MICRO-ANALYSIS IN MEDICAL BIOCHEMISTRY

(Earl J. King)

Fourth Edition

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(Originally written by Earl J. King, M.A., M.D., D.SC., F.R.I.C.)

By

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TABLE 1

Calculated normal values for blood, serum or plasma

Sodium	mEq. per litre	136 – 149	Normal
Potassium	mEq. per litre	3·8 – 5·2	Lognormal
Chloride	mEq. per litre	100 – 107	Normal
Bicarbonate	mEq. per litre	24 – 30	Normal
Calcium	mEq. per litre	4·7 – 5·5	Normal
Magnesium	mEq. per litre	1·4 – 1·8	Normal
Inorganic phosphate	mg. P per 100 ml.	2·8 – 4·2	Normal
Urea	mg. per 100 ml.	14 – 38	Lognormal
Glucose	mg. per 100 ml.	63 – 100	Normal
Creatinine	mg. per 100 ml.	0·1 – 1·4	Lognormal
Bilirubin	mg. per 100 ml.	0·1 – 0·5	Lognormal
Cholesterol	mg. per 100 ml.	140 – 280	Lognormal
Total protein	g. per 100 ml.	6·5 – 7·9	Normal
Albumin	g. per 100 ml.	4·2 – 5·5	Normal
Fibrinogen	mg. per 100 ml.	150 – 450	Normal
Alkaline phosphatase	K.A. units per 100 ml.	4 – 11	Lognormal
Total Acid phosphatase	K.A. units per 100 ml.	1·1 – 3·5	Lognormal
Amylase	Units per 100 ml.	80 – 180	Lognormal

TABLE 2

Estimated normal values

Blood, serum or plasma		
Tartrate labile acid phosphatase	K.A. Units per 100 ml.	0 – 0·8
Glutamic oxaloacetic transaminase	I.U. per 1.	2 – 20
Glutamic pyruvic transaminase	I.U. per 1.	2 – 15
Lactate dehydrogenase	I.U. per 1.	50 – 170
Hydroxybutyrate dehydrogenase	I.U. per 1.	40 – 100
5′-nucleotidase	I.U. per 1.	2 – 17
Serum iron	μg. per 100 ml.	110 – 130
Total iron-binding capacity	μg. per 100 ml.	250 – 400
Uric acid	mg. per 100 ml.	2 – 7
Haemoglobin	g. per 100 ml.	13·5 – 14·5
pH	at 38°	7·35 – 7·42
Pco_2	mm. of mercury	34 – 45
Cerebrospinal fluid		
Protein	mg. per 100 ml.	15 – 45
Lange		0000000000
Glucose	mg. per 100 ml.	50 – 70
Chloride	mEq. per litre	120 – 126

TABLE 3

Approximate composition and 24-hour output

	mg. per 100 ml.	*g. per 24 hours*
Urine		
Water	—	1500
Urea	1500	25
Ammonia	50	0·8
Creatinine	130	2
Amino acids	40	0·6
Uric acid	25	0·4
Total nitrogen	1000	15
Phosphate (as P)	100	1·5
	mEq. per litre	*mEq. per 24 hours*
Sodium	130	200
Potassium	45	70
Calcium	7	10
Magnesium	10	15
Chloride	140	200

	per 24 hours
Faeces	
Wet weight	60–250 g.
Dry weight	10–50 g.
Fat	less than 5 g.
Nitrogen	1·5 g.
Phosphate (as P)	0·5 g.
Sodium	3 mEq.
Potassium	10 mEq.
Calcium	30 mEq.
Magnesium	10 mEq.

PREFACE

WHEN the late Professor E. J. King died in 1962, he and I were about to start writing this 4th edition of 'Micro-analysis in Medical Biochemistry'. In the event, I have taken the responsibility alone, but I have tried to retain the spirit, and indeed much of the arrangement of King's own work. The book continues to represent the routine laboratory practice of chemical pathology at the Postgraduate Medical School. Although many special investigations have had to be omitted, because of a desire to keep the book to the reasonably small dimensions required in a laboratory manual, most of what we do is described here. As before, it is assumed that the reader has a reasonable knowledge of the fundamentals of chemical and analytical theory and practice.

During the eight years which have elapsed since the last edition, such important advances have occurred that almost complete rewriting has been necessary. Since 1956, the determination and interpretation of serum enzyme activities have become very much more important and this subject now merits a separate chapter. The technique of automatic analysis has emerged as almost indispensable in a busy laboratory and a detailed account of automatic methods has been included. However, not all laboratories possess the required apparatus, nor the demand to justify its purchase, so the corresponding manual procedures are fully described.

As well as these major new chapters, a number of shorter sections appear for the first time. These include the use of control sera and the preservation and preparation of specimens, the determination of blood pH and the acid base status of the blood, high temperature flame photometry of calcium and magnesium, the physical constants of cyanmethaemoglobin, a screening test and the rapid chromatography of barbiturates, cellulose acetate electrophoresis of plasma proteins, balance studies using chromium sesquioxide as a faecal marker, quantitative analysis of renal calculi and several new function tests. The inclusion of these sections has made it necessary to delete some of the material which appeared in earlier editions, including the radioactive isotope tests and the elementary information on volumetric analysis. It is felt that these topics are better covered in specialized texts.

In the task of preparing this volume, I have been greatly helped by my colleagues in the Postgraduate Medical School, to whom I extend my thanks. In particular, substantial contributions to the book have been made by

Dr. Dulcie Alldis (faecal and metabolic balance work, function tests).
Dr. Diana Barnes (blood methods, cerebro-spinal fluid and enzymes).
Dr. K. Fotherby (steroids).
Dr. Ruth Haslam (blood methods, calculus analysis and function tests).
Dr. D. W. Moss (enzymes, proteins and chromatography).

I am also indebted to Dr. Barnes, Miss Genevieve Leballeur and Miss Veronica Sullivan for their painstaking assistance in the preparation of the manuscript. Miss Leballeur, in addition, drew the new illustrations. Acknowledgement is made to the British Medical Bulletin for permission to use Figures 1 and 2.

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CHAPTER 1

NORMAL VALUES

BY normal value is meant the amount of a constituent present in the body fluid or excretion of a healthy human being. In fact, this amount varies over a range, and is affected by a number of factors, including age, sex, season, race, diet and genetic constitution. Thus, while most healthy persons can be included in a class having the accepted 'normal' amount, some individuals are found to show divergent figures. Such exceptional individuals may be entirely normal in all other investigated respects. The judgement, therefore, of whether a given analytical figure is normal will depend on the experience and total data at the command of the interpreter of the result.

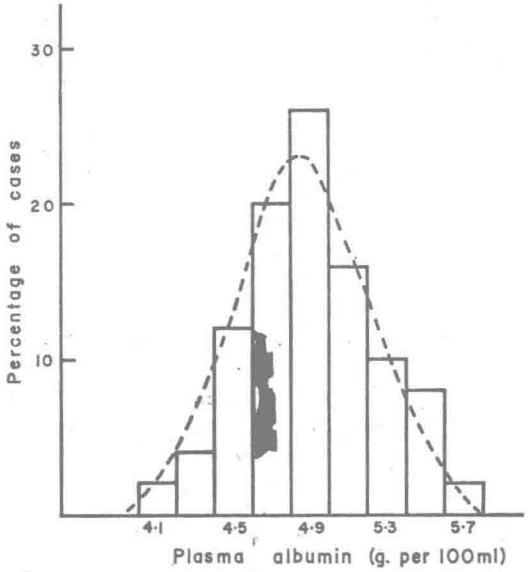

FIG. 1. Distribution of serum albumin levels in the population. This is a symmetrical Gaussian distribution.

The values given in this chapter are taken from our own accumulated results and from the literature. In most cases, they apply to the methods of analysis given here, or to a closely similar procedure. In some instances, we have carried out comparisons between the

method given and a method which was used when the standards for normal human beings was being determined. Blood values are for fasting persons.

The figures for whole blood, plasma and serum include information from surveys by Wootton, Maclean-Smith and King (1950) and Wootton and King (1953) of about 80 normal adults aged 20 to 50 years. These workers found that it was not always possible to express

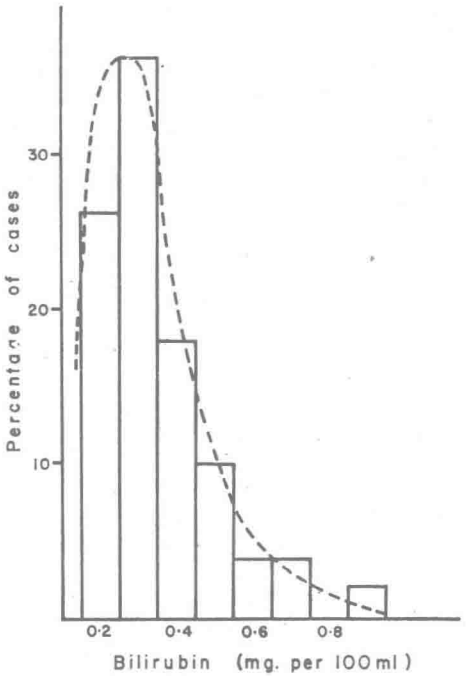

FIG. 2. Distribution of serum bilirubin levels. This distribution is 'lognormal' and hence not symmetrical.

their results as an average normal figure with a standard deviation because only in a minority of cases could the values be fitted by a symmetrical 'normal' distribution of Gaussian type (Fig. 1). Most of the remaining skewed distributions were satisfactorily fitted by log-normal curves (Fig. 2) of a type which has often been used for biological data (Gaddum, 1945*a*, 1945*b*). It is thus necessary to give these normal values in terms of a range, and we have chosen to calculate the range which encloses 90 per cent of the population (Table 1) so that the chances of a normal individual falling below the lower

limit of the range is one in twenty—a conventional statistical chance. Similarly, there is the same chance that a normal person will have a value above the upper limit. Other tables of normal values are included in Tables 2 and 3, constructed from other data not suitable for detailed mathematical treatment in which an attempt has been made

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Sodium	3 mEq.	
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Calcium	30 mEq.	
Magnesium	10 mEq.	

to estimate ranges with the same significance as the calculated ones. Further information is often given in the individual sections for each substance.

When considering the concentrations of ionic constituents in blood or urine, it is convenient to express them in terms of chemical equivalents so that variations in the concentration of one ion can be compared directly with variations in another. It is customary, therefore, to express the results for the anions (chloride, bicarbonate, phosphate, sulphate, proteins and organic ions) and the cations (sodium, potassium, calcium and magnesium) as milli-equivalents per litre of plasma. A normal solution (in the chemical sense) contains 1000 milli-equivalents per litre, so that a solution containing 1 mEq. per litre is 1 millinormal (mN) in concentration, and it is equally correct to express plasma concentrations as millinormality.

To convert concentrations into mEq. per litre, the constituent must first be expressed as mg. per litre and this figure is divided by

the equivalent weight of the ion. For simple monovalent ions, like sodium and chloride, the equivalent weight is the same as the atomic weight; for the divalent ions (calcium and magnesium) the equivalent weight is one-half of the atomic weight. The relationship may be expressed as an equation

$$\text{mEq. per litre} = \frac{\text{mg. per 100 ml.} \times 10 \times \text{valency}}{\text{atomic weight}}$$

In the case of plasma proteins, which act at blood pH as if they were weak acids, the equivalent weight has been determined experimentally. Phosphate equivalence is also complicated, in that two ionic species are involved at this pH. The resulting factors are given below.

To calculate mEq. per litre, divide	
Sodium (mg. per 100 ml.) by	2·3
Potassium (mg. per 100 ml.) by	3·9
Calcium (mg. per 100 ml.) by	2·0
Magnesium (mg. per 100 ml.) by	1·2
Chloride (mg. NaCl per 100 ml.) by	5·85
Chloride (mg. Cl per 100 ml.) by	3·55
Bicarbonate (volumes per 100 ml.) by	2·24
Phosphate (mg. P per 100 ml.) by	1·72
Protein (g. per 100 ml.) by	0·41

Variations in health and disease

In many cases, blood levels of certain constituents are raised (or occasionally lowered) as a manifestation of a disease state. Such changes are used for diagnostic and prognostic purposes and their detection makes up a large part of routine clinical chemistry. The extent of the deviation from the normal range which is needed to justify a confident diagnosis of abnormalities depends, of course, on the stability of the normal figure. In this respect, blood constituents are not all the same. Thus serum calcium, for example, almost never falls outside the range 4·5–5·6 mEq. per litre unless there is something amiss; similarly, a tartrate labile acid phosphatase higher than 0·8 K.A. units per 100 ml. is almost diagnostic of prostatic carcinoma, provided a suitable serum specimen is used. On the other hand, constituents such as glucose and urea vary widely even in a single individual as a result of dietary and hormonal influences, and similar fluctuations during the day also occur in the levels of serum potassium and inorganic phosphate.

TABLE 4

Common variations of plasma constituents in disease

Acid phosphatase	High in carcinoma of the prostate.
Alkaline phosphatase	High in bone disease, obstructive jaundice, in children
Amylase	High in acute pancreatitis.
Bicarbonate	High in metabolic alkalosis (pyloric stenosis, potassium depletion), respiratory failure. Low in acidosis (diabetic ketosis, renal failure).
Bilirubin	High in jaundice.
Calcium	High in hyperparathyroidism, invasive bone tumours sarcoids, myelomatosis, carcinoma of the breast. Low in tetany, parathyroidectomy, rickets, osteomalacia, malabsorption, renal failure, hypoproteinaemia.
Cholesterol	High in obstructive jaundice, nephrotic syndrome diabetes, pregnancy, myxoedema. Low in thyrotoxicosis.
Creatinine	High in renal failure
Glutamic-oxaloacetic-transaminase	High in cardiac infarction, hepatitis.
Glutamic-pyruvic transaminase	High in hepatitis.
Iron	Low in iron-deficiency anaemia. High in haemochromatosis.
Lactate dehydrogenase	High in cardiac infarction, hepatitis.
5′-nucleotidase	High in obstructive jaundice.
Protein	High in myelomatosis. Low in nephrotic syndrome.
Urea	High in renal failure, intestinal obstruction, cardiac failure, haematemesis. Low in pregnancy.
Uric acid	High in renal failure, gout.

CHAPTER 2

CONTROL OF LABORATORY ACCURACY

A NUMBER of surveys of laboratory accuracy have been conducted during recent years. The usual procedure was to distribute to several laboratories identical specimens of, say, blood or freeze-dried plasma. Each laboratory then analysed its specimen for the various constituents and the results were compared. In every survey, gross differences were observed in the values obtained. Thus Wootton and King (1953) reported that the highest blood urea result was four times the lowest, and a similar variation was found with other constituents. Similar results have been reported in the United States (e.g. Belk and Sunderman, 1947) and in every other country in which such surveys have been done. The problem appears to be universal as was shown by the results of an international trial (Wootton, 1956) while the most recent reports to hand, which are from Australia (Hendry, 1963), indicate that the present situation is still far from satisfactory.

The very large differences between the results from various hospitals prove that many clinical laboratories are less accurate than is generally supposed; and, indeed, their results may not be sufficiently precise to serve the purpose for which they are required. Fortunately, since attention was first directed to the control of accuracy, careful records have been kept in a number of hospitals and it is possible to maintain an acceptable standard provided suitable precautions are taken. The necessary measures are discussed here.

GENERAL LABORATORY PRACTICE

A good analyst is aware of the importance of his results and his procedures will be based on common sense and good scientific practice. Thus when setting up a new method in the laboratory, it is advisable to spend some time analysing sets of standard solutions, until one can define over what range the optical density (or other measurement) is linear with the concentration. Such repeated runs will also show whether the results are reproducible from batch to batch and from day to day. After this, a number of specimens from normal subjects should be analysed to confirm the normal range of the method. In some instances, it will also be advisable to add known quantities of a constituent to normal serum, perform the analysis