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CONTRIBUTIONS TO THE STUDY OF BLOOD CONSTITUENTS IN DOMESTIC ANIMALS IN SOUTH AFRICA. 5. THE DOG: NORMAL VALUES FOR RED CELL COUNTS, HAEMOGLOBIN, BLOOD SUGAR AND BLOOD UREA NITROGEN AND A PRACTICAL STATISTICAL METHOD FOR EVALUATING SUCH DATA

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A simple and reliable statistical method of discriminating between what may be termed normal and abnormal biological observations is essential in modern clinical laboratory practice (Wootton & King, 1953). Major difficulties are frequently experienced in the application of " statistical curve fitting " and in the transformation of frequency curves. Gaddum (1945) and Henry (1960) commented on the erroneous application of standard deviations to very skewed and asymmetric distribution curves. In their chapter on the subject King & Wootton (1959) consider as normal values 80 per cent of a population observed or of available data, when such data are grouped in an array and arranged as a frequency polygon or histogram. The upper and lower 9 per cent of the population are considered as being suspect of abnormality and the outer 1 per cent values are considered definitely abnormal.

A simple graphical method of obtaining this information, which will circumvent much of the difficult mathematics required by the more elegant statistical procedures, is often required by the biologist. Such a method must, however, yield information which is sufficiently accurate for all clinical and many research problems. The latter part of this paper is devoted to a method which is believed to possess these attributes. It is one which may be found in most text books on statistics and has been used extensively in social and welfare fields (Spiegel, 1961). It has recently been successfully applied by Brown and his co-workers in their biochemical studies on certain disease syndromes of sheep and goats (Wagner, 1964; Wagner & Brown, 1966a; Wagner & Brown, 1966b; De Wet & Brown, 1966). The application of the method in canine laboratory medicine is illustrated by some examples set out in the main body of this paper.

Materials and Methods

Red cell counts were performed by means of standard techniques and equipment. Haemoglobin was estimated photometrically with a Leitz clinical photo-electric colorimeter and the method outlined in the handbook for this instrument. Blood sugar was estimated by the method of Folin & Wu (1920) as modified by Lehman & Silk (1952) and blood urea nitrogen by the titrimetric method of Brown (1957).

Potassium oxalate was used in all instances as the anti-coagulant and all determinations were completed as soon as possible after collection of the blood samples.

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The dogs used in this work were adult animals taken from the immediate surroundings of this Institute or were drawn from the pool of animals available here for experimental purposes. Only those which were free from any clinical signs of disease were used. Dogs were fasted for approximately 16 hours before the collection of samples for blood sugar analyses.

The method of statistical analysis of the data collected is presented in the Appendix at the end of this paper.

Results

The data obtained from the various determinations have been tabulated as explained in the Appendix and are presented in Tables 1 to 4 at the end of this paper, together with the relevant cumulative relative frequency curves (Fig. 1–4).

The following have been established as the " normal " ranges for the blood constituents determined:—

Red cell count: $3 \cdot 96 \times 10^6$ to $7 \cdot 06 \times 10^6$ per mm^3 of blood.

Blood sugar: 54·0 to 70·4 mg per 100 ml of plasma.

Blood urea nitrogen: 4·0 to 25·0 mg per 100 ml of blood.

Haemoglobin: 11·45 to 16·65 gm per 100 ml of blood.

Discussion

The primary object of this work was to establish the " normal " values of certain blood constituents in dogs emanating from the immediate surroundings of this Institute, since these animals constitute the majority of patients presented at the clinics at Onderstepoort. Hitherto too much reliance has been placed on such figures which have been gleaned from the literature, the relevant work often having been performed on a population maintained under conditions vastly different from those pertaining here. Such values often fail to embrace differences which may exist due to age, sex, nutritional state or internal parasitism and are often taken as being acceptable for a method or procedure different to that used by the original author.

The dogs used in the present investigation were " adult " animals maintained under the same general conditions of nutrition and management. No differences have been observed with regard to sex in this work so far and the population from which the data were taken for presentation here consisted of animals of both sexes.

As with all other methods of statistical analysis, the validity depends upon strict adherence to the rules concerning the sampling of data and the choice of the population used. A histogram should be drawn in all work of this nature to establish the type of distribution one is dealing with. A fully homogeneous population should yield a unimodal distribution curve. The class marks referred to in the Appendix, which are considered to be the arithmetic means of the sums of the upper and lower boundaries of the classes under consideration should also be carefully determined.

Summary

The normal ranges for red cell counts, haemoglobin, blood sugar and blood urea nitrogen have been calculated for apparently healthy dogs emanating from the immediate surroundings of Onderstepoort. The statistical method involved makes use of cumulative relative frequency curves. A note is appended on the construction of these curves and the application of the " distribution-free tolerance limits test " to the results obtained.

References

BROWN, J. M. M., 1957. Some simple laboratory procedures for the practitioner. *J. S. Afr. Vet. Med. Ass.*, 28, 55.

DE WET, P. J. & BROWN, J. M. M., 1966. Contributions to the study of blood constituents in domestic animals in South Africa. 4. Normal values for haemoglobin, and plasma calcium, inorganic phosphate, magnesium, iron and copper in the blood of Angora goats in the Cape Midlands. *Onderstepoort J. Vet. Res.* This issue.

FOLIN, O & WU, H., 1920. A system of blood analysis. Suppl. 1. Simplified and improved method for determination of sugar. *J. Biol. Chem.*, 41, 367.

GADDUM, J. H., 1945. Lognormal distributions. *Nature*, 156, 463.

HENRY, R. J., 1960. Improper statistics characterising the normal range. *Amer. J. Clin. Pathol.* 34, 326.

KING, E. J. & WOOTTON, I. D. P., 1959. Micro-analysis in medical biochemistry. 3rd ed. London: J. and A. Churchill, Ltd.

LEHMAN, H. & SILK, E., 1952. The prevention of colour fading in the Folin and Wu estimation of blood sugar. *Biochem.* J., 50, XXXI.

SPIEGEL, M. R., 1961. Theory and problems of statistics. New York: Schaum Publishing Co.

WAGNER, A. M., 1964. Contributions to the study of blood constituents in domestic animals in South Africa. 1. Normal values for methaemoglobin reductase, erythrocyte fragility, blood glutathione, plasma catalse and the total erythrocyte pyridine nucleotides in sheep. *Onderstepoort J. Vet. Res.*, 31, 77.

WAGNER, A. M. & BROWN, J. M. M., 1966a. Contributions to the study of blood constituents in domestic animals in South Africa. 2. Normal values for the activity of some enzymes of the Embden-Meyerhof pathway and tricarboxylic acid cycle which occur in the plasma of sheep. *Onderstepoort J. Vet. Res.* This issue.

WAGNER, A. M. & BROWN, J. M. M., 1966b. Contributions to the study of blood constituents in domestic animals in South Africa. 3. Normal values for the activity of glyceraldehyde-phosphate dehydrogenase and glucose-6-phosphate dehydrogenase in the erythrocytes of sheep. *Onderstepoort J. Vet. Res.* This issue.

WOOTTON, I. D. P. & KING, E. J., 1953. Normal values for blood constituents—Interhospital differences. *Lancet*, 264, 470.

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TABLE 1.—*Red cell count in the normal dog*
(Figures of column 1 and 2 must be multiplied by 10^6)

Class range	Class mark (y)	Frequency per class f	fy	Relative frequency %	Cumulative relative frequency %
3–4	3·5	5	17·5	5·55555	5·55555
4–6	4·5	9	40·5	9·99999	15·55554
5–6	5·5	28	154·0	31·11108	46·66662
6–7	6·5	34	221·0	37·77774	84·44436
7–8	7·5	9	67·5	9·99999	94·44435
8–9	8·5	5	42·5	5·55555	99·99990
		f: 90	fy: 543	99·99990	

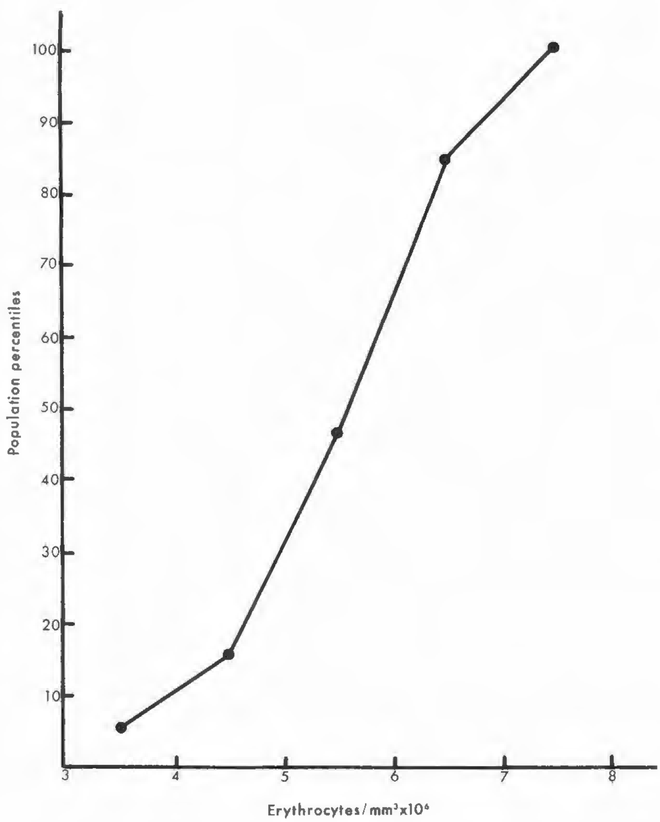

FIG. 1.—Red cell count

TABLE 2.—*Blood glucose level in the normal dog*
(As mg per 100 ml of plasma)

Class range	Class marks (y)	f	fy	Relative frequency %	Cumulative relative frequency %
50–54..................	52	3	159	3·84615	3·84615
55–59..................	57	12	684	15·38460	19·23075
60–64..................	62	21	1302	26·92305	46·15380
65–69..................	67	25	1675	32·05925	78·20505
70–74..................	72	14	1008	17·94870	96·15375
75–79..................	77	3	159	3·84615	99·99990
		78	4987	99·99990	

$$\text{Mean} = \frac{4987}{78} = 63,\ 93 \text{ i.e. } 64 \text{ mg}\%$$

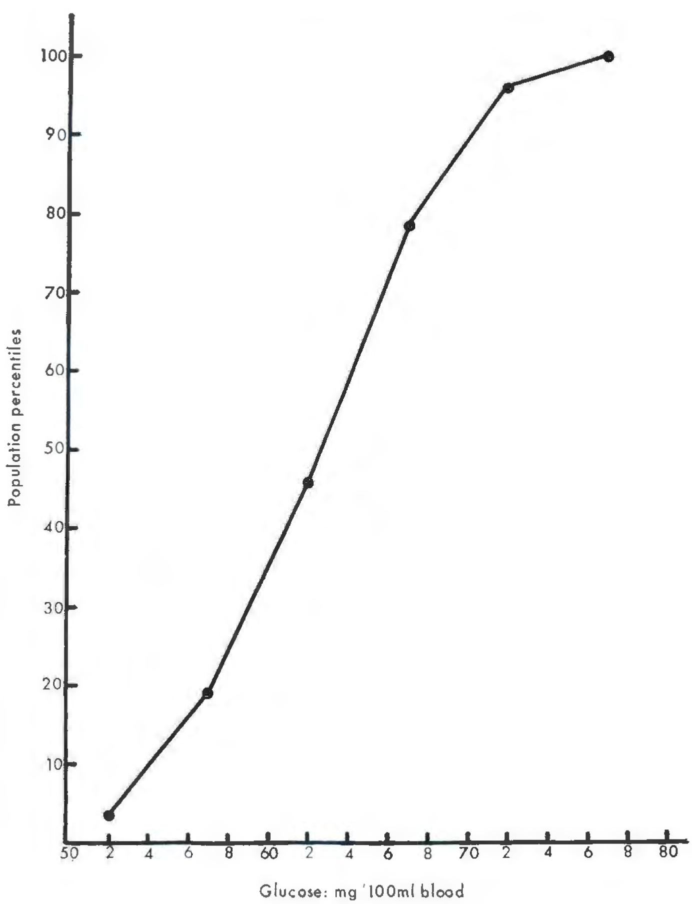

FIG. 2.—Blood glucose

TABLE 3.—*Blood urea nitrogen in the normal dog*
(Expressed as mg per 100 ml of blood)

Class range	Class mark	f	fy	Relative frequency %	Cumulative relative frequency %
1–5	2·5	2	5·0	1·73912	1·73912
6–10	7·5	27	202·5	23·47812	25·21724
10–15	12·5	38	475·0	33·04328	58·26052
16–20	17·5	19	332·5	16·52164	74·78216
21–25	22·5	15	337·5	13·04340	87·82556
26–30	27·5	9	247·5	7·82604	95·65160
31–35	32·5	5	162·5	4·34780	99·99940
		115	1762·5	99·99940	

$$\text{Mean} = \frac{1762 \cdot 5}{115} = 15 \cdot 32 \text{ mg\%}$$

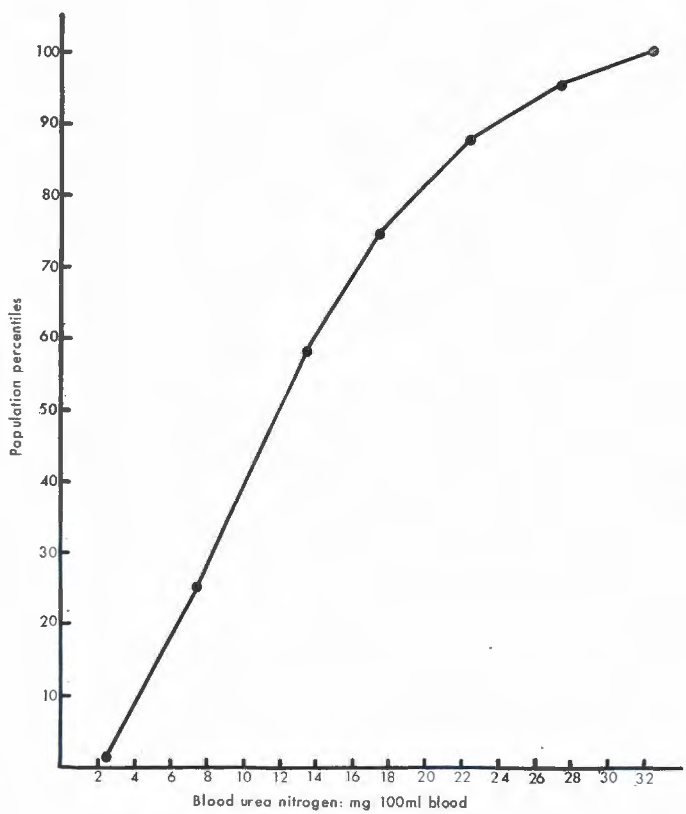

FIG. 3.—B ood urea nitrogen

TABLE 4.—*Haemoglobin blood level in the normal dog*
(Expressed as gm per 100 ml of blood)

Class mark	Class mark	f	fy	Relative frequency %	Cumulative relative frequency %
9·1–10	9·55	1	9·55	1·16279	1·16279
10·1–11	10·55	4	42·20	4·65116	5·81395
11·1–12	11·55	4	46·20	4·65116	10·46511
12·1–13	12·55	8	100·40	9·30232	19·76743
13·1–14	13·55	17	230·35	19·76743	39·53486
14·1–15	14·55	18	261·90	20·93022	60·46508
15·1–16	15·55	15	233·25	17·44185	77·90693
16·1–17	16·55	10	165·50	11·62790	89·53483
17·1–18	17·55	4	70·20	4·65116	94·18599
18·1–19	18·55	4	74·20	4·65116	98·83715
19·1–20	19·55	1	19·55	1·16279	99·99994
		86	1253·30	99·99994	

$$\text{Mean} = \frac{1253 \cdot 30}{86} = 14 \cdot 57 \text{ gm\%}$$

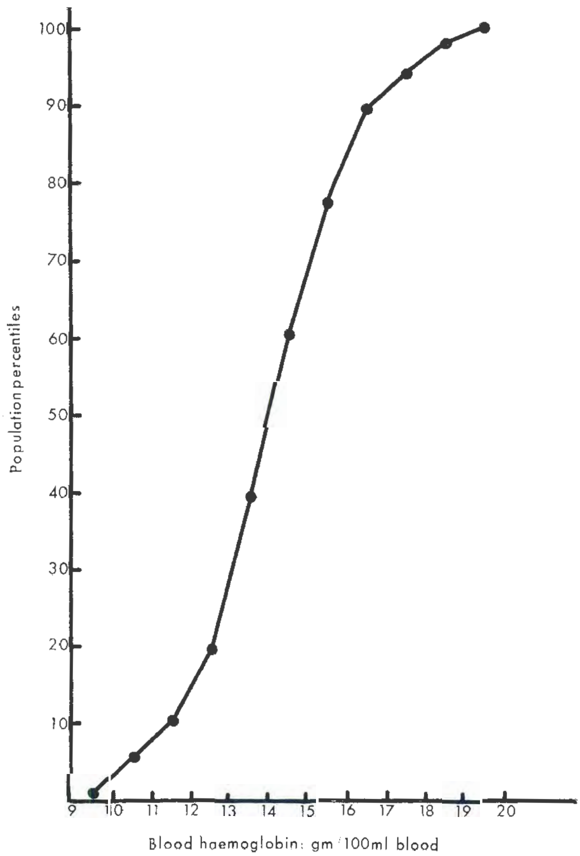

FIG. 4.—Haemoglobin

APPENDIX

Statistical analysis of the data collected

Since the 80 per cent of the population mentioned in the main body of the text is equally distributed on either side of the median of a frequency polygon, 40 per cent of the surface of the curve on either side of this median will contain the " normal " values for the population. The median is defined as the line perpendicular to the abcissa which divides the surface of the frequency curve into two equal parts, each containing 50 per cent of the given data.

The median and any percentage of the population can be determined graphically by constructing a percentage ogive (Galton's or cumulative frequency distribution curve). This curve is often sigmoidal when the cumulative relative frequencies are plotted on the ordinate axis and the observed values as " class marks " on the abscissa as shown in Fig. 5.

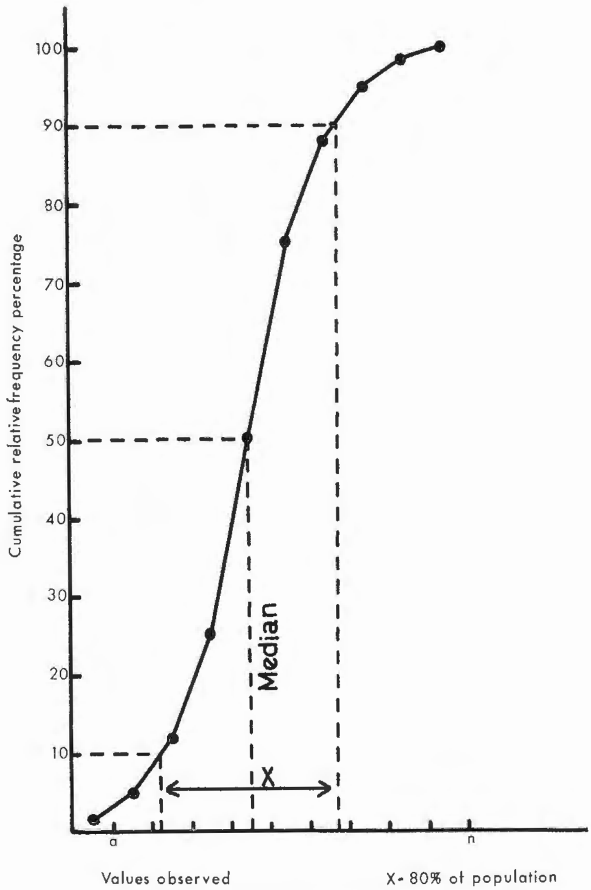

FIG. 5.—A cumulative relative frequency ogive—general type of curve obtained

Since the cumulative frequency curve gives the percentage of the population in percentiles, it is a simple matter to determine the " Normal Values " from it, i.e. the 40 per cent of the population on each side of the median.

The cumulative frequency curve is constructed as follows: A table is drawn up, the columns of which contain the following information: (i) the class range(s) classified in order of growing magnitude (classes of observed values); (ii) the class marks corresponding to the class ranges in the first column; (iii) the frequency (f), per class; (iv) the product of the class marks and the respective frequency per class (fy); (v) the relative frequency per class expressed as per cent; and (vi) the cumulative relative frequency expressed as per cent. The figures obtained in the final column, i.e. (vi), of the table are plotted on the ordinate axis against the class marks which are placed on the ab.cissa. A curve is then drawn through the points obtained in this manner.

Distribution-free tolerance limits

In order to ascertain that the figures obtained in the work reported earlier in the text are truly representative of the population from which the blood samples were drawn, the " 100 per cent tolerance limits " of the population was applied. By this is meant, for example, that if one takes a sample of 150 elements arranged in order of magnitude, one can be 96·3 per cent certain that 80 per cent of the population will lie between the 11th smallest and the 140th smallest elements of the sample. The tables used for applying this tolerance test were established for us by the National Research Institute for Mathematical Science of the Council for Scientific and Industrial Research. The formula used for computing these tables is the following:

$$(\text{Zr and Zs}) = \sum_{i=0}^{s-r-1} \binom{n}{i} \alpha^{i} (1-\alpha)^{n-i} \geqslant \beta$$

The results of our findings as they appear after the application of the " Distribution-free tolerance limits " test are presented in Table 5.

TABLE 5.—*Eighty per cent tolerance limits with confidence coefficient* 0·95 *for n samples given by Zr and Zs, the rth and sth order statistics of the samples*

	(n)	(r)	(s)	(β)
Red cell counts	90	4·5 × 10^6	7·5 × 10^6	96·2%
Blood glucose	78	57 mg%	72 mg%	97·0%
Blood urea nitrogen	115	7·5 mg%	27·5 mg%	96·5%
Haemoglobin	86	11·55 gm%	17·55 gm%	97·0%

The following conclusions may be drawn from this table:—

(i) Red cell counts: For a number of 90 determinations the probability is 96·2 per cent that the figures, 4·5 × 10^6 — 7·5 × 10^3 obtained from our sample, represent 80 per cent of the population from which this sample was drawn.

(ii) Blood glucose: For a number of 78 determinations the probability is 97 per cent that the figures 57–72 mg per cent obtained from our sample represent 80 per cent of the population from which this sample was drawn.

(iii) Similarly for blood urea nitrogen and haemoglobin the probabilities are 96·5 per cent and 97 per cent respectively that the ranges found represent 80 per cent of the population.

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