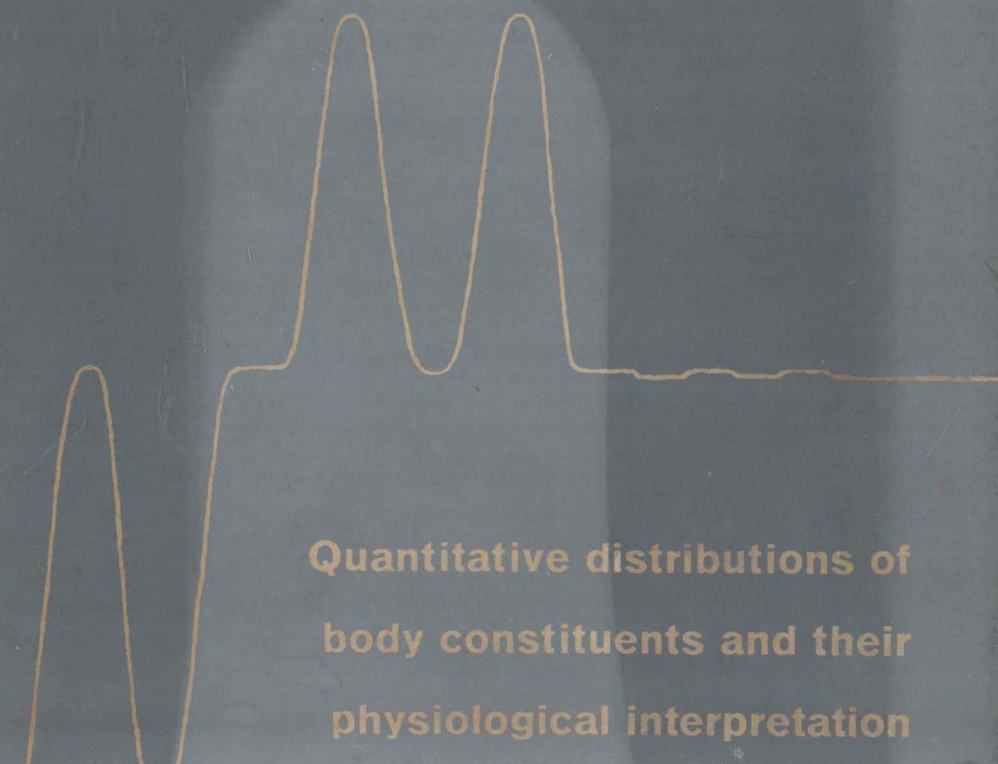
HALVOR N. CHRISTENSEN

Diagnostic Biochemistry



Quantitative distributions of body constituents and their physiological interpretation

DIAGNOSTIC BIOCHEMISTRY

Quantitative Distributions of Body Constituents and Their Physiological Interpretation

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FOREWORD

The present volume has grown out of past teaching at several levels of medical education; it may be recommended either as a supplementary text for a first course in biochemistry that penetrates vigorously into the diagnostic area, or for an advanced course for students who have had the more ordinary first course. At the same time the needs have been kept in mind of the physician who wants to reconsider the subject briefly from the ground up to improve his comprehension of biochemistry. The text may also serve to supplement hematology and clinical microscopy texts for instruction in laboratory diagnosis. Finally, the current tendency to explore all sorts of partitions and recombinations of medical education appears to create places for smaller presentations of special subjects like the present one.

In line with these objectives an attempt has been made to limit the present discussions to what may be more or less central areas of each subject, and to leave the discovery of some of the peripheral information to the student and to assign him questions that will cause him to manipulate the central ideas. The author has in mind that each student be assigned to think out some problem or to uncover some information that is available in the literature, and to communicate his result to his colleagues by a discussion, paper, or brief talk. Sometimes our students receive only one such assignment per semester; in other courses (for example, advanced elective courses) much of the didactic time may be spent with such problems. These assignments, some of which are proposed at the end of each chapter, require that the student know how to find papers in the original and derivative literature by subject or by author. Accordingly a preliminary discussion of library techniques has been included at the end of Chapter 1. In this way a simple course in library use is integrated with the central subjects.

Students are often deprived of the pleasure and benefits of the discovery for themselves of facts and relationships from various sources. Also they may be isolated somewhat from the actual process of the advance of knowledge, and from practice of their critical faculties, by the exclusive use of thoroughly prepared textbooks, lectures, and reviews (and now even curricula). As teachers we are supposed to *open doors* to the student. A question often holds the door open better than an answer (or a lecture), and at the same time may help the student to learn to find his own doors. One would suppose that such self-teaching, even if advantageous, would be inefficient; we are continually surprised that it is not.

Accordingly this book is written not only to serve as a sole source of information but also to guide the student to the library and to other counsel for some of the ancillary ideas. Obviously this arrangement gives priority to the needs of the medical student rather than to those of the medical scientist who may be looking for an extensive and profound review.

Indulgence needs to be requested in connection with a few of the assignments that ask the student to find small slips or inconsistencies, oral or typographic, in relation to case reports. The intent is to stimulate careful and critical reading and certainly not to lift eyebrows. Two types of library assignment not included in the lists may occasionally be useful: a student challenge of any account presented here, and a description by the student of a specific analytical procedure. In general, laboratory procedures have been discussed in detail only when required for an exposition of the significance of the results.

Acid-base balance is presented here in terms consistent with the chemical training that the entering medical student of today brings with him. Only those who are teaching first courses in biochemistry realize what a strain the student undergoes in inverting his thinking to some of the historic expositions. At the same time, however, the support to this subject provided by analyses for fixed anions and cations is also developed.

The list of references in the Appendix has been curtailed somewhat to encourage a conventional mode of attack on the library problems

included here, and also in recognition of the extensive bibliographies to be found in some of the treatises listed in Chapter 1.

The author wishes to acknowledge his indebtedness to his mentor, A. Baird Hastings, whose teaching and inspiration are reflected here. A debt of inspiration is also owed to Drs. Donald D. Van Slyke and James L. Gamble. I also want to acknowledge the suggestions and critical reading of all or portions of the manuscript by the following colleagues: Drs. Joseph P. Chandler, Adam A. Christman, Minor J. Coon, Stefan Fajans, Armand J. Guarino, Raymond Knauff, Lawrence Louis, Muriel Meyers, Saul Roseman, and David H. P. Streeten. Sketches and charts by Mr. Jamie Ross, and checking of all references by Mr. David Kronick, are also gratefully acknowledged. I am also particularly indebted to Dr. Benjamin Castleman and Dr. Joseph Garland for their generous permission for me to use the Case Records of the Massachusetts General Hospital, as published in the *New England Journal of Medicine,* for many of the library problems included.

H. N. C.

Ann Arbor, Michigan
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CONCENTRATION

Convenient references for the student of diagnostic biochemistry are the Interne's Handbooks, which give the normal values for a large number of blood and urinary constituents. Figure 1.1 illus-

Constituent	Material *	mg./100 cc. (mg. %) (or as noted)
Albumin	S	3.5–5.5 g./100 cc.
Amylase	P, S	70–200 units (Somogyi)
Ascorbic acid	P	0.4–1.0
Calcium	S	9–11 (4.5–5.5 milliequiv./liter)
Carbon dioxide (combining power)	S	56–65 vol. % (25–30 milliequiv./liter)
Carotenoids	S	100–500 Int. Units/100 cc.
Chloride	S	350–390 (100–110 milliequiv./liter)
Chloride, as NaCl	P	550–620
Cholesterol, total	S	110–300
Cholesterol, free	S	35–90
Cholesterol, esterified	S	75–210
Creatine	B	3–7
Creatinine	B	1–2
Fibrinogen	P	200–600
Globulin	S	1.5–3.4 g./100 cc.
Glucose	B	80–120
Glutamine	P, S	0–2

* B—Whole blood. P—Plasma. S—Serum.

Fig. 1.1 Excerpt from *A Pocket Book of Normal Laboratory Values . . .*, 1957, prepared by Smith, Kline & French Laboratories, Philadelphia.

trates a sample page from such a book. The information contained in the handbook will permit you to decide whether a laboratory result

you have obtained on a patient is *high, low, or normal.* You can then assimilate this finding and other relevant observations concerning the patient. If appropriate information already has been stored by you, you can come up with one or more answers, along with a feeling for the likelihood for each—perhaps also a suggestion of another line of inquiry that will increase your degree of certainty.

My question is: "What kind of information about the meaning of laboratory results will you store?" Will it be of these types: high blood sugar = diabetes; high NPN = renal insufficiency; low chloride = saline needed? Will the number 45 be an arbitrary numerical index that tells you have entered the region of elevated NPN or of elevated cerebrospinal fluid protein? Or will 45 mg. of NPN per 100 ml. give you a picture of a certain total concentration of an assortment of real substances that have reached this total level because of changes in the balance between their collective entry and exit from the solution which you have sampled? Perhaps, because many of the *morphologic* changes of disease are accepted more or less empirically, we are inclined to think of changes of *chemical concentrations* as empirical signs of disease and to overlook their subtle and dynamic character.

A simplified mental shorthand, like *NPN above 45* = renal insufficiency, may be convenient and safe for the experienced, but most of such equivalencies are "booby-trapped" for the unwary. For safety these notations need to be attended with a whole series of "feedback" qualifications (conscious or unconscious) arising from other information about *the patient* and about *this particular laboratory analysis.*

A colleague has suggested that the chemical laboratory should report only a percentile score for every analysis, for example *90* for the NPN to mean that only 10% of persons have a higher level. Thus one could have both a total serum protein and a protein-bound iodine of 68! The statistical aspect is protected, but the reality represented by the analysis becomes even more remote. We shall see subsequently that if a patient has a serum CO_2 of 10 millimoles per liter, a normal serum chloride *for him* would be 120 milliequivalents per liter rather than the usual value of about 103. What benefit would you receive from being told that this patient's

chloride analysis lies at the 99.99 percentile? To retain control of such feedback corrections, you must have the concentration values *per se.*

Concentration and Rate. We must first reconsider the concepts of concentration and rate. Perhaps we must stop to decide whether we really want to know the *concentration of a substance* or the *rate of a process.* In urinalysis we usually want to know a *rate,* the total amount of a substance excreted in 24 hr. or in some other interval, and we are not directly interested in the concentration. The urinary concentration tests are an exception, for in these the abilities to reabsorb and excrete water are under investigation.

Blood analysis on the other hand ordinarily yields *concentration of the substance,* which may be the most serviceable information. In many cases we are also interested in the total supply of the component; to obtain this we need to know the third dimension, the volume of the plasma or blood or extracellular fluid, or whatever the pool that our concentration measure represents. If we had this information we should know the total blood hemoglobin or the total serum albumin or the total extracellular fluid Na^+, and we should have a much better estimate of the actual deficiency. Despite our emphasis on blood, however, there is scarcely an instance where the circulating blood volume represents even closely the true pool size of a component.

We are so accustomed to the idea that it is the blood or serum *level* which we want, that as a shorthand expression we may ask, "What was the serum albumin? What was the urine calcium?" We take for granted that we shall be understood in one case to refer to a *concentration unit;* in the other, to an *excretion rate.* Note also another type of shorthand whereby the serum alkaline phosphatase activity may be recorded as 11 Bodansky units, when *concentration* (presumably 11 units per 100 ml.) is actually meant.

Preserving the Sense of Reality of Concentration Units. *Five per cent glucose* has an obvious meaning: 5 g. of glucose in 100 ml., or 50 g. per liter. If we know that 1 mole of glucose is 180 g., we can calculate that we have 50/180, or 0.278*M* glucose. A little laboratory experience in preparing solutions makes the *molar* unit

as real as the *per cent* unit. Similarly, 0.9% NaCl is 9 g. per liter, or 0.154 molar, or 154 millimolar.

The sense of reality is apt to be broken when we pass over to a Na^+ concentration of 154 *milliequivalents per liter*. Had the term *millinormal* gained acceptance, so that we could say 154 millinormal, half the obscurity would have been avoided. The other complication comes in deciding whether a gram atomic weight of a constituent like Na^+ or Ca^{++} represents *one, two,* or *more equivalents*. If we divide the weight of the solute by its milliequivalent weight, we obtain the number of milliequivalents.

Blood, Plasma, and Serum. Blood is at least a two-phase system, a fact we usually neglect when we state the blood concentration of a substance. The blood concentration is in effect a weighted mean concentration for the two phases. If the solute is restricted to the plasma or to the cells, its real concentration in that phase will be much higher than the average blood level. *Plasma* may be defined as the *extracellular phase* of the blood, or the same fluid modified so as to prevent its coagulation. *Serum* is a fluid produced from plasma by the coagulation reactions. In our discussions we shall encounter this ambiguity: Our analyses usually will be made on *serum,* but when we shall talk about the *circulating fluid* we shall speak properly only of the *plasma*. For example, we may say that the *serum* cholesterol is 210 mg. per 100 ml., but we shall refer to the mode of cholesterol transport in the *plasma*.

Significant Decimal Places. A reported concentration of 4.70 milliequivalents per liter is a claim of accuracy of better than one part in 47. The number should *not* be reported as 4.7 unless one wants to indicate that the uncertainty is still greater. Similarly, a reported cholesterol concentration of 198.8 mg. per 100 ml. represents a claim of a rather unlikely accuracy of better than one part in 199.

Osmolal Concentration. We know that it makes a great deal of difference what molecules are present in the blood or other body fluids. There is one attribute, however, that seems to be provided as well by one solute molecule as by another; that is, *osmotic pressure*. The *total* number of solute particles, whether they be molecules or ions, determines this property.

The *osmolal concentration* (usually in milliosmoles per kilogram of water) is just such an undiscriminating summation of all the particles present in a solution. One could analyze for all the likely components, but not very practically, and add them up, being sure to count $Na^+HCO_3^-$, for example, as two components. More practically one can determine very accurately how much the *freezing point* of serum or of a digestive juice or of urine is lowered from the freezing point of water. If this difference is divided by the *millimolal freezing point depression,* we get the milliosmolal concentration, the freezing point depression being another of the properties (*colligative* properties) that depend only upon the number of particles present.

The physician is so frequently concerned with the much smaller *colloidal* osmotic pressure or *oncotic* pressure that the very great sensitivity of cells and subcellular particles to the *total* osmotic pressure is perhaps overshadowed. Enormous pressures are set up by entering water when a cell is placed in a hypotonic solution, because of the difference in *total* osmotic pressure. Likewise, large amounts of work are required to produce a secretion with a higher or a lower *total* osmotic pressure than the body fluids.

Gas Concentration. We know the blood occasionally may contain, for example, as much as 16.5 g. of hemoglobin per 100 ml.—quite a large mass if separated out on a sheet of filter paper. If we know that 1 mole of oxygen combines with 16,500 g. Hb, or 1 millimole of oxygen with 16.5 g., we can understand that the oxygen capacity provided by the hemoglobin would be 10 millimoles per liter. If a millimole of oxygen under standard conditions is 22.4 ml., this would be 224 ml. per liter, or 22.4 ml. per 100 ml. (22.4 *vol. per cent*).

Suppose, however, you are told that a sample of venous blood has an O_2 pressure of 35 mm. of Hg. This is also in effect a *concentration unit.* You could reproduce this situation by bringing blood into equilibrium with a gas mixture in which the oxygen pressure is 35 mm.; for example, by using 4.6% oxygen in a gas mixture under a total pressure of 1 atm., or 760 mm. Hg. But even after the blood is separated from this gas phase, or even if we are

speaking of circulating blood, we can still say it has this O_2 pressure although there is no gas phase present.

You can perhaps make this designation more real to yourself if you stop to imagine that a tiny bubble of gas phase is caused to be formed over a large volume of the blood. In this bubble oxygen would then exert the stated O_2 pressure.

Let us now show that this is really a concentration unit. The oxygen pressure of blood gives us the concentration of physically dissolved oxygen because, according to Henry's law, the concentration of gas dissolved varies directly with the pressure, $C = KP$. If we know K, we can calculate C from P.

When given in milliliter of gas dissolved in 1 ml. of solution at 1 atm. of the gas, K is called α (the Bunsen solubility coefficient). The value of α for blood is 0.55 for CO_2 at 38°. Hence the physically dissolved CO_2 in blood at $P_{CO_2} = 40$ mm. Hg is $0.55 \times 40/760 \times 1000 = 29$ ml. per liter. Oxygen is less soluble, α being 0.024. Therefore, at $P_{O_2} = 100$ mm., by a similar calculation 0.32 vol. per cent will be dissolved.

The concept emphasized here is that we can record the concentration of physically dissolved oxygen in terms of O_2 pressure quite as well as in other concentration units. We cannot, of course, calculate the amount of *bound* oxygen from the P_{O_2} unless we have further information.

Partition. The foregoing concept can be extended one step further. During ether anesthesia we may know the *ether pressure* of the alveolar air with which the blood is approaching equilibrium. Or we may instead determine the *ether concentration* of the blood. Or conceivably, we may know the concentration of ether in a third phase; for example, the *fat droplets* of adipose cells. At an equilibrium distribution, either one of these should be equally successful in measuring the ether level of the organism. The ether level at every site in the organism may be expected to bear a definite relationship to that in the air or the blood if a steady state has been achieved.

This concept may be examined for applicability to the problem of the concentration of any metabolite or drug at a particular site. The cellular concentration may be *higher* or *lower* than that in the

blood plasma, but these concentrations are usually related. For example, the various amino acids are at much higher levels in the cells of the organism than in the extracellular fluid. Nevertheless, when the plasma level is artificially elevated for a short period of time, a corresponding elevation occurs in the cellular levels. Even before we know why this occurs, we can recognize the validity of this relationship and use it. Such recognized *partitions* between body compartments, where applicable, give far more value to serum analyses than they otherwise would have.

Sodium ions are largely confined to the extracellular compartment of the body; a rise of the extracellular Na^+ level, however, accelerates the movement of Na^+ into the cells, and the cell level must rise until sodium extrusion matches the rate of sodium entrance. Conversely, a fall of the extracellular K^+ level will cause K^+ loss from the cells. In these cases influences of changes of extracellular level are transmitted to the interior of the cell.

Examples of total lack of correspondence between cell levels and the circulating levels of a constituent are, of course, not hard to find. We do not expect to estimate tissue levels of enzymes or of triglycerides by serum analyses: Enzymes usually are confined to the cell of origin by their nature; triglycerides are highly insoluble, and a large fat globule in an adipose cell probably has no more tendency to dissolve or to enter the plasma than a small one.

Other substances become partially bound to cell constituents; the free form may be uniformly distributed, but the *apparent* concentration in cell will be increased by the amount of the bound form. If the amount of the binding agent is not too variable or the binding is not too stable, we may nevertheless note tendencies of the plasma level and tissue level to change together.

Such a substance as Cu^{++}, however, combines very stably with so many cellular and extracellular constituents that the free concentration probably is extremely small. Therefore Cu distribution presents a very complex problem, undoubtedly governed by the distribution, net movements, and affinities of the agents that bind it.

We must also understand that *large* amounts of a component may be transferred from one place to another without its appearing in appreciable concentrations in an *intermediate phase* (for example,