31-34.
- Nirmalan, G. (1967). Studies on physiological responses and blood constituents of domestic animals under varying conditions. M.Sc. Thesis, Kerala University, Trivandrum, pp. 85-134.
- Nirmalan, G. and Nair, S.G. (1969). Certain biochemical studies on the blood of Indian elephants (Elephas maximus). Res. vet. Sci., 10: 176-180.
- Nirmalan, G. and Nair, S.G. (1971). Plasma proteins and certain non-protein nitrogenous constituents in the blood of Indian elephants (Elephas maximus). Br. vet. J., 127: 207-213.
- Nirmalan, G., Nair, S.G. and Simon, K.J. (1967). Hematology of the Indian elephant (Elephas maximus). Can. J. Physiol. Pharmacol., 45: 985-991.
- Nockles, C.F., Yokel, J.W., Jackson, D.W. and Swanson, V.B. (1978). Factors affecting blood clotting in mature sheep and cattle. Br. vet. J., 134: 286-288.
- Ormian, M., Szarek, J., Ormian, W. and Felenezak, A.C. (1982). Contents of magnesium, copper, iron and zinc in blood serum and some indices and performance of cows in the Krakow region. Zezyty Nankome. Akademi., 175: 73-91.
- Nutr. Abstr. Rev. (1984), 54: 316.

- Osbaldiston, G.W. (1971). Erythrocyte sedimentation rate studies in sheep, dog and horse. Cornell Vet., 61: 386-399.
- Oser, B.L. (1965). Hawk's Physiological Chemistry. Tata McGraw Hill Publishing Company Ltd., Bombay, 14th Ed. pp. 1141-1143.
- *Osterhoff, D.R. and Cox, W.I.S. (1972). Serum polymorphism in three South African breeds of goats. Proc. XIIth Eur. Confr. Anim. Blood Grps. biochem. polymor., 576-582. Cited by Shamsuddin, A.K. (1984).
- Owen, A.C., Bowie, E.J.N., Zollman, P.E., Fass, D.N. and Gordon, H. (1974). Carrier of porcine Von Willebrand's disease. J. Am. vet. med. Ass., 35: 245-248.
- Parkes, A.S. (1956). Marshall's Physiology of Reproduction Vol. I. Longman, Green and Co., London, 3rd Ed. pp. 256.
- Patel, B.M., Vaidya, M.B., Patel, P.M. and Patel, C.A. (1969). Blood picture of Surti buffalo. Indian vet. J., 46: 861-864.
- Payne, J.M. and Leech, F.B. (1964). Factors affecting plasma calcium and inorganic phosphorus concentrations in the cow with particular reference to pregnancy, lactation and age. Br. vet. J., 120: 385-396.
- Payne, J.M., Rowlands, C.J., Manston, R. and Sally, D.M. (1973). A statistical appraisal of the results of metabolic profile tests on 75 dairy herds. Br. vet. J., 129: 370-381.
- Perkin-Elmer, (1976). Analytical methods for atomic absorption spectrophotometry. The Perkin-Elmer Corporation, Atomic Absorption production dept., Norwalk, U.S.A.

- *Pereschino, A., Macri, A. and Omer, M.A. (1973). Behaviour of serum lactate dehydrogenase and its isoenzymes in healthy buffaloes. Acta med. Vet., 19: 347-355. Abstr. Vet. Bull. (1975), 43: 485.
- Phillips, R.A., Sylke, V., Hamilton, D.D., Dole, P.B. and Emerson, V.P. (1950). Measurement of specific gravity of whole blood and plasma by standard copper sulphate method. J. biol. Chem., 183: 305-307.
- Pillai, M.G.R. (1972). Studies on the sedimentation rate and fragility of red corpuscles of animals. M.V.Sc. Thesis, Kerala Agricultural University, Trichur.
- Pillai, M.G.R. and Nair, S.G. (1974). The critical evaluation of the methods of assessing erythrocyte sedimentation rate in domestic animals. Kerala J. vet. Sci., 5: 56-57.
- *Pouling, L., Hans, H.A., Singur, S.J. and Wells, I.C. (1949). Sickle cell anaemia - a molecular disease. Science, 110: 543-548. Cited by Shamsuddin, A.K. (1984).
- Prasse, K.W. (1969). Lactic dehydrogenase activity and isoenzyme distribution in serum of normal cattle. Am. J. vet. Res., 30: 2181-2184.
- Purushotham, N.P. and Mahendar, M. (1963). A note on the comparative study of the blood picture in domestic animals. Indian Vet. J., 40: 553-558.
- Pyne, A.K. and Maitra, D.M. (1982). The biochemical constituents of blood of male buffalo calves. Indian vet. J., 59: 347-348.

- Raghavan, R.S. and Gaffar, A.A. (1960). Observations on the blood sedimentation rate and packed cell volume in buffaloes. Indian vet. J., 38: 349-352.
- Raghavan, R.S. and Gaffar, A.A. (1961). Specific gravity of blood and serum in relation to packed cell volume in buffaloes. Indian vet J., 38: 349-352.
- Roussel, J.D. and Stallcup, O.T. (1965). Effect of age on lactic dehydrogenase activity in blood serum of Holstein bulls. J. Dairy Sci., 48: 840.
- *Rubner, M. (1883). Cited by Brody, S. (1945).
- *Sato, S.A. and Vallenias, P.A. (1962). Serum transaminases in Thorough bred horses. Rev. Fac. Med. Vet. Lim., 15: 231-243. Abstr. Vet. Bull. (1963), 33: 3274.
- Schalm, O.W., Jain, N.C. and Carrol, E.J. (1975). Veterinary Hematology. Lea & Febiger, Philadelphia, pp. 87-209, 272-275.
- Schmitt, J. (1964). Haematological studies in elephants. Vet. med. Rev. Leverkusen., 2: 87-95.
- Seckington, I.M., Huntsman, R.G. and Jenkins, G.C. (1967). The serum folic acid levels of grass fed and stabled horses. Vet. Rec., 80: 158-161.
- Seiverd, C.E. (1973). Hematology for Medical Technologists. Lea & Febiger, Philadelphia, 4th Ed., pp. 363.
- Sen, A., Debdutta, R., Bhattacharya, S. and Deb, N.C. (1966). Haemoglobin of Indian Zebu cattle and Indian buffaloes. J. Anim. Sci., 25: 445-448.
- Simon, K.J. (1961). Haematological studies on elephants. Indian vet. J., 38: 241-245.

- Shamsuddin, A.K. (1984). Genetic studies on polymorphism of some blood protein in goats. M.V.Sc. Thesis, Kerala Agricultural University, Trichur, pp. 4-7.
- Singh, A.P., Joshi, H.C. and Singh, R. (1972). Studies on certain blood constituents in cattle and buffaloes. Indian vet. J., 49: 473-476.
- Snedecor, G.W. and Cochran, W.G. (1967). Statistical Methods. Oxford & IBH Publishing Co., Calcutta, Bombay, pp. 59-60, 147-148.
- Sonnenwirth, A.C. and Jarret, L. (1980). Gradwohl's Clinical Laboratory Methods and Diagnosis, Vol.I, The C.V. Mosby Company, London, pp. 263.
- Spector, W.S. (1956). Hand Book of Biological Data. W.B. Saunders Company, Philadelphia, pp. 51-53.
- Spooner, R.L. and Oliver, R.A. (1969). Albumin polymorphism in British cattle. Anim. Prod., 11: 59-63.
- Squibb, R.L., Gurman, M., Aguirre, F. and Scrimshaw, N.F. (1953). Ten constituents of the blood stream of well-fed white rats, chickens, swine, sheep, horses in Guatemala. Am. J. vet. Res., 14: 484-486.
- Stewart, G.H. and Clarkson, G.T. (1968). Serum iron and total iron binding capacity in the horse. Vict. Vet. Pract., 27: 25-26.
- Swenson, M.J. (1977). Duke's Physiology of Domestic animals. Comstock Publishing Associated, Cornell University Press, Ithaca and London, pp. 27-30, 395-399, 404-407.
- Tegeris, A.S., Earl, F.L., Curtis, J.M. (1966). Swine in Biomedical Research. Battelle, Richland, Washington, pp. 81-83.

- Tumbleson, M.E. and Kalish, P.R. (1971). Serum biochemical and hematological parameters in cross bred swine from birth through eight weeks of age. Can. J. comp. Med., 36: 202-209.
- Tumbleson, M.E., Burks, M. and Wingfield, W.E. (1973). Serum protein concentrations as a function of age, in female dairy cattle. Cornell Vet., 63: 65-71.
- Underwood, E.J. (1971). Trace Elements in Human and Animal Nutrition. Academic Press, New York, 3rd Ed., pp. 14-51, 57-106, 210-245.
- *Urbanyi, L. (1958). Calcium and phosphorus content of serum in domestic animals. Mag. allator. Lapja., 13: 45-46. Abstr. Vet. Bull. (1958), 28: 4056.
- Usami, S., Chien, S. and Gregerson, M.I. (1969). Viscometric characteristic of blood of the elephant, man, dog, sheep and goat. Am. J. Path., 217: 883-890.
- Van Vleet, J.F. and Alberts, J.O. (1968). Evaluation of liver function tests and liver biopsy in experimental carbontetrachloride intoxication and extra hepatic bile duct obstruction in the dog. Am. J. vet. Res., 29: 2119-2131.
- Verma, S.K. and Tyagi, R.P.S. (1974). The effect of caesarian section and normal parturition upon the concentration of blood electrolytes, glucose, and total proteins in goats. Res. vet. Sci., 16: 162-166.
- *Wantanbe, S. and Suzuki, S. (1967). Studies on serum albumin polymorphism in goats. Japan. J. Zootech. Sci., 38: 487-494. Abstr. Anim. Breed. Abstr. (1968), 36: 1636.

- West, E.S., Todd, W.R. and Bruggen, J.T.V. (1966). Textbook of Biochemistry. Oxford & IBH Publishing Co., New Delhi, 4th Ed., pp. 58-82, 108-109.
- Widdowson, E.M. and McCance, R.A. (1956). The effects of development on the composition of serum and extracellular fluids. Clin. Sci., 15: 361-365.
- Wintrobe, M.M. and Landsberg, J.W. (1935). A standardized technique for the blood sedimentation test. Am. J. med. Sci., 189: 102.
- Wroblewski, F. (1959). The clinical significance of transaminase activities of serum. Am. J. med., 27: 911-922.
- Young, E. and Lombard, C.J. (1967). Physiological values of the African elephant (Loxodonta africana). The Veterinarian, 4: 169-172.
- Zimmerman, H.J., Schwartz, M.A., Boley, L.E. and West, M. (1965). Comparative serum enzymology. J. Lab. Clin. Med., 66: 961-972.
- *Zurgilgen, H. and Ruther, M.J. (1962). Diagnostic and prognostic significance of serum transaminase in heart disease. Schewiz. Arch. Tierheilk., 104: 550-559. Abstr. Vet. Bull. (1963), 33: 3274.

* Originals not consulted

ANNEXURES

ANNEXURE 1

Linear measurements, observed body weight and height of Indian elephants

Sl. No.	Age (year)	Weight (kg)	Height at shoulder (cm)	Length between base of fore-head to base of tail (BFH-BT): body length (cm)	Length between point of shoulder to point of buttocks (PS-PB): body length (cm)	Chest girth (cm)	Neck girth (cm)	Right fore-foot circumference (cm)
1	2	3	4	5	6	7	8	9
1	9	2080	192	290	155	315	185	100
2	10	1615	198	260	160	290	177	100
3	11	1940	207	271	160	312	210	117
4	12	1750	206	260	129	303	192	112
5	12	2000	200	270	155	305	185	105
6	13	2170	230	285	230	337	205	140
7	14	2240	226	290	165	320	200	110
8	14	1780	212	265	132	298	188	105
9	15	2290	228	300	180	338	220	120
10	15	1740	225	282	130	284	178	108
11	15	2060	224	256	126	328	210	117
12	16	1630	211	260	155	285	191	103
13	16	2890	236	325	145	346	231	130
14	18	3445	255	315	215	380	250	135
15	18	1755	213	265	126	297	190	100
16	21	3870	277	335	216	385	265	135

(continued)

Annexure 1 continued

1	2	3	4	5	6	7	8	9
17	23	3625	273	340	230	376	240	145
18	25	3725	259	344	167	393	250	127
19	25	4130	231	340	220	422	267	132
20	28	3500	246	320	160	370	232	130
21	30	4950	280	380	259	429	303	159
22	30	3710	266	345	238	394	251	135
23	30	3300	250	335	215	356	244	135
24	30	4120	282	360	230	414	270	144
25	30	4370	290	362	160	408	277	147
26	30	3430	280	334	164	370	235	129
27	32	4430	269	370	240	421	365	130
28	32	3760	281	350	170	378	237	138
29	35	4660	295	370	235	410	300	152
30	38	4720	299	360	237	410	289	152
31	38	4510	305	390	235	415	269	149
32	40	4960	290	360	260	460	220	150
33	40	4420	287	375	230	410	275	150
34	45	4885	293	363	190	437	317	136
35	50	5250	289	400	250	420	300	149
36	50	4950	287	390	255	410	280	155
Mean		3352	252	326	192	367	242	130
\pm S.E.		\pm 201.61	\pm 5.79	\pm 7.42	\pm 7.35	\pm 8.49	\pm 2.59	\pm 3.00

ANNEXURE 2

Growth rate of elephants

Age in years	Number of animals	Body weight in kg	Percentage growth
0-5	1	460	360
5-10	5	1563	239.8
10-15	9	1997	27.77
15-20	4	2430	21.68
20-25	4	3838	57.94
25-30	7	3911	1.76
30-35	3	4283	9.51
35-40	4	4652	8.62
40-45	1	4885	5.01
45-50	2	5100	4.40

ANNEXURE 3

Measured body surface area and the areas of individual regions (m²)

Sl. No.	Measured surface area	Body area (BA)	Neck area (NA)	Hind-limb area (HLA)	Fore-limb area (FLA)	Trunk area (TA)	Tail area (TLA)	Fore-head area (FHA)	Ear area (EA)	Perineal area (PA)
1	2	3	4	5	6	7	8	9	10	11
1	20.48	6.79	1.49	4.21	5.25	1.19	0.32	0.63	0.45	0.15
2	17.97	7.40	0.34	4.54	3.11	1.00	0.35	0.55	0.56	0.12
3	15.31	4.59	0.94	4.04	4.05	0.56	0.39	0.45	0.16	0.13
4	15.32	4.98	0.86	3.84	3.96	0.67	0.20	0.47	0.25	0.09
5	18.40	6.12	1.07	4.04	4.60	1.07	0.29	0.67	0.39	0.15
6	21.11	6.31	1.74	4.85	5.61	0.88	0.39	0.80	0.36	0.17
7	20.83	5.53	1.31	5.95	4.74	1.18	0.64	0.80	0.53	0.15
8	20.30	5.79	1.43	4.84	5.70	0.74	0.46	0.75	0.36	0.23
9	16.66	4.93	0.95	4.04	4.74	0.79	0.31	0.50	0.28	0.31
10	28.82	6.61	1.83	7.14	8.90	1.88	0.66	0.96	0.68	0.16
11	25.39	7.76	1.67	5.61	7.41	1.22	0.35	0.77	0.44	0.16
12	31.47	11.14	3.87	6.05	7.28	1.33	0.53	0.60	0.49	0.18
13	12.20	4.76	0.55	2.58	3.07	0.56	0.21	0.25	0.18	0.04
14	13.02	4.52	0.98	2.08	2.50	0.64	1.75	0.26	0.25	0.04
15	18.25	5.86	1.44	3.99	3.99	1.70	0.36	0.45	0.36	0.10
16	21.76	6.03	1.49	5.59	5.78	1.16	0.54	0.65	0.31	0.21

(continued)

Annexure 3 continued

1	2	3	4	5	6	7	8	9	10	11
17	26.44	8.46	2.11	6.42	5.91	1.33	0.82	0.60	0.68	0.11
18	20.9	6.05	1.10	5.49	6.06	0.74	0.31	0.54	0.40	0.21
19	18.64	5.26	1.31	5.06	4.94	0.84	0.29	0.40	0.46	0.08
20	17.84	5.78	1.32	4.21	4.36	0.90	0.30	0.45	0.50	0.02
21	22.08	7.48	1.88	4.45	4.63	1.20	0.31	1.45	0.66	0.02
22	18.95	6.05	1.44	4.31	4.75	0.99	0.32	0.50	0.49	0.10
Mean	20.08	6.28	1.41	4.70	5.06	1.03	0.46	0.61	0.42	0.13
\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm
S.E.	1.01	0.32	0.15	0.25	0.32	0.07	0.07	0.05	0.03	0.02

ANNEXURE 4

Haematological parameters in the Indian elephant
Specific gravity of whole blood

	Baby elephants	Adult male elephants	Adult female elephants
1	1.054	1.056	1.056
2	1.050	1.059	1.069
3	1.052	1.059	1.062
4	1.054	1.061	1.050
5	1.052	1.059	1.052
6	1.054	1.066	1.052
7		1.052	1.052
8		1.054	1.052
9		1.053	1.054
10		1.053	1.055
11		1.050	1.054
12		1.054	1.055
13		1.054	1.052
14		1.050	1.047
15		1.051	1.053
16		1.052	1.055
17		1.051	
18		1.049	
19		1.051	
Mean	1.052	1.054	1.054
\pm S.E.	\pm 0.001	\pm 0.001	\pm 0.001

ANNEXURE 5

Specific gravity of plasma

	Baby elephants	Adult male elephants	Adult female elephants
1	1.027	1.027	1.025
2	1.025	1.028	1.030
3	1.026	1.030	1.027
4	1.025	1.026	1.030
5	1.027	1.028	1.026
6	1.027	1.030	1.025
7		1.028	1.024
8		1.024	1.034
9		1.027	1.032
10		1.028	1.035
11		1.025	1.028
12		1.025	1.025
13		1.027	1.030
14			1.030
Mean	1.026	1.027	1.028
\pm	\pm	\pm	\pm
S.E.	0.001	0.001	0.001

ANNEXURE 6

Relative viscosity of whole blood

	Baby elephants	Adult male elephants	Adult female elephants
1	6.973	5.449	7.060
2	5.474	5.470	6.690
3	5.317	6.464	5.707
4	6.018	5.510	7.317
5	8.053	6.730	6.293
6		5.881	5.466
7		5.487	5.474
8		6.306	5.317
9		6.068	6.718
10		6.732	6.694
11		5.870	6.718
12		6.249	5.880
13		6.114	5.153
14		6.625	7.289
15		6.771	7.674
16		5.896	
Mean	6.37	6.10	6.36
\pm S.E.	\pm 0.33	\pm 0.18	\pm 0.19

ANNEXURE 7

Absolute viscosity of whole blood (centipoise)

	Baby elephants	Adult male elephants	Adult female elephants
1	6.20	4.90	6.30
2	4.90	4.90	6.00
3	4.80	5.80	5.10
4	5.40	4.90	6.50
5	7.20	6.00	5.60
6		5.30	4.90
7		6.20	4.90
8		5.60	4.80
9		5.40	6.00
10		6.00	5.90
11		5.30	6.00
12		5.60	5.30
13		5.50	4.60
14		5.90	6.50
15		6.10	6.90
16		5.30	
Mean	5.70	5.54	5.68
\pm S.E.	\pm 0.28	\pm 0.14	\pm 0.16

ANNEXURE 8

Serum icterus index (Henry et al., 1952)

Principle: The proteins of serum are precipitated off with acetone and the intensity of yellow colour imparted to acetone by bilirubin is measured in a spectrophotometer using a solution of potassium dichromate as standard.

Reagents

1. Acetone mixture: Added 78 volumes of 'Analar' grade acetone to 22 volumes of distilled water. Mixed and kept in a refrigerator.
2. Stock Icterus standard: Dissolved 0.887 g of 'Analar' grade potassium dichromate in about 60 ml of distilled water and added a drop of concentrated sulphuric acid. This was then made upto 100 ml with distilled water.
3. Working Icterus standard: Diluted 1 ml of stock icterus standard to 95 ml with distilled water. The pH was adjusted to below 5 using concentrated sulphuric acid. This was made upto 100 ml using distilled water in a 100 ml volumetric flask and mixed well. The prepared working standard is equivalent to 10 units of icterus.
4. Blank: 9 part of acetone mixture was mixed with 1 part of distilled water.

Procedure: Added 1 ml of serum to 9 ml of cold (4°C) acetone mixture in a test-tube. Mixed and filtered using a Whatman No.42 filter paper. Placed a watch glass over the funnel. The collected filtrate was read in a spectrophotometer at 457 nm after setting it to zero reading with the blank.

Then read the optical density of the working icterus standard using distilled water as the blank for zero setting.

Calculation:

$$\frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 10 = \text{units of icterus}$$

ANNEXURE 9

Serum icterus index (units)

	Baby elephants	Adult male elephants	Adult female elephants
1	2.50	3.13	2.50
2	2.50	1.25	3.13
3	1.88	2.50	2.50
4		2.50	1.88
5		2.50	1.25
6		2.50	
7		2.50	
8		2.50	
9		3.75	
10		1.25	
11		1.25	
12		1.25	
Mean	2.29	2.24	2.25
\pm S.E.	\pm 0.44	\pm 0.22	\pm 0.34

ANNEXURE 10
pH of whole blood

	Baby elephants	Adult male elephants	Adult female elephants
1	7.22	7.15	7.08
2	7.35	7.22	7.08
3	7.16	7.20	7.18
4	7.19	7.13	7.15
5	7.15	6.99	7.22
6	7.22	7.13	7.24
7		7.32	7.24
8		7.12	7.23
9		7.11	7.32
10		7.25	7.02
11		7.26	6.99
12		7.15	6.96
13		7.20	7.02
14		7.30	
15		7.28	
16		7.42	
17		7.28	
18		7.34	
19		7.33	
Mean	7.21	7.22	7.13
\pm S.E.	\pm 0.04	\pm 0.02	\pm 0.03

ANNEXURE 11
pH of plasma

	Baby elephants	Adult male elephants	Adult female elephants
1	7.19	7.01	7.05
2	7.24	7.04	7.06
3	7.14	7.07	7.12
4	7.18	7.08	7.15
5	7.15	6.98	7.12
6	7.16	7.08	7.23
7		7.07	7.19
8		7.05	7.20
9		7.24	7.27
10		7.21	7.01
11		7.10	6.95
12		7.19	6.94
13		7.20	6.98
14		7.27	
15		7.28	
16		7.23	
17		7.22	
18		7.28	
19		7.22	
Mean	7.18	7.15	7.10
\pm S.E.	\pm 0.04	\pm 0.02	\pm 0.03

ANNEXURE 12

Whole blood coagulation time (sec.)

	Baby elephants	Adult male elephants	Adult female elephants
1	435	361	390
2	258	310	294
3	378	390	264
4	248	480	382
5	342	373	414
6	280	339	409
7		433	354
8		428	251
9		349	295
10		353	256
11		512	259
12		339	299
13		419	265
14		437	
15		483	
16		413	
17		355	
18		475	
Mean	323.5	402.7	317.8
\pm S.E.	\pm 26.0	\pm 15.0	\pm 17.0

ANNEXURE 13

Erythrocyte sedimentation rate in baby elephants

	mm/5 min	mm/10 min	mm/15 min	mm/20 min	mm/30 min	mm/40 min	mm/50 min	mm/60 min
	2	3	8	15	27	38	43	46
	1	4	10	21	38	46	48	50
	7	15	35	43	52	53	55	56
	8	16	44	55	57	59	60	61
	2	10	21	40	42	54	55	56
	3	4	6	13	25	34	39	41
Mean	3.83	8.67	20.67	31.17	40.17	47.33	50.0	51.67
\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm
S.E.	1.0	2.8	4.5	4.8	3.2	2.2	2.4	2.0

ANNEXURE 14

Erythrocyte sedimentation rate in adult male elephants

	mm/5 min	mm/10 min	mm/15 min	mm/20 min	mm/30 min	mm/40 min	mm/50 min	mm/60 min
2	-	30	40	-	53	59	60	
2	-	-	-	43	49	-	53	
2	8	15	35	50	55	-	57	
8	-	45	53	61	62	64	65	
1	11	-	-	-	-	48	50	
2	12	-	24	-	-	-	51	
4	13	21	27	41	48	51	52	
3	7	14	-	46	51	52	65	
5	14	30	45	56	58	59	59	
2	7	36	44	53	55	55	57	
-	-	41	46	50	52	53	54	
2	12	36	54	57	58	59	60	
2	12	33	45	48	52	54	56	
3	12	29	42	58	61	61	62	
7	19	36	52	56	59	60	60	
2	11	43	52	58	59	60	61	
5	10	16	31	40	52	53	54	
7	18	30	50	55	58	59	60	
2	11	-	30	45	53	54	56	
Mean	3.89	11.73	30.33	40.25	51.06	55.0	56.31	57.47
\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm
S.E.	0.58	2.0	2.9	3.0	1.9	1.3	1.3	1.1

ANNEXURE 15

Erythrocyte sedimentation rate in adult female elephants

	mm/5 min	mm/10 min	mm/15 min	mm/20 min	mm/30 min	mm/40 min	mm/50 min	mm/60 min
	2	6	24	35	-	-	-	52
	1	4	25	30	-	-	-	50
	2	6	26	43	55	56	57	58
	3	13	43	47	54	56	57	57
	4	20	44	45	58	58	60	60
	5	26	41	55	57	58	59	60
	1	9	35	53	58	60	60	61
	3	18	29	42	52	54	56	57
	8	32	46	55	59	60	60	60
	9	20	41	56	59	61	61	61
	6	14	37	48	55	57	58	60
	2	5	16	35	42	47	48	52
Mean	3.83	13.92	33.92	44.5	54.9	56.7	57.6	57.82
\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm
S.E.	0.70	1.9	3.2	3.4	2.4	1.7	1.6	1.4

ANNEXURE 16

Distribution of serum proteins and A/G ratio in Indian elephants
Total proteins (g/dl)

	Baby elephants	Adult male elephants	Adult female elephants
1	7.15	5.51	7.83
2	9.00	6.13	7.30
3	6.50	5.35	6.13
4	6.12	6.61	7.75
5	6.64	6.37	6.12
6	6.13	6.61	6.75
7		6.37	5.43
8		7.40	9.00
9		7.15	9.00
10		6.75	8.00
11		7.25	7.20
12		5.63	6.00
13		5.14	5.40
14		5.14	6.00
15		5.14	5.40
16		5.14	6.00
17			6.00
Mean	6.92	6.17	6.78
\pm S.E.	\pm 0.43	\pm 0.26	\pm 0.26

ANNEXURE 17

Albumin (g/dl)

	Baby elephants	Adult male elephants	Adult female elephants
1	2.27	2.00	2.31
2	1.67	1.92	1.73
3	2.17	1.54	2.16
4	2.39	2.00	1.96
5	2.16	2.20	1.96
6	1.82	2.80	1.74
7		2.00	2.50
8		1.82	1.67
9		1.70	1.67
10		1.63	1.67
11		2.39	2.80
12		2.17	2.50
13		2.29	2.20
14		2.08	2.78
15		2.29	2.22
16		2.29	2.50
17			2.50
Mean	2.08	2.07	2.17
\pm S.E.	\pm 0.14	\pm 0.09	\pm 0.18

ANNEXURE 18
Globulin (g/dl)

	Baby elephants	Adult male elephants	Adult female elephants
1	4.88	3.51	5.52
2	7.33	4.21	5.57
3	4.33	3.81	3.97
4	3.73	4.61	5.79
5	4.48	4.17	4.16
6	4.31	3.81	5.01
7		5.34	2.93
8		5.58	7.33
9		5.45	7.33
10		5.12	6.33
11		4.80	4.40
12		3.46	3.50
13		2.57	3.18
14		3.06	3.22
15		2.85	3.18
16		2.85	3.50
17			3.50
Mean	4.86	4.08	4.61
\pm S.E.	\pm 0.52	\pm 0.31	\pm 0.31

ANNEXURE 19
Albumin-Globulin Ratio

	Baby elephants	Adult male elephants	Adult female elephants
1	0.465	0.570	0.418
2	0.230	0.456	0.311
3	0.501	0.404	0.544
4	0.640	0.434	0.338
5	0.482	0.528	0.471
6	0.422	0.735	0.347
7		0.374	0.853
8		0.326	0.230
9		0.312	0.230
10		0.318	0.260
11		0.491	0.636
12		0.627	0.714
13		0.891	0.698
14		0.680	0.863
15		0.804	0.698
16		0.804	0.714
17			0.714
Mean	0.46	0.55	0.53
\pm S.E.	\pm 0.08	\pm 0.05	\pm 0.05

MIXTURE 20

Reagents for agar-gel electrophoresis

Preparation of the following reagents:

1. Tris-barbiturate buffer pH 8.6 (vessel buffer)

The buffer was prepared by dissolving 17.7 g of Tris (Trihydroxymethyl amino-methane) and 9.9 g of barbitone sodium in about 1000 ml of water and then added 0.3 g of sodium azide, which dissolved slowly with shaking. The mixture was made upto 2 litres with distilled water. The pH of the buffer was adjusted to 8.6 and checked the pH with a digital pH meter (BCIL-India).

2. 1.5% agar solution

To 1.5 g of pure Japanese agar powder, added sufficient Tris-barbiturate buffer pH (8.6) to make up the volume to 100 ml.

3. Amido black B stain

Solution A: 16.4 g of sodium acetate was dissolved in distilled water and made upto 1 litre.

Solution B: 11.55 ml of acetic acid was made upto 1 litre using distilled water.

46.3 ml of solution B and 3.7 ml of solution A were taken and the total volume was made upto 100 ml with distilled water. The pH of the solution was adjusted to 3.6.

Dissolved 0.1 g of Amido black in 100 ml of this buffer solution of pH 3.6.

4. Fixative I

Acetic acid	- 1 part
Methanol	- 7 parts
Distilled water	- 2 parts

(continued)

Annexure 20 continued

5. Fixative II

Acetone	- 9 parts
Distilled water	- 1 part

6. Destaining fluid

Methanol	- 4 parts
Acetic acid	- 1 part
Distilled water	- 5 parts

7. Boric acid buffer pH (7.8) (Vessel buffer)

37 g of boric acid and 4 g of sodium hydroxide was dissolved in 500 ml of distilled water and the final volume was made upto 1 litre using distilled water. The pH of the buffer was adjusted to 7.8 and checked with a digital pH meter (ECIL-India).

8. Tris-citric acid buffer pH 5.8 (gel-buffer)

Solution A: 10.5 g of citric acid was dissolved and made upto 1 litre using distilled water.

Solution B: 23 g of Tris (Trihydroxy-methyl amino-methane) was dissolved in a little of water and finally made upto 1 litre with distilled water.

The gel buffer was prepared by adding 15 ml of solution A and 250 ml of solution B and adjusting the pH of the resultant solution to 5.8. The pH was checked using a digital pH meter (ECIL-India).

9. 1.5% agar solutions: To 1.5 g of pure Japanese agar powder, added sufficient gel buffer to make the volume to 100 ml.

10. Haemoglobin buffer system pH (8.9) (Vessel buffer):

Tris (Trihydroxymethyl amino methane)	- 20.2 g
EDTA (disodium salt)	- 2 g
Boric acid	- 1.5 g
Distilled water	- 1 litre

(continued)

Annexure 20 continued

The pH was adjusted to 8.9 and checked using a digital pH meter (ECIL-India).

11. Benzidine stain:

250 g of benzidine, 0.4 ml of hydrogen peroxide and 1.4 ml of glacial acetic acid were mixed properly and made upto 100 ml with water. This is then heated to boil and then cooled and used freshly.

Preparation of the following reagents:

12. Barbitone (vernal) buffer pH (8.6) (vessel buffer)

The buffer was prepared by dissolving 10.3 g of sodium barbitone in about 900 ml of water and then adding to it 1.83 g of diethyl barbutonic acid, which dissolved slowly with shaking. The mixture was made upto 1 litre with distilled water. The pH of the buffer was adjusted with a digital pH meter (ECIL-India).

13. Preparation of gel:

120 mg of agarose was dissolved in 20 ml of vernal buffer pH (8.6) by boiling in a water bath. 24 mg of agar was also dissolved the same way in 4 ml of vernal buffer pH (8.6). The agarose and agar were mixed in the ratio 4:1 and then again mixed well by keeping in the boiling water bath. This is layered on the microscopic slide (about 3 ml/slide).

(continued)

Annexure 20 continued

Procedure:

Slits were made on the agar-agarose coated slide about 2 cm away from one edge and layered about 0.05 ml of serum. A current of 5 mA/slide was given for 5 hours, after keeping the whole electrophoretic chamber in a refrigerator. The fixing was done with ethanol:acetic acid:water mixture (80:10:10) for 30 minutes. It was then kept in contact with the staining solution (saturated filtered solution of oil red O in 60% alcohol) overnight. The slides were then washed gently in tap water and dried at room temperature.

ANNEXURE 21

Serum glutamic oxaloacetic transaminase-SGOT (i.u./l)

	Baby elephants	Adult male elephants	Adult female elephants
1	6.960	8.160	10.56
2	16.80	13.44	9.600
3	16.80	9.600	38.88
4	3.60	10.56	19.68
5	6.960	12.48	33.12
6		13.44	9.600
7		5.760	13.44
8		33.12	19.20
9		28.80	16.80
10		19.68	19.20
11			18.24
12			14.88
13			16.80
Mean	10.18	15.65	18.46
\pm S.E.	\pm 3.8	\pm 2.7	\pm 2.4

ANNEXURE 22

Serum glutamic pyruvic transaminase - SGPT (i.u./l)

	Baby elephants	Adult male elephants	Adult female elephants
1	8.800	6.000	6.000
2	5.280	5.280	6.000
3	6.000	6.000	6.000
4	2.400	6.720	6.720
5		2.640	5.280
6		2.640	6.000
7		2.400	5.760
8		4.560	2.640
9		5.280	4.560
10		6.000	2.400
11			4.560
12			3.840
Mean	5.64	4.75	4.98
\pm S.E.	\pm 0.85	\pm 0.54	\pm 0.49

ANNEXURE 23

Lactic dehydrogenase - LDH (i.u./l)

	Baby elephants	Adult male elephants	Adult female elephants
1	330.0	275.0	330.0
2	330.0	695.0	410.0
3	440.0	550.0	255.0
4		275.0	600.0
5		440.0	
6		600.0	
7		640.0	
8		275.0	
Mean	366.67	468.75	398.75
\pm S.E.	\pm 90.04	\pm 55.14	\pm 77.98

ANNEXURE 24

Creatine phosphokinase - CPK (i.u./l)

	Baby elephants	Adult male elephants	Adult female elephants
1	18.19	26.68	20.01
2	47.64	53.36	76.96
3	25.01	33.35	34.42
4		75.04	
5		83.38	
6		47.76	
7		42.44	
8		47.64	
Mean	30.28	51.12	43.80
\pm	\pm	\pm	\pm
S.E.	12.0	7.4	12.0

ANNEXURE 25

Estimation of trace elements in serum of elephants

Procedure for preparation of trichloroacetic acid protein-free serum filtrate:

To 10 ml of elephant serum in a boiling tube, added equal quantity of 20% trichloroacetic acid (BDH-Analar). Gently mixed the contents and the tube was loosely capped. It was then heated in a water bath at 90°C for 15 min, cooled and then filtered through a Whatman 41 filter paper into a clean dry test-tube. This protein free serum filtrate was used for the estimation of trace elements (Iron, Copper and Zinc).

ANNEXURE 26

Major elements in the serum of Indian elephants
Sodium (mmol/l)

	Baby elephants	Adult male elephants	Adult female elephants
1	100.0	117.4	117.4
2	121.8	121.8	119.6
3	124.0	119.0	109.2
4	193.6	117.4	124.0
5	110.9	119.6	109.2
6	119.6	119.6	115.3
7	124.0	117.4	121.3
8	109.0	117.4	193.6
9		119.6	158.8
10		109.2	160.9
11		109.2	119.6
12		109.2	124.0
13		124.0	119.6
14		124.0	124.0
15		121.8	109.2
16		115.3	124.0
17		117.4	109.2
18		121.8	
19		121.8	
Mean	125.36	118.24	126.99
\pm S.E.	\pm 6.7	\pm 4.3	\pm 4.6

ANNEXURE 27
Potassium (mmol/l)

	Baby elephants	Adult male elephants	Adult female elephants
1	4.220	5.243	4.987
2	5.371	4.475	4.475
3	4.603	4.731	3.964
4	4.731	4.348	4.987
5	4.731	4.220	4.475
6	5.243	5.115	4.092
7	4.860	4.475	4.220
8	6.010	4.603	4.475
9		4.220	4.731
10		4.731	4.603
11		4.731	4.731
12		4.731	5.115
13		5.115	5.371
14		4.859	6.001
15		4.731	5.115
16		4.919	5.754
17		4.731	5.371
18		5.243	
19		5.493	
Mean	4.97	4.77	4.85
\pm S.E.	\pm 0.17	\pm 0.11	\pm 0.12

ANNEXURE 28
Calcium (mmol/l)

	Baby elephants	Adult male elephants	Adult female elephants
1	2.465	2.250	2.269
2	2.250	2.126	2.209
3	2.978	2.076	2.082
4	2.183	2.115	2.176
5	2.033	2.182	2.227
6	2.362	2.438	2.161
7	2.229	1.999	2.058
8	2.349	2.005	2.182
9		2.156	2.249
10		2.137	2.083
11		2.211	2.000
12		2.264	2.333
13		2.274	2.252
14		2.342	2.213
15		2.448	2.070
16		2.525	2.206
17		2.362	2.051
18		2.385	
19		2.201	
Mean	2.36	2.24	2.17
\pm S.E.	\pm 0.058	\pm 0.038	\pm 0.046

ANNEXURE 29

Magnesium (mmol/l)

	Baby elephants	Adult male elephants	Adult female elephants
1	1.053	1.004	1.022
2	1.238	0.935	1.039
3	1.368	0.955	0.798
4	0.942	1.026	1.053
5	1.041	0.985	1.084
6	0.967	1.113	1.008
7	0.888	0.936	1.024
8	0.909	0.952	0.736
9		1.127	0.888
10		1.047	0.971
11		1.275	0.823
12		1.055	0.827
13		1.129	0.962
14		1.233	1.061
15		1.014	1.034
16		0.948	1.220
17		0.804	0.958
Mean	1.05	1.03	0.97
\pm S.E.	\pm 0.046	\pm 0.031	\pm 0.032

ANNEXURE 30
Trace elements in the serum of Indian elephants
Iron (μ mol/l)

	Baby elephants	Adult male elephants	Adult female elephants
1	32.45	33.09	55.83
2	30.01	47.16	47.20
3	31.87	31.48	32.59
4	49.10	29.37	29.23
5	33.59	36.71	23.99
6	31.66	44.66	21.27
7		27.50	59.84
8		34.74	57.01
9		38.21	44.51
10		35.06	37.60
11		23.35	52.11
12		27.75	40.79
13		26.82	40.07
14		23.60	60.38
15		26.50	46.41
16		31.05	
17		28.61	
Mean	34.78	44.48	43.26
\pm S.E.	\pm 14.0	\pm 8.3	\pm 8.9

ANNEXURE 31
Copper (μ mol/l)

	Baby elephants	Adult male elephants	Adult female elephants
1	24.08	26.57	43.56
2	21.09	31.92	34.62
3	25.50	25.59	30.75
4	22.06	34.66	18.76
5	31.76	27.86	18.07
6	18.57	31.35	28.93
7		22.44	27.95
8		27.92	30.66
9		29.65	29.93
10		28.96	54.17
11		41.36	25.46
12		32.74	43.37
13		27.92	50.39
14		26.50	50.36
15		28.39	30.94
16		22.73	21.09
17		21.97	
Mean	23.84	28.74	33.69
\pm	\pm	\pm	\pm
S.E.	3.4	2.0	2.1

ANNEXURE 32
Iron:copper ratio

	Baby elephants	Adult male elephants	Adult female elephants
1	1.35	1.25	1.28
2	1.42	1.48	1.36
3	1.25	1.23	1.06
4	2.23	0.85	1.56
5	1.06	1.32	1.33
6	1.70	1.42	0.74
7		1.23	2.14
8		1.24	1.85
9		1.29	1.49
10		1.21	0.69
11		0.56	2.05
12		0.85	0.94
13		0.96	0.80
14		0.89	1.20
15		0.94	1.50
16		1.37	
17		1.30	
Mean	1.50	1.14	1.33
\pm	\pm	\pm	\pm
S.E.	0.17	0.06	0.11

ANNEXURE 33

zinc (μ mol/l)

	Baby elephants	Adult male elephants	Adult female elephants
1	27.44	28.24	30.29
2	29.00	27.90	43.41
3	33.84	28.03	56.91
4	35.18	32.61	42.28
5	30.26	40.78	33.29
6	31.05	40.48	45.25
7		48.10	30.07
8		30.60	44.88
9		50.79	47.48
10		25.09	54.40
11		18.39	42.53
12			40.42
Mean	31.13	33.73	42.61
\pm S.E.	\pm 3.4	\pm 2.5	\pm 2.4

CERTAIN PHYSIOLOGICAL STUDIES ON INDIAN ELEPHANTS

By

K. P. SREEKUMAR

ABSTRACT OF A THESIS

submitted in partial fulfilment of the
requirement for the degree

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

Department of Physiology and Biochemistry
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
Mannuthy - Trichur

1986

ABSTRACT

Clinically healthy 44 Indian elephants, of varying ages and both sexes, maintained under ideal conditions of management formed the subjects for the study.

Prediction equations to estimate body weight and height at shoulder from linear measurements had been derived. True body surface area in elephants was measured.

Formulae for computation of total body surface area from areas of individual regions of the body and from body weight and height had been presented and its usefulness discussed.

Normal values for haematological parameters viz., specific gravity, viscosity, icterus index, pH, coagulation time and erythrocyte sedimentation rates had been established.

At 15 minutes, 50 per cent of erythrocytes had settled. Use of erythrocyte sedimentation rate as a clinical test is discussed.

Elephants had low albumin and high globulin levels and the A/G ratio was less than one.

The electrophoretic fractionation of total proteins and lipoproteins have been discussed. Polymorphism in albumin was observed but no variant of haemoglobin could be detected.

Levels of glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, lactic dehydrogenase and creatine phosphokinase have been assayed.

The mineral status of elephants had been established. Normal levels of sodium, potassium, calcium, magnesium, iron, copper and zinc as well as the iron:copper ratio had been worked out.