Pathology

Quantitative Chemical Techniques of HISTO- and CYTOCHEMISTRY

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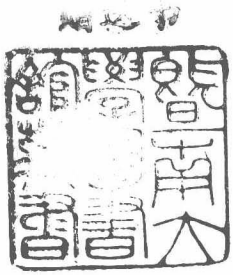

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Volume II

IN MEMORY OF

KAJ ULRIK LINDERSTRØM-LANG

He was the kind of "giant" of whom Claude Bernard (1865, translation by Green, 1957, p. 41) had written, "Each great man belongs to his time and can come only at his proper moment, in the sense that there is a necessary and ordered sequence in the appearance of scientific discoveries. Great men may be compared to torches shining at long intervals, to guide the advance of science. They light up their time, either by discovering unexpected and fertile phenomena which open up new paths and reveal unknown horizons, or by generalizing acquired scientific facts and disclosing truths which their predecessors had not perceived. If each great man makes the science which he vitalizes take a long step forward, he never presumes to fix its final boundaries, and he is necessarily destined to be outdistanced and left behind by the progress of successive generations. Great men have been compared to giants upon whose shoulders pygmies have climbed, who nevertheless see further than they. This simply means that science makes progress subsequently to the appearance of great men, and precisely because of their influence. The result is that their successors know many more scientific facts than the great men themselves had in their day. But a great man is, none the less still a great man, that is to say,—a giant."

Preface

The preface to Volume I included a definition of the scope of the treatment of the techniques presented and certain points of view that apply to this volume as well. In his foreword to these two volumes, Heinz Holter, like Robert R. Bensley before him, emphasized the ultimate responsibility of the worker to employ these techniques with critical consideration. Usually, the individual analytical method has been developed for a specific use, and when it is applied to another, e.g., in which a different kind of sample is employed, appropriate adjustments are often required, and the discrimination of the analyst must be exercised.

A reflection of the greater development and use of spectrophotometric techniques, compared to others, is found in the greater proportion of this volume devoted to them. Fluorometric techniques are being used increasingly to replace certain spectrophotometric ones, largely because of the greater sensitivity of the former, but the number of the latter described to date is still far greater. Commercial availability of instrumentation is an important factor in determining the use of a technique. The early emphasis on titrimetric methods was diminished to a great degree by the introduction of photoelectric apparatus, which made spectrophotometric methods relatively simple and convenient.

In this volume, certain earlier titrimetric methods are given, even though subsequent spectrophotometric or other methods for analysis of the same constituent are also presented. Laboratories in many parts of the world are variously equipped, and the simpler titrimetric apparatus can often provide means for reliable and accurate analysis when other equipment is not at hand, and, apart from this, the titrimetric technique is sometimes the one of choice.

Although this volume is devoted to chemical techniques, flame photometric and microbiological techniques are also included because they offer unique opportunities of providing quantitative data of interest for histo- and cytochemical investigation within their own limited areas of application. This extension of the subject mat-

ter is feasible since these techniques have been exploited relatively little as yet and they can be included without making the volume too unwieldy.

A sincere attempt has been made to bring the methods up-to-date as far as possible. An occasional paper that would have been included appeared after the manuscript was completed, but the methods given are a rather complete selection of those considered by the writer to be the more important of those described to 1963.

Part of this volume, as well as of Volume I, was written during a leave in Europe, supported by The Commonwealth Fund; and the author is grateful for this essential help, and for that provided by Prof. Heinz Holter, Physiological Department, Carlsberg Laboratory, Copenhagen, Prof. Peter Dohrn, Zoological Station, Naples, and Prof. Arne Engström, Institute of Medical Physics, Karolinska Institute, Stockholm, who made their libraries and other facilities available. The careful critical review and suggestions offered by Prof. Oliver H. Lowry, Department of Pharmacology, Washington University, St. Louis, of the section on spectrophotometric techniques are gratefully acknowledged.

Many authors, journals, and commercial organizations kindly permitted use of figures and other material from their publications; this assistance and that of the staffs of the Lane Medical Library, Stanford University, of the University of Minnesota Libraries, and of the publisher are fully appreciated. This volume was started during the author's tenure as Professor of Physiological Chemistry, at the University of Minnesota, Minneapolis.

Unit Abbreviations

ml	10^{-3} liter
μl	10^{-6} liter
mμl	10^{-9} liter
$\mu\mu$l	10^{-12} liter
gal	gallon
g	gram
mg	10^{-3} gram
μg	10^{-6} gram
mμg	10^{-9} gram
$\mu\mu$g	10^{-12} gram
lb	pound
oz	ounce
d.c.	direct current
a.c.	alternating current
rpm	revolutions per minute
emf	electromotive force
m	meter
cm	10^{-2} meter
mm	10^{-3} meter
μ	10^{-6} meter
mμ	10^{-9} meter
A	10^{-10} meter (Angstrom unit)
diam.	diameter
vol.	volume
in.	inch
ft	foot
STP	standard temperature and pressure
abs.	absolute
yr	year
hr	hour
min	minute
sec	second
cp	10^{-2} poise
amp	ampere
mamp	10^{-3} ampere
μamp	10^{-6} ampere
μF	10^{-6} farad
mv	10^{-3} volt
Kev	10^{3} electron volts
MΩ	10^{6} ohm (megohm)
$\mu\Omega$	10^{-6} ohm
M	molar
mM	10^{-3} molar
N	normal
eq	equivalent
meq	10^{-3} equivalent
p.p.m.	parts per million
conc.	concentrated
dil.	dilute
soln.	solution
sp. gr.	specific gravity
d	density
C.P.	chemically pure
h.p.	horse power
m.p.	melting point
b.p.	boiling point
E	extinction
N.A.	numerical aperture
rel.	relative
approx.	approximate
std. dev.	standard deviation

All temperatures are given in degrees centigrade.
If not otherwise indicated, all solutions are understood to be aqueous.
If not otherwise indicated, the term *alcohol* refers to 95% ethyl alcohol.

Compound Abbreviations

ATP	adenosine triphosphate
ADP	adenosine diphosphate
AMP	adenosine monophosphate
PP	pyrophosphate
G-1-P	glucose-1-phosphate
G-6-P	glucose-6-phosphate
G-1, 6-DP	glucose-1, 6-diphosphate
6-PG	6-phosphogluconate
F-6-P	fructose-6-phosphate
HDP	fructose-1, 6-diphosphate
MDH	malic dehydrogenase
LDH	lactic dehydrogenase
GDH	glutamic dehydrogenase
6-PGDH	6-phosphogluconate dehydrogenase
G-6-PDH	glucose-6-phosphate dehydrogenase
GAPDH	glyceraldehyde phosphate dehydrogenase
PGK	phosphoglycerokinase
CoA	coenzyme A
CoQ	coenzyme Q
PN	pyridine nucleotide
PNH	reduced pyridine nucleotide
DPN	diphosphopyridine nucleotide
DPNH	reduced diphosphopyridine nucleotide
TPN	triphosphopyridine nucleotide
TPNH	reduced triphosphopyridine nucleotide
TCA	trichloroacetic acid
DNA	deoxyribonucleic acid
RNA	ribonucleic acid
NT	neotetrazolium chloride
INT	iodonitrotetrazolium chloride
BAL	British Anti Lewisite
BTNA	*N*-benzoyl-L-tyrosine *p*-nitroanilide

DNFB	dinitrofluorobenzene
QH	3-quinolylhydrazine
GSSG	oxidized glutathione
GSH	reduced glutathione
Tris	tris (hydroxymethyl) aminomethane
P–H	phenolate–hypochlorite

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