CLINICAL BIOCHEMISTRY PRINCIPLES AND METHODS

Editors
H.Ch.Curtius and Marc Roth

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Abbreviations

A	absorbance
Ac	acetyl-
a. c.	alternating current
a. m.	ante meridiem
ad lib.	ad libitum
ADP	adenosine diphosphate
AMP	adenosine monophosphate
amu	atomic mass unit
approx.	approximately
A_r or at. wt.	(relative) atomic weight
atm	atmosphere
ATP	adenosine triphosphate
b. p.	boiling point
c	concentration
ca	circa
cat. no	catalogue number
CM	carboxymethyl
conc.	concentration, concentrated
c. p. m.	counts/min
CSF	cerebrospinal fluid
d	relative density
D	configurational prefix
d. c.	direct current
DEAE	diethylaminoethyl
dis.	disintegration
d. f.	degree of freedom
DNP	2,4-dinitrophenyl-
d. p. m. or dis/min	disintegrations/min
DOC	deoxycorticosterone
E_o	electrode potential, standard
EC	Enzyme Code Number
ECTEOLA	epichlorhydrin triethanolamine
EDTA	ethylene diamine tetracetate
EI	electron impact
eV	electron volt
fructose 6-P	fructose 6-phosphate
g	acceleration of free fall
GC	gas chromatography
GLC	gas-liquid chromatography
glucose 6-P	glucose 6-phosphate
Hb	hemoglobin
HV	high voltage
I	ionic strength
i. d.	internal diameter
i. r.	infrared
K_m	Michaelis constant
L	configurational prefix
LAP	leucine amino peptidase
M^+	molecular ion
m-	meta-
Me	methyl-
M_r or mol. wt.	relative molecular weight
MS	mass spectrometry
m. p.	melting point
N, n	number
NAD, NADH	nicotine adenine dinucleotide (oxidized, resp. reduced form)
NMR	nuclear magnetic resonance
o-	ortho-
p-	para-
p. a.	pro analysi
p. m.	post meridiem
p. p. m.	parts per million
Pi	inorganic phosphate
POPOP, PPO	see chapter III B 1
PTFE	poly(tetrafluoroethylene)
PVC	polyvinyl chloride
Ref.	reference
rev./min	revolutions per minute
R_f	ratio to front
R_t	retention time
s. d. or s	standard deviation
s. e. m.	standard error of the mean
S_f	flotation rate (in Svedberg units)
S_r	relative standard deviation (= coefficient of variation)
syn.	synonym
t	time
TLC	thin-layer chromatography
TRIS, tris	tris-hydroxymethyl-aminomethane
U	unit
u. v.	ultraviolet
UDPG	uridine diphosphoglucose
USP	United States Pharmacopoeia
v/v	by volume
vol.	volume
wt.	weight

Preface

In the evolution of medicine, a remarkable feature is the progressive influence of experimental sciences, in particular biochemistry and molecular biology. Function and malfunction of the human organism are for an important part determined by biochemical phenomena, and the interpretation of illness is greatly aided by data supplied by the laboratory.

Clinical biochemistry, which was formerly included as a minor branch in such fields as physiological chemistry or pathology, has experienced an enormous growth in the past 20 years. Its scope lies essentially in the analysis of chemical compounds in human fluids and tissues, for the purpose of aiding in the prevention, diagnosis and treatment of disease.

At present, one can distinguish between two main groups of compounds being submitted to analysis. The first includes substances such as glucose, urea, creatinine, total protein, cholesterol, sodium, calcium and aminotransferases. Variations in their blood level reflect a consequence rather than a cause of disease. Many of them occur in concentrations superior to 0.1 mmol/l and can be assayed by relatively simple methods.

The development of suitable automatic equipment permits their analysis in large series.

Another group consists of substances intimately connected with the cause of an illness: hormones, vitamins, and often enzymes or other proteins. For example, the determination of the activity, in liver tissue, of an enzyme whose lack indicates an inherited metabolic disorder, or the estimation of a steroid hormone in a disturbance of its biosynthesis, can permit a direct insight into the state of the illness and, in addition, perhaps assist in explaining unknown metabolic processes. Such determinations are often of greater complexity, because minute amounts of substance (sometimes inferior to the nanomole) have to be specifically determined. High-precision instrumentation for physicochemical and biochemical analysis as well as qualified scientific personnel are required to solve the most difficult analytical problems.

Whether an assay is simple to perform or not, to scrupulously follow the instructions of a published technique and to employ a good instrument is not sufficient to warrant satisfactory results; in addition, the operator should be aware of the principles of the method he uses and be capable

of discerning sources of error. It is one of the aims of this book to provide the necessary information for a good understanding of the principles governing analytical procedures.

Good practice of clinical chemistry also requires an awareness of the diagnostic and therapeutic problems for the solution of which the clinician needs laboratory results. The size of this book has precluded a detailed discussion of this important aspect, but clinical commentaries and relevant literature references have been included whenever possible.

This book is written by authors representing various kinds of qualifications: chemists, biochemists, physicians, physicists and others. This variety just reflects what makes clinical biochemistry such a stimulating field, for diversity excludes dogmatism, favours exchange of ideas and promotes scientific progress. We hope that this work will prove useful to the many people who either work in the laboratory or depend on some way on its results. In addition, we feel that the subject coverage is such that it should offer a valuable help for the teaching in clinical chemistry.

It is of course not possible, in a book of this size, to present a detailed and complete description of all the recommended methods. One has often just cited easily available texts on techniques of well-known reliability, preferring to put more emphasis on newer techniques and areas on which little had been written hitherto. It was also almost unavoidable that some excellent methods escaped our attention, or were not mentioned because of lack of space. We kindly ask their authors for indulgence.

We owe our thanks to the authors who gave so much of their precious time to contribute to this work, to Drs. Brewer and Scott who translated some of the manuscripts, to our secretaries, Miss Loosen, Miss Raatikainen and Mrs. Zollet, for their excellent assistance, and to the publishers for their encouraging support.

Zürich and Geneva, Spring 1974

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Quantities and Units

M. Roth

The present development of science is marked by growing coherence among its multiple subdivisions. As knowledge increases, phenomena which were formerly considered to be entirely unrelated can be linked together by causal relationships. The understanding of physiology and medicine requires a knowledge of the more basic sciences like physics and biochemistry. This interdependence makes the development of a common way of expressing concepts and quantities highly desirable, and probably unavoidable.

Clinical chemistry is in special position since it forms a link between biochemistry and clinical medicine. It is understandable that clinical chemists be especially concerned with the replacement of the arbitrary modes of expression in clinical medicine with rational ones from the logical and unambiguous language of the basic sciences.

In the last century, the results delivered by the medical laboratory were mostly qualitative, but they are now mainly quantitative. Unfortunately, the quantities have hitherto been expressed in a multitude of units, depending on habits, methods and individuals.

Concentrations are the common form of presentation, but for such an important constituent as urea, for example, no less than five modes of expression are currently used: mg/100 ml, mg/l, mg urea nitrogen per 100 ml, mg urea nitrogen per l, mmol/l.

International bodies such as the international Union of Pure and Applied Chemistry (IUPAC) and the International Standards Organization (ISO) have been concerned with the problem of standardization of units and issued recommendations which are generally accepted in the basic sciences. Two clinical chemists, R. Dybkaer and K. Jørgensen, have carefully studied to which extent these recommendations could eventually be followed to improve the chaotic situation prevailing in clinical chemistry. They wrote their conclusions in the form of the book "Quantities and Units in Clinical Chemistry" (1), the essentials of which have now been approved by the international Federation of Clinical Chemistry (IFCC) and the clinical chemistry section of IUPAC.

The readers of this book are encouraged to use the new system of quantities and units. It was unfortunately not possible to convert the

manuscripts in all respects to the system, so that non-coherent quantities and units will still be found in this edition. We wish, however, to present here the principal features of the recommended system, because we are convinced that its advantages will lead to its wide acceptance in the future. For more details the reader is referred to the literature (1−3).

A basic concept is that amounts of substances should be expressed in moles instead of mass units. Disease involves biochemical reactions, which themselves involve molecules. Many physiological and pathological processes become much clearer if they are explained in molecular terms. For example, the degradation of hemoglobin produces one mole each of CO, Fe and biliverdin per mole heme. One mole of bilirubin is firmly bound by one mole of albumin, whereas one mole transferrin binds two moles of iron.

While the mole was formerly employed only for molecules, the new system extends its use to other formula units such as atoms, ions, and electrons. This permits one, for example, to add the mole concentrations of the ions to those of the undissociated molecules contained in a solution to obtain the osmolar concentration of the solution.

Tab. 1 lists the factors to convert molar amounts into mass amounts, and vice-versa, for a number of substances.

Another feature is the definition of basic kinds of quantities and base units and of their coherent combined (derived) forms. Tab. 2 shows a number of such expressions which may be used in clinical chemistry. Units may be preceded by prefixes denoting decimal factors; these are restricted to powers of 10 with exponents which are integer multiples of 3 (Tab. 3).

An expression such as mg% is no longer acceptable and should be appropriately converted to mg/l or, if possible, mol/l. The correct units for μ and mμ are μm and nm respectively.

Care should be taken to avoid ambiguous mathematical or fractional expressions. More than one solidus (/) should never be used in the same expression unless parentheses are used. Thus, mol/min/l is ambiguous while mol/(min × l) is correct.

On the inside cover, a number of abbreviations used in this book are listed. They have been made to agree whenever possible with the recommendations of the IUPAC and IUB-IUPAC nomenclature commissions. Other abbreviations specific to particular subjects are defined in the corresponding chapters. They are not necessarily consistent with a system.

Symbols for elements may be followed by a right subscript to indicate the number of atoms in the molecule or ion, and by a right superscript to

denote the state of ionization. Examples: Cl^-, SO_4^{2-}, Ca^{2+}, O_2. To specify a nuclide, the mass number is placed in the left superscript position. Example: ^{14}C. The atomic number may be placed in the left subscript position. Example: $^{12}_{6}C$.

Standardization of scientific expressions is a difficult task, as inadequate traditions are difficult to eradicate, and scientists may have different opinions on what is the best standard. Clarity and coherence are criteria which should help to define a better language for scientific communication.

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(1) Dybkaer, R. and K. Jørgensen: Quantities and Units in Clinical Chemistry. Munksgaard, Copenhagen (1967).

(2) Dybkaer, R.: Standard Meth. Clin. Chem. 6, 223 (1970).

(3) Manual of Symbols and Terminology for Physicochemical Quantities and Units. Pure and Appl. Chemistry 21, 1 (1970).

Table 1

to convert mmoles to mg
μmoles to μg
nmoles to ng
pmoles to pg
multiply by the relative molecular mass (M_r) given below.

to convert g to mmoles
mg to μmoles
μg to nmoles
ng to pmoles
multiply by 1000 × the reciprocal molecular mass ($1000/M_r$) given below.

Substance	Formula	M_r	$1000/M_r$
Acetoacetic acid	$C_4H_6O_3$	102.1	9.80
Acetone	C_3H_6O	58.1	17.22
Adrenaline	$C_9H_{13}NO_3$	183.2	5.46
Alanine	$C_3H_7NO_2$	89.1	11.22
Albumin		68000	0.0147
Aldosterone	$C_{21}H_{28}O_5$	360.4	2.77
p-Aminohippuric acid	$C_9H_{10}N_2O_3$	194.2	5.15
δ-Aminolevulinic acid	$C_5H_9NO_3$	131.1	7.63
Ammonia	NH_3	17.0	58.71
Angiotensin II (human)		1046	0.96
Arginine	$C_6H_{14}N_4O_2$	174.2	5.74
Ascorbic acid	$C_6H_8O_6$	176.1	5.68
Aspartic acid	$C_4H_7NO_4$	133.1	7.51
Bilirubin	$C_{33}H_{36}N_4O_6$	584.7	1.71
Bromosulfophthalein	$C_{20}H_8Br_4Na_2O_{10}S_2$	838.0	1.19
Calcium	Ca	40.1	24.95

Table 1 (Continued)

Substance	Formula	M_r	$1000/M_r$
Carbon monoxide	CO	28.0	35.70
Carbon dioxide	CO_2	44.0	22.72
Carotene	$C_{40}H_{56}$	536.7	1.86
Cholesterol	$C_{27}H_{46}O$	386.7	2.59
Citric acid	$C_6H_8O_7$	192.1	5.21
Copper	Cu	63.5	15.74
Coproporphyrin	$C_{36}H_{38}N_4O_8$	654.7	1.53
Cortisol	$C_{21}H_{30}O_5$	362.5	2.76
Creatine	$C_4H_9N_3O_2$	131.1	7.63
Creatinine	$C_4H_7N_3O$	113.1	8.84
Cysteine	$C_3H_7NO_2S$	121.1	8.25
Estrone	$C_{18}H_{22}O_2$	270.4	3.70
Fructose	$C_6H_{12}O_6$	180.2	5.55
Galactose	$C_6H_{12}O_6$	180.2	5.55
Glucose	$C_6H_{12}O_6$	180.2	5.55
Glutamic acid	$C_5H_9NO_4$	147.1	6.80
Glycerol	$C_3H_8O_3$	92.1	10.86
Glycine	$C_2H_5NO_2$	75.1	13.32
Hemoglobin		64500	0.0155
Histidine	$C_6H_9N_3O_2$	155.2	6.44
β-Hydroxybutyric acid	$C_4H_8O_3$	104.1	9.61
5-Hydroxyindole acetic acid	$C_{10}H_9NO_3$	191.0	5.24
Hydroxyproline	$C_5H_9NO_3$	131.1	7.63
Insulin (human)		5807	0.172
Iodine	I	126.9	7.88
Iron	Fe	55.8	17.91
Isoleucine	$C_6H_{13}NO_2$	131.2	7.62
Lactic acid	$C_3H_6O_3$	90.1	11.10
Lactose	$C_{12}H_{22}O_{11}$	342.3	2.92
Leucine	$C_6H_{13}NO_2$	131.2	7.62
Lysine	$C_6H_{14}N_2O_2$	146.2	6.84
Magnesium	Mg	24.3	41.12
Methionine	$C_5H_{11}NO_2S$	149.2	6.70
Nitrogen	N	14.0	71.43
Noradrenaline	$C_8H_{11}NO_3$	169.2	5.91
Phenolsulfonphthalein	$C_{19}H_{14}O_5S$	354.4	2.82
Phenylalanine	$C_9H_{11}NO_2$	165.2	6.05
Phosphorus	P	31.0	32.29
Porphobilinogen	$C_{10}H_{14}N_2O_4$	226.2	4.42
Pregnanediol	$C_{21}H_{36}O_2$	320.5	3.12
Progesterone	$C_{21}H_{30}O_2$	314.4	3.18
Proline	$C_5H_9NO_2$	115.1	8.69
Pyruvic acid	$C_3H_4O_3$	88.1	11.36

Table 1 (Continued)

Substance	Formula	M_r	$1000/M_r$
Serine	$C_3H_7NO_3$	105.1	9.52
Serotonin	$C_{10}H_{12}N_2O$	176.2	5.68
Testosterone	$C_{19}H_{28}O_2$	288.4	3.47
Threonine	$C_4H_9NO_3$	119.1	8.39
Thyroxine	$C_{15}H_{11}NO_4I_4$	776.9	1.29
Triiodothyronine	$C_{15}H_{12}NO_4I_3$	651.0	1.54
Triolein	$C_{57}H_{104}O_6$	885.4	1.13
Tryptophan	$C_{11}H_{12}N_2O_2$	204.2	4.90
Tyrosine	$C_9H_{11}NO_3$	181.2	5.52
Urea	CH_4N_2O	60.1	16.65
Uric acid	$C_5H_4N_4O_3$	168.1	5.95
Vitamin A	$C_{20}H_{30}O$	286.4	3.49
Vitamin B_{12}	$C_{63}H_{88}N_{14}O_{14}PCo$	1355.4	0.737
Water	H_2O	18.0	55.51
Xylose	$C_5H_{10}O_5$	150.1	6.66

Table 2

List of Quantities and Units

Quantity		Unit		
Name	Symbol	Name	Symbol	Equivalent to
length	l	metre	m	
mass	m	gramme	g	10^{-3} kg
time	t	second	s	
electric current	I	ampere	A	
amount of substance		mole	mol	
volume	V	liter	l	10^{-3} m^3
force		newton	N	kg · m · s^{-2}
energy		joule	J	kg · m^2 · s^{-2}
power		watt	W	kg · m^2 · s^{-3}
electric charge		coulomb	C	A · s
electric potential difference		volt	V	kg · m^2 · s^{-3} · A^{-1}
frequency		hertz	Hz	s^{-1}
mass concentration	c		g/l	
mass fraction			g/kg	$\times 10^{-3}$
volume fraction			l/l	$\times 1$
substance concentration	c		mol/l	
number concentration		reciprocal liter	l^{-1}	

Table 2 (Continued)

List of Quantities and Units

Quantity Name	Symbol	Unit Name	Unit Symbol	Equivalent to
molality			mol/kg	
mole fraction			mol/mol	× 1
Celsius temperature		degree Celsius	°C	
absolute temperature	*T*	kelvin	K	
pressure[1]	*p*	pascal	Pa	$N \cdot m^{-2}$
relative density[2]	*d*			× 1

[1] The same unit can be used for partial pressure defined as the product of the mole fraction of a gaseous component and the pressure of the gaseous mixture.

[2] Defined as the density (kg/l) of the object divided by the density of water under the same conditions.

Tab. 3. Prefixes denoting decimal factors.

Factor	Prefix	Symbol	Examples *l*	*m*	*t*	*I*	V	
10^{12}	tera	T						
10^{9}	giga	G						MHz
10^{6}	mega	M						kHz
10^{3}	kilo	k	km	kg	ks			Hz
1			m	g	s	A	l	
10^{-3}	milli	m	mm	mg	ms	mA	ml	
10^{-6}	micro	μ	μm	μg	μs	μA	μl	
10^{-9}	nano	n	nm	ng	ns		nl	
10^{-12}	pico	p	pm	pg			pl	
10^{-15}	femto	f					fl	
10^{-18}	atto	a						

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General Methodology