UNIVERSIDADE D
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HEALTH DIAGNOSTICS FROM HEPHÆSTIAN FLAME:
ROOTS AND BRANCHES IN CLINICAL CHEMISTRY DEVELOPMENT

Tese no âmbito do Doutoramento em História das Ciências e Educação Científica, orientada pelos Professores Doutores Ana Leonor Pereira, Cláudia Cavadas e João Rui Pita e apresentada ao Instituto de Investigação Interdisciplinar da Universidade de Coimbra.

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I was adopted by a woman, born in 1908, and a man, born in 1914, who became my parents. Elizabeth Gaillard Malloch and John Howard Davis nurtured an appreciation in me of many cultures. My curiosity might have remained dormant had other parents with less eclectic interests raised me. Between them, they ensured that I had experienced the plays of Chekhov, Sheridan, and Shakespeare, the music of Beethoven, Bach, Brahms, Prokofiev, Ralph Vaughan Williams, Tchaikovsky, and Burl Ives, among many others, that I had lived in a foreign country (Malta) and visited Rome, Pompeii, Stuttgart, Paris, the Louvre, London, and Edinburgh, and been a passenger in a drive across the U.S., all before I was eight. While they shuffled off this mortal coil long ago, I thank them. I was raised curious, which led me to Coimbra at sixty-four years of age and to complete a doctorate at seventy.

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The past six years have been a great experience, and I intend to expand upon what I have learned until I can no longer think clearly or type well.

A Personal Search for a Path to the History of Science

Researching the history of a field of medical science variously known as quantitative diagnostic medicine, laboratory medicine, clinical pathology, or clinical chemistry occurred to me for a few reasons, the principal reason being practical and mundane. I had retired from corporate work managing multinational laboratory operations and needed something to occupy my time. Learning has always been a critical focus, although full-time employment leaves little time for learning new disciplines. In retirement and between executing a few laboratory consulting assignments, I had been reading works addressing aspects of the history of science and found them very interesting. I also received some motivation from two key scientific mentors.

Although I worked for two summers as a maintenance worker in a low-country South Carolina chemical factory that manufactured dye intermediates, my first experience as a chemist was in the laboratory of Dr. John H. Dawson at the University of South Carolina (USC) (Dawson et al., 1983). After completing a doctorate with Dr. Carl Djerassi (1923—2015) at Stanford University and a postdoctoral fellowship with Dr. Harry Gray (1935—present) at California Institute of Technology, Dr. Dawson joined the USC Chemistry Department. He had received a grant to study the biophysics of cytochrome p450cam induced in *Pseudomonas putida* bacteria. Although I met him while asking for a laboratory dishwashing job, he hired me instead to perform the multi-step purification of the enzyme to homogeneity (Gunsalus & Wagner, 1978; O'Keeffe et al., 1978). In a brief time, I was introduced to the formulation of buffers, preparation, and use of several types of chromatographic columns, and the use of a spectrophotometer to determine the homogeneity of the enzyme once it had been purified from the bacterial cells. The cytochrome p450cam I purified was then used in ligand titration experiments conducted by our postdoctoral fellow, Dr. Masanori Sono, and other research group members. Dr. Dawson also took the purified enzyme to the Stanford Linear Accelerator (SLAC), where Dr. Dawson collaborated with Stanford's Dr. Keith Hodgson to conduct spectral probes of the enzyme with various ligands using extended x-ray absorption fine structure (EXAFS) spectroscopy (Penner-Hahn et al., 1983). Most of all, I had the experience of working in a group where everyone conducted their research, but all supported each other's efforts. To me, it was fascinating work and an exciting time.

In 2016, after I had worked in the pharmaceutical industry for over thirty years, I contacted Dr. Dawson. He was transitioning to emeritus status at the university and was chairing a retrospective symposium as part of the Southeastern Regional American Chemical Society (SERMACS) meeting. The symposium was focused on research in bioinorganic chemistry. Dr. Dawson was also hosting a dinner party with many of the colleagues he had accumulated over his career. The experience of hearing pure science discussed among world-class researchers reminded me that I had studied chemistry because it was a pure science rather than an applied science. Visiting Dr. Dawson and his colleagues inspired me to find a doctoral program to study the history of science, mainly as it related to my interests in science. I found an ideal program at the University of Coimbra.

I had the good fortune to work for Dr. Robert Powell (P. E. Warner et al., 1995; Grosse et al., 1994; Koch et al., 1996), a Glaxo clinical pharmacologist who understood that it was essential to study both the pharmacokinetics[1] and the pharmacodynamics[2] of new drugs as soon as possible during clinical drug development. As a bioanalytical chemist—an analytical chemist using sample preparation, chromatography, mass spectrometry, and immunoassay methods to determine the concentration of drugs, metabolites, and biomarkers in biological fluids and tissues—I was tasked with deploying best-in-class analytical methods so that blood, serum, plasma, and other samples obtained during execution of a clinical trial could be turned into concentration/time data and returned to the pharmacokineticists in the shortest amount of time possible. The drug development process tends to be long and expensive, so the time saved lowers the development cost. Working in a clinical pharmacology research and development group for Dr. Robert Powell was a transformative experience for me, not only because Dr. Powell expected excellence from us all, which was inspiring, but because his vision for clinical drug development was based on the best practices from academic, industry, and regulatory minds at that time. He was particularly interested in the ideas of Dr. Carl Peck (Peck et al., 1992). Dr. Powell led us to adopt the drug development practices Dr. Peck recommended into work performed by Glaxo clinical pharmacology. It was natural for me to research the roots of the science and technologies that would ultimately enable the quantification of drugs and metabolite concentrations from biological fluids and tissues. While at Glaxo and Alza, I contributed to several new drug applications, including those for supplemental or abbreviated regulatory filings and representing *de novo* chemical entities.

Research in the sciences is time-consuming. It is common for research on current topics to consume all the time available, leaving little time to investigate how current practices evolved. What developments in commerce, astronomy, navigation, or medicine led to measurement, lens-making, timekeeping, and illness treatment innovations? How did scientists reach the point where sophisticated electronic instruments could perform unimaginably sensitive analyses of previously unknown substances?

When I started the program at the University of Coimbra, I thought my research would lead me to investigate the innovations that led to creating and refining photomultiplier tubes. These sophisticated devices convert light impulses or the impact of ionized molecular fragments into measurable amounts of electrical current that can be recorded using chart recorders or computers. I came into the program with an open mind. While I always thought of ways that might lead me to investigate the history of photomultiplier tubes, other gaps in the literature led me in different directions.

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[1] The study of the dynamic movements of foreign chemicals (xenobiotics) during their passage through the body encompasses the kinetics of absorption, distribution, biotransformation/metabolism, and excretion (ADME).

[2] The study of the biochemical, physiologic, and molecular effects of drugs on the body involves receptor binding, post-receptor effects, and chemical interactions.

For instance, when I started the program, I knew little about Antoni van Leeuwenhoek. What I had learned was incorrect. As I was writing one of several essays required for completion of the first year of classes, I discovered a biography of Leeuwenhoek written by a British protozoologist named Clifford Dobell (Dobell, 1932). The passion for discovery Leeuwenhoek, a haberdasher with almost no formal education, embodied compelled me to learn more and ask questions the existing literature had yet to answer. Pursuing these questions resulted in the publication of three peer-reviewed papers. The papers filled gaps in Leeuwenhoek studies on how he had devised ways to measure microscopic objects and creatures while comparing them with ordinary objects, like sand grains and millet seeds. This led me to an understanding of 16th and 17th-century micrometry and an assessment of how Leeuwenhoek used proportions to explain to his readers how small objects were. I also found that texts addressing the history of hematology had under-represented the significant number and extent of Leeuwenhoek's observation of red blood cells and arthropod blood, which is blue due to the oxygen-binding protein hemocyanin.

Leeuwenhoek studies were not what I had in mind when I started the program, so I started investigating the history of the analysis of alkaline phosphatase, an essential protein measured in the clinical chemistry panels that your physician might request from a clinical chemistry laboratory. References from some articles published by Aararon Bodansky in the 1930s (Bodansky, 1932) led me to 19th Century innovations in physiology and the roots of hematology, particularly the works of Karl von Vierordt (1818—1884), Carl Schmidt (1822—1894), Louis-René Le Canu (1800—1871), Gabriel Andral (1797—1876), Louis Jules Gavarret (1809—1890) and Jean-Baptiste Dumas (1800—1884). To my surprise, studying their research led me back to Leeuwenhoek and microscopy. However, their research also touched upon the roots of colorimetric measurement, which would ultimately nurture the development of photomultipliers. The rich history of physiology and hematology made investigating these matters worthwhile. Discovering how little had been written about their work in the context of the history of medical science made the research a valuable addition to the body of knowledge.

As time passed, other topics intrigued me. A viral pandemic swept the earth, resulting in two papers, one still under peer review. The papers addressed the history of the 2003 SARS-CoV epidemic and the 1833 cholera epidemic that swept Portugal from Porto to the Algarve. I became intrigued by the methods developed by Auenbrugger and Laennec, both because they were non-chemical methods of quantifying health status and because each had accumulated a set of legends about how they had arrived at their practices.

This work has been invigorating because it has been full of surprises. The research touches on many topics to create a context for discussing quantitative diagnostic medicine.

We are creatures who have developed a fondness, a need, a compulsion to define. We define ourselves and our surroundings and will probably be involved in this fascinating and elusive endeavor until our final moments as a species. Some resist the compulsion to define, attempting to pretend everything is a mystery. Others pretend that ancient prophets knew

everything worth knowing and that there is no further reason to examine our beautiful and complex universe. As we play out our lives, the definitions often create a range of possible explanations rather than a single truth or an absence of possibilities. We are more often successful at defining by tending towards a consensus among thinkers rather than by single individuals arriving at reasonable explanations. Sometimes, individual thinkers arrive at an unexpected insight, which catalyzes other thinkers and research groups to prove or reject it, embellish its implications, or carve it away to nothingness. This work strives towards definition by introducing reasonable explanations rather than pretending to find singularities of truth. It is a work in which ranges—a continuum of possible values bracketed by increasingly improbable values—are celebrated at every turn. Like the blind men and the elephant (*Elephant and the Blind Men*, n.d.), we will wrestle with the enormous creature, reduce it to manageable elements, reconstruct it into a living, breathing whole, and hope the result resembles the magnificent creature we came to behold.

Let there be nothing untried; for nothing happens by itself, but men obtain all things by trying.

- Herodotus (~484—~425 B.C.E.)

History, as nearly no one seems to know, is not merely something to be read. And it does not refer merely, or even principally, to the past. On the contrary, the great force of history comes from the fact that we carry it within us, are unconsciously controlled by it in many ways, and history is literally present in all that we do. It could scarcely be otherwise, since it is to history that we owe our frames of reference, our identities, and our aspirations.

- James Baldwin (Baldwin, 1966)

To embrace the medical problem, experimental medicine must include three basic parts: physiology, pathology, and therapeutics. Knowledge of causes of the phenomena of life in the normal state, i.e., PHYSIOLOGY, will teach us to maintain normal conditions of life and to conserve HEALTH. Knowledge of diseases and of their determining causes, i.e., PATHOLOGY, will lead us, on the one hand, to prevent the development of morbid conditions, and, on the other, to fight their results with medical agents, i.e., to cure diseases.

- Claude Bernard (Bernard, 1957)

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ABSTRACT

This thesis explores roots, branches, inflection points, and under-explored areas of quantitative diagnostic medicine, also known as clinical chemistry, laboratory medicine, and clinical pathology. In doing so, it will define biomarkers and their importance in diagnosing human health. It will attempt definitions of health and illness, particularly in terms of homeostasis, a concept first introduced in the 19th century and applicable today. It will offer a top-level review of diagnostic methods and illustrate that health diagnostics are essential to physicians and a global marketplace.

The thesis will describe that clinical chemistry, as with so many other technological and scientific advances, has relied on the earliest mysteries of material science—the fact that without humanity employing fire in increasingly subtle ways, metal and glass objects would never have come into existence.

The thesis will introduce the importance of photodetectors and photomultipliers, objects that have come to serve us all as surrogate eyes, capable of objectivity our eyes could never achieve. The photomultiplier is a supreme *object d'art* resulting from the use of flame. For instance, the photomultiplier has allowed the subjective practice of colorimetry to become sensitive across a wide range of electromagnetic radiation wavelengths and enabled the detection of ionized molecular fragments, entities we could only imagine might exist under certain conditions. It could be argued that the precursor to photomultiplier tubes were the telescope and microscope—instruments fashioned from refined glass and molded metal but also instruments that provided the human eye with powers it did not possess previously.

The thesis will introduce a common practice in modern communities—a health check-up with a physician—to demonstrate quantitative diagnostic medicine's central place in

modern health-focused societies. The thesis will briefly introduce how diagnostic technologies developed from fundamental scientific discoveries before moving on to applying science and technology to diagnostic issues that human health poses. The section will conclude that while much is known, we are often presented with surprising information that provides new challenges to diagnostics and therapeutics.

The remaining sections of the thesis will discuss work from various individuals who represent essential developments in the history of quantitative diagnostic medicine. Chapter Three provides a brief history of percussion techniques and mediate auscultation.

Chapter Four suggests an unlikely union between Newton's definition of the visible spectrum and Leeuwenhoek's discovery of "animalcules"—bacteria and protozoans, although he did not know them as such—and the physical and chemical behavior of red blood cells.

Chapters Five, Six, and Seven are the first of four peer-reviewed and published articles in the thesis (I. M. Davis, 2020a, 2022a, 2022c).

Chapter Eight is a work in progress and has not been submitted for publication. It introduces the work of Gabriel Andral, Jules Gavarret, and Louis-René Le Canu, upon whom Andral and Gavarret based aspects of their 1840 study of blood components in the presence of various maladies.

Chapter Nine introduces the work of Carl Schmidt and Karl von Vierordt, two mid-19th-century German physiologists who studied blood components and the number of red blood cells in a defined blood volume, respectively. The work is in peer review and is published on the Cambridge Open Engage website to establish the priority of the research (I. et al., 2022b).

Chapter Ten is another work in progress. It follows the development of clinical chemistry methods for alkaline phosphatase.

Chapters Eleven and Twelve address aspects of epidemiology. Chapter Thirteen is a historical review of the Portuguese cholera epidemic of 1833 and 1834. Chapter Fourteen, peer-reviewed and published in Reviews in Medical Virology, reviews the SARS-CoV epidemic of 2003.

In summary, this thesis has addressed various gaps in the record, particularly concerning Antoni van Leeuwenhoek, Carl Schmidt, Karl von Vierordt, and Gabriel Andral. In addition, it uses the example of a clinical chemistry method to address the importance of maintaining persistent records of laboratory activities that form the foundation of reproducible science in all fields. In general, the thesis addresses the importance of biomarkers and surrogate endpoints to establish an understanding of patient health. Diagnosis has inevitably been a practice associated with assisting humans since before recorded history. In the 18th, 19th, and 20th centuries, diagnosis was increasingly associated with quantifying chemical entities unknown in earlier eras.

Keywords: History of science and technology, history of medicine, Leeuwenhoek, Vierordt, clinical chemistry

SUMÁRIO

Esta tese explora as raízes, pontos de inflexão e áreas pouco exploradas da química clínica, medicina laboratorial e patologia clínica. Ao fazê-lo, definirá biomarcadores e sua importância para o diagnóstico da saúde humana. São abordadas definições de saúde e doença, particularmente em termos de homeostase, um conceito introduzido pela primeira vez no século XIX e aplicável hoje. Faz-se ainda uma revisão dos métodos de diagnóstico e da sua relevância.

A tese descreve que a química clínica, assim como tantos outros avanços tecnológicos e científicos, baseou-se nos primeiros mistérios da ciência material - o fato de que, sem a humanidade empregando o fogo de maneiras cada vez mais sutis, os objetos de metal e vidro nunca teriam surgido.

Nesta tese descreve-se a importância dos fotodetetores e fotomultiplicadores, equipamentos que passaram a dar informação objetiva que nossos olhos jamais alcançariam. O fotomultiplicador é um objeto de arte resultante do uso da chama. Por exemplo, o fotomultiplicador permitiu que a prática subjetiva da colorimetria se tornasse sensível em uma ampla gama de comprimentos de onda de radiação eletromagnética e permitiu a deteção de fragmentos moleculares ionizados, entidades que poderíamos imaginar que poderiam existir sob certas condições. Pode-se argumentar que os precursores dos tubos fotomultiplicadores foram o telescópio e o microscópio — instrumentos feitos de vidro refinado e metal moldado, mas também instrumentos que forneceram ao olho humano poderes que ele não possuía anteriormente.

A tese descreve o lugar central do diagnóstico clínico e quantitativo nas sociedades modernas focadas na saúde. A tese apresentará brevemente como as tecnologias de

diagnóstico se desenvolveram a partir de descobertas científicas básicas antes de passar para o uso da ciência e tecnologia aplicadas às questões de diagnóstico que a saúde humana. A seção será concluída com as observações de que, embora muito seja conhecido, existem informações que fornecerão novos desafios para diagnósticos e terapêuticas.

As seções restantes da tese discutirão o trabalho de vários indivíduos que representam desenvolvimentos importantes na história da medicina diagnóstica quantitativa. O Capítulo Três fornece uma breve história das técnicas de percussão e ausculta mediata.

O Capítulo Quatro sugere uma união improvável entre a definição de Newton do espectro visível e a descoberta de Leeuwenhoek dos "animalcules" – bactérias e protozoários, embora ele não os conhecesse como tais –, bem como o comportamento físico e químico dos glóbulos vermelhos.

Os capítulos cinco, seis e sete são os primeiros dos quatro artigos publicados e revisados por pares apresentados na tese.

O Capítulo Oito apresenta o trabalho de Gabriel Andral, Jules Gavarret e o trabalho de Louis-René Le Canu, sobre quem Andral e Gavarret basearam aspectos de seu estudo de 1840 sobre componentes do sangue na presença de várias doenças.

O Capítulo Nove apresenta o trabalho de Carl Schmidt e Karl von Vierordt, dois fisiologistas alemães de meados do século XIX que estudaram os componentes do sangue e o número de glóbulos vermelhos em um volume definido de sangue, respetivamente. Este trabalho foi depositado no repositório Cambridge Open Engage para estabelecer a prioridade da pesquisa (I. M. Davis, 2020a, 2022a, 2022c).

O Capítulo Dez segue o desenvolvimento de métodos de química clínica para fosfatase alcalina.

Os Capítulos Onze e Doze abordam aspetos da epidemiologia. O Capítulo Onze é uma revisão histórica da epidemia portuguesa de cólera de 1833 e 1834. O Capítulo Doze, revisado por pares e publicado em Reviews in Medical Virology, analisa a epidemia de SARS-CoV de 2003 (I. M. Davis, 2020c).

Em resumo, esta tese abordou várias lacunas no registo da história da ciência, particularmente no que diz respeito a Antoni van Leeuwenhoek, Carl Schmidt, Karl von Vierordt e Gabriel Andral. Além disso, aborda a importância de manter registos persistentes das atividades de laboratório que formam a base da ciência reproduzível em todos os campos. Em geral, a tese aborda a importância de biomarcadores e *endpoints* substitutos como meio para estabelecer uma compreensão da saúde do doente ou se se está a iniciar uma doença. O diagnóstico tem sido inevitavelmente uma prática associada à assistência humana desde antes da história registada. Nos séculos XVIII, XIX e XX, o diagnóstico tornou-se uma prática cada vez mais associada à quantificação de entidades químicas desconhecidas em épocas anteriores.

Palavras-chave: História da ciência e da tecnologia, história da medicina, Leeuwenhoek, Vierordt, química clínica

CHAPTER 1 | General introduction

This thesis will attempt to describe and evaluate developments in the history of quantitative diagnostic medicine that were important to developing this extensive range of investigative techniques. Some quantitative diagnostic techniques are well-known within science but may not be well understood within the scope of laboratory medicine. Other techniques originated as methods within the scope of medical practice but have not been given recent or sufficient attention as they are historical developments and do not fall within the scope of contemporary medical practice. Other inquiries explore the development of knowledge that would be pivotal to the growth of quantitative techniques and methods.

1.1 What is Quantitative Diagnostic Medicine?

Quantitative diagnostic medicine—and medical diagnostics in general—relies on establishing definitions of health to understand how and why a state of health has been affected by the broad range of endogenous (i.e., genetics, organ failure) and exogenous (i.e., bacteria, viruses, poisons) threats capable of degrading the quality of life. Definitions of health have, over centuries, established normal ranges for many measurements such as body temperature, respiration, pulse, heart rate, skin appearance, eye and pupil response, speech patterns, body and limb movement, reflexes, and other physiological characteristics. The normal ranges and the assessment of aberrant values largely depended on the healthcare provider's experience. They may have been limited in relevance to other locations or people. This changed once healthcare providers wrote out their findings and circulated their observations to other providers in other locations.

What are the quantitative diagnostics used in medicine? In aggregate, there are various methods, both simple and innovative, intended to assess a dimension of human health by determining a value or number with a health function (e.g., the systolic and diastolic values from a blood pressure assessment or the amount of potassium or alkaline phosphatase in a blood sample). The values are accumulated for a representative population of "normal" human subjects or patients. Variations within that population establish a normal range. Once the normal range of results is determined, abnormal results can be evaluated, and possible causes for the abnormality can be assessed. For instance, the average resting heart rate or pulse for adults falls between 60 and 100 beats per minute (bpm). Resting heart rates lower or higher than values within this range may indicate bradycardia (lower than typical heart rate) or tachycardia (higher than normal heart rate). However, both are typically associated with other symptoms.

Assessments may be as complicated as developing three-dimensional maps of the brain or other organs using magnetic resonance imaging (MRI) or positron emission tomography (PET). Physicians request standard diagnostic testing to determine whether a patient is healthy or ill. However, physicians sometimes require sophisticated diagnostic methods when the patient's status remains unclear after routine testing.

As humans developed an increasingly complex knowledge of the universe and what defines life within that universe, we also developed sophisticated tools for assessing and recording measurements. Often, a technology would emerge from studies of astronomy, chemistry, physics, and biology that did not necessarily start as a method of aiding an assessment of health. Physicians, physiologists, pathologists, and a long list of medical specialists were able to extend their understanding of physiological functions among humans and other beings. Developments in optics and lens making, color analysis, electricity, electronics, magnetism, ionization, vacuum and pressure pumps, fluid mechanics, materials sciences, gravimetric, volumetric, and time measurement, along with the consolidation of rules and regulations governing measurement all proceeded and sometimes paralleled developments in our growing understanding of how life functions at the organism, organ, cellular, metabolic, mechanistic, microbial, genetic, and molecular levels.

Once a function is understood, at least in part, its range of function can be determined. A range of functions implies quantitation of function, and this is where quantitative diagnostics enters the arena of medicine. These functions can be understood as systems much like chemical engineering systems can be evaluated, i.e., if appropriate reactants and reagents enter a vessel with a suitable solvent and are maintained at the correct temperature and pressure for some time, chemical products will result. To extend the analogy, the

reactants, and reagents (system input), time, solvent, temperature, and pressure (system conditions), along with product and by-product generation (system output), generally can be measured by one or more quantitative methods. Too low a reactant or reagent concentration, too high a temperature, pressure, or reaction time, and the product and by-product profile changes. The product may not form, or by-products might form instead of the intended product. The product may be formed but in a lower or higher yield than expected. Each reaction stage can be evaluated and used to learn something potentially useful about the nature of the process. If this analogy is applied to quantitative diagnostic medicine, the reaction products and by-products may indicate something useful about fluctuations in health.

1.2 What is a Surrogate Endpoint or Biomarker?

The substance or substances measured in quantitative diagnostic medicine are sometimes called surrogate endpoints or biomarkers. A surrogate endpoint is "a laboratory measure or a physical sign intended to be used as a substitute for an endpoint measuring a direct, meaningful clinical outcome. A surrogate endpoint does not measure the expected clinical benefit of the therapy being studied directly, but rather is expected to predict that clinical benefit" (U.S. Food and Drug Administration, n.d.). A biomarker

> is an objective measure that captures what is happening in a cell or an organism at a given moment. Biomarkers can serve as early warning systems for your health. For example, high lead levels in the bloodstream may indicate a need to test for nervous system and cognitive disorders, especially in children. High cholesterol levels are a common biomarker for heart disease risk" (National Institute of Environmental Health Sciences, n.d.).

To extend the definitions further,

> [although] …all surrogate endpoints are a type of biomarker, not all biomarkers are surrogate endpoints. For a biomarker to be a functional surrogate endpoint, the relationship between the biomarker and the "direct" or clinically meaningful endpoint must be firmly established" (U.S. Food and Drug Administration, n.d.).

These definitions have been refined due to their importance in conducting clinical trials and environmental health assessments. They apply to the clinical chemistry tests a physician order from a central, reference, clinical, hospital, or specialty laboratory.

A list of biomarkers has been collected into an accessible database called MarkerDB (Wishart et al., n.d.). The biomarkers are subdivided and cross-referenced under the following categories for ease of use: chemical, genetic, protein, and karyotype biomarkers (Wishart et al., 2021).

Humans, or any entity from viruses[3] to whales, are in a reductive sense comparable to autonomous or semi-autonomous engines performing enormous numbers of chemical engineering feats every instant. These reactions occur throughout an organism's viability; when they are no longer viable, they are degraded to simple chemical components that other living things may use. Living things in all domains express chemicals that are a part of life, indicate when a process has gone awry, and mark the passage of time until individuals cease to execute their functions. They also mark the *post-mortem* state as an indicator for pathologists and forensic scientists. Chemical markers known as biomarkers are indicators of functionality or health and dysfunctionality or illness, injury, and disease. How these markers are measured and what they indicate about health is central to this work.

[3] Whether viruses can be considered living creatures is a matter of debate among scientists (Rybicki, 1990).

1.3 What is Health?

To understand the processes reflected in quantitative diagnostic medicine, one must come to an understanding of health. Health seems like an indisputable quality, an easily understood condition until a search is conducted for definitions of health. Health is defined differently by different physicians, scientists, and philosophers and in different eras worldwide. It is a concept that is actively debated. It may be discussed until our understanding of the nature of life at the molecular, mechanistic, cellular, tissue-based, organ-based, and organism levels is complete—if complete understanding is possible. Thinking about life and health are necessary elements of understanding. None of the individual ways of thinking about health provide sufficient knowledge to understand the whole. What if one or more functions are outside the normal range? Is this considered healthy? Is there an acceptable range for abnormal values or abnormal functions? When does health tip into illness?

The Hippocratics described their understanding of health, disease, and the physician's role centuries before the current era.

(1) Diseases have a natural course, which the physician must know thoroughly, so as to decide whether the issue will be favourable or fatal.

(2) Diseases are caused by a disturbance in the composition of the constituents of the body. This disturbance relates to atmospheric and climatic conditions.

(3) Nature tries to bring these irregularities to a normal state, apparently by the action of innate heat, which "concocts" the "crude " humours of the body.

(4) There are "critical " days at fixed dates when the battle between nature and disease reaches a crisis.

(5) Nature may win, in which case the morbid matters in the body are either evacuated or carried off in an abcession, or the "coction" of the morbid elements may not take place, in which case the patient dies.

(6) All the physicians can do for the patient is to give nature a chance, to remove by regimen all that may hinder nature in her beneficent work (Hippocrates, (trans. W.H.S. Jones), 1957, p. xvi).

1.4 What is Homeostasis?

The alignment of health and physiology concepts with chemistry, engineering, and physics was reflected in Arthur Guyton's use of control systems theory and systems analysis—concepts from engineering—in the mid-1960s. When these systems functioned within normal parameters, homeostasis was maintained; when acute or chronic disruptions occurred, the systems were no longer homeostatic, and a path to normal function had to be found (Guyton, 1967, pp. 179-201).

The notion of homeostasis is grounded in the 1857 lectures of Claude Bernard (1813—1878) (Bernard, 1973, pp. 84-100) and the writings of Walter Cannon (1871—1945) (Cannon, 1929), who credited Eduard Pflüger (1821—1910), Léon Fredericq (1831—1955), and Richet (1850—1935) for their writings in 1877, 1885, and 1900, respectively, as contributors beyond Bernard's work. Cannon converted Bernard's descriptor of the *milieu intérieur* to homeostatic and homeostasis, linking the term to the mechanical study of statics as "a steady state produced by the action of forces" (Cannon, 1929, p. 401). Richet's explanation of homeostasis, although rendered archaic by current physiologists, is particularly evocative:

The living being is stable. It must be in order not to be destroyed, dissolved, or disintegrated by the colossal forces, often adverse, which surround it. By an apparent

contradiction it maintains its stability only if it is excitable and capable of modifying itself according to external stimuli and adjusting its response to the stimulation. In a sense it is stable because it is modifiable—the slight instability is the necessary condition for the true stability of the organism" (Cannon, 1929, p. 399).

Homeostasis serves as an organizing analogy for physiological processes. It also serves as one of several concepts underlying a thorough definition of health. Other authors have proposed different terms and explanations, including heterostasis (Selye, 1973), allostasis (Ramsay & Woods, 2014), and hormesis (Calabrese & Baldwin, 1998), along with other notions[4]. Kelvin Davies has recently proposed adaptive homeostasis, extending Cannon's concept to control for various stressors (Davies, 2016). Davies defines the term as the "transient expansion or contraction of the homeostatic range in response to exposure to sub-toxic, nondamaging, signaling molecules or events, or the removal or cessation of such molecules or events" (Davies, 2016, p. 1). In effect, he proposes an elaboration of homeostasis to account for contemporary knowledge of cellular mechanisms such as "signal transduction pathways that transiently alter transcription/translation" (Davies, 2016, p. 5).

Health, then, can be defined as a homeostatic system, while illness or disease can be defined as a nonhomeostatic system. When a biological function is perturbed, homeostasis is disrupted. If the physician is knowledgeable about what ions or proteins to assess, it may be that the degree to which homeostasis has been disrupted can be measured.

[4], including predictive homeostasis, reactive homeostasis, homeorhesis, homeorheusis, homeokinetics, rheostasis, homeodynamics, teleophoresis, poikilostasis, and allodynamic regulation.

1.5 Methods for Diagnosis

It is reasonable to speculate that the earliest methods of diagnosis relied on using at least some of the five senses: sight, sound, touch, smell, and taste. As experience grew among the healers, intuition, and experience, particularly experience with individual patients, developed in some healers as ancillary senses. The patient's appearance compared to their regular aspect (flushed, pallid, shivering, sclera color), the sound of their voice, breathing, heart, and gastrointestinal tract, the feel of their skin as to warmth, wetness, chill, goosebumps (*cutis anserine*), the speed and quality of their pulse at various points, whether they discharged any sweat, breath, or flatulence with particular qualities, and less commonly whether they discharged any fluids with abnormal flavor or odor, may have all helped a healer understand whether the patient was healthy or ill.

First and foremost, among the senses is humankind's ability to create a *gestalt*, an integrated whole or big-picture sense. A healer's mind blends disparate elements, from interacting with the patient, their family, and the community to understanding their living conditions. Thinking about the information collected and deriving plausible causes, treatments, and outcomes is a very human behavior. Sometimes, we arrive at correct assessments in all three categories, and sometimes we miss the goal entirely.

One of the earliest documented diagnostic methods was examining urine and dates to Babylonian, Egyptian, and Far Eastern medical texts (Eknoyan, 2007, p. 865). In the *Prognostics*, Hippocrates based urine evaluation on "color (white, red, black), consistency (thin, thick, watery, clear, cloudy), sediment (smooth, leafy, farinaceous, absent), odor (fetid), and volume (deficient)" (Eknoyan, 2007, p. 866). The process of urine examination was called uroscopy. The extent to which urine color, clarity, and taste indicated anything medically useful about a patient is less obvious. Color and clarity variations rely on a

healer's knowledge of the patient's typical urine sample. In the case of uroscopy, the practice devolved into uromancy, a practice adopted and shaped by charlatans, comparable to reading tea leaves or an animal's organs—haruspicy—for signs of the future.

The medieval concept of uromancy lies behind us, except for its practice by natural healing advocates and fringe groups practicing divination. However, quantitative diagnostic information, like any other information, can be transformed into misleading or fraudulent information. Incorrect interpretation can result from insufficient quality control measures through result normalization within or between laboratories, use of unproven or inadequately validated technologies, or misuse of reagents provided by instrument manufacturers (Hammerling, 2012; Plebani, 2010). Quantitative diagnostic methods should measure the concentrations of various substances in bodily fluids and tissues, allow comparison of concentrations with values obtained from patients who have been assessed as healthy, and help support a diagnosis of patient health or illness.

1.6 Diagnostic Testing Has a Huge Global Footprint

Testing modalities have increased over recent decades as more clinical sample testing is initiated in point-of-care (POCT) or near-patient testing, where the testing is performed by qualified personnel at or near the site of a patient at home, in their physician’s office, or at their bedside in a clinic. While the benefits of POCT are significant, the benefits also pose risks to patient management (Shaw, 2021).

Self-monitoring or at-home diagnostic devices provide convenient and relatively inexpensive means for patients to track fluctuations in their health. These innovations range from blood pressure and blood glucose testing devices to cholesterol, infectious disease, and fertility testing kits. Various watch and phone hardware and software applications have even brought near-constant quantitative monitoring of some health metrics to consumers

with sufficient means to use devices ranging from simple exercise-tracking applications to more innovative ones. The U.S. Food and Drug Administration (FDA) has cleared only one watch-based application for implementation in the new Apple Watch models. The applications monitor simple electrocardiogram-type fluctuations and irregular heart rhythm metrics. The FDA clearance letters indicate that the applications “are not intended to replace traditional methods of diagnosis or treatment” (Angela C. Krueger, 2018, p. 1).

Critically, quantitative diagnostics should be interpreted in the context of similar populations, meaning groups of people with similar genetics in similar stages of life and who are of the same gender, ethnicity, and national origin. The emerging multidisciplinary approach of precision medicine attempts to focus on therapeutics and doses in this manner (Chang & Colonna, 2018). If the values obtained by quantitative diagnostic methods are refined similarly, therapies may more accurately and precisely determine the patient's condition.

Enormous improvements in precis

ion diagnostics and therapeutics will happen when assessing individual genetic expression in healthy and ill subjects becomes possible. Although the initial stage of the human genome project was completed in 2003, an updated "complete sequence" was published in 2022, which followed completion efforts by the Genome Reference Consortium in 2013 and 2019 (Nurk et al., 2022). Still, a long list of unknowns associated with the 2022 sequence remains, so it is not easy to know when accurate individual genetic analysis will occur as a central feature of human healthcare. Variation at the level of individual genetics ultimately may not be helpful except for grouping subjects for treatment by precision medicine therapies.

While market research is not a direct evaluation of the medical value of quantitative diagnostic testing, it provides some insight into the use of clinical laboratory services and at-home diagnostic testing by physicians, hospitals, pharmaceutical companies, and individuals concerned about monitoring their health. A 22 July 2022 article on the industry website BioSpace referred to a report by the market research firm Vision Research Reports indicating that the clinical laboratory services market was valued at US$238 billion in 2021 and is projected to reach US$415 billion by 2030. BioSpace also cited a Vision report for at-home diagnostic testing, indicating that it was valued at US$5.4 billion in 2020 and is expected to grow to US$8.5 billion by 2030. In both cases, fast growth is expected in the Asia Pacific market (Clinical Laboratory Services Market Size, Growth, Forecast Report 2021 to 2030, 2022; Home Diagnostics Market Size to Reach US$ 8.5 Billion by 2030, 2022).

Determining quantitative data to assist physicians in evaluating a patient's health has become an enormous global business expected to grow substantially. While it is reasonable to talk about the current and future benefits of patient access to quantitative medical diagnostics, it is essential to note that according to the 2021 Lancet Commission on Diagnostics "47 % of the global population has little to no access to diagnostics." Access to diagnostic imaging methods is "similar or even worse" (Fleming et al., 2021, p. 1997). As the global population gains increased access to diagnostic testing, revenue will likely grow for decades. As testing grows, the need to control variables will become increasingly crucial to the correct use of instrumentation, reagents, and personnel.

CHAPTER 2 | How Quantitative Diagnostic Medicine Became Possible

2.1 The Prehistory of Quantitative Diagnostic Medicine

It may seem obvious, but we would not be the species we have become without a means to mold and mix the raw materials of our earth and atmosphere. The deepest roots of technological and material science, methods, and techniques that fit collectively under the umbrella of clinical chemistry may have started when someone making bricks decided to take a divot of clay out of the wet brick, pare away the sides, and make a bowl, cup, or similar item. If a brick or a clay piece were placed in a fire, the clay would have hardened while the waters of hydration were forced out, the clay becoming impermeable to liquids in the annealing process. The ceramic would be used at the hearth, and the bricks were used for housing. Cupped hands preceded these clay cups and bowls, dried gourds, carved wood, and sculpted stone. River water had been sculpting wood and stone long before humans crafted either. These models could have suggested pottery before clay was placed in fire.

It may have been that these early clay vessels, these crucibles, yielded raw forms of glass as an unexpected artifact. Many varieties of clay are composed of minerals principally of silicates and various cations, anions, and hydrates. If wet clay was rested in quartz sand, it might have emerged from the fire with a more lustrous finish—a rudimentary form of glazing—than was generally the case. Specific clays with silicates mixed with soda ash (sodium carbonate) or potash (potassium carbonate, plant ash, or wood ash) may have produced the same result. However, brickmaking and pottery crafting are thought to have preceded glassmaking by millennia. Somebody must have noticed the glazing and the vitrification and decided that they would like to reproduce the artifact. The oldest pottery known at present was used by hunter-gatherers somewhere between 20,000 and 15,000 years before the present (the dates are calibrated against radioisotopes) (Craig et al., 2013). The earliest form of glazing dates to 8,000 B.C.E., while the formulation of purer forms of

glass dates to 3,000 to 2,000 B.C.E. in Mesopotamia and Egypt (Macfarlane & Martin, 2004). Early historians indicate that glass was first formed by accident from the sand on the river Belus in current-day Israel; other writers suggest that glass was formed when a forest fire took place on a sandy hill, thus mixing potash and sand (Freestone, 2008; Macfarlane & Martin, 2004).

While certainty about when and how metallurgy and glassmaking first became routine practices, histories of glass and metal inevitably start with fire. Many cultures viewed fire as a supernatural gift, something fearsome and divine. The Greeks imagined Hephæstus and punished Prometheus for sharing the secret with humankind. To demystify the allusion from the thesis title, humanity and human health have benefited beyond measure from the allegorical role that Hephæstus and his fiery forge played in the pantheon of Greek gods, hence his Hephæstian flame. While these are understood as stories told to explain the inexplicable, Aristotle's four elements are all involved in these early crafts: fire, earth (ore, minerals, clay), water (hydrates), and air (fire requires air, and forced air causes fire to burn hotter).

Brick and pottery crafts resulted in the crucible, a vessel for melting solids. Metal-containing ore and quartz pebbles ground into fine powder—frit—were placed in crucibles and heated in fires, ovens, and furnaces. The metals separated from the raw ores, and the quartz frit melted into glass. Metallurgy and glassmaking emerged from the oven through the crucible, from the simplicity of an item made from clay and heat. The first crucibles may have resembled something a nephew once formed in a beginners' pottery class—more a lump with a dent than the ceramic labware we use today. Making these items—brick, cup, crucible, metal, glass—may have resulted from the time-honored act of throwing items into a fire to see what happened. Wood became air and ash. The fire died away. What other

transformations might occur? Will it burn and disappear, or will it survive the flames? What disappeared and what did not? Did the item change into some other form? Some changes would be noticeable—charring, shrinkage, liquefication, melting. Other changes—degassing, desiccating—would have been more challenging to detect.

Glass and metal would eventually allow the invention and construction of varying designs of lenses, telescopes, microscopes, colorimeters, hemoglobinometers, hemocytometers, vacuum tubes, and photodetectors. In each case, raw materials existed in nature long before humankind became human and started a series of serendipitous experiments. Materials were discovered in their various raw forms. Through some cognitive process that differentiates us from all other creatures on the planet, we began to understand their properties and usefulness as materials for creating other materials that resembled natural objects or did not exist in nature at all. Various elements included as trace substances in glasses of pure silicon dioxide—sand—gave glasses color, made them more transparent, translucent, or opaque, made them harder or softer, brittle, increased their melting points, and changed their refractivity indices, first described mathematically by Claudius Ptolemy in the 2nd Century C.E.

As we know now, metals doped into silicon in minute quantities can allow for the creation of semiconductors, while glasses can also act as insulators between metals. In these ways and many others, the combination of nonmetals and metals created materials that have made our era possible in ways unimaginable to the merchants who first traded them. They were baubles, shiny substances that intrigued our eyes and created wealth for those sufficiently powerful to hoard them. When translucent glass was crafted, observant citizens noted that a convex glass—a vase or vessel filled with water, for instance—could be used

to focus the sun into a hot beam of light sufficient to start fires. When metals were melted and reshaped, they might be used as cooking and farming tools or weapons.

Innovations in fire-making had to come first, before metals, before glass. How did our ancient minds realize the fire that warmed them or cooked their food was sufficient for making some materials and insufficient for making others? How did they learn that fire could be coaxed beyond its default temperatures? It could be the realization that different segments of a flame, demarcated by different colors of the flame, reached different temperatures. Maybe it was a by-product of the brickmaking process—once bricks were made, they could be used to create ovens and kilns, which could be heated to higher temperatures if air could be force-fed into the base of the fire.

How does brickmaking correlate to quantitative diagnostics? The development of optical systems that could measure a stream of photons, electrons, or ions would rely on the use of glasses crafted to provide an unbiased evaluation of electromagnetic radiation within a range of wavelengths. The glasses used in scientific instruments should introduce as little bias as possible into the data capture and generation process. While photodetectors were a vital innovation, photon and electron-emitting materials were also necessary for limited-bias measurements. For these enormous leaps in technological sophistication to occur, centuries of knowledge and technique had to evolve until glasses and metals were created and refined to suit the needs of humans.

2.2 The Importance of the Photodetector

At first glance, the fields of immunochemistry and mass spectrometry do not have much in common. Immunochemistry relies on the intermolecular binding of ligands and receptor molecules. When some metals, proteins, antibodies, drugs, and metabolites are bound to photoreactive molecules, the resulting chemical species is easier to detect and quantify

using wavelengths in the ultraviolet and visible spectrum. Although it is an entirely different technology, mass spectrometry relies on ionizing and separating chemical compounds and detecting the resulting fragments as a function of their mass-to-charge ratio. If we examine the two chemistries more closely, several similarities appear. Both rely on energetic interactions.

For immunochemistry, the interactions are partially characterized by binding constants and are unique for any specific ligand-molecule interaction. Some ligands and molecules do not bind to each other at all—have no affinity for each other and therefore have a binding constant of zero—and some ligands and proteins bind irreversibly or covalently. Between the extremes lies a variable series of interactions from weak to strong binding, all based on the presence of ionic, dipole, and covalent bonds, along with varying amounts of help from hydrogen bonding, van der Waals interactions, and the like. The interactions depend on optimal pH and temperature conditions for ligand-target pairs. These changes can be monitored, quantified, and interpreted as proportional to the binding between ligand and protein, antigen, and antibody, drug, and receptor. The photomultiplier measures the light, fluorescence, phosphorescence, luminescence, absorption, or transmission of light once a solution of the material has been stimulated in some way.

For mass spectrometry, ionization requires electrons to be added or removed from one or more bonds in analyte atoms or molecules. The ionization energy required for a molecule to lose or gain an electron varies by orbital (sigma, pi, or delta orbital) or bond (ionic, covalent) type. Ionization energy must be added to an atom or bond so that an electron's "binding constant" is exceeded. Ionization typically results in the fragmentation of the intact molecule into several smaller molecular species, some of which have a positive or negative charge, and some have no charge (neutral fragments). In some types of mass

spectrometry, “parent” molecular ions can be fragmented further by interactions with specific gases introduced into a collision cell; “daughter” ions unique to the “parent” ion are formed and can be detected. These ions travel through various ion optics—metal plates like light transmission gates, slits, or collimators that hold very specific electrical charges that keep the ions within a constrained flight path—and collide with one of several types of detectors, all of which transform the molecular charge into an electronic response. In some detectors, photons are emitted from a scintillating surface—a material that emits luminescence in response to the impinging ions—and enter a photomultiplier tube (*Figure 2.1.*) to produce an amplified cascade of secondary electrons. The ionization and detection process results in a measurable current proportional to the accumulation of each ion over time; only if the cumulative amount of an ion impinging on the photomultiplier rises above the electronic instrument noise threshold is an ion registered as measurable. It is worth noting that ion optics, except for the optics in play at the photomultiplier tube, differ from those enabled by lenses. These optics use metal, and transmission occurs through gaps in metal defined by current (Watson & Sparkman, 2007).

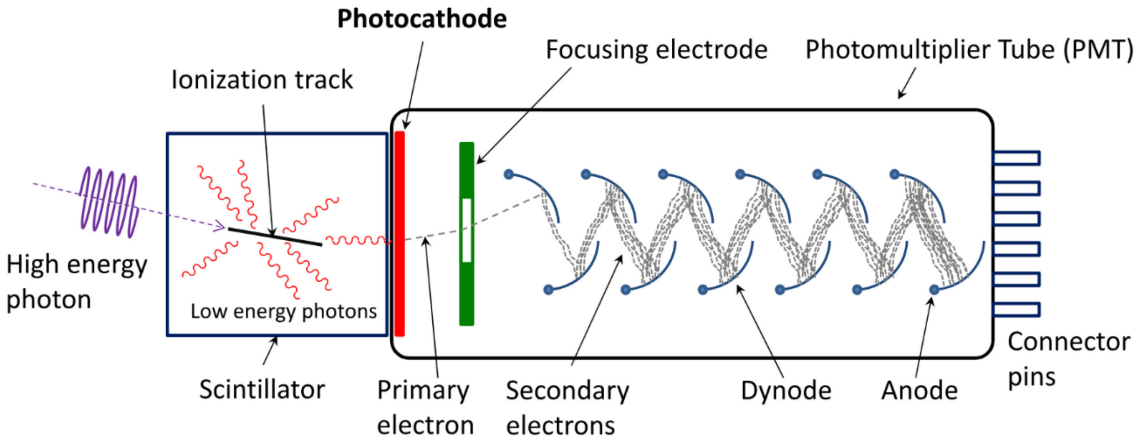

Figure 2.1. *General photomultiplier tube with scintillator design for amplifying incident light by electron cascade (Qwerty123uiop, 2022) (This is the pseudonym of the artist responsible for this figure, borrowed under Creative Commons attribution).*

Both methodologies assist in perceiving molecular objects that are too small to be visible using our eyes or a light microscope. They become visible because some of their properties are determined through nuanced uses of electromagnetic radiation (E.M.R.) and, ultimately, detection by a photodetector. While it is possible to say that they are "seen," what we see represents the events occurring as energy interacts with molecules. A beam of light is passed through a solution containing binding and target molecules for immunochemistry. The extent to which the initial light is attenuated by absorbance at characteristic wavelengths can be considered proportional to the concentration of the ligand-antibody complex. For mass spectrometry, as each ion collides with the photodetector, the accumulating amount of that ion can be determined above a noise threshold defined by the limitations of the materials from which the instrument has been designed and constructed. Because the ions that arrive at the photodetector have been separated from each other—typically both by some chromatographic process as well as by various ion optics—and because the ions detected have a mass-to-charge ratio (m/z) that is partially representative of their identity, the structure of the molecules can be determined. In both cases, something invisible to us has become, if not visible, knowable in essential ways. The quantity of molecules in a sample volume is often critical information. It can relate to efficacy or toxicity in the case of drug therapy.

The general purpose of photodetectors is to register a photoelectric event—photodetector substitutes for the human eye. While our eyes register photoelectric events very well, there is a threshold below which an event is not registered. The threshold is thought to be three photons (Holmes et al., 2015). Wavelengths beyond the visible wavelengths are not detected, although our eyes experience wavelengths in the infrared and ultraviolet regions as heat and harm. Recent studies suggest that some infrared wavelengths may be experienced through one of several possible phenomena (Palczewska et al., 2014).

Photodetectors, which are analogous to artificial eyes, avoid the natural organ's flaws, limitations, and subjectivity in favor of providing objective data on the properties of various materials. However, even photodetectors of various designs can have their own set of flaws and biases. Photodetectors can be designed to detect wavelengths above and below the visible range of E.M.R., as well as within narrow or broad bands of wavelengths in any range, using optical or mechanical gates, slits, or collimators. The photodetectors used with mass spectrometers are tuned to detect the impact of a molecular or atomic ion on various electron-emitting surfaces.

2.3 The Theoretical Basis of Colorimetry

As with many other areas of early science, the need to measure light was driven by the necessity of improving navigation through observation of celestial objects and by commercial concerns. Pierre Bouguer (1698—1758) measured the light of our moon in 1725 by comparing its intensity to that of a candle. One of the phenomena of light that fixed human imagination was that of color. How did colors mix to form other colors? How did we perceive color? Was it a property innate to specific materials, was color a phenomenon within the eyes themselves, or was it something our brains did with the light that entered our eyes? Was perception of color a result of a combination of these? Aside from Isaac Newton's thoughts on light, the visible spectrum, and color, followed by Michael Faraday's color wheel experiments, some early research into the nature of color and light was in service of the dye and pigment industries (L.-G. Oltra et al., 2001). While early versions of the colorimeter relied on the ability of the human eye to differentiate between pigment colors, designs based on the use of photodetectors would make measurements of incident and filtered light and determine the differences. Improved photodetector designs depend on innovations in materials science and are a subject of continuing research (Bartley, 2023;

Glebov et al., 2021). They are a complex technology and depend on principles developed since the photoelectric effect was discovered by Heinrich Hertz and illuminated by Albert Einstein (Polyakov, 2013).

The evolution of photodetectors lies at the center of our improved understanding of the universe—its physics, chemistry, biology, medicine, and quantitative diagnostic chemistry methods used in laboratories worldwide. Precise and accurate wavelength measurement from electromagnetic radiation became possible through a convergence of materials availability, technical skills, tool refinement, and an ever-expanding understanding of science. Along with these remarkable developments, it became possible to convert differences in the incident and absorbed light into data, ionized chemical species into photons, electrons into secondary electrons, electrical current, and then information about the amount and type of a chemical moiety and its properties. Monochromatized wavelengths and ionized molecules, by way of photodetectors, resulted in an enhancement of knowledge.

2.4 Colors, Colorimetry, Chemistry, and Microscopy

The color of bodily fluids was observed and noted as far back as the Hippocratic works, although urine, vomit, pus, and feces were described more vividly than blood. Skin and eye color were also evaluated. The humors (blood, phlegm, yellow bile, black bile) were known for their colors, along with other properties, as indicators of patient health status, as noted by one of the Hippocratic writers: “How could they be like one another, when their colours appear not alike to the sight nor does their touch seem alike to the hand?” (Hippocrates (W.H.S. Jones, trans.) 1959, p. 13). Blood is not usually characterized by color but by physical characteristics, e.g., congealed, thin, watery, hot, and chilled, although the colors of arterial and venous blood must have been noted. An exception is found in Volume IV,

where “when men are cut, the blood that flows is at first very hot and very red, and then it flows with more phlegm and bile mixed with it” (Hippocrates (W.H.S. Jones, trans.) 1959, p. 19). While this description does not address the darkness of venous blood, it permits a description of blood as very red or “mixed.” In Volume IV, stool or feces is described as “black like (black) blood,” although this is soon attributed to “a discharge of black bile, or as it were of black blood” (Hippocrates (W.H.S. Jones, trans.) 1959, p. 141).

While it is logical to assume that each of the humors and related excreta was viewed after being diluted, tint, texture, and nuances of color were observed by physicians, priests, and barbers practicing the medical arts, these nuances were not described sufficiently in these translations of Hippocrates. They may not be described sufficiently in the original Greek. Overall, when comparisons are made between degrees of biliousness, for instance, they seem to rely on the observer's impression of ideal humor color rather than a side-by-side comparison. In other instances, the patient's color is described as "good," "healthy," "better," "light," "dark," "ruddy," "yellowish," "yellow-green,” “livid,” “bad,” or “poor.”

In *Affections*, Hippocrates allows that the blood may become dilute due to some inner process that produces an abundance of phlegm, which causes the blood to appear “more watery than usual” and the “patients appear whiter” (Hippocrates (P. Potter, trans.) 1988, p. 34). Later in *Affections*, Hippocrates indicates that tenesmus[5] causes dark blood and mucus to “pass in the stools” (Hippocrates (P. Potter, trans.) 1988, p. 47); elsewhere, this is described as “burnt-up blood” (Hippocrates (P. Potter, trans.) 1988, p. 44). Sciatica is attributed to “when bile and phlegm are deposited in the blood,” although no color is described for this mixture, while blood texture is described as “congealed” (Hippocrates (P. Potter, trans.) 1988, p. 53). Gout is also attributed to this mixture of blood, bile, and

[5] A cramping pain in the bowels whether your bowels are empty or not (*Tenesmus*, n.d.).

phlegm, but no description of the blood or skin is given (Hippocrates (P. Potter, trans.) 1988, p. 55). In *Diseases I*, Hippocrates indicates that blood turns into pus when it "flows from a wound or a vessel into the upper cavity" (Hippocrates (P. Potter, trans.) 1988, p. 107). It is unclear whether this is a color description as the blood "must turn to pus" and may no longer be described as blood. Later passages describe blood as turning to pus or putrefying and suppurating. Whatever might have led to this description, we know that it simplifies other changes and does not describe blood color.

Lung suppuration caused by "small vessels" rupturing is described as resulting in small tears in some vessels, which can release "at first little and darkish, but then more and of a purer kind" of blood (Hippocrates (P. Potter, trans.) 1988, p. 125). It is enticing to think of this description as differentiating between venous "darkish" and arterial "purer" blood, particularly given the bleeding location in the lung, but this may be a stretch. In several places, vomit is described as "charged with blood" but can contain "livid material, also phlegm and bile" (Hippocrates (P. Potter, trans.), 1988, p. 139). Again, it may be that the "livid material" describes venous, deoxygenated blood, which is now understood as the basis of lividity (Hayman & Oxenham, 2016, p. 5).

In Chapter 20 of *Diseases I*, the livid blood is described as "livid not with pure blood, but with thin watery blood of a small amount" (Hippocrates (P. Potter, trans.) 1988, p.147). As a result of phrenitis, bile that has been "set in motion enters the vessels and the blood, it stirs the blood up, heats it, and turns it to serum, altering its normal consistency and motion," which suggests that the blood turns to serous straw-colored liquid in the presence of bile. Pleurisy and pneumonia result in sputum that is "bloody and livid," thus raising the possibility that both arterial and venous blood are being observed. However, the livid nature of the sputum is seen when "a small amount of blood is mixed together into much sputum,

and this is not expectorated at once" (Hippocrates (P. Potter, trans.) 1988, p. 179). In *Diseases II*, Hippocrates describes what happens when blood overfills "vessels in the head":

[I]f you make an incision into an arm suffering from the condition, or into the head or any other part of the body, the blood that flows forth is dark turbid, and diseased; and yet not rightly so according to the name, but the blood should flow red and pure (Hippocrates (P. Potter, trans.) 1988), p. 197.

Whether this describes venous and arterial blood or whether it describes clotted blood and oxygenated blood is unclear from the text.

In section 73 of *Diseases II*, a Hippocratic writer describes one of the so-called "dark diseases,"

[T]he patient vomits up dark material that is like the lees of wine, sometimes like blood, sometimes sharp like vinegar, sometimes saliva and scum, sometimes yellow-green bile" (Hippocrates, Hippocrates Vol. V, 1988, p. 329).

These passages from the "Hippocratic Collection," as translator Paul Potter describes the volumes in an introduction to *Diseases II*, underline the importance of color to some healers more than two millennia before the advent of colorimetry. While the descriptions have few nuances, they wrestle with how best to provide other healers practicing in that era a guide on physiological colors and what might have caused them. This rudimentary guide to the importance of color is rewarded when our knowledge of visible light wavelengths and frequencies, colorimetry, and spectrophotometry expands. However, the Hippocratics understood that important information was present in the colors the body presented, whether in health or illness.

Aside from William Harvey, who was the first person to describe blood circulation accurately, little chemistry was done to the blood except by alchemists such as the Spanish alchemist Geber (~14th C.) and Roger Bacon (~1220 to ~1292). Among the first to describe the effect of physical and chemical experiments on red blood cells was Antoni van Leeuwenhoek (I. M. Davis, 2022c).

It is thought that the work of Hermann Boerhaave (1668—1738) changed the perception of how chemistry could be helpful to the practice of medicine (Christie, 1994a; Verwaal, 2020). For instance, Boerhaave's two-volume *Elementa Chemiae (Elements of Chemistry)* represents an introductory chemistry course compiled from lectures he presented from 1718 to 1729 at the University of Leiden. The first representations of these courses were assembled from his students' notes, which upset Boerhaave as they did not accurately represent his material. He eventually produced an authorized version, which was translated into English by Timothy Dallowe, although Christie wonders whether this is a complete representation of the material presented in lectures (Christie, 1994a, p. 6). The two volumes provide examples of chemical interactions with blood (Boerhaave, 1735a, 1735b).

Conversely, Boerhaave credits other "physics" (i.e., physicians) and natural philosophers for their insights. He started the two-volume work with a substantial section titled "The History of the Art," meaning the history of chemistry. The section lists virtually anyone who ever performed metallurgy, written on alchemy, or used the Greek or Egyptian word "khēmia" up to his time. After listing contributors, he believed enabled the medical arts, Boerhaave provided brief biographies of Theophrastus von Hohenheim (Paracelsus; 1493—1541) and Jean-Baptiste van Helmont (1580—1644). He considered them directly responsible for blending chemistry and medicine, although each had flaws. Boerhaave notes that although both professed to have created remarkable cures for many maladies,

neither lived to old age. In addition, Helmont failed to save his sons from the plague, his daughter from leprosy, his wife, his maid, or himself from death (Boerhaave, 1735a, p. 16). Although Boerhaave does not offer a biography of Friedrich Hoffman (1660—1742), he finishes the introductory section with the following homage:

But above all, the very ingenious Frederic Hoffman, in his *Observationes Physico-chemicæ selectiores, libris tribus comprehensæ*, publish'd at *Hall* [Halle, Germany], 1722; a Gentleman, who has done a vast deal of service to the chemical Art, and enriched both Chemistry and Physic, with abundance of beautiful observations (Boerhaave, 1735a, p. 18).

Boerhaave also cites Robert Boyle (1627—1691), a founder of the Royal Society and considered one of the first "modern" chemists. Boerhaave does not mention Jan Swammerdam in either volume, despite Boerhaave overseeing the posthumous publication of Swammerdam's massive *Bybel de Natuure* (*The Book of Nature*) (Swammerdam et al., 1737), Boerhaave cited Antoni van Leeuwenhoek's observations on spermatozoa and ova but did not mention his observations of red blood cells or "animalcules" (I. M. Davis, 2022c). However, Boerhaave cites the importance of Isaac Newton's work on light and optics over twelve times, referring to him as the great, illustrious, or incomparable Newton in each case (Boerhaave, 1735a, pp.105, 132, 142, 143, 152, 165, 232, 233, 251, 324, 363, 413).

Boerhaave, then, serves as a bridging intellect between the works of Newton and Leeuwenhoek, each of whom wrote their first letters to the Royal Society in 1672 and 1673, respectively, and various 18th and 19th Century chemists and physiologists. Boerhaave passed his approach to blood directly to his student Hieronymus David Gaubius (1705—1780), who separated blood into three constituent parts—serum, red blood, and fiber—then into four elements—water, bitter oil, volatile salt, and black earth—and finally into the four

principles of water, phlogiston, salt, and earth, “which all imparted characteristic properties to the red fluid” (Verwaal, 2020, pp. 74-76). The approach favored by Boerhaave and Gaubius did not go unchallenged, however. Thomas Schwencke (1694—1767), another Boerhaave student and the individual who coined the term haematology, found similar constituents when blood was distilled but argued that no chemist could “recombine them to form human blood, not in colour, smell, or any other property,” introducing doubt into the value of Boerhaave’s approach to the chemical analysis of blood. Schwencke’s concerns were echoed by French physician Théophile de Bordeu (1722–1776), who was focused on practical observations of the state of the patient and particularly how the pulse could reflect characteristics of the blood more appropriately than the distillation of blood (Verwaal, 2020, p. 84).

The individuals who pursued investigations into the nature of blood and its components used new analytical chemistry techniques introduced by Jöns Jakob Berzelius (1779–1848), Justus von Liebig (1803–1873), and William Prout (1785–1850), all of whom were focused on the analysis of “animal fluids” (Coley, 2001, p. 2168). In 1806, Berzelius published the first volume of *Vorlesungen über Tierchemie* (*Lectures in Animal Chemistry*) and the second in 1808. These texts focused primarily on physiological chemistry and the methods of chemical analysis he developed to satisfy his fastidious nature (Winderlich, 1948, p. 501). Liebig, a couple of decades younger than Berzelius, was motivated to improve techniques of organic chemical analysis. He obtained a grant to study in Paris starting in 1822. He attended lectures by Joseph Gay-Lussac (1778–1850), Louis Jacques Thenard (1777–1857), and Louis-Nicolas Vauquelin (1763—1829), leading figures in the development of organic analysis techniques, each influenced by Antoine Laurent Lavoisier (1743–1794); Liebig worked with Gay-Lussac during this time (Rosenfeld, 2003). While Liebig and his students at the University of Giessen developed analytical chemistry

techniques, some praised him. Others thought he had overreached by constructing baseless hypotheses. To quote from the 1965 introduction to a reprint of Liebig's 1840 *Die organische Chemie in ihrer Anwendung auf Agricultur und Physiologie*, translated and published two years later in English as *Animal Chemistry*, "[w]hen Liebig's ideas appeared, the leading physiologists of the time avowed that they offered deep insights concerning the internal processes of the entire animal economy. An equally eminent chemist complained, however, that Liebig was merely encumbering physiology with erroneous hypotheses presented as facts" (Ihde, 1965, p. 795).

In parallel with the work emerging from the laboratories of Berzelius and Liebig were methods of inquiry enabled by colorimetry and modern optics. Ironically, colorimetry was initiated by the discovery of chlorine and the bleaching properties of hypochlorite. Initial methods that employed titrimetric measurement of indigo dye sought to find the amount of bleach that would diminish the characteristic blue color to clarity, a technique pioneered by François-Antoine-Henri Descroizilles (1781-1825) (Duval, 1951, pp. 513-8; L.-G. Oltra et al., 2001) (Szabadváry, 1966, pp. 197-227). Variations in bleaching methods evolved to produce colorimetry instruments from the workshops of Jules Dubosq and others (Brenni, 1996; D. J. Warner, 2006). By the 1860s, these innovations led to instruments that could volumetrically determine the amount of hemoglobin in a blood sample by way of its color based on research performed by William Hyde Wollaston (1766—1828), Joseph von Fraunhofer (1787—1826), Robert Bunsen (1811—1899), and Gustav Kirchoff (1824—1887) on the fundamental principles of spectroscopy (Thomas, 1991, pp.631-2), and the spectral characteristics of hemoglobin by Felix Hoppe-Seyler (1825—1895), and Karl von Vierordt (1818—1884) (Perutz, 1995; K. von Vierordt, 1871; K. von Vierordt, 1869).

Ideas about the complicated nature of blood and its functions made significant advances starting in the 18th Century (Sheftel et al., 2012, p. 166). They accelerated with Johann Friedrich Engelhardt's identification of a fixed ratio between iron and protein in red blood cells (Engelhardt, 1825). This idea, in turn, built off the knowledge that there were identifiable and quantifiable entities with the ancient name "iron," combined with the more modern word "protein"—a term suggested by Berzelius and formally introduced by Dutch chemist Gerhard Johan Mulder (1802-1880)—each of which represented an advance of knowledge. Engelhardt wrote about the ratio of iron to protein he found in red blood cells. His finding was disputed, as he suggested that there were 16,000 units of protein to each unit of iron, and few believed there were any chemical entities as large as this ratio suggested. His calculations were remarkable for their prescient accuracy; hemoglobin has an average formula weight of about 65,000 daltons (4 times 16,000); Engelhardt's calculation equals 64,000, a difference of 1.6 %. The principal activity of the red blood cell is concentrated in hemoglobin. This oxygen transport protein contains four iron atoms, each bound within an individual porphyrin ring in each hemoglobin unit.

Microscopy fell into general disuse and disfavor after the initial uses by Galileo Galilei (1564—1642), Pierre Borel (Petrus Borellius; ~1620—1671), Athanasius Kircher (1602—1680), Giuseppe Campani (1635-1715), Robert Hooke (1635—1703), Jan Swammerdam (1637—1680), Marcello Malpighi (1628—1694), and Antoni van Leeuwenhoek (1632—1723). Eighteenth Century anatomists, such as Giovanni Battista Morgagni (1682–1771), John Hunter (1728–1793), and Matthew Baillie (1761–1823) avoided the microscope entirely in their works (Hajdu, 2002). A few 18th-century physiologists, such as Father Josephus Maria de Turre (birth and death unknown) and William Hewson (1739—1774), maintained an interest in microscopy, particularly blood cells. This changed through the efforts of Alfred Donné (1801—1878), who, in 1836 and

1837, successfully demonstrated the utility of microscopy to colleagues and students at the Medical School of Paris (*Ecole de Médecine de Paris*). He gained their interest by paying for the installation of twenty microscopes from his funds (Diamantis et al., 2009). In 1844, Donné published a manual on the use of microscopy in medicine titled *Cours de microscopie complémentaire des études médicales*, thereby creating a formal text for others interested in using microscopes in the service of healthcare (Alfred Donné, 1845).

These techniques, while powerful in their ability to confirm results obtained by newer means, were also on their way to being eclipsed by new spectrometric techniques, which relied on the colors of solutions and our ability to measure the solution's transmission and absorbance of light. Spectrometric techniques owed their emergence to influences from Pierre Bouguer (1698—1758), August Beer (1825—1863), and Johann Lambert (1728—1777), who, over a century, constructed a theoretical and mathematical correlation between the absorption and transmission of light and the concentration of a substance in solution (Mayerhöfer et al., 2020). Their theories of the interaction of light and chemical species developed the relationship between the intensity of incident light and transmitted light, along with expressions for path length and a constant related to individual materials. The evolution of new scientific instruments capable of measuring the transmission or absorbance of light under particular conditions was advanced by Jules Dubosq. However, Pliny described a colorimetric method that may be the first analytical test ever performed. Nierenstesein explains the historical chemistry of gallnut dyes and states that in 1751, Johann Teodor Eller (1689—1760) used a gallnut dye preparation to determine that blood contained iron (Nierenstein, 1931).

In effect, microscopy and colorimetry methods enhance the human eye's abilities. We could not distinguish the hairs upon a flea's leg until magnifying glasses were fashioned

into microscopes. We always could see color, but we could not see the atomic or molecular basis for color—the absorbance and transmission of wavelengths by concentrations of molecules. For semi-quantitative and quantitative comparisons, the development of colorimetry and spectroscopy was required. In the case of microscopy and colorimetry, refinement resulted in knowledge about details of our world that were beyond our eyes' ability to perceive.

2.5 A Visit to the Physician[6]

Those of us who visit a physician regularly answer various questions about our health (Frankel, 1999; Danielle Ofri, 2018). The visit may start with a questionnaire provided by office staff. The patient fills out personal information and is asked about their current health. Sometimes, the questionnaires ask for information about health issues within their family, which may point to genetic conditions the physician should consider. The patient then meets with a nurse or physician's assistant, who determines the patient's height and weight. Suppose the patient has been taking medications, vitamins, or supplements, prescribed or not, by the physician we are visiting, other physicians, specialists, or on our own. The assistant asks the patient to list the medicines. We may be questioned about our use of alcohol, tobacco products, and other "recreational" drugs. The interactions become less verbal after these initial determinations. Our pulse, body temperature, and blood pressure are assessed by touching the wrist, introducing a thermometer into our mouth or ear canal, and using a sphygmomanometer and stethoscope; newer models work simultaneously as pressure cuff and stethoscope.

[6] This section describes a typical visit to a physician in a group practice in the United States of America. The nature of a visit to a personal physician for an annual examination may vary by country.

The assistant may ask the patient to disrobe. The nurse, physician's assistant, or physician leaves the room to let the patient disrobe privately. The patient waits for the physician, who typically knocks before entering. The physician has reviewed the information provided by his assistant, who may be in attendance. Hopefully, they have placed new information in the context of the information provided from earlier visits and clinical chemistry and imaging results. The physician will check heart and lung sounds, touch and tap parts of the abdomen for tenderness, and hear sounds that provide helpful information regarding the patient's status. The physician may check reflexes, neurological function through eye movements, and oral cavity and ear canal health. Whether the visit is for a specific patient complaint or the latest in a pattern of health check-ups, the physician will ask questions to elicit information not covered explicitly in the documentation gathered so far. If warranted, the physician may request follow-up tests. The collection of blood and urine samples can be acquired or scheduled for the following morning; many tests require a twelve-hour fast before sampling. These samples will undergo chemical analysis using various types of clinical chemistry methods. Various specialized tests may be scheduled at sites that perform X-rays, colonoscopies, eye exams, hearing tests, or other tests. The primary physician may refer the patient to another physician if they believe a medical specialty is appropriate.

Depending on healthcare practices in individual countries, the physician’s office or a clinical laboratory may have a phlebotomist on staff who places a tourniquet on the patient’s upper arm, punctures the patient’s skin with a small-bore needle, and angles it into a vein, hopefully without causing discomfort. Blood flows into one or more collection tubes, where one or more sample stabilizing ingredients wait to be mixed with the blood. The types of tubes used are part of the specification for the blood biomarker analysis requested by the physician. Sometimes, the blood sample must be tested as whole blood to

obtain the relevant information. Sometimes, it is transformed into plasma—whole blood minus red blood cells (erythrocytes), leukocytes (white blood cells), and thrombocytes (platelets). The plasma collection tubes include an anticoagulant to remove blood cells without a clot forming. Sometimes, the whole blood is transformed into serum—whole blood from which the blood clot and clotting factors, such as fibrinogen and prothrombin, have been removed by centrifugation. The serum collection tubes include a clotting activator so that a clot will form relatively quickly. These are more useful for the conduct of some quantitative tests.

The urine may go through similar processing steps. In some circumstances, a urine preservative is included in the collection container. Collection tubes and containers for blood, blood plasma, serum, and urine must all be sterile and free of microbes and other contaminants, such as plasticizers.

Other samples might be collected, but it is sufficient to focus on blood and urine testing. Some tests may be performed in the office, while others are sent to a clinical laboratory. These clinical laboratories are called reference, central, or clinical pathology labs. Typically, clinical laboratories may do far more than analyze blood samples from physicians' patients (e.g., tissue samples, microbiological samples, and samples collected from clinical trial participants). However, the focus of this thesis will remain on blood sample testing.

Whether the testing is performed at the physician's office or is sent out for testing, the instruments and reagents used for tests are complicated pieces of engineering that perform various types of chemical analysis in a "hands-off" manner—samples are introduced at one end of the instrument, data and waste products are the products of the process. The instruments use various kinds of fluidics, robotics, optics, electronics, and best-in-class

materials to render blood products or urine into numbers to help assess the patient's health during testing. The test values are made possible by various scientific developments involving buffer chemistry, immunochemistry, electrochemistry, dye chemistry, chemical reactions, and spectroscopy, all with histories related to their development.

The test results seem straightforward—a gravimetric or activity-related numerator value paired with a gravimetric or volumetric denominator within a range of values (the reference interval) for that test previously determined as expected for a healthy population. Even the determination of a healthy population is a complicated proposition, as it requires the collection of samples from subjects selected based on some combination of inclusion and exclusion criteria, much as is the case with clinical trial design and conduct.

When the results are reviewed by the central laboratory and released to the physician and patient, a review of their meaning to the patient should occur. The meaning of the tests should be shared with the patient, especially if the patient is unsophisticated. Sometimes, the results affirm that there are no abnormalities in your test results and that the cause of your complaint needs to be clarified by other means. Sometimes, the test results may indicate a problem, and the physician can adjust your treatment plan and prognosis. Sometimes, the test results may be faulty due to problems with the sample collected, sample storage and shipment, sample testing, or sample reporting (Houben et al., 2010). Problems of this type occur in many cases; samples should be drawn fresh from the patient, and these samples should be tested to ensure meaningful results. In all cases, it is beneficial for the patient to obtain a current set of clinical chemistry results. If future results show a change from the initial results, the initial results provide a baseline assessment of what "normal" looked like for the patient at the previous sampling time.

2.6 The Evolution of Quantitative Diagnostic Technologies

The idea that success has many parents is attributed to Count Ciano, a son-in-law of Mussolini (*Count Galeazzo Ciano 1903–44*, 2016). At the time, Ciano did not know that failure would visit Mussolini soon enough or that he would be put to death due to his vote to depose *Il Duce*. The general idea applies to many developments in science and technology. It is undoubtedly true of quantitative diagnostic medicine, where an immunological test depends on theories of immune response that developed over the centuries or where mass spectrometric confirmation of an endogenous compound relies on developments in physics and chemistry spanning most of the 20th Century.

These notions, along with increasingly specific, less speculative discoveries in the minds and laboratories of Louis Pasteur (1822—1895), Claude Bernard (1813—1878), Elie Metchnikoff (1845—1916), Robert Koch (1843—1910), Paul Ehrlich (1854—1915), and Emil von Behring (1854—1917) developed into theories of the immune response and immunology. An increased understanding of the immune system led to the development of immunochemical analysis methods. The work of Michael Faraday (1791—1867), James Clark Maxwell (1831—1879), William Crookes (1832—1919), J. J. Thomson (1856—1940), and Ernest Rutherford (1871—1937), among many others, laid the foundation for mass spectrometric identification of organic and inorganic substances. So, quantitative diagnostic technologies are based on technologies that were developed for whatever initial reason and became helpful in assisting the diagnosis of health or illness. Immunochemical and electrochemical methods could quantify the amount of a specific protein or ion present in blood, serum, or plasma; the value could be compared to values obtained by quantitating the protein from other patients. Mass spectrometric methods might assist a physician in determining whether a patient had too much of a potentially lethal substance in their system that was making them ill.

These advances—technological catalysts to a better understanding of human health—were aided immeasurably by the development of optical theory, improved glasses, lenses, and microscopes, and the development of technologies for measuring the amount of light absorbed. Technologies that measured the absorbance and transmission of light also required a theory of light that recognized frequencies and wavelengths. An understanding of optics, electromagnetism, chemistry, and the construction of technologies based on new theories enabled advanced diagnostic technologies unimaginable before the 19th and 20th centuries.

The development of methods for measuring endogenous and exogenous substances also relied on improved optical systems. Optical systems extend the functionality of the human eye beyond what nature has provided. The microscope has served to extend the powers of the human eye, which resolves objects at 0.1 millimeters (mm) to about 0.0002 mm (0.2 micrometers (μm)) with the best light microscopes using an oil immersion objective lens (Murphy & Davidson, 2012, p. 22). Electron microscopes extend resolution down to 0.1 nanometers (nm) (0.0000000001 mm)—resolution at the single atom scale (Murphy & Davidson, 2012, p. 24). Whereas the range of human vision is limited to objects that emit or transmit light at wavelengths between about 750 to 380 nm, optical systems can detect wavelengths in the infrared and ultraviolet spectral ranges, along with radio and microwaves in the long wavelengths, x- and γ-rays in ever-shorter wavelengths. Optical systems can detect the range of electromagnetic radiation with greater precision and accuracy than the human eye could ever achieve. Optical systems are designed to provide objective information unhindered by the limits of retinal and opsins—molecules that enable sight—along with the cone and rod cells embedded in the back wall of the eye.

Although each manufactured optical system has biases like our eyes, the various materials and electronics that make these tools possible can be calibrated to overcome their limits (Webster & Eren, 2014, pp. 46-51). Data sets gathered by these tools also can be mathematically adjusted to minimize the importance of bias. Once an artificial optical system is developed, the absorbance and transmission of light within key ranges of wavelengths can be measured.

2.7 Human Physiology and Quantitative Diagnostic Technologies

While materials, methods, and technologies have changed profoundly since the earliest diagnostic technologies, definitions of human health and illness have also changed. The diagnostics practiced by healers, shamans, priests, alchemists, magicians, Aesculapians, Hippocratics, Galenists, Islamists, apothecaries, herbalists, ayurvedic and traditional Chinese practitioners, barbers, surgeons, physicks, physicians, all had one significant strength when practiced well—the “healer” paid attention to the patient in a manner that is sometimes difficult in our era of brief office visits (Chen, 2009, pp. 1866-72)[7]. Archaic medical practices were eventually replaced by an era's "modern" medicine, although traditional medicine is still the primary healthcare practice in many world areas. Placing the ear against the thorax was replaced by the stethoscope. Observing the color of urine or blood was replaced by microscopic examination and immunochemical methods. Relying on the patient's family history and recitation of woes, while remaining essential, has been supplemented by the objective facts of clinical chemistry test results, whether the tests are pertinent or not.

[7] There is virtually no reliable epidemiological data regarding the safety and efficacy of ancient medical practices.

Each passing year teaches us that while we may know more than we did in the previous year, much remains unknown. Medical scientists are caught in a real-world instance of Zeno's Paradox, where we are always halfway closer to knowing everything there is to know about human health, only to have that remaining body of knowledge slide away from us as new complexities arise.

2.8 Current Limitations to Quantitative Diagnostic Medicine

An emerging research area focuses on species microbiomes—the complement of microbial life humans, other creatures, and plants—that cluster inside us. Since Antoni van Leeuwenhoek's examination of matter dislodged from his teeth or collected from pond water in the 1670s, we have known that odd and previously inconceivable creatures lived amongst us (Dobell, 1932). However, it took many other scientists many years of formal training and improved technologies to learn how pervasive these "animalcules" were.

Scientists in the 19th and early 20th centuries began to understand that mitochondria, chloroplasts, and other organelles were the result of symbiotic relationships between microbes and other life forms, an idea extended by Lynn Margulis (Sapp, 1994; Gray, 2017), other biologists studying horizontal gene transfer between viruses and eukaryotes (Irwin et al., 2021), and more generally among microbial populations (Haudiquet et al., 2022). More broadly, American historian William McNeill has written about the interplay between "parasites" and populations of people during the evolution of our species (McNeill, 1998). Some microscopic creatures came to be understood as entirely responsible for the worst of our afflictions. In contrast, others enable our health in surprising ways that we do not fully understand (Aggarwal et al., 2022). Our health has always been a negotiation between resident parasites—a term that previously implied some impairment and now includes viruses, archaea, bacteria, fungi, and protozoans—and our human cells,

each with their unique complement of genetic material. An additional 3.8 x 10^{13} bacterial cells are estimated to be packed in among our cells (Sender, 2016), along with another ten times that many viruses. No reliable estimate of our Archaean, fungal, or protozoan complement is known. These "parasites" are part of our daily functioning reality, as they were to our hominin and hominid ancestors. While we have come to understand their presence, we do not understand how and why they work with and against us to facilitate health and illness (Mousa et al., 2022).

While an initial pass at sequencing the human genome was completed in the early 21st Century, many secrets remain among its approximately three billion base pairs (Bentley, 2000). Long sections do not seem to be used as templates or codes for any transcription or replication, which may be vestigial accumulations of sequences that once were important in human evolution but do not serve a function anymore. The order in which various enzymes read genome sections may conceal significant mysteries. The recent completion of a telomere-to-telomere (T2T) sequence of the human genome has opened fresh opportunities for an increased understanding of genome structure and function and the epigenome associated with it (Gershman et al., 2022; Nurk et al., 2022). This T2T sequence represents 225 million base pairs added to the previous model. In brief—and quoting from Gershman:

The epigenome refers to D.N.A. modifications (e.g., CpG methylation), protein-DNA interactions, histone modifications, and chromatin organization that collectively influence gene expression, genome regulation, and genome stability(Gershman et al., 2022).

Each of these epigenomic categories—and others that may exist beyond these, for instance, non-CpG methylation—represents a field of continued study unto itself and indicates how much remains to be understood about our genetic code (Arand et al., 2012).

The current era has also shown an explosion of increasingly sensitive and specific technologies and methods for the quantitative determination of biomarkers (various surrogate indicators of health that include metal and organic ions, small molecules such as ureas, creatinine, and steroids, peptides, proteins, and nucleic acids), metabolites (metabolomics), proteins (proteomics), nucleotides, cell types (cellomics), intra- and intercellular signaling molecules, viruses, bacteria, fungi, protozoans, all with their by-products, and virtually any other plausible indicator of health or incipient illness and disease. It is common to open medical and chemical journals dedicated to various specialties and find a new set of biomarkers, methodologies, assays, antibodies, detector modalities, and data analysis methods touted as the latest and best way of determining the concentration of a biomarker and the progression of an illness. The biomarkers either indicate that the health status of the subject remains within normal parameters for their age, gender, and sometimes race, or it indicates that one or more biomarkers are indicating a deviation from health.

There are a few problems with this approach. When a biomarker deviates from its reference interval or range by approaching the upper or lower limits, the subject may have already entered a state of illness or disease. Rescuing patients becomes more difficult once their biomarkers trend beyond the reference interval. This suggests that the biomarkers serve as a late indicator of disease progress rather than an early warning system. Additionally, core biomarkers in clinical chemistry are assessed during virtually any clinical check-up. More exotic biomarkers are only assessed if one or more symptoms manifest among the core assessments. Some tests are based on evolving medical knowledge or through innovations in reagents or technologies that allow the detection of previously undetectable biomarkers. New tests must be communicated in reputable journals, validated in new laboratories, and perceived as reliable (i.e., accurate, precise, reproducible, and

replicable) and helpful. Some tests are so new that they may be unknown to typical general practitioners or specialists in related fields. Insurers may vary on whether they will reimburse a patient for some tests. Some fields of medical inquiry, such as inquiries into the interplay between the human microbiome and our health, are so new that methods of testing the interactions still need to be studied and understood.

Researchers have recently claimed that they have discovered new structures that could be characterized as new organs or extensions of previously known structures. For instance, in 2018, researchers identified a human tissue system they named the interstitium, composed of what was previously considered connective tissue with various cell types and fluid conduits permeating the body; in their estimation, it became the largest of our human organs (Benias et al., 2018). While others dispute the novelty of the claim (Neumann, 2018), it is remarkable that a system as pervasive as the interstitium has been marginalized by many. Neumann suggests it has been noticed and claims that Benias and journalists are engaged in hyperbole.

More recently, an unnoticed system of “bilateral macroscopic salivary gland locations in the human nasopharynx” or tubarial salivary glands was reported (Valstar et al., 2021). Discovered by an imaging methodology named molecular imaging modality of positron emission tomography (P.E.T.)/computed tomography with radio-labeled ligands, this is a complicated type of quantitative diagnostic that depends on firing anti-electrons—positrons—into human flesh pretreated with radioactively labeled chemicals which are then "seen" or registered by photoelectric sensors and scintillation crystals once positrons have impacted them. The technique also depends on increasingly sophisticated data capture and analysis methods that produce a multi-dimensional map of the imaged area. P.E.T. can be paired with time-of-flight (T.O.F.) and magnetic resonance imaging (M.R.I.) techniques to

build more thorough maps of targeted areas of the body (M. S. Lee et al., 2021). In this case, the use of emerging technologies to visualize the structure has been commended, and the novelty of the discovery is being debated, complete with references to 19th-century anatomy literature (Mudry & Jackler, 2021).

Whether increased knowledge of our microbiome and genetics will yield significant improvements in healthcare remains to be seen. Even now, there have been new insights. Knowledge of the role of mRNA in human biology and the impact it could have on the *in situ* synthesis of antibodies to the SARS-CoV-2 virus has empowered new mRNA applications (Gote et al., 2023). Small interfering R.N.A. has been added to a list of emerging techniques for silencing genetic sequences (Alshaer et al., 2021; Khvorova, 2023). Knowledge of clustered regularly interspaced short palindromic repeats (CRISPR)-Cas-9 (CRISPR-associated protein 9) sequences found in the genomes of prokaryotic organisms such as bacteria and archaea have been used in various forms to edit human genetic sequences. However, editing genetic sequences requires caution (Horodecka & Düchler, 2021). To rephrase, scientists have learned a lot between prehistory and the present about what keeps us healthy and what makes us ill (Lange et al., 2022; Lorenzo et al., 2022; Spencer & Fullerton, 2022).

CHAPTER 3 | Auenbrugger and Laennec: Qualitative and Quantitative Diagnostic Methods

René Théophile Laennec (1781—1826) invented the stethoscope, which enhanced what we can hear within the human body. Auscultation—listening to bodily sounds—can be considered a simple methodology, but it represents an innovation in qualitative diagnostics. While auscultation does not detect anything as nuanced as changes in the amount of a biomarker within a patient's blood or tissues, the experienced physician may detect changes in patient status without needing a blood sample, complex imaging methods, or surgery.

Humans (*Homo sapiens*) and our ancestors (hominins and hominids) must have done an adequate job of caring for each other's health for hundreds—or thousands—of millennia before anyone called themselves a physician. If we had not cared for each other, we would have succumbed as a species to any one of the numerous health challenges we faced as we spread across the globe. Instead, some of our ancestors died, while others fought off threats, resisted illness, and survived. Some of us survived due to traits of our species—our ability to adapt to various environments. Some of us learned how to differentiate illness and health, support our kind's health, and treat those of us who became ill. Our species played a numbers game against all other species inimical to our health. More of us needed to survive gestation, birth, maturation, and quotidian threats than died. More of us had to survive and produce surviving children than died, whatever the cause.

The survival challenge must have involved creating a body of knowledge relevant to our location on the planet. What plants, animals, minerals, animal products, and waters supported health and banished illness, and what materials harmed us? As species of plants and animals, and even the populations of unknown species hidden in the water, varied by locale, the knowledge must have required updating as we traveled. In addition to these bodies of knowledge, there would have been a growing sense of how our bodies function when healthy and ill. This knowledge must have included heart, pulse, and respiratory rate

assessments. In the history of human health assessments, initial health checks were probably performed using qualitative rather than quantitative endpoints. The pulse or heartbeat was fast or slow or typical, rather than a specific number of pulses per minute. Breaths were evaluated as long and deep, short, slow, rapid, or shallow, with unusual or typical sounds, rather than quantified.

However, these are speculations, although backed with simple logic. We will never know when caring for each other started or who the first person was to administer care among our species. We will never know, much as we will never know the exact date, year, century, or millennia in which our species distinguished itself from our predecessors. Who was the first person to notice the heartbeat and pulse? Who first noticed they varied in speed—fast after exertion and slow upon waking? Who noticed that breathing sounds also varied or that skin color and temperature varied with relative health? Who first noticed that beating on the sternum produced different sounds than beating on various ribs or abdominal zones? We will never know who these people were, but they must have existed.

Thoracic and abdominal percussion seem to be such simple methods. Anyone who has struck their chest in various places would notice different tones. We have seen Tarzan and his friendly apes doing this in movies (if we are of a certain age) or Bobby McFerrin using body percussion, a practice that may be as old as humanity. Josef Leopold Auenbrugger (1722—1809) was the first to formally study the different sounds and their implications—how the sounds correlated with health or illness in the underlying structures—and write down his findings. He started his research at the Spanish Military Hospital in Vienna, Austria, about seven years before he published his work in 1761. The result was his monograph, *A New Discovery from the Percussion of the Human Thorax* (*Inventum Novum ex Percussione Thoracis Humani)* (Auenbrugger, 1761).

Auenbrugger was born in Graz, Austria, the son of a wealthy innkeeper (Borghi, 2018). His father kept barrels of fermented beverages in the inn's cellar. In his Leopold Auenbrugger biography, Max Neuburger speculated that Auenbrugger would watch his father tapping on the barrels to determine their fullness (Neuburger, 1909). Others have repeated the legend. Did this lead to Auenbrugger's thoracic percussion technique? Auenbrugger is cited as an accomplished musician who wrote a libretto for Antonio Salieri. It may be that his ear for music helped him distinguish between nuances in the sounds elicited from his tapping on the human body (Jarcho, 1961). However, Auenbrugger does not mention tapping beer casks in his father's inn as the method's origin. Auenbrugger compares it to "the stifled sound of a drum covered with a thick woolen cloth or other envelope" (Willius & Keys, 1941, p. 195). Further on, he states:

These varying results depend on the greater or lesser diminution of the volume of air usually contained in the thorax (lung). The cause which occasions this diminution, whether solid or liquid, produces analogous results to those obtained by striking a cask, for example, in different degrees of emptiness or fulness, the diminution of sound being proportioned to the diminution of the volume of air contained in it (Willius & Keys, 1941, p. 198).

Auenbrugger does not explicitly state the source of his experience with casks. It is unclear who started the legend of Leopold and his father's beverage containers or whether the legend has any basis.

What is known with certainty is that Auenbrugger's method generally was ignored by his fellow physicians in Vienna, except by Maximillian Stoll (1742—1787), a physician who succeeded Anton De Haen (1704—1776) as head of the University of Vienna medical clinic and Albrecht von Haller (1708—1777). This Swiss biologist was later considered the father of experimental pathology (Cummins, 1945). Both recommended Auenbrugger's

technique to others. Roziere de la Chassagne, a physician based in Montpellier, translated Auenbrugger's work into French in 1770, only nine years after its initial publication. He gives the Auenbrugger method the faintest recommendation:

Having learned that a German physician had published a new method for showing the existence and seats of diseases of the chest by percussing that cavity, I procured the work, a translation of which appears at the end of this volume. But no one should imagine that I give full confidence to the author's doctrine; it seems to be another method we can use without risk. ... I have not tested it, and there are not many hospital physicians who are able to make such a trial. I consider myself happy if the public approves of my zeal, still more so, to be the first to announce a useful discovery to physicians in my own country (Dock, 1935, p. 445).

Still, Roziere's mention of Auenbrugger's work appeared around the beginning of Jean-Nicolas Corvisart's (1755—1821) medical training. Chest percussion went unmentioned by Corvisart's professors in medical school. Corvisart added commentaries on Auenbrugger's original observations while accumulating twenty years of experience teaching the method to numerous pupils using living and patients and cadavers. In his review of Roziere's and Corvisart's contributions to Auenbrugger's work, Dock found Corvisart's commentaries insightful. Dock also judged it unusual that Auenbrugger, who stopped working at the Spanish Military Hospital one year after the publication of *Inventum Novum* and despite living forty-eight years after its publication, did not attempt to increase interest in chest percussion among physicians (Dock, 1935).

However, Auenbrugger and Laennec were linked through Corvisart's twenty-year interest in chest percussion and translation with commentary of *Inventum Novum*. Corvisart became one of Laennec's professors in medical school.

The practice of listening to body sounds must predate history. It took millennia until Laennec recognized that placing one end of a tube to the ear and the other against the chest provided more nuanced information about the heartbeat and other internal utterings than placing the ear directly against the chest. Laennec had several names for his device before settling on "stethoscope" (Donoso & Arriagada, 2020). The historical record says that René-Théophile-Hyacinthe Laennec in 1817 was the first person to use such a tube for auscultation (Hanna & Silverman, 2002), the use of which he described in detail in his work *De l'Auscultation Médiate ou Traité du Diagnostic des Maladies des Poumons et du Coeur* (R.-T.-H. Laennec, 1819). Laennec cited as a basis for his invention the use of wood as an amplifier for sounds:

… I happened to recollect a simple and well-known fact in acoustics, and fancied, at the same time, that it might be turned to some use on the present occasion. The fact I allude to is the augmented impression of sound when conveyed through certain solid bodies, —as when we hear the scratch of a pin at one end of a piece of wood, on applying our ear to the other. Immediately, on this suggestion, I rolled a quire of paper into a sort of cylinder and applied one end of it to the region of the heart and the other to my ear, and was not a little surprised and pleased, to find that I could thereby perceive the action of the heart in a manner much more clear and distinct than I had ever been able to do by the immediate application of the ear. From this moment I imagined that the circumstance might furnish means for enabling us to ascertain the character, not only of the action of the heart, but of every species of sound produced by the motion of all the thoracic viscera. With this conviction, I forthwith commenced at the Hospital Necker a series of observations, which has been continued to the present time. The result has been, that I have been enabled to discover a set of new signs of diseases of the chest, for the most part certain, simple, and prominent, and calculated, perhaps, to render the diagnosis of the diseases of the lungs,

heart and pleura, as decided and circumstantial, as the indications furnished to the surgeon by the introduction of the finger or sound, in the complaints wherein these are used (R. T. H. Laennec & Forbes, 1821).

Laennec tells us that it is a property of acoustics in wood that allows mechanical energy introduced at one end of a piece of wood to be amplified along its length rather than dampened. This property varies with the wood type and size and whether the frequency introduced into the wood resonates. This recollection led him to create a paper tube from a *cahier* or notebook, translated into English as "quire" by John Forbes, and hold it to someone's chest. His first subject might have been someone known to him rather than a new patient, who might have found Laennec's actions perplexing. Laennec said that immediate auscultation—placing the ear directly to the body—was confounded by the obesity of one patient, as their fatty tissues made hearing heart and lung sounds difficult. Then again, one stated motivation for Laennec's experiment was that placing his ear against the chest of a female patient was too intimate. Was it an attempt at placing some distance between his patient and himself that inspired his thinking? Was it an impulse to respect his patients' space? Was it, as he said, a recollection of the acoustic properties of wood that led him to his discovery? Was it all these considerations? Several stories exist in the literature regarding how and why Laennec invented the stethoscope (*Figure 3.1.* and *Figure 3.2.*). In any event, and for whatever reason, by establishing a distance between the patient and himself, Laennec's technology enhanced the information available to diagnosticians.

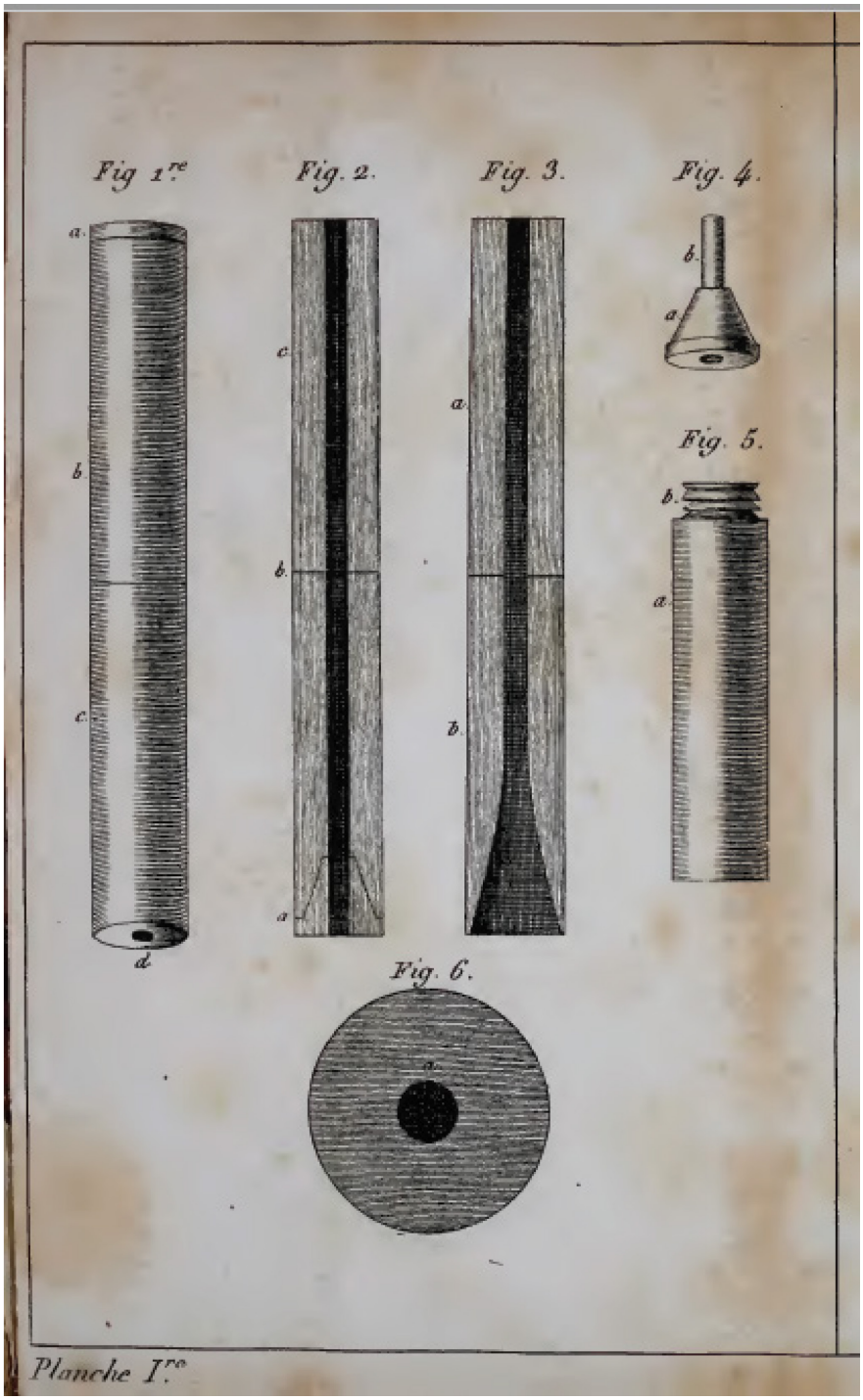

Figure 3.1. *The first drawing of a stethoscope by Rene Laennec, its inventor (1819). (Laennec, De l'Auscultation Médiate ou Traité du Diagnostic des Maladies des Poumons et du Coeur, 1819).*

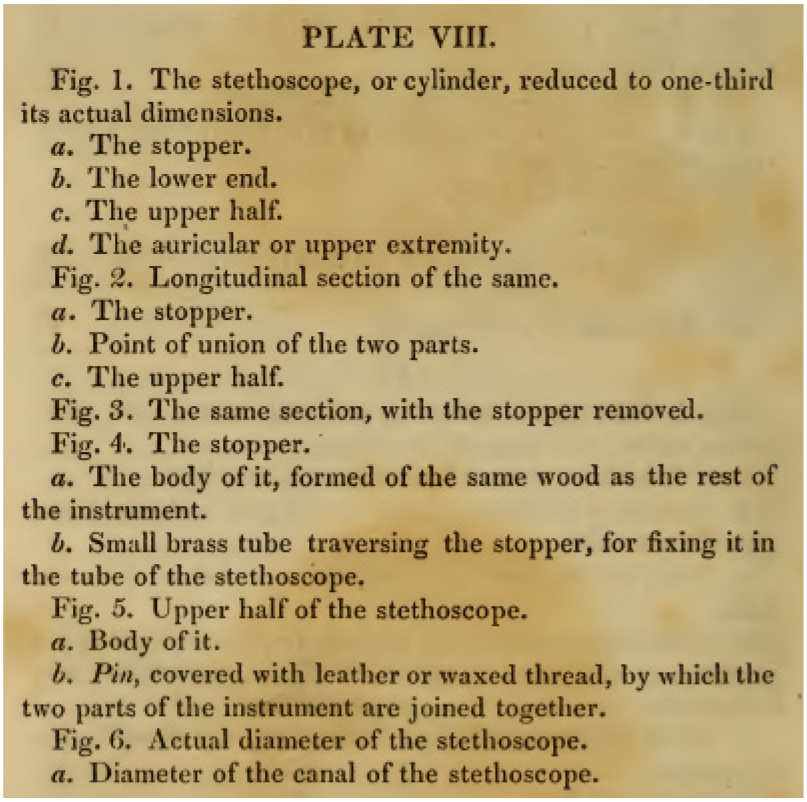

PLATE VIII.

Fig. 1. The stethoscope, or cylinder, reduced to one-third its actual dimensions.
a. The stopper.
b. The lower end.
c. The upper half.
d. The auricular or upper extremity.
Fig. 2. Longitudinal section of the same.
a. The stopper.
b. Point of union of the two parts.
c. The upper half.
Fig. 3. The same section, with the stopper removed.
Fig. 4. The stopper.
a. The body of it, formed of the same wood as the rest of the instrument.
b. Small brass tube traversing the stopper, for fixing it in the tube of the stethoscope.
Fig. 5. Upper half of the stethoscope.
a. Body of it.
b. *Pin*, covered with leather or waxed thread, by which the two parts of the instrument are joined together.
Fig. 6. Actual diameter of the stethoscope.
a. Diameter of the canal of the stethoscope.

Figure 3.2. *Translated description of the parts of the Laennec stethoscope, 1829. Some design changes occurred between the publication of Laennec's design in 1819 and the design described in the Forbes translation (R. T. H. Laennec & Forbes, 1821, p. 437*

Laennec said it was surprising that no one had combined the two ideas—listening to chest sounds and using a tube—before he did. Hippocrates had cited immediate auscultation as a method. There are confounding issues in both Auenbrugger's and Laennec's tales.

Laennec was considered an accomplished flutist. He owned a lathe and kept various wood blocks in his workshop to facilitate his hobby as a woodworker. His lathe could create flutes, and Laennec fashioned his first versions of "*Le Cylindre*" using this tool. After he published his masterwork, he would sell the book and cylinder together to physicians interested in his technique. He named it "stethoscope" once he heard what other physicians

called it instead of his preferred “the cylinder.” “Stethoscope” is from Greek words for “I see” (*skopos*) and “the chest” (*stethos*) (Donoso & Arriagada, 2020, p. e446).

The phenomenon upon which Laennec depended for his insight into auscultation was the ability of wood to conduct and amplify sound. Idiophones—percussion instruments dating back to the slit drum—depend on this property. Of course, the flute is also known to be an ancient musical instrument, but the resonance and amplification of a tone in a tube make the flute viable. Theories regarding how humans developed music suggest that it was part of how we experienced our environment, with its winds, rains, and thunder, along with the sounds of the animals that lived with us among the trees and grasses, rivers, and lakes, within and upon the ground beneath our feet. Maybe it was a species of woodpecker that implied that trees could become drums or that we could clap our hands or beat our chests and create a shared sound that suggested other percussion instruments. Maybe other birds suggested that the human voice, then early flutes, could be used to imitate their astonishing songs, or maybe our voices suggested melody as we varied tone to express meaning and emotion. In any event, our capacity for music, our interest in the sounds that surround us, and our ever-increasing familiarity with the materials that make up the world in which we live all resulted quite indirectly in both Auenbrugger’s and Laennec’s series of insights, techniques, and inventions. Although there are no references to the connection, it may be that Auenbrugger’s percussive technique, along with any informally communicated backstory that he learned his technique on barrels, led to Laennec’s insight that wood transmitted and amplified sound.

A stunning amount of knowledge had to accumulate regarding material properties, technologies, and sciences before the stethoscope became such an omnipresent medical tool. These included, at least partially, properties of wood (e.g., deal pine, boxwood, cedar,

ebony, cherry), woodworking and various woodworking tools, the invention of the lathe, the invention of woodwind instruments, the discovery of natural rubber and *gutta-percha*, vulcanization, the invention of Ebonite, the discovery of metalwork and alloys, the creation of pewter, brass, steel, stainless steel, and aluminum, and the invention of plastics.

Between humankind's discovery of naturally occurring tubes, e.g., bamboo, the discovery that wood conducts and amplifies sounds, and Laennec's lathe work creating relatively simple cylinders, millennia and many heartbeats came and went. However, once Laennec's cylinder arrived, the modifications to his idea branched out like a tree of life. Over time, new designs would introduce functional improvements to his monaural cylinder using various other materials, some of which were created after the initial cylinder was used. Ebonite, a hard rubber product invented by Charles Goodyear, latex rubber, lead, then steel, pieces that facilitated the invention of the binaural stethoscope allowed further convenience to the physician and privacy for the patient. An inflexible tube might have implied to users that a flexible tube could provide improved ease of use. Using latex rubber for surgical gloves in the 19th C. (Ownby, 2002) might have inspired the use of flexible tubing. What thought processes were required to transform an idea based on a rigid material into working with a flexible material such as rubber? It may have been a motivation as simple as portability. Carrying a rigid cylinder around for long shifts could not have been ideal. A tube pressed to the auricle might have suggested that earpieces resting in the external acoustic meatus would be an improvement. Coiled springs covered in woven silk were used in some models designed by C.J.B. Williams (1845) and others. Ivory and horn were used as chest pieces for other types, but these had to be harvested from one of the creatures so endowed, their properties understood and formed into useful bits. In 1894, Dr. Robert Bowles patented adding a plastic diaphragm to existing stethoscope designs, although other physicians had mentioned the possible utility of membranes or

"microphones" from the mid-19th century (Bishop, 1980, p. 454). Today, the flexible binaural stethoscope is as common a representation of the healthcare worker as the caduceus.

Aside from revealing nuances of the heartbeat, lung sounds, and other previously unappreciated internal mutterings, the stethoscope is an excellent example in the complicated history of diagnostics. Of course, the number of heartbeats, breaths, and abnormalities in either are powerful quantitative diagnostic techniques further enhanced by the invention of the electrocardiogram (AlGhatrif, 2012) and many biomarker tests. They are based on the mechanical properties of sound rather than the physicochemical properties elicited when electromagnetic radiation interacts with materials and molecules.

The primary focus of this work is the evolution of quantitative tools that allow the quantification of endogenous chemical species that serve as markers of normal and abnormal physiological functions. The evolution of materials, technologies, techniques, and sciences that have facilitated quantitative diagnostics is at least as long and nonlinear as that of the stethoscope. There is little overlap between the methods used to query our internal chemical processes and those used for auscultation. However, the general focus is the same: know as much as possible about human health in as minimally invasive a way as possible.

CHAPTER 4 | The Roots of Modern Clinical Chemistry

In the last third of the seventeenth century, two remarkable natural philosophers worked at teasing apart the fabric of the universe. One is hailed as the natural philosopher who defined the maturation of mathematics and physics; the other was a diligent observer of previously unseen structures in nature. Both were focused on phenomena that would lead, in distinct ways, to revolutions in our perceptions of life. Both brought new understanding and applications to the ancient art and science of optics. Their work would eventually provide a foundation for discoveries in chemistry, biology, and medicine that would change the nature of human healthcare. However, Antoni van Leeuwenhoek (1632-1723) and Isaac Newton (1643-1727), born eleven years apart, could not have been more different. Neither could have understood how far-reaching their work would be, although both were determined to observe whatever their sense-based, intellectual, and physical tools allowed.

They had at least one person in common: the secretary of the Royal Society, Henry Oldenburg (~1619—1677), who received Newton's 1672 letter (Newton, 1672, pp. 3075-87), as well as Leeuwenhoek's first letter written on 28 April 1673 (Leeuwenhoek, 1939, p. 31). Leeuwenhoek's original letter has been lost, but its revelatory contents were published in an extract in May of that year (Leeuwenhoek, 1673). In this first letter, written at roughly forty years of age, Leeuwenhoek added new details to microscopic observations made in *Micrographia* by Robert Hooke (Hooke, 1665). Newton and Leeuwenhoek were also innovators in the use of optical glass, as well as implacable observers, in distinct ways, of nature. However, Newton innovated as a theoretician. Leeuwenhoek's innovations were as an obsessive sample collector, observer, and craftsman of tiny single-lens microscopes, along with sample holders, vessels, and other apparatus that facilitated his observations.

Newton's early innovations involved using a prism to dissect white light into the visible spectrum and then recombine the spectrum into white light. He designed and constructed a

reflecting telescope, which he sent to The Royal Society for their inspection. Newton's work with prisms laid the foundation for using portions of the spectrum—colors we think of as red through violet—as a probe of solutions and materials in analytical methods such as spectroscopy and colorimetry. Leeuwenhoek, empowered by the lenses he created, could observe and provide relative measurements for objects that were far smaller than those previously seen by humans. His observations of bacteria, protozoans, spermatozoa, and red blood cells opened possibilities for using microscopes in many areas of science. While links between microbes and disease had been and continued to be suggested until the matter was resolved in the 19th Century, no unequivocal link was established by Leeuwenhoek or others at the time (L. A. Robertson, 2022). Advances in the understanding of light and "animalcules" would eventually lead to the creation of measurement technologies that would advance how human health was understood. Along with other antecedents, several mid-nineteenth-century scientists and physicians would eventually create an instrumental bridge between counting objects and determining the concentration of active moieties.

It is also interesting that Newton and Leeuwenhoek induced a passing fascination with prisms and microscopes among less serious naturalists who wanted to see what had been described and yet were either too impatient, unskilled, or careless to observe prismatic spectra or "animalcules." Although Dr. Patricia Fara addresses only aspects of Newton's insights into the use of a prism to conduct studies into the nature of light, what she says applies to those who attempted to use Leeuwenhoek's lenses as well:

In retrospect, Newton's experiments seem to expose natural reality in a delightfully clear and incontrovertible way, but they proved extremely hard for his readers to replicate. Achieving his results demanded a sophisticated grasp of glass technology and a delicate and patient experimental hand (Fara, 2015, p. 3).

…by concealing vital details, such as the type of glass or shape of the prisms, Newton renders replication extremely difficult (Fara, 2015, p. 5).

As in Fara's description of Newton's prism experiments, Leeuwenhoek was evasive about how he produced his tiny single-lens microscopes. However, he shared details of his many lenses and sample holders, even sending examples of these to the Royal Society. He left instructions with his daughter to send some of the lenses after his death to Sir Isaac Newton, president of the Royal Society at the time. Despite their shared interests in using glass to achieve new understandings of nature, neither mentioned the other in known letters. However, Newton annotated his copy of *Micrographia,* and Leeuwenhoek, although unschooled in the English language, commented on Hooke's observations of book mold in his first letter; Newton's interest in *Micrographia* was probably more related to optics than to objects Hooke had documented. As the Society's president, Newton must have been aware of Leeuwenhoek's existence, if not the details of his observations. They shared an interest in Hooke's work, but for Newton, the interests in microscopy and social engagement were passing matters. If his critics could not keep up with him, that was their problem to resolve, not his. Leeuwenhoek spent his life engaged in commerce as a merchant, burgher, *wijnroeier*, host to curious scientists, notables, and the occasional regent. He added to and corrected his observations until he died in 1723. Leeuwenhoek sometimes wrote in letters that he wished his curious visitors would leave him to his observations. Still, if they were sufficiently important, he shared his findings with them, hoping that doing so would improve the acceptance of his observations.

By some accounts, Newton's letter received undue credit for teasing apart white light into an initial five colors (red, yellow, green, blue, purple), which he later amended to seven colors so that they could be understood as analogous to the notes on a diatonic musical

scale (Fara, 2015, p. 6). Analysis of sunlight and its color components had several precedents. These included writings by Athanasius Kircher (1602—1680) in 1646, Jan Marek Marci (1595—1667) in 1648, Robert Boyle (162—1691) in 1664, and Francesco Grimaldi (1618—1663) in 1665, with Marci, a physician at the University of Prague, describing the colors and that each color corresponded to a specific refraction angle 0(Burns, 1987; Ure, 1986). It might have been that Boyle's 1664 publication of *Experiments and Considerations Touching Colours* influenced Newton's experiments and ruminations on the nature of color (Boyle, 1664). Boyle cited Kircher's work, which predated Marci's publication (Burns, 1987, p. 3). Newton may have been aware of the other works, or at least he might have been aware of Boyle's publication about eight years before, but he cited none in his 1672 publication. He mentioned Boyle many times in his letters and was known to have corresponded with him, but many of those letters were lost by a printer. Newton's opinion of Boyle's work seems respectful. Boyle does not seem to have challenged Newton regarding his light theory.

On the other hand, Newton and Robert Hooke became embroiled in a bitter argument over several points of optical science when Newton's first letter to the Royal Society was read and published in 1672. It is known that Hooke's *Micrographia* was in part responsible for Newton's refutation of Hooke's ideas. Hooke forcefully criticized Newton's findings. Newton responded four months later in a letter characterized as "viciously insulting" (Purrington, 2009, pp. 135-7).

Nonetheless, Newton's work with the prism and his insights into the properties of sunlight and the heterogenous, spectral properties of white light became critical milestones in the wide-ranging work for which he became famous. His insights also opened a way of thinking about light that made it possible for future scientists to create better lenses,

establish the dual nature of light as both particular and wave-like, and create an understanding that light was not just a spectrum of seven colors but a continuous spectrum of wavelengths that could be resolved into frequencies and amounts of energy as well.

Between Leeuwenhoek and Newton, new ways of viewing natural phenomena became possible. While Newton did not invent the colorimeter and Leeuwenhoek did not indict microbes as a cause of disease, their insights may have opened the minds of other natural philosophers and physicians yet to be born.

CHAPTER 5 | Antoni van Leeuwenhoek and the Context of 17th-Century Micrometry

5.1 Introduction

Development of the microscope dating to the late 16th century and throughout the 17th century required improved lens-making and an entirely new scale for the measurement of tiny, previously invisible objects. When Antoni van Leeuwenhoek (1632—1723) started his series of microscopic investigations sometime before 1673 using the lenses he manufactured, he developed methods of comparing the "animalcules" he observed to objects that were within our ability to comprehend. His intuitive sense that this would be a critically important approach to communicating with the Royal Society and an ever-expanding list of naturalists in the European community led to simple comparisons and elegant analogies. His favorite objects for comparison were grains of sand, although he also compared the new-found objects to millet seeds, vinegar eels, lice and water-flea eyes, and various hairs and threads. Leeuwenhoek's micrometry took place in the context of accelerating interest in measurement that started as far back as the beginning of human commerce but became more formal with Pythagoras and Euclid, amongst others. The design and use of the quadrant by Ptolemy around 150 CE created a guide that eventually produced a series of 16th and 17th-century refinements, starting with Pedro Nunes and the publication of *De Crepusculis* in 1542. The paper will provide examples of the refinement of precision in micrometry and how these developments might have assisted Leeuwenhoek in his measuring observations and methods, which succeeded in defining a previously unknown world.

5.2 Leeuwenhoek's Measurement Methods

In 1932, a British biologist and protozoologist, Clifford Dobell, FRS (1886—1949), published *Antoni van Leeuwenhoek and his 'Little Animals.'* The publication year was important; it was the 300th anniversary of Leeuwenhoek's birth in Delft, Holland. He had

spent much of his spare time researching and writing the homage while conducting protist research focused on human health at the British National Institute for Medical Research. It was, as Dobell says on the title page, an “account of the Father of Protozoology and Bacteriology and his multifarious discoveries in these disciplines, collected, translated and edited from his printed works, unpublished manuscripts, and contemporary records” (Dobell, 1932).

Dobell's monograph added a historical perspective to the growing recognition of the importance of microbial life. Among its many attributes, Dobell illuminated the methods Leeuwenhoek used in discovering various objects, especially microscopic animalcules. A vital feature of these methods was Leeuwenhoek’s attempts at measuring the creatures he witnessed through his uniquely powerful single-lens microscopes. Leeuwenhoek compared the creatures and many other objects to everyday items his readers would find familiar. He paid significant attention to sharing his measurements and methods in numerous letters to the Royal Society and others interested in his work. In some ways, his comparisons have a childlike simplicity, like “How many marbles would it take to fill up a jar?" The objects he observed were not marbles; the animalcules were previously unseen and critically important life forms that would have been difficult for anyone to appreciate had Leeuwenhoek not provided his comparisons.

It is unclear from his letters (17 vol published to date) how formally Leeuwenhoek was trained in mathematics, particularly the mathematics of measurement. Still, he knew accounting and the quality control methods common to 17th-century cloth merchants. In his time, there was an increasing interest in refining the precision of measurement methods, particularly from the mid-16th C. through to 1673, when Leeuwenhoek wrote his first letter to the Royal Society. Observers from the Royal Society and other European scientists came

to accept what he witnessed through his lenses. Current methods have verified that his micrometric methods produced reasonable measurements for erythrocytes and some bacteria. While it will remain unknown how deeply Leeuwenhoek was influenced by the micrometric methods developed for navigation, surveying, ballistics, and Cartesian geometry, people he knew and interacted with regularly were familiar with these topics. It may be that the conversations and correspondence he had with figures from William Davidson to Regnier de Graaf, Robert Hooke, Jan Swammerdam, and Christiaan Huygens, not to mention his colleagues in commerce and city hall, gave him reasons to consider his animalcules as objects worthy of measure.

5.3 Antoni van Leeuwenhoek Joins the Scientific Community.

Leeuwenhoek submitted his first scientific letter to the Royal Society under a cover letter drafted on 28 April 1673 by his friend Dr. Regnier de Graaf (1641–1673). This Delft physician corresponded with the Royal Society secretary, Henry Oldenburg. Graaf felt it was essential to inform the Society of Leeuwenhoek's investigations. Leeuwenhoek believed that his observations would be compared to those made by Robert Hooke, whose *Micrographia* had been published in 1665, and to other persons in Europe's scientific community. He said as much in the first paragraph of this first letter:

I have several times been pressed by various gentlemen to put on paper what I have seen through my recently invented microscope. I have constantly declined to do so, first because I have no style or pen to express my thoughts properly, secondly because I have not been brought up in languages or arts, but in trade, and thirdly because I do not feel inclined to stand blame or refutation by others (Leeuwenhoek, 1939a, p. 43).

Leeuwenhoek launches into his observations of mold (“mould”), “the sting and some articulations of the bee, and also the sting of a louse” (A. Leeuwenhoek, 1939a, 1939b, p.

43). He also states that the observations noted in this letter were not his first regarding the bee stinger apparatus, as he had made some notes "…about two years ago…." He visited England on holiday in 1668 and is thought to have brought a microscope with him. It may be that this visit also brought familiarity with Hooke's second edition (1667) of *Micrographia*. Leeuwenhoek's comments regarding the blue mold found on the covers of books are noteworthy as they address observations made in *Micrographia* but improve on the details Hooke made available with his lower-power compound microscope.

With this first observation, Leeuwenhoek knew that his observations would be met with skepticism. With this first letter, he began to offer a means by which his readers could appreciate the details within structures already observed by Galileo and Hooke. Leeuwenhoek took his new Society colleagues into an invisible realm by comparing objects his lenses revealed with everyday items. He subdivided the known to measure the unknown.

5.4 Subdividing the Visible Universe

In a footnote[8] that clarifies this observation, Dr. A.J. Kluyver, a professor of microbiology at the Technical College of Delft in 1939, supplied the following information:

There is every reason to conclude that this is the first observation concerning the formation of spores in moulds. Although the description is not quite correct, there can be no doubt that L.[9] here observed the formation of a sporangium and the liberation of the sporangiospores in one of the species of moulds belonging to the *Mucoraceae* (Leeuwenhoek, 1939a, p. 31).

[8] Footnotes of this sort are provided throughout all volumes of *The Collected Letters of Antoni van Leeuwenhoek* and provide valuable context for van Leeuwenhoek's observations. It is important to note that the language used in the footnotes is that of academic specialists in 1930s Europe, thus introducing terms and grammatical idiosyncrasies consistent with that time.

[9] The notes use L. as an abbreviation for van Leeuwenhoek's name

It may have been various hunter-gatherers who first started subdividing and enumerating their surroundings into units. Foraging may have involved counting—of themselves, their community members, the number of trees or plants of various types, the units of food which could be harvested from a tree or plant, nuts and seeds they ate and used in farming, the number, and types (running versus still, sweet versus foul) of water sources, the distances to important locations. Of course, early peoples were surrounded by small—and large—objects in their environment, from dust motes, which became a unit of measure in Vedic scriptures, to grains of sand, up through mountains, valleys, the skies, and stars. All must eventually have become sources of wonder instead of mere objects in their surroundings.

Subdivision became more formal with the advent of traders and merchants. Merchants would have expected payment proportional to the items they delivered; both parties might have kept a mental record until the scope of trade made memory insufficient. Whatever counting was employed in the management of harvests, sales, and payment is thought to have been a driving force for the creation of written languages in Sumer (Kramer, 1963, p. 1609) and Egypt (Wilkinson, 2010, p. 883), where large rivers would have funneled trade to the most significant cities sitting along their banks.

Other types of counting involved surveying and navigation. Farmers and merchants would have to find each other. Creating larger communities may have involved following rivers, observing the sun's and star's positions, and reckoning their location relative to their destination. Mathematicians such as Pythagoras (~570—~495 BCE), Euclid (~325—~270 BCE), Archimedes (~287—~212 BCE), and Claudius Ptolemy (~100—~170 CE), along with their antecedents in Sumerian, Egyptian, and Greek cultures, helped provide a basis for surveying and navigation by collating, improving, developing, and documenting

rational methods for allocating land, designing and building roads and structures, and exploring the seas and oceans that surrounded them.

The quadrant became a principal instrument for surveying, navigation, and determining time relative to location. Ptolemy developed an early version of the quadrant. The fundamental idea went through various changes over the next twelve hundred years. Among other valuable approaches was the application of transversal lines, which introduced greater precision into quadrant measurements. The basic methodology dates to Euclid but was updated by Levi Ben Gerson (1288–1344), who seems to have based his method on an instrument called Jacob's staff, invented by Jacob Ben Makir a century earlier. Significant improvements to the quadrant evolved from interactions between Pedro Nunes, an esteemed Portuguese mathematician, and various Portuguese navigators during the Age of Discovery (Bleichmar et al., 2009, pp. 84-88).

5.5 Pedro Nunes and the Nonius

The 16th century brought about significant refinements in metrology. Mathematician Pedro Nunes (1502—1578), born in the Alentejo town of Alcácer do Sal, Portugal, created a scaling methodology to assist navigators in determining location and direction by observing the stars. Nunes created a measurement concept called the *nonius* and improved existing methods for sailors using quadrants. They could make observations of a star's altitude in the sky relative to the horizon, stars, or other celestial objects. The *nonius* was defined using a series of concentric arcs on which a series of dividing marks were made (*Figure 5.1.*). Each concentric arc—from outermost to innermost—had one less dividing mark than the previous arc, such that the first arc held ninety marks, the next, somewhat shorter arc held eighty-nine, and so on until the last arc was etched with forty-five marks. The effect was to divide degrees into a subdivision of sixty minutes per degree (Kwan,

2011). The subdivisions provided users of the quadrant with refined precision for determining the altitude of their reference points. Movement of the alidade or index arm sighting on one star could be correlated with calculation tables Nunes had developed for use with his scaling system.

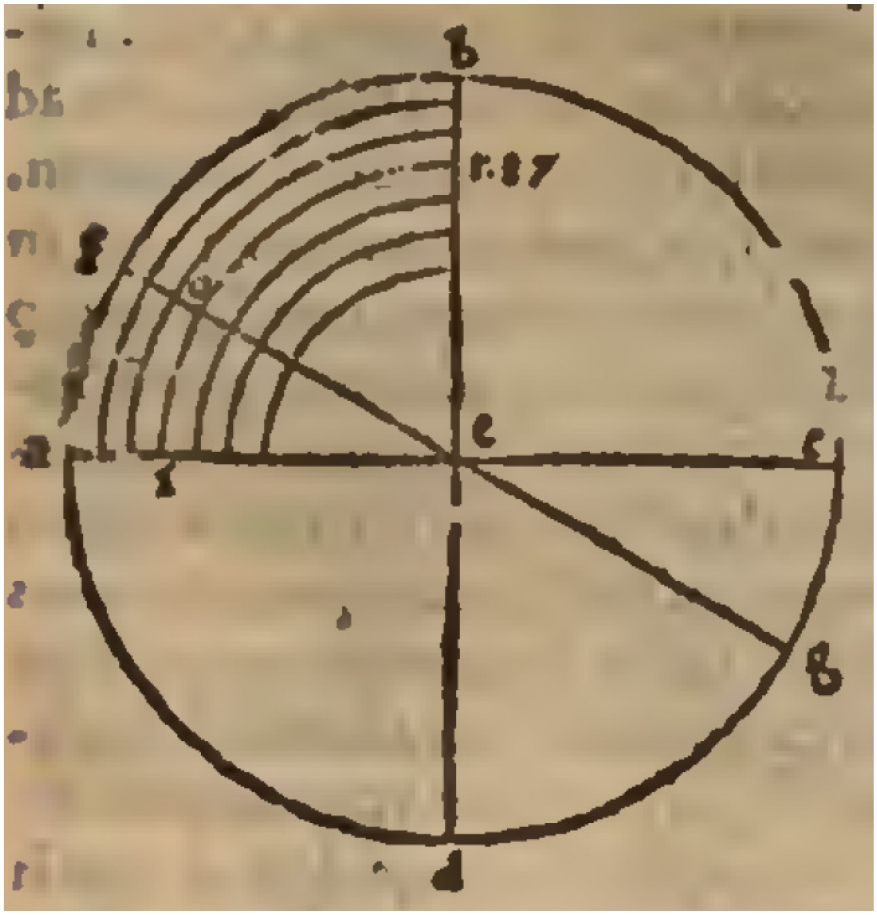

Figure 5.1. *The nonius scale as published in Pedro Nunes's 1573 edition of De Crepusculis Postulate III (Nunes, 1573).*

In brief, Nunes' work provided a means of dividing the enormous sky into comprehensible loci defined by relative altitudes in Euclidean space and time if the objects were being observed throughout a voyage. While astronomers were interested in three-dimensional relationships among the stars, planets, and moons, the night skies could be understood as a plane across which light sources were scattered and appeared to move. Our

sense that objects moved across the sky over time on any given night is primarily due to the rotation of our planet on its axis within a relatively static sky, except for planetary and moon movement. Planets that could be seen in the 16th and 17th centuries described their orbits around *Sol*, as did our planet, and the moons we could see were also orbiting their planets. These movements were a source of various competing navigational proposals to the surveyors and navigators of the world. The *nonius* supplied a means of assigning objects a numerical location at a specific time. The *nonius* thus established a way for navigators and natural philosophers to think about spaces, large or small, celestial or earthbound. His work responded to practical needs articulated by Portuguese navigators such as D. João de Castro and Martim Afonso de Sousa (Bleichmar et al., 2009).

The precalculated tables that *nonius* users depended upon were updated by Christopher Clavius (1538—1612), a student of Nunes at Universidade de Coimbra (O'Connor & Robertson, 2010). However, some dispute the extent of their interactions (B. Almeida & Leitão, 2009). Clavius' refinement of the *nonius* divided each subordinate arc into 128 parts, primarily so that each arc could be subdivided by repeated bisection) (Kwan, 2011). While this approach may have improved precision beyond what Nunes had presented, the labor required remained intensive for creating each arc with 128 subdivisions.

The nonius was adopted briefly by Tycho Brahe (1546—1601), who referred to Pedro Nunes as "*Hispanicus*." After Brahe moved from Denmark to Prague, Jacob Kurz (Jacobus Curtius, 1554—1594) developed a quadrant that extended Nunes' concept, but it was even more difficult to manufacture; it involved "locating and engraving over 5,000 dots (Kwan, 2011, p. 370). Brahe came to prefer the transversal line system for cosmography (*Figure 5.2.*), a conceptually straightforward system that achieved the same goal. The application of transversal and diagonal lines to linear grids and concentric circles was used to achieve

improved measurement precision. Brahe's use and preference for this conceptually more straightforward system seem to achieve similar ends—it was easier to divide an enormous sky.

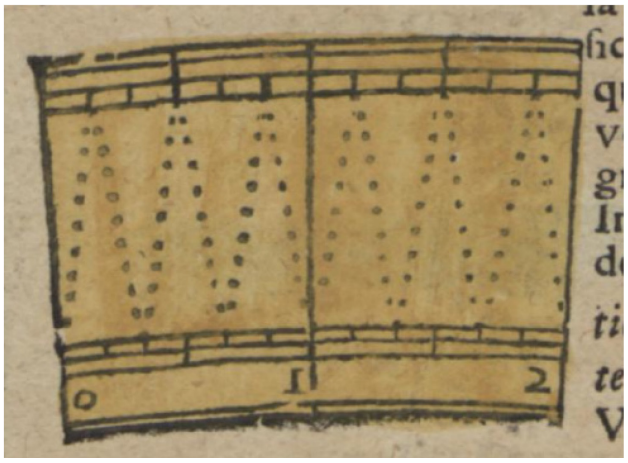

Figure 5.2. *Part of a dotted diagonal scale is shown in Tycho's Mechanica. The dots divide these two degrees of arc into individual minutes (Brahe, 1598).*

Brahe's Astronomiae instauratae mechanica, published in 1598, includes an illustration of a quadrant subdivided by the *nonius* system (*Figure 5.3.*).

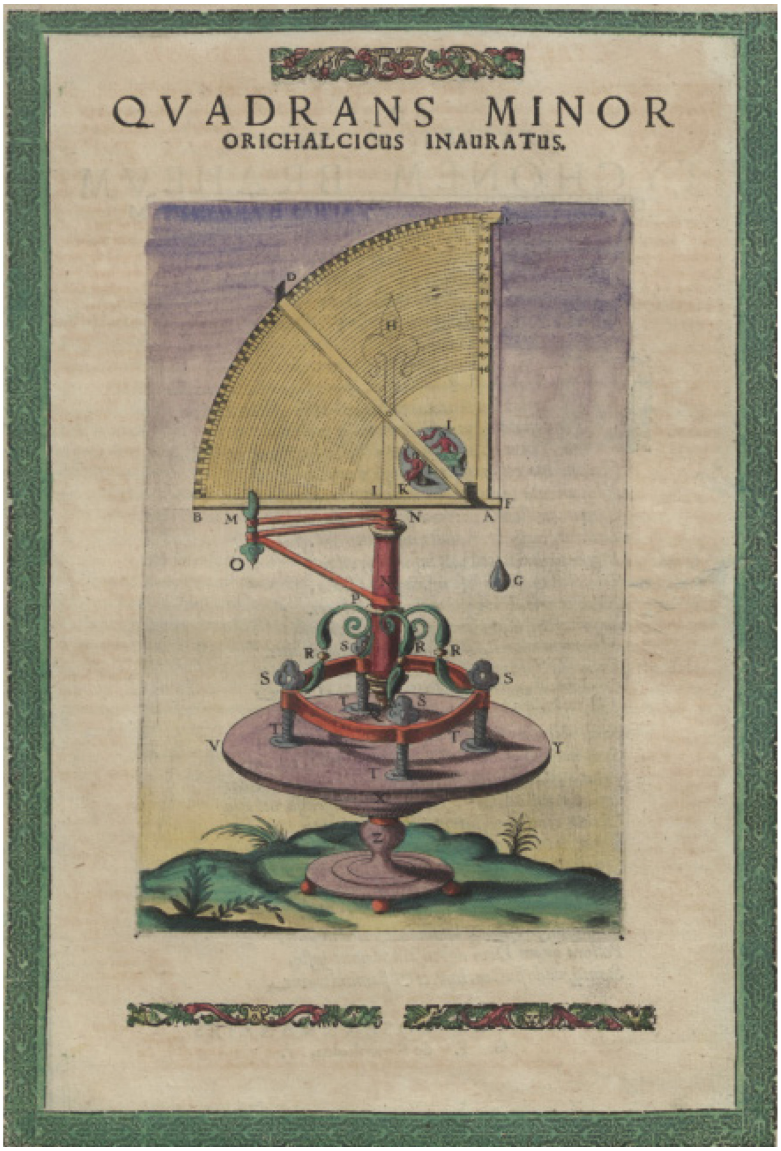

Figure 5.3. *Tycho Brahe made and used a quadrant with the nonius system (Brahe, 1598).*

Shortly after Kurz's use of the nonius in a quadrant, instrument maker James Kynuyn built the nonius into a quadrant (*Figure 5.4.*) presented a half-century later in Robert Dudley's *Dell'Arcano del Mare* (1646—1647). Kynuyn is remembered for fashioning other navigational instruments, such as the astronomical compendium from 1593, which included "nocturnal; a latitude table for 39 world locations; a magnetic compass; a list of ports and harbours; a perpetual calendar and table of fixed feast days; a high tide computer

for several European ports; lunar phase and age indicator *vovelles* and a planetary aspectarium. The remains of an equinoctial sundial can also be seen" (The British Museum, n.d.). Although the compendium was a Swiss army knife sort of device, it is unclear whether anyone used the nonius quadrant created by Kynuyn. Recently, the Kynuyn device has been reproduced through the efforts of the late António Estácio dos Reis (1923—2018) of the Academia de Marinha and Jorge Leitão (*Figure* 5.5.).

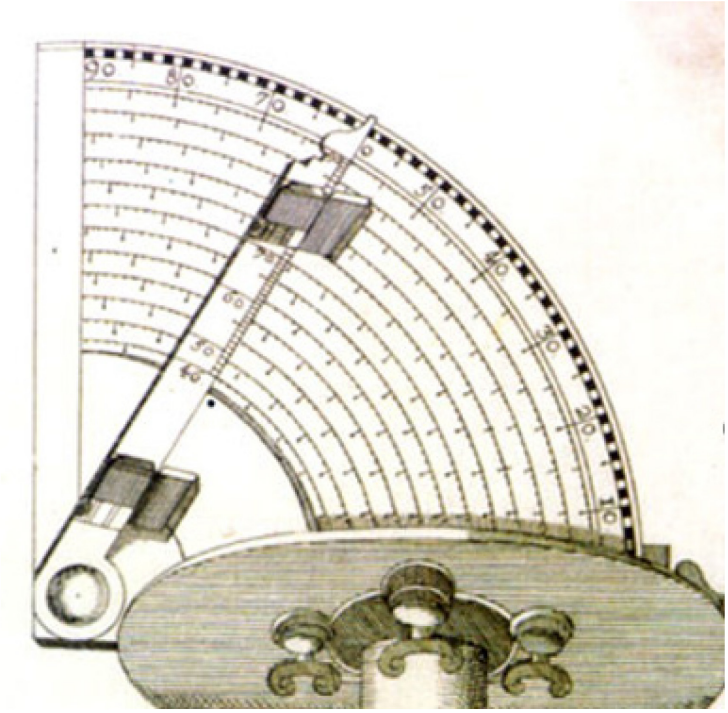

Figure 5.4. *The illustration presented by Robert Dudley in Dell'Arcano del Mare, edited in 1646-7, reproduces James Kynuyn's 1595 quadrant before the alidade and the compass were lost (Dudley, 1647). The nonius scale is displayed on the concentric arcs.*

Figure 5.5. *Reconstruction of the Kynuyn quadrant with alidade and compass, supervised by Estácio dos Reis and forged by Jorge Leitão from original molds. The reproduction is in the Museu de Marinha, Lisboa (Chance Makes History, n.d.).*

George Weymouth (~1585—1612) adopted the nonius for making azimuthal readings in his publication *The Jewell of the Artes* (1604). A navigator and shipbuilder known for explorations on behalf of the East India Company for a northwest passage from the Atlantic to the Pacific Ocean and for early explorations of Maine, Weymouth wrote *The Jewell* as a tribute to King James I (1567—1625), as he hoped to receive support for future explorations. It is unknown whether he used the nonius with quadrants in his possession during his voyages, only that he thought sufficiently highly of the system to cite it in his masterwork (*Figure 5.6.*).

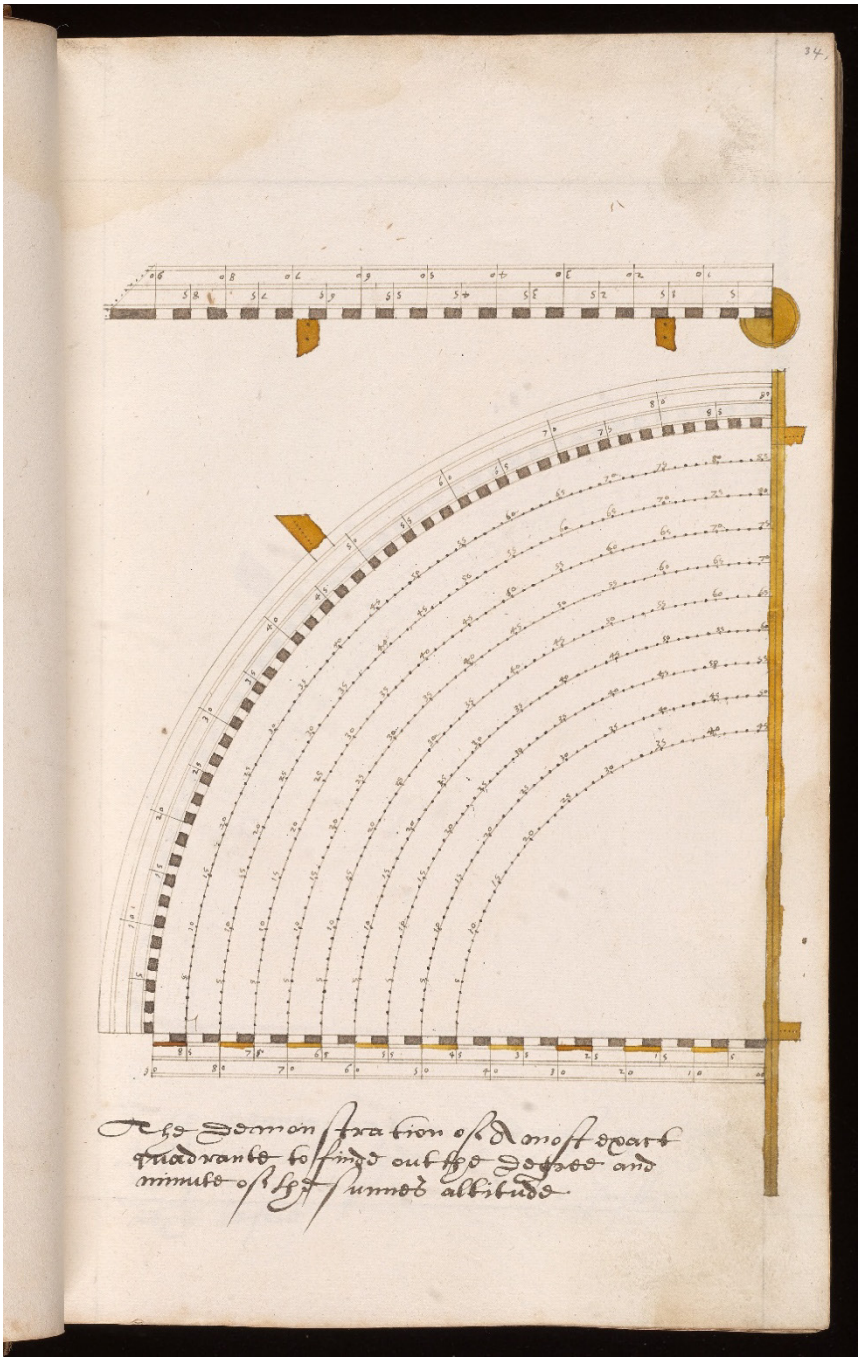

Figure 5.6. *Page from The Jewell of the Artes by George Weymouth (1604) showing the subdivision of a semicircle using the method of Pedro Nunes (Weymouth, 1604).*

Further improvements in the functionality of measurement instruments and micrometry came with Lucas Brunn (1572—1628). In 1609, he developed a micrometer that used a screw-based adjustor to improve the accuracy of quadrant use 1(Brooks, 1991, pp. 127-173). It is thought that the primary use for which he intended his adjustment system was to

improve gunsight designs. He worked with Christof Treschler, a Dresden instrument and gunsight maker, to fashion his screw-based system. Brunn's system owed much to the mathematics of Leonhard Zubler's Nova Instrumentum Geometricum (1607) (Swetz & Katz, 2011), which changed surveying methods of the time, and to Treschler's skills at precision machining, but Brunn, while court mathematician in Dresden, also worked in Dresden's armories. Creating accurate and precise weapons may have been his principal motivation. In effect, Brunn helped turn the battlefield into a grid. However, Brunn was not the first person to use screws as adjustors. This method was used as far back as Nasir al-Din al-Tusi (1201—1274) and Ulugh Beg (1394—1449), both Iranian-born mathematicians and astronomers. These screw adjustors, as well as those used by Regiomontanus (1436—1476), were not exclusively intended for measurement applications (Brooks, 1991).

Galileo Galilei (1564—1642) was also intrigued by the problems of micrometry. In 1611, he proposed using a micrometric grid attached to the telescope's body to subdivide the sky into sectors (*Figure 5.7.*). He was particularly interested in creating a technology that would assist navigators in using the moons of Jupiter as a reference for longitude measurements on Earth. Galileo was so enamored of this idea that he created a combined telescope and micrometer[10]—the celatone—that navigators could use while standing on a ship's deck (*Figure 5.8.*). The practicality of using such a device while the ship pitched and rolled beneath the navigator's feet led to the rejection of this concept. However, Galileo returned to the idea about ten years later with a celatone that could be worn as the navigator sat in a hemisphere placed in an oil-lubricated tub, thus allowing the navigator to retain

[10] The term micrometer will be used in two senses within the text: the first refers to a device that allows the division of dimensions into small amounts; the second refers to a unit of measurement—the micrometer—introduced in the 17th and 18th centuries with the creation of the metric system of measurement. One micrometer, micron, μ, or μm equals 1×10^{-6} m.

their relative position to the sky much as gimbal systems would allow (*In-Depth Celatone*, 2010).

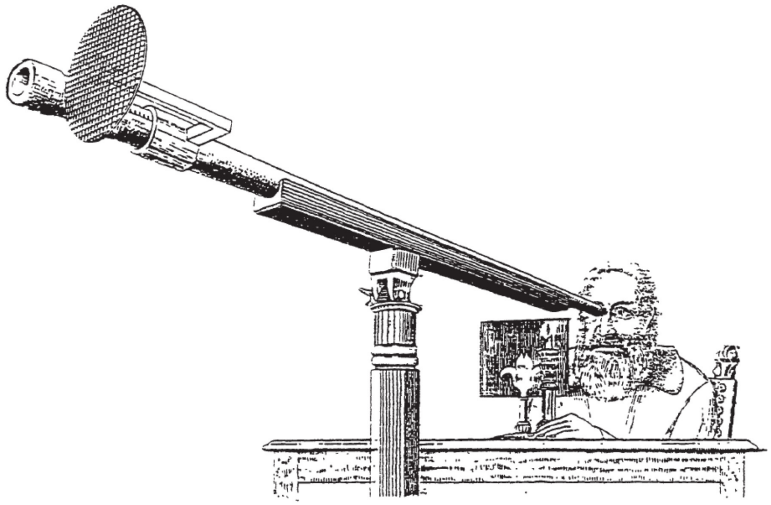

Figure 5.7. *Illustration of Galileo using a micrometric grid attached to a telescope (Celatone (Ricostruzione) IF 102793.Jpg, 2012).*

Figure 5.8. *A celatone intended for use as a micrometric sky-measuring telescope and worn as part of a helmet. The practicality of this device was defeated by the constant motion of the ship's deck (Celatone (Ricostruzione) IF 102793.Jpg, 2012).*

In 1631, Pierre Vernier (1580—1637), citing Pedro Nunes, created an improved, consistent scale (*Figure 5.9.*) to interpolate fractional measurements (Kwan, 2011). This scale followed Nunes's approach with the *nonius* about ninety years before. The Vernier

scale often was called the *nonius* scale until the 19th century (Estácio dos Reis, 1999). When the Vernier scale was used as an adjustment device with a quadrant (*Figure 5.10.*), it used a screw-based adjustor on the alidade like those designed by Brunn to improve the accuracy and precision of gunsights.

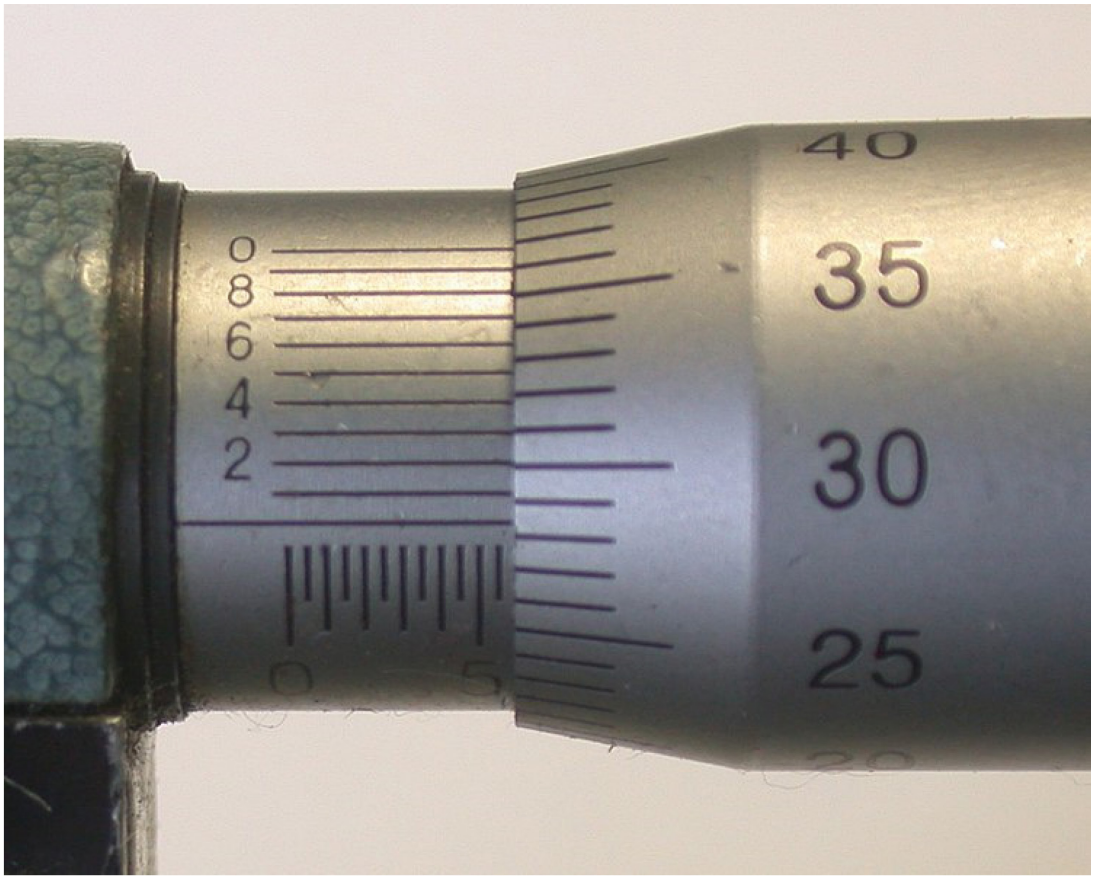

Figure 5.9. *A micrometer using the Vernier scale, also known as the nonius scale until the 19th century (McKechnie, 2019).*

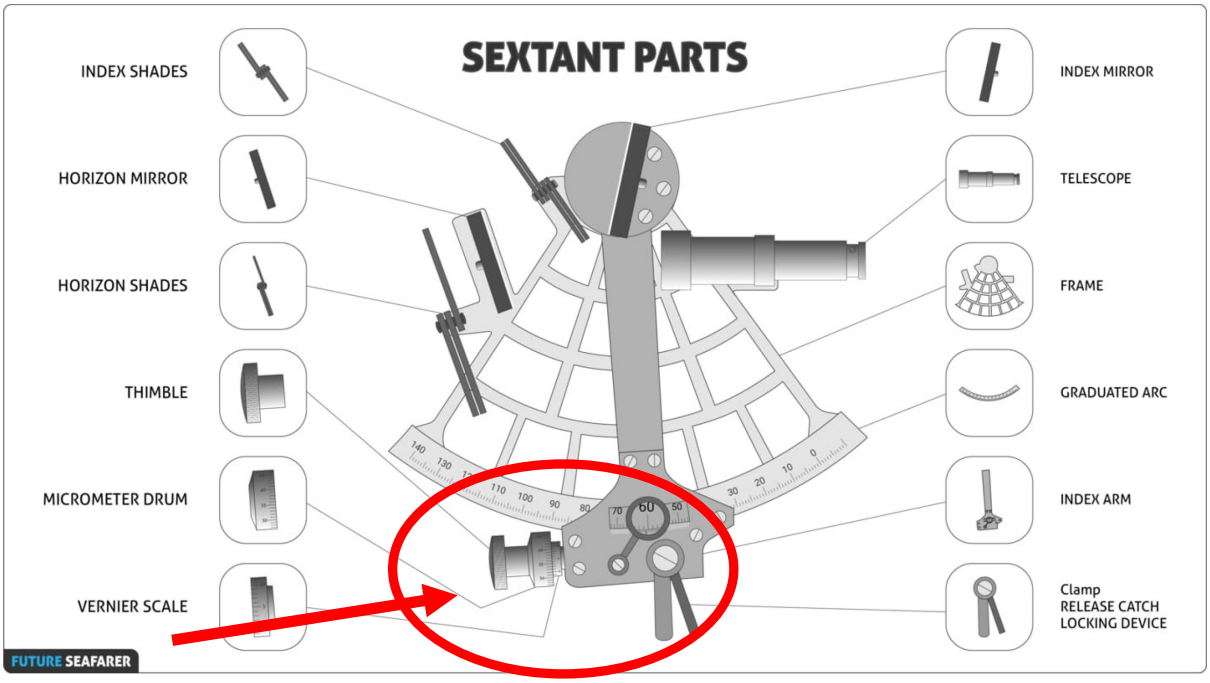

Figure 5.10. *Diagram of a sextant highlighting a screw-based adjustor using the Vernier or nonius scale (Future Seafarer, n.d.).*

Following Galileo, William Gascoigne (~1612[11]—1644) developed a way of dividing a measured foot into "near 30000 parts" and using these divisions, as others had done, to improve the precision of his terrestrial and celestial observations using his telescope micrometer (Sellers, 2012, p. 18). In correspondence, Gascoigne attributed his insight to a spider spinning its web within his telescope (Dijksterhuis, 2004). In his book *The Perfectionists*, Simon Winchester describes Gascoigne's innovation as follows:

He had embedded a pair of calipers in the eye-glass of a telescope. With a fine-threaded screw, the user was able to close the needles around each side of the image of the celestial body (the moon, most often) as it appeared in the eyepiece. A quick calculation, involving the pitch of the screw in inches, the number of turns needed for the caliper to fully enclose

[11] Brooks (Brooks, 1991) lists Gascoigne's birth as occurring in 1620; Sellers goes to lengths to show Gascoigne was one-half year old in 1612 (Sellers, 2012).

the object, and the exact focal length of the telescope lens, would enable the viewer to work out the "size" of the moon in seconds of arc (Winchester, 2018, p. 77).

Strangely, Gascoigne's achievements as an astronomer and metrologist were not adequately recognized until Adrien Auzout (1622—1691) sent a letter late in 1666 to Henry Oldenburg (~1619—1677), secretary of the Royal Society, describing Auzout's telescope micrometer, which allowed division of a foot into "29000 or 30000 parts" (Sellers, 2012, p. 18). Auzout's scientific *bona fides* were solid. He was a founding member of the Académie Royale des Sciences who had communicated with Oldenburg on other occasions, thus establishing himself with the Royal Society. In this instance, however, his methodology was found by Robert Hooke and Christopher Wren, both eminent Royal Society members, to be strongly reminiscent of Gascoigne's work. Gascoigne's importance to advances in micrometry was not to be supplanted by M. Auzout.

Further support for Gascoigne's posthumous claim to the novelty of his device was received from Richard Towneley (1629—1707), who owned three of Gascoigne's instruments. Towneley claimed that Gascoigne had prepared a treatise on optics that had gone missing. All that Towneley had in his possession were some letters and notes from Gascoigne.

The cross-channel spate of controversy started by Auzout's letter to the Society led to other investigators reasserting or revealing their work on astronomical micrometers. Christiaan Huygens (1629—1695), the Marquis Cornelio Malvasia (1603—1664), Pierre Petit (1594—1677), Chérubin d'Orléans (1613—1697), and Ole Rømer (1644—1710) all wrote letters or published papers documenting their micrometric techniques between the years 1659 (Huygens) and 1672 (Rømer). Huygens's 1659 publication describing a type of micrometry is characterized in *Lenses and Waves* as "not a real micrometer," although it

"did produce reliable, accurate data" (Dijksterhuis, 2004, p. 44). In 1666, Huygens adopted a micrometer of "cross wires arranged in squares" (Bell, 1950, p. 197). In *Micrographia*, Robert Hooke wrote that micrometry is well-suited for use with telescopes:

These should be fitted with a *Rete*, or divided Scale, plac'd at such a distance within the Eye-glass, that they may be distinctly seen, which should be the measure of minutes and seconds… (Hooke, 1665, p. 237).

To summarize, the micrometers were used to determine the relative positions of objects in the sky and their movement in time. The motion was in three dimensions: altitude, relative position, and time. The sky became a surface of "microscopically"-divided segments, subdividing observation angles into their smallest reproducible parts. Their motion in space defined the objects in relationship to the movement of people across bodies of water.

In this intellectual environment, Leeuwenhoek matured as a microscopist and natural philosopher.

5.6 Leeuwenhoek's Connection to Micrometry

It is unclear from Leeuwenhoek's letters what his connection to formal micrometry might have been. Some biographical notes make an informal connection. Leeuwenhoek was born into a family that made its living making baskets, an essential means of commercial packaging. He received little institutional education beyond five years of grammar school at the Latin School in Warmond. Leeuwenhoek also studied with his uncle, Benthuizen's sheriff and bailiff. When he was sixteen, Leeuwenhoek started a five-year apprenticeship (1648-'54) with international merchant William Davidson (~1614—~1689) while boarding with another uncle, a wool buyer. Davidson's interests included trade in

Denmark, Sweden, Norway, Virginia, and Scotland and his business in the Netherlands. Whatever education Leeuwenhoek had received prepared him sufficiently that he was able to pass the master cloth merchant examination within six weeks of starting his apprenticeship. During these years with Davidson, Leeuwenhoek may have learned about the problem of determining longitude using nautical instruments. The relationship between Davidson and Leeuwenhoek appears to have been very trusting. Davidson thought so well of Leeuwenhoek that he granted him power of attorney in 1653. This level of trust suggests that Leeuwenhoek learned something about international business. Communication between the two must have been significant (Leeuwenhoek, 1989, p. 297).

Leeuwenhoek leveraged this relationship to open his own business. He would have relied on subdivision and micrometric measurement techniques to evaluate the cloth quality he bought and sold. For that matter, the baskets his family made and traded were woven articles, although they are made from grasses, leaves, roots, and fibers from various trees. Thread count is measured in the number of threads per square inch and can vary between the fabric's length (warp) and width (weft). The quality and identity of the cloth fibers would have been determined by using a draper's lens (*Figure 5.11.*).

Figure 5.11. *Thread counting magnifying glass (L. Robertson et al., 2016b).*

It is not known when or how Leeuwenhoek became interested in glass lenses, whether it was from his uncle's tutoring, cloth merchant Davidson's likely interest and knowledge of telescopes and quadrants, or whether it was the draper's lens—surely used in Davidson's business, as well as Leeuwenhoek's. It may have been that the middle 17th century was the era in which optical devices matured into widespread use. It was the Dutch opticians Hans and Zacharias Janssen, as well as Hans Lipperhey, who, in the closing decade of the 16th century and early 17th century, ground lenses of sufficient quality that magnifying glasses, telescopes, and microscopes became useful and popular devices across Europe. Johannes Hudde (1628—1704), an eminent Dutch mathematician and mayor of Amsterdam from 1672-1703), was involved in a Leiden-based mathematics study group that discussed Cartesian geometry, amongst other mathematical trends. Some members also acquired compound microscopes in the mid-1650s, and it may be that Hudde started experimenting with them in the late 1650s. It is thought that his experiments with single-lens microscopes did not start until around 1660. By 1663, Hudde shared his lens-making methods with

Balthasar de Monconys, a French scholar who visited Hudde in Amsterdam. While Leeuwenhoek may have been aware of Hudde's interests, there is little evidence that the two knew each other. Jan Swammerdam (1637—1680), a Dutch physician, anatomist, and microscopist who did know both parties, wrote that he is certain Leeuwenhoek "initially followed Hudde's invention" (Zuidervaart & Anderson, 2016, pp. 260-1). In short, 1655 through 1673 was a period in the history of Dutch natural philosophy when increasing attention was given to optics-based topics, resulting in the development of single-lens microscopes. Cartesian geometry also piqued interest in Hudde's circle of colleagues. Frans van Schooten, the leader of the Hudde mathematics discussion group, translated Descartes' *La Géométrie* into Latin in 1649 (Burton, 2011).

In 1699, Leeuwenhoek wrote that "[A]s for myself, although glasses of extreme smallness were made by me already about 40 years ago, they are seldom used by me" (Leeuwenhoek, 1989, p. 297). Whether this was an attempt at claiming early eminence in single-lens microscopy or not is unknown, but this would place his first fabrication of lenses around 1659. In 1668, he visited England and observed the "chalk cliffs and chalky lands at Gravesend, Rochester, etc" (Leeuwenhoek, 1939a, p. 159). His trip followed Robert Hooke's publication of *Micrographia*, which ignited a growing interest in microscopy, particularly after the second edition was published in 1657. In a letter dated 7 September 1674, he wrote about his examination of English chalk substances that he may have collected while visiting. If he had been making lenses since 1659, he might have taken a lens of some description with him on the trip, although this is debated by the *Collected Letters* editors in footnote 39 of the first volume.

In 1669, Leeuwenhoek was licensed by the court of Holland as a land surveyor. He would have studied the text for non-academics in use since 1600. The course would have

exposed him to the mathematics and tools of surveying and might have been Leeuwenhoek's introduction to subdivision methods. Vernier's work, published in 1631 and referencing the nonius, would have been integral to the coursework. He might have learned some Cartesian concepts from colleagues affiliated with Hudde since René Descartes published *Discours de la Méthode* in 1637. We know that surveying instruments and measuring tools were among Leeuwenhoek's personal effects inventoried after he died in 1723 by Delft notaries (Zuidervaart & Anderson, 2016, pp. 23–24).

While a provincial Dutch merchant by some criteria, he was friends with intellectuals such as Reiner de Graaf and Jan Swammerdam, physicians, and Constantijn Huygens, an eminent Dutch composer, musician, and writer. Delft physicians and surgeons thought so well of him that he was invited to attend dissections in the nearby anatomy theater. In 1679, after establishing himself as a lensmaker and naturalist focused on the fine structure of objects, he became a *wijnroeier*—wine gauger—weights and measures inspector of wine, oil, and vinegar. He became responsible for using a wine rod, a specialized measuring stick, to determine the volume of liquid in barrels. The position would also have required a demonstrable knowledge of volumetric measurement, a technique he had applied in his first letter to the Society. It was typical for the *wijnroeier* and land surveyor professions to be linked as both were positions of civic responsibility, integrity, and trust. While this occurred after his initial letters to the Society, it demonstrates a broad and continuing relationship with metrology.

These trends may have influenced Leeuwenhoek, but he seems to have created techniques entirely on his own. He defined his micrometry by using everyday items. His "sky" was a water droplet, a volume. While he might have observed creatures in two dimensions, he understood that he was seeing them in three. He watched them move over

time and described their movements, which proved they were living beings. He divided the volumes in which his animalcules skittered about by how many of them might fit the shape of a seed or sand grain. He imagined his animalcules into positions in a Cartesian volume, an x, y, z coordinate system. His methods were verified by Robert Hooke, Christiaan Huygens, and others in his own time and sometimes would be proven accurate centuries later.

5.7 Leeuwenhoek's Measurement Models

In the first letter, in a passage on mold stalks and spores, Leeuwenhoek makes estimates of relative size while providing details regarding the life processes of a fungus that Robert Hooke seemed to have missed using a compound microscope with less resolving power. Leeuwenhoek created a scientific problem by seeing what others had not ventured to discover. This method echoed an approach seen in the earliest documentation of measurement, that of the cubit, which was a measurement based on "arm-measures":

The earliest measures were those of length, and they were derived from the rough-and-ready standard afforded by the limbs of man. The readiest of these measures were those offered by the length of the forearm, and by parts of the hand; these formed a natural series of far-reaching importance.

These arm-measures were—

The Cubit, the length of the bent forearm from elbow-point to finger-tip, about 18 to 19 inches.

2. The Span, the length that can be spanned between the thumb-tip and little finger-tip of the outstretched hand. It is nearly half of the cubit, about 9 inches.

3. The Palm, the breadth of the four fingers, one-third of the span, one-sixth of the cubit, about 3 inches.

4. The Digit or finger-breadth at about the middle of the middle finger, one-twelfth of the span, one-twenty-fourth of the cubit = 1 inch.

From this division of the cubit into six palms and 24 digits, and of its half, the span, into 12 digits, came the division of the day into watches and hours, of the year into months; came also the consecration of the number 12 in legend, history, and social institutions—came in short duodecimalism wherever we find it.

Add to the above measures the outstretch of the arms, the fathom, we have the five primitive limb-lengths (Nicholson, 1912, p. 1).

The cubit and its associated measures assisted humankind in measuring the environment we lived in and in measuring materials for construction and trade. Houses might have been quite lopsided without the cubit, and a horse or cow might have been described as large or small without comparing it to others. The cubit helped impose order on what otherwise might have been the chaos of an unmeasured, undivided universe.

As part of the work performed by the committee of Dutch scientists, a table (*Table 5.1.*) was compiled from measurements used by Leeuwenhoek, along with extensive notes explaining the more archaic measures, as well as measurements that have changed meaning in the time since the 17th C.

Table 5.1. *Weights and measures used by Leeuwenhoek (Leeuwenhoek, 1939a, p. 379).*

Linear Measures		***Weights***	
Mile	7.4074 km	Pound	476 g
Rhineland rood	3.767 m	Grain	65 mg
Rhineland foot	31.4 cm	"Ace"	47 mg
Inch	2.61 cm	***Measures of capacity***	
A coarse grain of sand	870 µm	A shipload of sand	4.45 m^3
A fine grain of sand	260 µm	A cartload of wine	900 L
A hair from L's beard	100 µm	A "toelast" of wine	500 L
A hair from the head	60—80 µm	A Sevilla pipe	435 L
A hair from L's wig	43 µm	A Bourdeaux hogshead	220 L
The eye of a louse	50—60 µm	A quart	7.5 L
The hair of a louse	3—9 µm	A stoup	2.3—2.5 L
A thread from the cocoon of a silkworm	8 by 16 µm	A pint	0.35—0.9 L
A red globule of the blood (erythrocyte)	8.5 µm diameter	A millet seed	2 mm diameter
The smallest animals (i.e., bacteria) in pepperwater	2—3 µm	Counter	1/25 or 1/30 of a quantity of water "as large as a grain of millet"
Elucidatory notes from Arnold Hoogvliet (1687—1763): "Here the dimensions of the invisible are determined with fixed measures."			

Lists of this type of date back to those published by Dino di Garbo (~1280—1327) in the early 14th Century (Welborn, 1935, pp. 15–35). Di Garbo compiled his extensive list of "the weights and measures most often used by apothecaries, doctors, and men of the market-place" as he noted the variations in the measurements and, it was assumed, the impact of these variations on using measurements to understand medical writings of his and earlier periods.

The following explanations of metrics are taken from the notes provided in Volume 1 (Leeuwenhoek, 1939a, pp. 381–385):

Inch. The length of an inch differs in the 17th C. according to the part of the country. We can deduce the length of LEEUWENHOEK's inch from the measuring-rod of 5 inches figured, original size, in his letter of 28 September 1716. However, the measurement is not very accurate for the inches differ from 2.555 to 2.615 cm. with an average of 2.61 cm. DOBELL found a mean of 2.615 cm. derived from 20 measurements (Dobell, 1932, p. 334). For convenience' sake we have dropped the third decimal in this edition, taking an inch of 2.61 cm. for reducing the following measurements. [S.][12, 13]

Coarse sand grains are Leeuwenhoek's common standard of comparison. In his letter of 25 July 1684, he says he took "a coarse sand-grain with an axis approximately 1/30 of an inch." This will give a mean of c. 870 µ for a "coarse sand-grain." Occasionally, Leeuwenhoek uses this measure for measuring capacity. [S.]

A fine sandgrain. The diameter of a fine sandgrain is fixed by L. at a 1/80 of an inch in his letter of 20 May 1679 and at 1/100 of an inch on 3 March 1682. This will give an average of 260 µ. [S.]

The eye of a louse, used in the letter of 9 October 1676 as a standard for comparing the size of "little animals" in rainwater, proves to measure 50 to 60 µ. Dobell (Dobell, 1932, p. 337) found the average to be about 70 µ, ranging from 64 to 80 µ. Cf., however, B. Cohen, (Cohen & Leeuwenhoek, 1937)gives 80—85 µ. [H.]

[12] The "S." in brackets represents the editors' shorthand approach to identifying the author of various footnotes and explanatory passages throughout the volumes. In this instance and elsewhere, "S." indicates that Dr. Abraham Schierbeek, lecturer in the history and methodology of biology at the University of Leiden, provided context. Dr. Schierbeek also authored biographies of van Leeuwenhoek and Jan Swammerdam.

[13]The following is quoted directly from a footnote in Volume 2 of the Collected Letters: "As appears from the letter of 12 January 1680, L. uses as measure the "Rhineland" foot, which is 0.314 meters in length. In that case an inch is 2.62 centimeters. Cf. Oeuvres Completes de CHR. Huygens, XIII; p. 133, note 1, and R.K. Kuipers, Geill. Wdb. Der Ned. Taal. There are occasional deviations from the above figures (cf. Dobell, AvL and his Little Animals, but these differences are so slight as to be negligible." [M. = Judica I. H. Mendels, qualified teacher of Dutch in Secondary School, Amsterdam.] (Leeuwenhoek, 1939a, p. 49).

A red globule of the blood or "globule that makes the blood red" (red-blood corpuscle). In his letter of 1 June 1674 L. says: "I judge the red globules of the blood to be quite 25,000 times smaller than a fine sand-grain." This means that according to L., a red blood corpuscle has a diameter of 8.5 μ. This agrees with what he says in the letter of 25 June 1684: "the complete globules that make our blood red are so small that 100 of them laid lengthwise would not make up the axis of a course sandgrain" (circa 870 μ see above). We now know that the diameter of a red blood-corpuscle is circa 7.2 μ. [H.]

Animalcules in pepperwater (bacteria). In his letter of 26 December 1678 L. says: "thirty million of these animalcules do not cover as much space as a coarse sandgrain." We can consequently estimate these "little animals" at 2 μ or 3 μ. [S.]

A millet seeds. The volume of a millet seed, used by L. for determining a volume of water, is calculated by him as follows in his letter of 23 March 1677. He estimates its diameter at circa ¼ of that of a green pea ("for I estimate that if the axis of a millet seed is 1, the axis of a green-pea will be quite 4½; in which case a quantity of water, equaling a millet seed, will be approximately 1/91 part of a drop of water, according to the common rules of mathematics"). Dobell fixed the diameter of a seed of *Panicum miliaceum* L. at circa 2 mm. [S.][14]

Counter. L. found a still smaller measure of capacity by sucking up a quantity of water "as large as a grain of millet" into a capillary tube and dividing this into 25 or 30 or more parts." Counting infusoria in this manner, he really became the inventor of the principle on which the counter is based. [S.][15]

[14] It is worth noting that van Leeuwenhoek's lenses typically had a diameter of about 3 mm, slightly larger than a millet seed (~2 mm).

[15] Dr. Schierbeek is alluding to what became known as cell counters or hemocytometers in this description.

With this list of descriptions, any interested person could comprehend how Leeuwenhoek arrived at the estimated sizes of microscopic "animalcules." In addition to the reports in his letters and the notes written by the specialists enlisted by the Dutch committee, Leeuwenhoek used arithmetic and a small amount of geometry to help subdivide the new universe he had discovered.

5.8 How Leeuwenhoek Measured the Microscopic World

Throughout his lifetime, Leeuwenhoek remained elusive about the techniques he used to create his lenses. Somehow, he knew he had devised methods superior to those used by other microscopists. He knew of Jan Hudde's and Jan Swammerdam's apparatus and is thought to have seen Robert Hooke's *Micrographia*. Nonetheless, a queue of notables from Delft and beyond visited him and used the lenses he had created to view the phenomenon he had observed using devices like that shown in the *Figure 5.12.*.

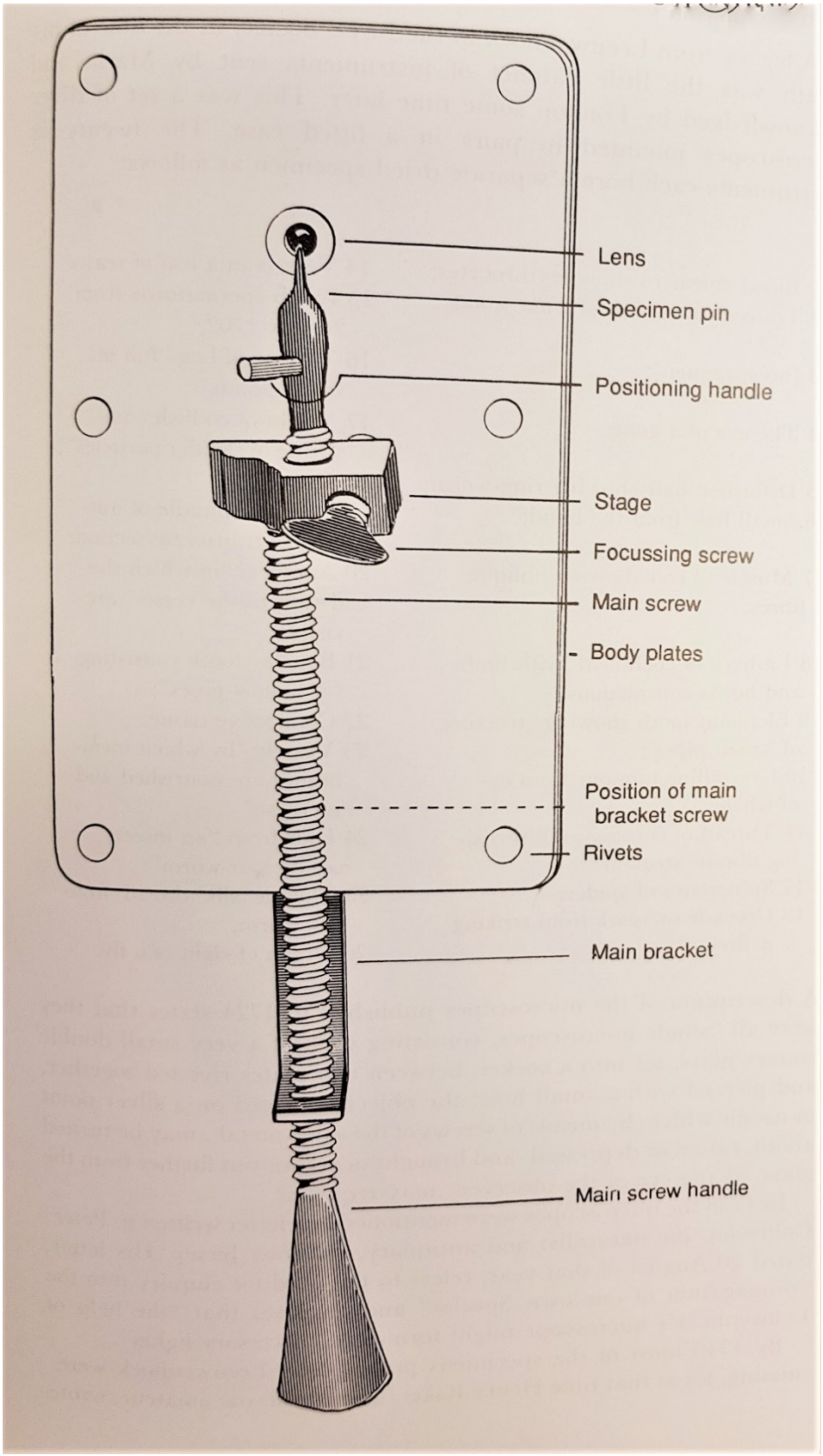

Figure 5.12. *Diagram of a typical Leeuwenhoek microscope (Ford, 1991).*

Leeuwenhoek created two initial measurement models to evaluate his microscopic observations and to assist the Royal Society in evaluating his work. The first model employs a circle Leeuwenhoek describes as "ABGC" with an axis "AG." The circle contains two circles D and E and a point F (*Figure 5.13.*). Having defined his circle ABGC, he then assigns identities. The big circle should be understood as a sand grain with a diameter of about 400 micrometers (1 micrometer, micron, or μm equals 1 x 10^{-6} meters). Within the compass of that imaginary sand grain, he can visualize an animalcule with a size of circle D, which is $1/12^{th}$ the diameter of the large circle. Using geometry provides Leeuwenhoek with a comparison: the large circle has 1,728 times the volume as circle D, or the sand grain is much larger than this initial animalcule. He then suggests that circle E represents a second type of animalcule; this creature is between $1/4^{th}$ and $1/5^{th}$ the size of animalcule D (Leeuwenhoek often uses the more conservative estimate in his models, in this case, that animalcule E is $1/4^{th}$ the size of D). Geometry provides that D is 64 times larger than E; therefore, 110,592 animalcule E can fit within circle ABGC (64 x 1,728 = 110,592), a moderately sized sand grain. However, Leeuwenhoek has also seen an animalcule of size F with one of his lenses. Animalcule F is only $1/10^{th}$ the size of animalcule E, so it would take 1,000 animalcule F to be as large as animalcule E. The multiplication is as clear as before: 1,000 x 64 x 1,728 = 110,592,000 animalcules would fit in the circle ABGC. To ensure the reader understands, he then works the puzzle from the smallest animalcule up through animalcules E and D to the sand grain, which could fit over 110 million creatures (Leeuwenhoek, 1939a, pp. 335–341).

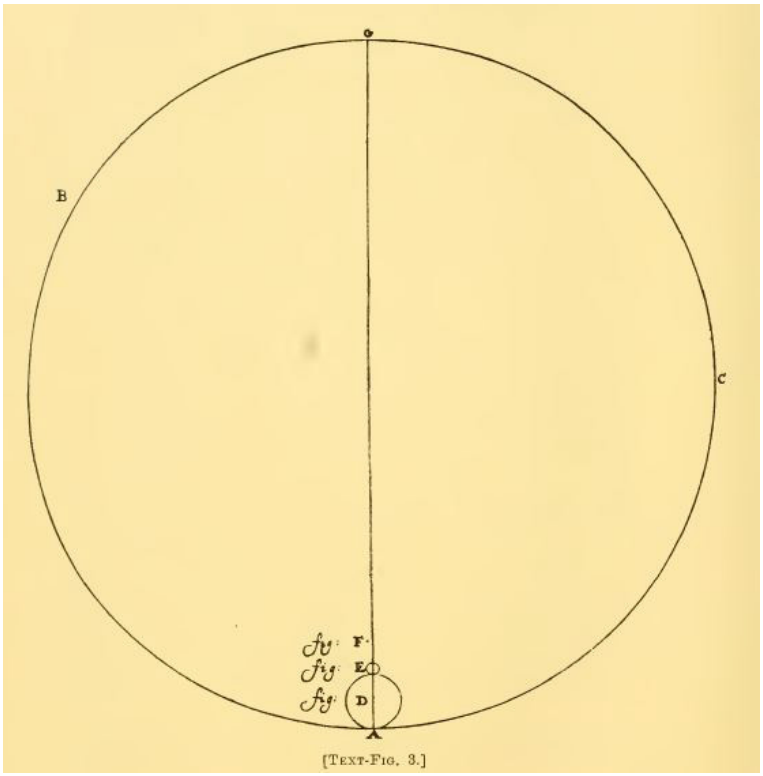

Figure 5.13. *Circles illustrating proportions (a pen-and-ink drawing by Leeuwenhoek, Letter 65) (Leeuwenhoek, 1939a).*

Having worked through the model top-to-bottom and bottom-up, Leeuwenhoek then assigns circle ABGC a size equivalent to a coarse sand grain (about 860 µm) such that the axis AG is 20 times the axis of D. In this model, he states that D has five times the axis of E (rather than four times), but that E has ten times the axis of F. Still using this simple method, he calculates that over 1 billion animalcules could fit within the larger grain.

Dr. Schierbeek provides a footnote regarding the micrometry Leeuwenhoek conjured in his model. Dr. Schierbeek concludes that animalcule F is smaller than one micrometer (µm) in diameter, a believable size for some of the creatures Leeuwenhoek described. Microbiologists later identified the animalcules and assigned genera and species to the creatures they saw. Additionally, the University of Utrecht in 1933 possessed an original Leeuwenhoek microscope and achieved resolution of objects of about one µm in diameter

(van Cittert, 1933). For his 1981 publication, Zuylen undertook a comprehensive optical examination of eight Leeuwenhoek lenses and determined that measured resolving power ranged from 1.35 to 4 μm for seven of the lenses, slightly above the measurement made by Cittert (Zuylen, 1981, pp. 309-28), although some of the measurements had been conducted previously by earlier scientists (Rooseboom, 1939; van der Star, 1953). Until recently, light microscopes were limited by diffraction to resolutions of about 0.25 μm (Czajkowsky et al., 2015, pp. 119–125). In 2016, Zuidervaart and Anderson made the critical point that Leeuwenhoek's observations were linked inextricably to the samples, sampling tubes, pins, adjusting screws, and other devices that Leeuwenhoek designed and created to fix the objects of interest within the limited focal plane of his lenses (Zuidervaart & Anderson, 2016, p. 15). It is thought that drove Leeuwenhoek's creation of over 500 lenses: many were made with a specific observational target in mind. In 1739, H. Baker disputed this (Wilson, 1997). However, the current thinking is that most of Leeuwenhoek's lenses were discarded or destroyed when, after purchasing them at estate auctions, their various new owners found that they did not have the patience or eyesight to replicate Leeuwenhoek's observations.

Perhaps Leeuwenhoek's most straightforward method for measuring the invisible involved estimating the number of animalcules that could fit into a cube 1 inch (2.61 cm)[16] on each side (*Figure 5.14.*). In this exercise, Leeuwenhoek also affirms his comparison model—his standardization method focused on sand grains. In a letter dated 20 May 1679 to Constantijn Huygens, Leeuwenhoek states the number of sand grains that fit in an inch and that the volume of sand grains in a square inch amount to 6,400, thus giving 512,000 sand grains in a cubic inch. From there, Leeuwenhoek indicates that he "judges that three or four hundred of the smallest animalcules, laid out one against another, would reach to

[16] The van Leeuwenhoek inch was 2.76% larger than the current inch, which is 2.54 centimeters.

the length of an axis of a common grain of sand." He uses the more conservative of these estimates—300 of the smallest animalcules—to build his argument for determining the size of the animalcules. Today, we might do both calculations and develop some statistical rigor from several observations, but Leeuwenhoek uses what is familiar and knows he must persuade the skeptical.

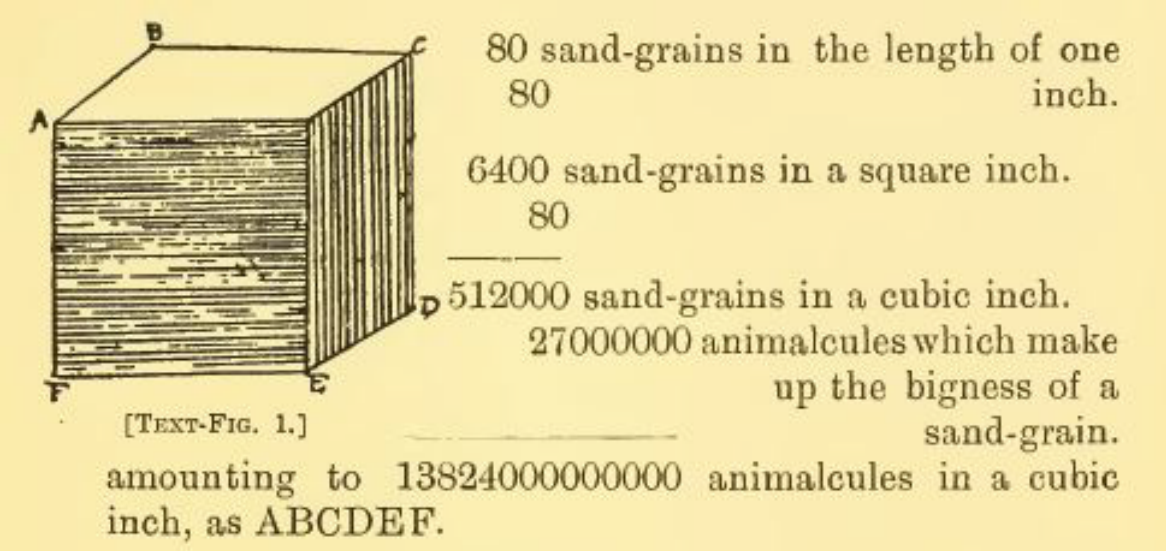

Figure 5.14. *A cubic inch as drawn by Leeuwenhoek, along with his calculations, in a 20 May 1679 letter to Constantijn Huygens (Dobell, 1932, p. 189).*

His model suggests that the volume of a single sand grain could contain 300^3, or 27 million of these "smallest animalcules." This number could easily be somewhere between 300^3 and 400^3, or 64 million, but perhaps he was correct to use the more conservative three hundred; this gives a size estimate for this class of microbe that has stood the test of more sophisticated inquiries by current microbiologists. Using 300^3, Leeuwenhoek arrives at a stunning estimate that 13,824,000,000,000—almost 14 trillion animalcules—would fit in a cubic inch (Dobell, 1932, pp. 188–189).

While Leeuwenhoek chose the more conservative number, which gave him estimates that comport with our present understanding of his lenses' resolving power, some luck was

involved in his calculations. Without other tools, his use of estimation and approximation for these hitherto unknown creatures seems like an appropriate approach to the problem he faced.

He provides a pedagogical example to his audience by asking his readers to do the following:

This number of animalcules is so great, that if one had as many sand-grains, of the bigness aforesaid, then one could lade with them more than 108 of our ordinary sandlighters; that is, reckoning one *schagt* of sand (which is 144 cubic feet)[17] to every lighter (Dobell, 1932, p. 189).

Comparison models are often used in introductory science classes to help students visualize small objects. While Leeuwenhoek's comparison is unlikely to have been the first size comparison, it provides an example of the comparative sizes of his animalcules when sized up to grains of sand.

An early example of his method is found in his comparison of red blood cells ("red Globuls of the Blood") as being "25000 times smaller than a [fine] grain of sand" (Diez-Silva et al., 2010; Leeuwenhoek, 1939a, p. 103). From Table 7.1, we can recall that a fine sand grain is estimated to be about 260 μm in diameter. The most critical element of this comparison is that this estimate gives us a measurement of red blood cells as about 8.5 μ in diameter, within the range of sizes found for red blood cells using current methods (Diez-Silva et al., 2010, pp. 382–388). Leeuwenhoek included capillary tubes of blood with this letter and a description of procedures so that the Royal Society might replicate his findings.

[17] 108 sandlighters would hold 15,552 cubic feet of sand.

Leeuwenhoek often invited peer review, which is remarkable for a person without scientific training.

In Letter 26, written 9 October 1676, Leeuwenhoek compares a protozoan to a full-grown louse eye—about 50 µm broad and 60 µm long[18]—and the protists 1,000 times smaller than the louse eye. Dobell thought the protozoan was a *Monas* species, perhaps *Monas minima*, but *Bodo parvus* or *Bodo minimus* might be possible. Letter 26, Page 75, says that

"…ten hundred thousand [1 million] of these little Creatures do not equal an ordinary grain of Sand in bigness: And comparing them with a Cheese-mite (which may be seen to move with the naked eye) I make the proportion of one of these small Water-creatures to a Cheese-mite, to be like that of a Bee to a Horse: For, the circumference of one of these little Animals in water, is not so big as the thickness of a hair in a Cheese-mite" [the hair of a cheese-mite is about 1-3 µ thick[19]] (Leeuwenhoek, 1939a, pp. 73–75). If we are skeptical of Leeuwenhoek's ability to see very small objects, current verification of these objects as being in the low µm range should diminish our skepticism.

5.9 The Historical Basis of Leeuwenhoek's Micrometry

One of Leeuwenhoek's favored size comparisons is to the millet seed. It is unknown why he chose this type of seed; except they were small and readily available. Many of his audience likely knew the relative size of various seeds. Seeds are one of the oldest units of measurement, having a history traceable to India, China, and the Americas. Common grains and seeds used included rice, corn, millet, barley, and carob (our word "carat," a weight

[18] Dr. Heringa [H.] provides this size comparison (*vide supra* footnote 8)
[19] Dr. Heringa [H.] provides this size comparison (*vide supra* footnote 8)

value of 200 mg, comes from “carob”). From *The Laws of Manu*, Robert Crease provides the following passage (probably 2nd or 3rd C. CE):

The very small mote which is seen when the sun shines through a lattice, they declare to be the least of all quantities and to be called a trasarenu (a floating particle of dust). Know that eight trasarenu are equal in bulk to a likshâ (the egg of a louse); three of those to one grain of black mustard (râgasarshapa) and three of the later to a white mustard-seed. Six grains of white mustard are one middle-sized barley-corn and three barley-corns one krishnala (a retti or rati seed) (Crease, 2011, p. 23).

Chinese documents dating to the 2nd or 3rd century B.C.E. describe the use of millet seeds to define the *chi*—a length measurement that varied from 16 to 24 centimeters in its early history—and capacity measurements. The method of standardization for the *chi* settled on the length of a *huangzhong* pitch pipe, then standardized to the number of millet seeds required to make up the length of that musical standard and the number of seeds required to fill the pipe. This standard was documented in the *Hansu*, which meant *Historical Documents of the Han Dynasty*. The *chi* was equivalent to ninety millet seeds placed end-to-end, while 1,200 seeds filled the *huangzhong* pitch pipe. The number of millet seeds varied depending on their size, but in the *Hansu,* it was standardized as stated. It is worth mentioning that this convergence of length and capacity measurements with a pitch pipe also meant that the measurements were tied to courtly music and rituals, as well as the music interval systems (Crease, 2011, pp. 43–44).

Leeuwenhoek uses the millet seed and the green pea as alternative comparators to help his readers understand the number of animalcules he sees in water. In his 23 March 1677 letter to the Royal Society, he states:

My division of water, and my counting of the animalcules, are done after this fashion. I suppose that a drop of water doth equal a green pea in bigness; and I take a very small quantity of water, which I cause to take on a round figure, of very near the same size as a millet-seed. This latter quantity of water I figure to myself to be the one-hundredth part of the foresaid drop; for I reckon that if the diameter of a millet-seed be taken as 1, then the diameter of a green pea must be quite 4½. This being so, then a quantity of water of the bigness of a millet-seed maketh very nearly 1/91 part of a drop, according to the received rules of mathematicks (as shown in the margin). This amount of water, as big as a millet-seed, I introduce into a clean little glass tube (whenever I wish to let some curious person or other look at it). This slender little glass tube, containing the water, I divide again into 25 or 30, or more, parts; and I then bring it before my microscope, by means of two silver or copper springs, which I have attached thereto for this purpose, so as to be able to place the little glass tube before my microscope in any desired position, and to be able to push it up or down according as I think fit (*Figure 5.15.*) (Dobell, 1932, p. 169).

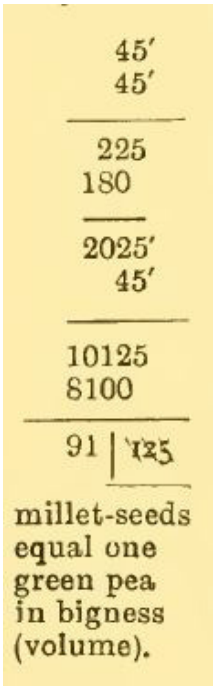
45′
45′
225
180
2025′
45′
10125
8100
91 | 125
millet-seeds
equal one
green pea
in bigness
(volume).

Figure 5.15. *Calculations by Antoni van Leeuwenhoek of the number of millet seeds equaling one "green pea in bigness" (volume)" (Dobell, 1932, p. 169).*

Leeuwenhoek goes on to say that the millet-seed comparator allows him to estimate the number of animalcules in a drop of water—about 4½ millet seeds in volume—which contained an estimated 2.7 million animalcules (Dobell, 1932, p. 170).

Why didn't Leeuwenhoek use the standard measurement system for microbiology and all other scientific disciplines? The metric system, the basis of the SI (Système International d'Unités) system, had not been created. It took the Enlightenment era, the French Revolution erupting in 1789, and scientific meetings in Paris in September and on 28 November 1798 to propose an alternative to the patchwork of measurement systems tied to tradition and the monarchy. A committee of scientists in the late 17th C would take the first steps toward standardization. The pan-European committee of scientists further studied the metric proposals in the first half of 1799, and the system would be ratified by the Commission on Weights and Measures appointed by the Academy of Sciences on 22 June 1799 (Crosland, 1969, pp. 227–230). It would take another 75 years to adopt the metric system and make significant headway (Cox, 1958, pp. 358–379; Cox, 1958, pp. 358–379). As Frederick A.P. Barnard said in his address to the State University of New York at Albany on 1 August 1871: "I know the strength of early associations and the power of rooted habits; I know how fondly men will hug the evil which is familiar and reject the good that is strange" (Barnard, 1872, p. 70).

Leeuwenhoek's comparisons between familiar items and previously unobserved and undocumented materials and creatures permeate his letters. Whether advances in navigation, surveying, ballistics, and other measurement systems using micrometry directly or indirectly inspired Leeuwenhoek is still unknown.

What we witness in the evolution of measurement from the ancient era through the development of micrometry to Leeuwenhoek's measurements is the creation and convergence of terms and methods towards a consistent metrology.

It is difficult to know what drove Leeuwenhoek to look deeply into drops of water or mold growth on a book. It is also challenging to know what inspired his nascent metrology. Indeed, he was raised in an environment that valued weaving, whether baskets or cloth. He was repeatedly placed in, or selected, environments where he learned more than expected, particularly in his passage of the master exams for cloth merchant. He was skilled at his business and impressed his fellow Delftians to the extent that he gained positions of responsibility in local government as a surveyor, *wijnroeier*, and burgher.

An exciting aspect of Leeuwenhoek's observations is that he examined everything from crystal and mold growth to the reproduction of vinegar eels. It did not matter to him because he was not raised in a specific discipline with its compartmentalization and prejudices. If there was something to view, he found it and viewed it with his tiny lenses. If it moved, he wrote about it. If it grew, he described it. If it was dissolved, he watched it disappear, then watched it recrystallize out of the cooling water. If he managed to form a salt of some compound in circumstances that controlled exposure to air, he would observe its color changes (i.e., oxidation) when he allowed air to interact. He sometimes returned to the object later if he had documented something in his early observations. He observed it again, sometimes changing the descriptions and the drawings he made to reflect his improved experience, knowledge, skills, or improvements with his lenses.

Although he had no formal scientific training, he was generally accepted by the best European scientists[20] during and after his 91 years of life. His methods were models for

[20] He had some critics.

future microbiologists, protozoologists, and microscopists to build procedures and follow him. His willingness to improve his observations based on a better lens or more experience with the objects he observed serves as a model for scientists doing what they can with the tools they have to determine the nature of the world around us.

Many of Leeuwenhoek's observations and measurements involved objects somewhat visible to the unassisted eye. His microscopes provided an improved sense of their structure. The most remarkable of his observations were of the invisible world, which had not been adequately imagined before he saw, described, measured, and drew his animalcules.

As Nick Lane, a biochemist from University College London, once wrote, "More than being the first to see this unimagined world of 'animalcules,' he was the first even to think of looking—certainly, the first with the power to see" (Lane, 2015, p. 1).

5.10 Acknowledgements

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CHAPTER 6 | Antoni van Leeuwenhoek: Defining proportion in the microscopic realm

6.1 Abstract

While most microscopists know of Antoni van Leeuwenhoek's contributions to single-lens microscopy and his historical observations of bacteria and protozoa, the methods he developed to measure these and other previously unseen structures in the natural world may be underappreciated. These methods and the overall extent of Leeuwenhoek's observations are generally unknown to the lay audience, even for those who follow the history of natural philosophy and science. As with the historical development of other technologies and scientific disciplines, the roots of Leeuwenhoek's micrometry tap into innovations in astronomy and commerce. Astronomers and navigators have searched for and defined methods for measuring the relative positions of objects in the sky. These, in turn, advanced methods for land surveying and other areas of commerce. Leeuwenhoek's measurement of the microscopic world may have grown out of his surveying interest, drapery business, or other commercial experiences. He compared animalcules, blood cells, fat globules, veins and arteries, insect, plant, and mineral structures to a range of conventional, minuscule objects such as fine and coarse grains of sand, millet grains, human hairs, and other items. Examples of Leeuwenhoek's comparisons will be provided.

Keywords: Leeuwenhoek, History of Microscopy, Micrometry, Measurement, 17th Century

6.2 Introduction

Antoni van Leeuwenhoek's fascinating life starts with the fact that he built a brilliant body of work in natural philosophy despite little formal education. What education he did receive was probably due to early schooling in Warmond, followed by mentoring from his uncle, an attorney and town clerk in Benthuizen. His education was followed by a successful apprenticeship from 1648 to 1654 with William Davidson in Amsterdam. An international merchant, Davidson granted Leeuwenhoek power of attorney over Davidson's affairs in 1653 while he was sidelined during the First Dutch War. During his apprenticeship, Leeuwenhoek was undoubtedly trained in using a draper's glass, a type of magnifying glass. It may be that he also learned to use quadrants and telescopes during that time. This time may have initiated his interest in optical instruments and fascination with the fine structure of our world. However, he would eventually see more of it through his microscopes than anyone imagined was there.

Leeuwenhoek returned to Delft in 1654 after completing his apprenticeship. He started his business at the *Hippolytusbuurt* address, which served as his home and laboratory until he died in 1723. Leeuwenhoek was a draper and haberdasher in Delft commerce. He would have needed to control the quality of his merchandise. This gave him the incentive to examine the structure of threads. Although the cloth seems well-ordered to the untrained eye, it has messy structures — frayed threads, inconsistent diameters, and fuzz balls. These disordered nuances might have piqued his curiosity and made him wonder whether an improved glass would help his work.

In 1660, he was appointed Chamberlain to the Sheriffs of Delft, a position that likely had no impact on his microscopy but may have assured a steady income, always desirable for the natural philosophers of the time. The position may also have increased his

interactions with others with knowledge of mathematics and medicine (L. Robertson et al., 2016b, pp. 24–29).

His interactions with highly educated Dutch citizens may have led to learning about the single-lens microscopes used by Johannes Hudde and Jan Swammerdam. Hudde's use of a single-lens microscope is thought to have started around 1660. In 1699, Leeuwenhoek wrote: "As for myself, although glasses of extreme smallness were made by me already about 40 years ago, they were seldom used by me" (Leeuwenhoek, 1989, p. 297). That would have placed his initial lens crafting at about 1659. It is unknown whether he was motivated to make them due to word-of-mouth information passed indirectly from Hudde or Swammerdam or his consideration of the draper's lens. Swammerdam wrote that he was certain Leeuwenhoek "initially followed Hudde's invention" (Zuidervaart & Anderson, 2016, pp. 260–261). Whatever Leeuwenhoek did with lenses in 1659, they became an obsession as years passed.

6.3 Sources of Proportions and Micrometry

A stunning aspect of Leeuwenhoek's observations is his comparison of tiny objects with objects that his colleagues in The Netherlands and at the Royal Society might have found familiar. His relatable comparisons used a combination of novel micrometry and defined proportions.

Aside from counting or enumerating objects, defining proportions might have some of the most ancient history in mathematics. Enumeration came from knowing the number of people or the number of trees that yielded food. Proportion might have come from the number of children from one mother or the number of fruits from a single tree. Proportion is almost inextricable from counting, as a proportion is established in comparing counts of two similar or dissimilar entities. As such, proportion provides a relatable way of

understanding numbers or sizes; a gatherer might carry 40 of a small fruit but only 10 of a larger variety. Leeuwenhoek understood that proportions could help his colleagues and correspondents understand what he saw through his tiny lenses.

Leeuwenhoek's micrometry—his novel comparison of tiny structures to known objects—may have had roots in several sources. The influences may be conceived of as follows:

1. Commercial and cultural: From some time before the current era (~400 BCE) to ~200 C.E. Innovators included China and India; weight tables using dust motes, millet, and other grains and beans;

2. Mathematics: ~3000 BCE to 200 CE; from Mesopotamian, Egyptian, and Greek mathematics; Known innovators in measurement include Pythagoras, Euclid, and Archimedes;

3. Navigation, astronomy, surveying, and ballistics: These disciplines were developed from the mid-16th century to the mid-17th century. Innovators in micrometry include Pedro Nunes, Pierre Vernier, Lucas Brunn, and William Gascoigne.

Whether he drew inspiration from ancient commercial measurements, such as the comparison of dust motes and various seeds from India and The Laws of Manu or Manu-smriti, is unknown. However, this or a similar system likely infiltrated global commerce as trading expanded. The Manu-smriti is thought to have originated sometime between 200 BCE and 200 CE. Among its many applications is weighing gold and silver for commercial purposes. The smallest unit in the system described in The Laws of Manu is the dust mote or trasarenu. Eight dust motes were said to be the size of a louse egg, a comparator that

Leeuwenhoek used in some instances. Other comparators are described in the following table (Manu, 2016, pp. 8.132-8.134) (*Table 6.1.*):

Table 6.1. *Unit conversion table from the Manu-smriti.*

Smaller unit	***Larger unit***
8 trasarenu (1 dust mote ~ 83 micrograms)	1 likshâ, louse egg, or râgasarshapa
3 likshâ (~0.67 milligrams (mg) each)	1 black mustard seed (~3 mg each)
3 black mustard seeds	1 white mustard seed (9 mg)
6 white mustard seeds	1 middle-sized barley corn (~50 mg)
3 barley corns	1 reti seed or krishnala (~150 mg)
5 krishnala	1 masha bean (a type of lentil (~750 mg))
16 masha	1 suvarna (unit of gold (~12 grams))

While we might consider the dust mote weightless, these laws evaluated them as weighing about 80 micrograms each. One cannot envy the mote-collector his position in the marketplace.

A less likely source for Leeuwenhoek's proportional comparisons is the Chinese use of millet seeds to calibrate the fundamental tone of the *huangzhong* or "yellow bell" pitch pipe. While it is improbable that Leeuwenhoek knew of this calibration method, he used millet seeds in his comparisons, and the Chinese system illustrates the general concept of establishing proportions between small and larger items. The system, refined in the 2nd or 3rd century BCE, used 90 millet seeds placed end-to-end to define the chi, which also defined the length of a yellow bell pitch pipe made from bamboo. While millet seeds vary in size, the system also determined a standard size for the seeds used in this measurement. Once the length of the pitch pipe was defined, the pipe's capacity was further defined by ensuring that 1200 seeds filled the pipe(Crease, 2011, p. 23).

Another example of comparing seeds and commercial standards is the establishment of the carat, currently standardized to 200 mg but initially, a unit based on a localized standard for carob seed weight (Turnbull et al., 2006).

Non-standardized comparisons were — and are still — used in various types of commerce and some activities dominated by tradition. Horse racing still uses the furlong, while ships may still use the fathom, league, and knot. The adoption and refinement of the metric system, starting in the 19th and continuing into the 21st century, has had an enormous impact on international commerce and the conduct of science.

Leeuwenhoek's practice in his letters was to cite a proportion between the objects he saw and those more well-known to his audience. While he probably did not derive this directly from the Manu-smriti, many such conversion tables existed in localized markets worldwide. Leeuwenhoek compared anatomical and plant structures to human hairs, for instance, or his animalcules — previously unseen creatures now known to be protozoans and bacteria — to millet seeds and grains of sand.

The most apparent Leeuwenhoek influence on developing proportions between the micro- and macroscopic worlds was his use of "the common rules of mathematics." His use of various geometrical figures, theorems, and calculations originated primarily with Euclid and Archimedes. Leeuwenhoek's use of math is discussed in an article by E. J. Dijksterhuis in Volume 3 of the Collected Letters (Leeuwenhoek, 1948, pp. 443–453).

Dr. Dijksterhuis does not suggest a possible connection to cartesian geometry. However, we know that Johannes Hudde studied with Frans van Schooten, a Leiden professor of mathematics who translated Descartes' *La Geometrie* into Latin. Schooten also advanced the practice of cartesian and analytical geometry with his research group, including Christiaan Huygens, Constantijn Huygens's son. We also know that Descartes had

recommended Schooten to Constantijn Huygens as a math tutor to his son Christiaan. Hudde was included as an appendices author in a two-volume edition of the translation published between 1659 and 1661 (O'Connor, J.J.; Robertson, 2009). While there is no evidence linking Hudde and Leeuwenhoek directly, we know that Leeuwenhoek wrote Constantijn Huygens in April 1674 and referred to an earlier visit to the senior Huygens home in Zuilichem (Leeuwenhoek, 1939b, p. 67). They remained correspondents for a decade afterward. It may be that cartesian geometry was part of everyday discourse among members of the community of natural philosophers and that Leeuwenhoek benefited from his friendship with the senior Huygens, the preeminent poet and composer of the Dutch Golden Age. The senior Huygens also supported Descartes's efforts around 1638 to build an apparatus for grinding hyperbolic lenses so he may have been interested in Leeuwenhoek from that perspective (Nolan, 2016, pp. 380–381).

There is another aspect to Leeuwenhoek's proportional comparisons that seems cartesian. Leeuwenhoek imagined his various animalcules into the three dimensions they might occupy in a grain of sand or a millet seed. Leeuwenhoek's approach resembles the notion of the close-packing of spheres or other objects in a cube or pyramid, later becoming important in solid-state chemistry. Stacked cannonballs are an example of close-packing of spheres.

This idea is shown in Leeuwenhoek's illustration of a cubic inch and calculation of how many animalcules might fit, i.e., 13,824,000,000,000 or 13.8 trillion (*Figure 6.1.*).

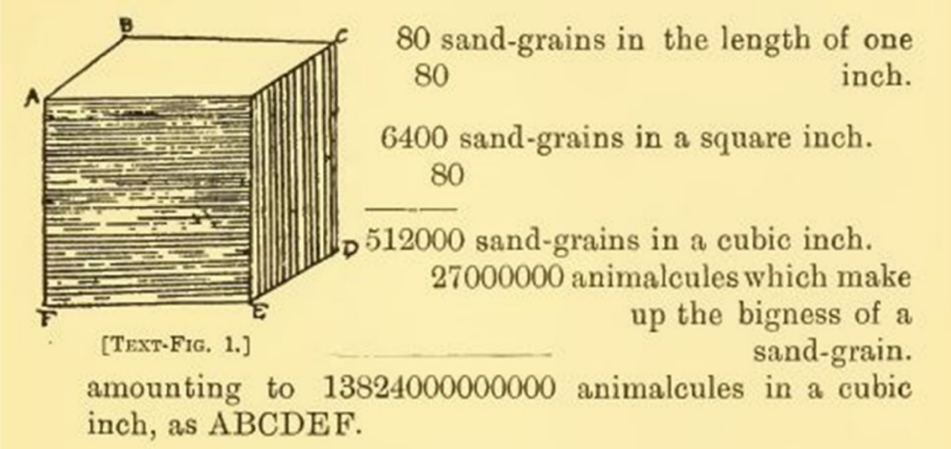


Figure 6.1. *Leeuwenhoek calculates the number of animalcules that could fit in a cubic inch (Dobell, 1932, p. 189).*

The more formal influence for Leeuwenhoek's micrometry probably started with his apprenticeship with William Davidson, who might have introduced him to the quadrant, which, along with the alidade — its moveable arm — the astrolabe, and time measurement, helped navigators find their position and direction during voyages. Micrometry helped subdivide the sky into small, reproducible parts that could serve as reference points. It did so by establishing a set of proportions, i.e., if the marks on the alidade are *a* and *b* and the objects in the sky are *x* and *y*, then our location on earth is *c* and *d*, an approximate set of coordinates on the map. The finer the divisions possible using the alidade, the more precise the relative positions of stars, planets, moons, and the corresponding location on earth.

Between 1660 and 1669, Leeuwenhoek studied surveying and was licensed to practice in 1669. He would have become familiar with the *nonius*, a measurement system used with quadrants and other surveying tools. Pierre Vernier's 1631 publication cited Pedro Nunes, the Portuguese mathematician who, in 1573, developed the *nonius* system to help Portuguese navigators determine their location and direction using star positions. Vernier and others referred to these subdivisions as *nonius* well into the 19th century. The

development of Nunes' system and other early micrometric techniques is covered in depth in *Antoni van Leeuwenhoek and measuring the invisible: The context of 16th and 17th century micrometry* (I. M. Davis, 2020b).

We have no exact sense of what influenced Leeuwenhoek's drive to measure and compare the objects he observed with known objects. It is a matter of conjecture that his exposure to the earlier types of micrometry led him to communicate as he did. We know that something drove him to compare these previously unseen objects and objects that were likely to be familiar to his correspondents. The diligence required for Leeweunhoek to make these observations and then imagine them into the shapes and volumes of fine or coarse grains of sand or a millet seed makes them extraordinary.

6.4 Examples of Proportional Comparisons

This discussion brings us to the seventh decade of the 17th Century. In a letter dated 28 April 1673, Leeuwenhoek wrote to Henry Oldenburg at the Royal Society. Although the original letter is lost, we rely on Oldenburg's translated summary in *Philosophical Transactions*. Leeuwenhoek described structures of mold, bees, and lice that others had described previously.

He also makes his first size comparison. He describes the louse's sting as being "five and twenty times less than one single hair" (Leeuwenhoek, 1939b, p. 35). Elsewhere, Leeuwenhoek states that "20 hairbreadths are equal to 1/30th of an inch, which works out to 600 hairbreadths in an inch," defining a single hair as approximately 44 micrometers in thickness (Leeuwenhoek, 1948, p. 59). An inch in Leeuwenhoek's time and locale was equal to 2.61 centimeters. If the assumptions about Leeuwenhoek's hair thickness are correct, the louse's sting is approximately 2 microns thick. Several hair diameters are listed in Volume 1 of Alle de Brieven, the broadest being a hair from Leeuwenhoek's beard,

which he estimated to be 100 microns (Leeuwenhoek, 1939b, p. 379). Even if the hair thickness is taken as 100 microns, this would give a thickness of the louse's sting as 4 microns. These are close to sizes determined by current methods of microscopy. Furthermore, the similarities between human hairs and fabric threads are apparent.

In his 15 August 1673 letter, Leeuwenhoek expanded on the observations summarized in *Transactions* and sent along figures drawn under his supervision. Leeuwenhoek finds another opportunity to use hair as a comparator when examining the "pipes" or channels in coniferous wood. In this comparison, he indicates that the pipes are "about the thickness of a hair" (Leeuwenhoek, 1939b, p. 49). Later in the same letter, he describes the opening of a “Glass-pipe” or capillary tube as having “a hole as small as a hair” (Leeuwenhoek, 1939b, p. 59). In his letter of 1 June 1674, Leeuwenhoek describes separating the filaments in a cow’s flesh and discovering that any of the individual filaments were, again, “some 25 times thinner and finer than a hair” (Leeuwenhoek, 1939b, p. 111). It bears mentioning that Leeuwenhoek perceives hairs as composed of globules, an object descriptor that varies in size, shape, and arrangement and is present throughout his letters.

Leeuwenhoek initiates his comparisons with sand in a 7 September 1674 letter by comparing “English Earth” to a grain of sand and finding that the earth is “[a hundred] thousand times smaller than a common grain of sand, after Geometrical computation, by which the axis of a grain of Sand is many hundred times bigger than the axis of one of these particles which compose the said English Earth” (Leeuwenhoek, 1939b, p. 161). By English Earth, Leeuwenhoek means a type of clay and discusses other types of clay and their comparison to grains of sand. Not surprisingly, the clay is formed of globules.

In his 1 June 1674 letter to Henry Oldenburg, Leeuwenhoek gives a proportion describing red blood cells and sand grains: "The red Globuls of the Blood I reckon to be

25000 times smaller than a [fine] grain of sand" (Leeuwenhoek, 1939b, p. 103). This comparison is extended in a letter of 25 July 1684: "I determined that the perfect globules, which colour our blood red, are of such a size that if 100 of them were put end to end they would not attain the diameter of a coarse grain of sand, so that a 1.000.000 blood-globules are the same size as one coarse grain of sand" (Leeuwenhoek, 1952, p. 267). These eyeball estimates of the size of a red blood corpuscle give a size within one micron of an average human red blood cell measured by current methods. It is important to note that what Leeuwenhoek imagines here is that the blood cells are packed together rather than suspended in serum. His count is not a concentration of red blood cells in fluid but gives an estimate of size by way of proportion to grains of sand, both fine and coarse. It is almost a close-packed imagining of red blood cells. Marcello Malpighi and Jan Swammerdam are prioritized for seeing red blood cells before Leeuwenhoek, but neither gave any information regarding their size, relative or otherwise.

Leeuwenhoek's first mention of algae and animalcules comes in a 7 September 1674 letter to Oldenburgh. Near the end, he comments that the "green streaks spirally ranged" were about the "thickness of a man's hair on his head" (Leeuwenhoek, 1939b, pp. 163–165). Clifford Dobell, writing about Leeuwenhoek's work in 1932, thought the "green streaks" were a type of Spirogyra — "the earliest recorded observations on this organism"(Dobell, 1932, p. 110). Of course, Leeuwenhoek acted as a diligent observer and did not know the identity of the "green streaks," only that they had different shapes and behaviors, which he reports as honestly as he knows how.

He writes that he sees an…

abundance of little animals, some of which were roundish; those that were somewhat bigger than others, were of an oval figure: On these latter I saw two leggs near the head,

and two little fins on the other end of their body: Others were somewhat larger than an Oval, and these were very slow in their motion, and few. These animalcula had divers colours, some being whitish, others pellucid; others had green and very shining little scales: others again were green in the middle, and before and behind white, others grayish. And the motion of most of them in the water was so swift, and so various, upwards, downwards, and roundabout, that I confess I could not but wonder at it. I judge that some of these little creatures were above a thousand times smaller than the smallest ones, which I have hitherto seen in [the rind of] chees, wheaten flower, mould, and the like (Leeuwenhoek, 1939b, p. 165).

Here, in his first sighting of microscopic creatures, he gives an initial sense of their proportion to cheese mites, which vary in size from 220 to 600 microns.

In a 9 October 1676 letter to Oldenburg, Leeuwenhoek wrote about the many types of small creatures he had seen, enumerated them, and described their shape and motion. He gives their proportion to reference objects as follows: "… so small, that I was not able to give them any figure at all. These were a thousand times smaller than the eye of a [full-grown] Louse: For I judge, the axis of the eye of such a Louse to be more than ten times as long as the axis of any of the said little creatures" (Leeuwenhoek, 1941, p. 73), with the eye of a louse being about 50 by 60 microns. In another passage, he uses the louse hair as the comparator: "…some that were 3 or 4 times as long as broad; but their whole thickness did, in my estimation, not much exceed that of the hair [covering the body] of a Louse" (Leeuwenhoek, 1941, pp. 91–93) (*Figure 6.2.*). The hairs on a louse vary from about 3 to 9 microns.

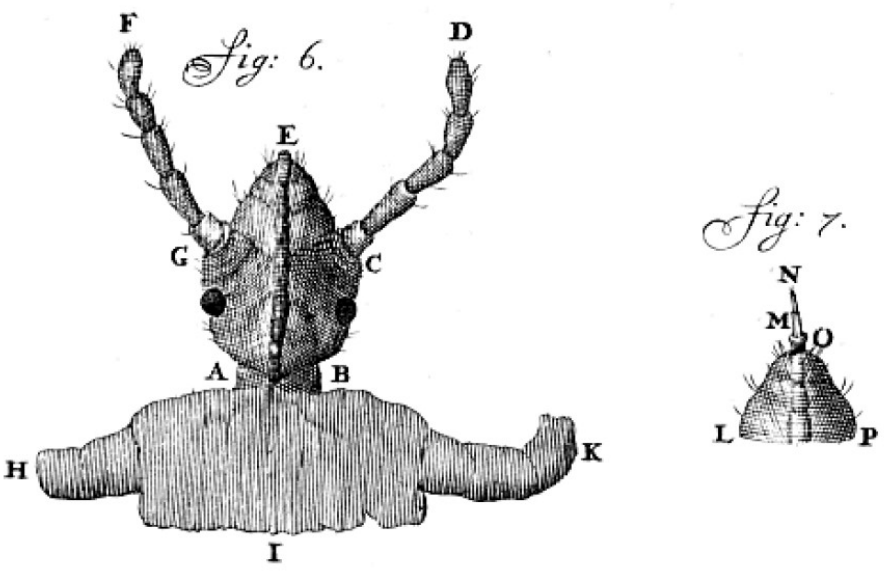


Figure 6.2. *Illustration of the head and tail of a louse (Leeuwenhoek, 1976, p. 498).*

In this letter, Leeuwenhoek observes bacteria, although no one would know them as such for centuries to come. His description, though, includes the following proportion: "…if 100 of them lay [stretched out] one by another, they would not equal the length of a grain of coarse Sand; and according to this estimate, ten hundred thousand of them could not equal the dimensions of a grain of such coarse Sand" (Leeuwenhoek, 1941, p. 95). Here, he goes back to the louse eye comparison: "These round animalcules I imagine to be more than 50 times smaller than the eye of a louse" (Leeuwenhoek, 1941, p. 113), which would make these creatures about 1 micron each.

Leeuwenhoek also estimates how many creatures he has seen in a sample, making a comparison with sand but not in terms of their size. He writes: "…I found great plenty of them in one drop of that water, [8 or 10,000 more or less hardly mattering], and they looked to my eye, through the Microscope, as common sand doth to the naked eye" (Leeuwenhoek, 1941, p. 105).

There is a cautious element to what Leeuwenhoek relates to the Royal Society as if he understands that his observations might beggar their imagination as they might have his own if he had not repeatedly observed them. For instance, he writes, "And even if I said that there were a hundred thousand animalcules in one drop of water which I took from the surface of the water, I should not err. Others, seeing this, would estimate the number at quite ten times more, of which I have instances, but I give the lowest numbers" (Leeuwenhoek, 1941, p. 115). He does not say how he arrives at these estimates in the letters. If he used quantitative methods here, he might provide different numbers, but somehow, his estimating approach works well. He might have done well to adapt a device like one of the telescope micrometers in limited circulation at the time.

Leeuwenhoek provides a couple of rather ingenious proportions. In his 5 October 1677 letter, he uses curved calipers to make his size measurements. While referring to an earlier letter, he uses both volumetric and gravimetric methods to assign a proportion between comparison materials (*Table 6.2.*):

'In my letter of 23 March 1677, I have shown that the quantity of water of 92 1/8 grains of millet equal the size of a drop of water (which is as large as a green pea). To demonstrate this clearly to some friends who could not comprehend this, I took six grains of millet and fixed them side by side in some cobbler's wax. I then measured with a pair of curved compasses the length of the axis of these grains of millet. The opening of the compasses was as large as the axis of a big red currant. The third power of six is 216. Let us now take an uncertainty for a certainty and assume that a red currant sinks in water. Now the grains of millet also sink in water, so they must have the same specific weight. This being the case, 216 grains of millet and the red currant must be equal in weight. I put the red currant

in a very accurate pair of scales and found that it was equal in weight with 212 grains of millet' (Leeuwenhoek, 1941, p. 253).

Table 6.2. *Proportions of millet seeds to a red currant and drop of water (Leeuwenhoek, 1941, p. 253).*

Comparator	***Equivalence***
92 1/8 grains of millet	One water drop or one green pea
Six grains of millet in cobbler's wax	Big red currant axis
Six millet grains cubed ((millet grains)^3)	216
One red currant and 216 millet grains	Same weight in water
Red currant weighed alone	Weight of 212 millet grains
Water as big as a millet seed	No less than 45,000 animalcules
Ordinary drop of water	No less than 4,140,000 animalcules

He reveals the relevance of this information later. He can affirm that

… there were more than 1000000 living Creatures contained in one drop of Pepper-water. I should not have varied from the truth of it, if I had asserted that there were 8000000; for if according to some of the included testimonials there might be found in a quantity of water as big as a millet seed, no less than 45,000 animalcules. It would follow that in an ordinary drop of this water there would be no less than 4,140,000 living creatures. [I add that, at several times, I saw as many more animalcules, which were so diminutive in size that they were invisible to the Gentlemen who gave these attestations. In that case the above] number if doubled will make 8280000 living Creatures seen in the quantity of one drop of water.

'This exceeds belief. But I do affirm, that if a larger grain of sand were broken into 8000000 of equal parts, one of these would not exceed the bigness of one of those little

creatures, which being understood, it will not seem so incredible to believe that there may be so great a number in the quantity of one drop of water.' (Leeuwenhoek, 1941, p. 255).

In his letter to Constantijn Huygens dated 20 May 1679, he compares his observation of animalcules to grains of sand after finding that 14 trillion animalcules would fill one cubic inch. He proposes an astonishing comparison: "…if one had so many sand-grains of the afore-said size, one would lade with them more than 108 of our ordinary sand-barges, that is to say if one reckons one ship-load, that is 144 cubic feet, to one barge" (Leeuwenhoek, 1948, p. 59).

To demonstrate the extent to which Leeuwenhoek used his micrometric comparisons, consider the following from 4 April 1687: "The Width of this Tooth was very nearly two-fifths of the length of an Inch; and according to my best reckoning I judged that I saw 120 tubules in the length of one-45th part of an Inch, that is, then, 5,400 tubules in the length of one Inch width. Now if we assume a molar such as referred to above, to be round, then the length of the Diameter of the molar is 2,150 times thicker than one of the tubules of which the same is composed; and when we multiply this number by itself then the outcome will be the number 4,822,500; to sum up, therefore, the Proportion of one bone-tubule is 1-to-4,822,500 to the thickness of the Tooth" (Leeuwenhoek, 1961, p. 205) (*Figure 6.3.*).

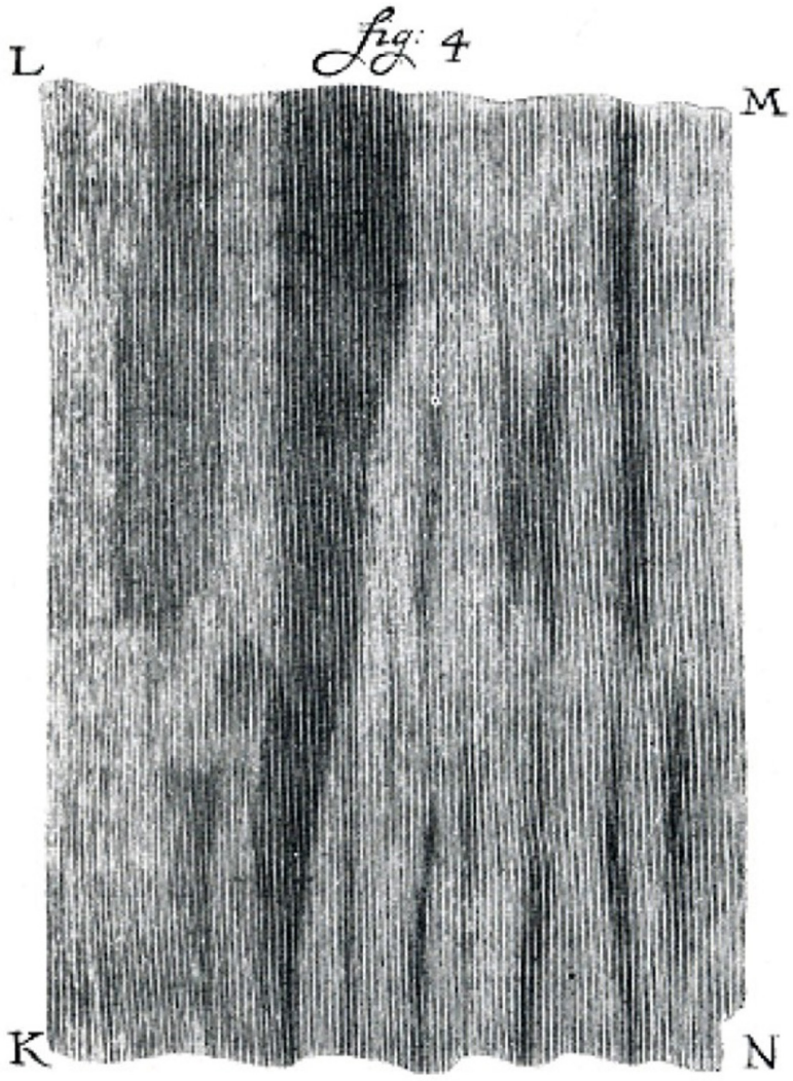


Figure 6.3. *Enamel of the crown of a human molar (Leeuwenhoek, 1961, p. 440).*

This example provides an object that we know from our own bodies and gives a sense of its structure; there are nearly five million tubules in the thickness of one of our molars.

Ultimately, Leeuwenhoek was a keen observer, using tools he created, tools he refined past any other microscopes of his day, to answer questions posed by his own. He broadened his queries as others questioned what he could see in various substances and structures. His information gave a relatable sense of proportion — a sense of unity — between the structures he viewed and objects that his correspondents could imagine, even if the comparable objects were often tiny.

Nick Lane, a biochemist from University College, London, summarized the brilliance of Leeuwenhoek's insights as follows: "More than being the first to see this unimagined world of "animalcules," he was the first even to think of looking — certainly, the first with the power to see" (Lane, 2015, p. 1). Nevertheless, Leeuwenhoek also wanted us to understand the incredibly small size and structural diversity of what he saw and how those objects compared to ordinary objects in our relatively large lives. He almost reflexively enlisted the idea of proportions between objects that were new, unknown, and entirely unexpected to his audience with objects they might know or at least comprehend. It was the sort of pedagogical insight that might occur naturally to a man who matured as a merchant of cloth but became one of the most profound observers of nature that has ever existed.

CHAPTER 7 | “Round, red globules floating in a crystalline fluid” – Antoni van Leeuwenhoek’s observations of red blood cells and hemocytes

7.1 Abstract

This article addresses a deficit in the cell biology and hematology literature, specifically regarding Antoni van Leeuwenhoek's central role in observing and describing red blood cells and hemocytes. While the existing literature on the history of hematology usually mentions Antoni van Leeuwenhoek, typically, it is an incomplete summary of his contributions. Leeuwenhoek is cited as one of the three individuals who first saw and described red blood cells through their microscope lenses. Jan Swammerdam and Marcello Malpighi also documented red blood cells in human blood before Leeuwenhoek. The literature fails to mention that Leeuwenhoek commented on red blood "globules," as well as arthropod hemocytes, at least thirty-five times in thirty-one letters spanning thirty-nine years of correspondence to The Royal Society and others. Some of his descriptive passages were extensive. His observations on blood circulation are a separate set of observations in his letters and are not covered here. Leeuwenhoek viewed various creatures to see if there were characteristics of their blood cells that he could share with the recipients of his letters. He also would view the cells in different chemical and physical environments to understand their properties. Leeuwenhoek's observations of blood corpuscles are discussed in chronological order. Comments included in footnotes by the Committee of Dutch Scientists, who edited the published seventeen volumes of Leeuwenhoek's *Alle de Brieven*, are also discussed when their comments are relevant.

Keywords: Antoni van Leeuwenhoek, 17th Century, red blood cells, erythrocytes, hemocytes, microscopes.

7.2 Introduction

Antoni van Leeuwenhoek's letters are essential documents in the maturation of science and historical microscopy. He addresses structures and objects that are typically sequestered within specific scientific disciplines. Leeuwenhoek often returned to subjects and samples discussed in earlier letters. Sometimes, it is due to his innate curiosity, perhaps because of an unresolved question that persisted from previous letters. At other times, one or more of his correspondents and colleagues had asked him for more details. Among his many areas of interest, fluid circulation — in plants and animals — and particularly the structures found in the blood of creatures, including mammals, fish, birds, amphibians, and arthropods, saw their share of his attention.

Leeuwenhoek does his best to explain, sometimes in metaphor, how the red blood cells — his blood globules or those of warm-blooded creatures — appear to him. He also dissected several arthropods and made the first known observations of hemocytes in their hemolymph. Leeuwenhoek sees objects beyond his understanding but notes their peculiarities and moves on to other matters. He discusses his observations so thoroughly that there is little doubt left to the reader about what Leeuwenhoek has observed. In other cases, room for interpretation remains. The editors of the letters come to the reader's aid with comments on what Leeuwenhoek has reported.

Between 1673 and 1712, Leeuwenhoek's *(Alle de Brieven (Collected Letters*) Deel 1-17 (Volumes 1 through 17)) included at least thirty-five descriptions of blood globules and hemocytes in insects and crustaceans, structures that he described as colorless and "only less transparent than the fluid in which they floated" (Leeuwenhoek, 1967, p. 111; Leeuwenhoek, 1979, p. 149).

7.3 Recognition of Leeuwenhoek's Contribution to Hematology

Aside from a 1931 article by Dr. John Stein in the journal that preceded *Science* (Stein, 1931) and the masterly work of Clifford Dobell (Dobell, 1932), Leeuwenhoek received scant credit for the extent of his observations of red blood cells and little mention for his notes on hemocytes. This oversight was addressed partially in Chapter 8 of *Antoni van Leeuwenhoek: Master of the Miniscule*, which focuses primarily on Leeuwenhoek's circulatory observations but includes some commentary on the blood corpuscles (L. Robertson et al., 2016a, pp. 146–148).

Nonetheless, recognition of Leeuwenhoek's inquiries into the nature of red blood globules was not proportional to the scope of his work. In 1974, Douglas Surgenor 1974 work The Red Blood Cell, a two-volume work*, was published. He* provided only the following description of Leeuwenhoek's work: "The description of the red blood cells of a man was finally accomplished in 1674 by Anton van Leeuwenhoek," followed by a brief quote from his letter to Henry Oldenburg from 7 April 1674 (James M. Stengle, 1974, p. 5).

Although his comments were limited, Dr. Maxwell Wintrobe, one of the 20th century's preeminent hematologists, is best at citing Leeuwenhoek's contribution. Wintrobe's first work on hematology came in *Blood, Pure and Eloquent*, a collection of essays addressing various topics in the field. In his opening essay, Wintrobe writes:

> The Dutchman Jan Swammerdam (1637—1680) and the Italian Marcello Malpighi (1628—1694) are often credited with describing the red blood corpuscles before Leeuwenhoek. However, Swammerdam only referred to "ruddy globules" in the blood and doubted that blood in its vessels contained such globules, while Malpighi's interest seems to have been only casual. It is Leeuwenhoek, therefore, who deserves the credit

> for having provided the first adequate description of the red blood cells (Wintrobe, 1980, pp. 7–9).

Wintrobe goes on to state that "…the tiny constituents of the blood, the so-called red corpuscles (the erythrocytes), which carry oxygen about the body, were first seen; and it was with similar rudimentary equipment that the cells concerned with the defenses of the body … the "white" or colorless corpuscles (the leukocytes) may have been observed, perhaps first by Malpighi and by Leeuwenhoek…" (Wintrobe, 1980, pp. 10–11).

Several other essayists in *Blood, Pure and Simple* remark on Leeuwenhoek's contribution, yet none of them expand on the extent of his observations or mention his work describing arthropod circulation of hemocytes.

In *Hematology, the Blossoming of a Science*, Wintrobe adds to his 1980 work:

> Antonj van Leeuwenhoek (1632—1723) of Delft made the first complete account of the red cells. This City Hall custodian's hobby was grinding lenses, which he did with exceptional skill; moreover, he was curious and industrious and lived and worked until the age of ninety-one… Unlike Malpighi, he did not mistake the red corpuscles for fat globules (Wintrobe, 1985, pp. 10–11).

It is worth noting that Malpighi recognized his error later in his life and realized that what he had thought were fat globules were blood cells, although he did not go further than this correction (Leeuwenhoek, 1939a, pp. 408–409).

Leeuwenhoek ekes out slightly more recognition in an article summarizing the achievements of Wallace H. Coulter, an innovator in cell counting technologies. Dr. Robinson, the author of the article, cites Dr. Stein's 1931 Leeuwenhoek article:

> Interestingly, the key to Van Leeuwenhoek's role as an instigator of discovery was his ability to design and manufacture technologies — in his case, a microscope-based one that was transformational in his time, being easy to manufacture even though somewhat challenging to use.

While these accounts provide some context to Leeuwenhoek's work and credit him with the "first complete account of the red cells," they significantly understate the scope of his contribution while placing the Swammerdam and Malpighi observations in perspective.

7.4 Leeuwenhoek and Blood Studies

The first letter mentioning blood was written to Henry Oldenburg, the first secretary of the Royal Society and editor of *Philosophical Transactions* until 1677. The letter was dated 15 August 1673 and described the imbibition, digestion, and excretion by a louse of human blood. In the letter, Leeuwenhoek describes interspecies (human-to-louse) blood circulation as a source of nutrition rather than an oxygen carrier (Leeuwenhoek, 1939b, pp. 55–57). Leeuwenhoek eventually will describe red blood cells' relative size, color, and shape, as well as hemolymph and hemocytes in various arthropods. He burrows into the details of the circulation of blood fluid and red blood “globules” in many mammals, fish, frogs, and birds, although his numerous observations of blood circulation go beyond the scope of this review.

“Globules” is a term often used by Leeuwenhoek to describe structures he views through his lenses. In this single letter, he uses “globules” to describe structures in blood, milk, breasts, hair, fingernails, and “unnatural tumors.” He probably observed fat droplets in milk, breasts, and tumors, while he may have observed structures formed by proteins in hair and fingernails.

In a letter dated 5 April 1674 to Constantijn Huygens, Leeuwenhoek addressed some observations he had made during a visit to Huygens' house in Zuilichem "some time ago." Leeuwenhoek states that he

> had observed blood from my hand, which I found to consist of red globules, also floating about in a wheylike fluid. This is to inform you that I have since examined my blood once more, this time with greater accuracy. I now find that the fluid in which the globules float are more like a crystalline than like a wheyish fluid" (Leeuwenhoek, 1939b, p. 67).

In a footnote, the *Letters'* editors clarify that by writing "crystalline," Leeuwenhoek implies clear, whereas whey is turbid.

In a letter dated 7 April 1674, just two days later, he followed up with Oldenburg and confirmed the fluid's crystalline nature, but this letter adds some subtle details. He allows that he has "divers times endeavoured to see and to know, what parts the Blood consists of" — he has observed blood and thought about it more times than he has written about in his letters. He writes that the "small round globuls" are "driven thorough [sic] a Crystalline humidity or water," that is, they are no longer floating but are driven, are in motion. He also says that when he views his blood cells in "very small parcels," they yield "very colour (weijnich couluer)," which is an archaic way of saying "little color" (Leeuwenhoek, 1939b, p. 75). Between his letter to Huygens and to Oldenburg, Leeuwenhoek reveals several "new" observations about the blood, at first allowing that the globules are red, then that in small numbers they have little color.

On 24 April, Leeuwenhoek wrote to Huygens again. He shared that "four or five globules" have "very little colour" and notes that the globules "can only pass in one thickness and singly through some subtle veins," perhaps being his first observation of red

blood cells passing through capillaries or arterioles feeding the capillaries. When the cells pass through these tiny vessels, "one cannot expect the red globules to impress the eye with the sensation of a red colour," indicating that it is even less surprising when the cells are seen individually.

In a 1 June 1674 letter, Leeuwenhoek responded to a series of questions from Robert Boyle, the eminent natural philosopher, and Royal Society member. In 1684, Boyle published *Memoirs for the Natural History of Human Blood and planned a revision* (Boyle, 2000, pp. 3–102). *Leeuwenhoek comments on blood color changes, the relative heaviness of blood globules compared to the serum in which they are suspended, and color differences of blood cells within the diameter of a vein. Leeuwenhoek then makes nine numbered observations, the first of which directly addressed Boyle's question. The second response is related to Leeuwenhoek's methods for blood sample observation, to his first description of the relative size of blood globules to a fine grain of sand. It further clarifies that the globules are "white and colourless" as single cells. Leeuwenhoek states that "Mr. BOILE" advised him to continue pursuing the observations he has provided to the Royal Society and anything more regarding "...the red, florid colour which blood acquires as soon as it is drawn from the veins and exposed to the air, and also to the blood under the surface, as being distinct from the other blood in colour." T*he entirety of his first observation is included below, and he responds directly to Boyle's queries while expanding on related matters. The third note discusses the behavior of blood globules in a gently heated capillary. The rest of the numbered observations are related to globules in other materials, particularly in various kinds of bone.

1. The small Red Globuls in the Blood, formerly spoken of are heavier than the Crystalline liquor in which they are carried, because soon after that the Blood is let out

of the Veins, those Globuls by little and little subside towards the bottom; and being made up of soft fluid Corpuscles, and many lying upon one another, they do unite themselves close together, and by this close conjunction the Blood that is under the surface alters its colour, and becomes dark-red or blackish, as I have observed several times: of which I take the reason to be, (with submission to better Judgments) that the Air cannot move every way round about the Globuls, and hits as 'twere against a close darkish body. Touching the Florid red colour of the surface of the Blood exposed to the Air, that comes, in my opinion, from hence, that the uppermost Globuls are not press'd, and therefore retain their nature, and the Globuls subjacent to the uppermost lye close together, by reason of which close conjunction the Light cannot penetrate through them, but is reflected, and so gives a greater light to, and about, the uppermost Globuls, than they had before the union of the inferiour Globuls; and [consequently it is this] that makes [their red colour brighter and more florid].

This passage received footnoted comments from the editorial committee working towards publishing *Alle de Brieven* Volume 1. The first comment is from Professor Doctor Gerard Heringa, a histologist working at the time (the late 1930s) in the medical school at the University of Amsterdam. He interprets Leeuwenhoek's comment regarding the blood corpuscles uniting "themselves close together" as referring to the formation of *rouleaux* or stacks of blood cells and that these formations of blood cells result in the venous or deoxygenated color of the blood deeper in the vein. Heringa also comments that if Leeuwenhoek is referring to *rouleaux* as the reason why the blood "… that is under the surface alters its colour, and becomes dark-red or blackish," the explanation is unnecessary, as the deeper blood cells might have been exposed to less oxygen and the hemoglobin, unknown to Leeuwenhoek, would be in its dark, deoxygenated form. It is difficult to assess

whether Leeuwenhoek's description and Heringa's explanation are aligned, as Leeuwenhoek does not provide us with an illustration of the phenomenon.

Another footnote expounds on the translation of a single word - *lucht*. Dr. Heringa and Judica Mendels, a "qualified teacher of Dutch in a secondary school, Amsterdam," introduce an alternative translation (Leeuwenhoek, 1939b, p. 375). While the Royal Society interpreted the passage to mean that Leeuwenhoek believed that “air” was responsible for the bright red color of blood near the surface of a sample, Heringa and Mendels believe that this was due to mistranslation of the word “lucht,” which meant “light” in Leeuwenhoek’s dialect (Leeuwenhoek, 1939b, p. 95). If they are correct, Leeuwenhoek was merely commenting on the surface blood reflecting light differently than the deeper portion of the sample rather than on whether the surface blood was different in the presence of "air." Again, arriving at a definitive interpretation of what Leeuwenhoek saw or meant by his description is difficult.

There is much to note in this letter, but perhaps the most stunning statement comes with Leeuwenhoek’s estimate that the red blood globules are “25000 times smaller than a [fine] grain of sand” (Leeuwenhoek, 1939b, pp. 103–105). In a footnote, Heringa and Dr. Abraham Schierbeek comment that this would put the diameter of a red blood cell at approximately 8.5 microns, “which strikingly agrees with the now accepted computation of 7,2” (Leeuwenhoek, 1939b, p. 103). Leeuwenhoek goes on to affirm previous observations that blood cells "…when they are single, and stick within to the sides of the Glass-pipes, will appear white and colourless," along with minor statements regarding how blood cells rise in a gently heated capillary tube (Leeuwenhoek 1939a: pp. 103–5), and that "…bloody Globuls did issue from between…" muscle filaments observed in cow flesh (Leeuwenhoek 1939a: p. 109). Schierbeek, a lecturer in the history and methodology of

biology at the University of Leiden and a prolific author with over 150 books, brochures, and articles to his credit, became an essential biographer for both Leeuwenhoek and Jan Swammerdam (Luyendijk-Elshout, 1979).

One month later, Leeuwenhoek wrote Oldenburg on 6 July 1674 with more information from his work. He sent along some glass tubes so that the “Curious Gentlemen may share my observations of Blood etc” (Leeuwenhoek, 1939b, p. 119). He also states that he has created "a more convenient Glass-pipe" for "seeing the motion of the Globuls in the Crystallin liquor of the Blood." He comments that he has sent Constantijn Huygens some tubes to forward to his son, Christiaan, and that Christiaan has responded that he saw only "other particles," which Leeuwenhoek speculates might be the "red Globuls" that "come to stick to one another" and "exhibit odd and misshapen particles." Here again, Heringa suggests that some of the particles may be rouleaux of blood cells, which tend to form serpentine piles of cells. Leeuwenhoek's response is to Christiaan Huygens' vague comment, so Heringa may be creating an explanation unsupported by the letter (Leeuwenhoek, 1939b, pp. 121–125).

On occasion, Leeuwenhoek veers into stunning metaphors to explain what he sees. In his 22 January 1675 letter to Oldenburg, he describes:

> "the globules in the blood as clearly as if we saw with our eyes without the help of glasses the grains of sand on a piece of black taffeta, some of them lying in a heap, others spread apart, and it is curious to see how the globules of the blood draw together when they lie scattered" (Leeuwenhoek, 1939b, p. 213).

“The grains of sand on a piece of black taffeta,” is thought by Clifford Dobell and others to be an observation aided by some form of dark-ground or dark-field microscopy (Dobell, 1932, pp. 330–332). Additionally, Leeuwenhoek provides a beautiful metaphor drawn from

the drapers' profession. Dobell extends his basis for the comment to include Leeuwenhoek's observations of "flagella and cilia and spirochaetes and micrococci with only a magnification of some 200-300x diameters" (Dobell, 1932, p. 331). A joint footnote by Schierbeek and Dr. Ir. Albert J. Kluyver, a microbiology professor at the Technical College of Delft, expands on Dobell's hypothesis by enumerating several additional microscopists who agree with Dobell and have developed methods for understanding what Leeuwenhoek might have been doing to achieve the dark-field effect (Leeuwenhoek, 1939b, p. 213).

Leeuwenhoek goes into detail in his letter on 26 March 1675 regarding serum color and the nature of blood. He observes that, while the water evaporates from the blood, "…in the clear substance, that was slightly yellow of colour, I could see nothing but some few particles that are not worth noticing and that looked like globules." Leeuwenhoek could not have centrifuged the "clear substance," so it may be that some of the globules were other particles that had avoided the clotting process. As Leeuwenhoek evaporates the substance, he sees a

> very clear and transparent matter as if it had been glass except where it had been spread somewhat more thickly, where many peculiar figures could be seen, as if oblong crenulated leaves had sprung from one central point, each leaf having a peculiar shape with ribs and crenate sides, and from those leaves sprung other leaflets and all those parts seemed to be composed of globules.

Dr. Heringa comments that this set of observations is "far from clear" but attempts to unravel what Leeuwenhoek has reported. Heringa supposes that the globules or particles Leeuwenhoek witnessed are typical in serum that has gone through a thickening,

evaporating, or inspissating[21] process. Leeuwenhoek's poetic invocation of crenulated leaves with ribs and crenate sides Heringa puts down to the growth of "linked crystals forming along the fissures which soon appear in the above-mentioned film" (Leeuwenhoek, 1939b, pp. 281–285).

In his last blood-related letter of 1675, Leeuwenhoek again made remarkable observations to Oldenburg. He amended comments from the previous letter that he had not seen "plain globuls move in that waterish matter without any evaporation made" in the serum, although there "were but very few of them." The core of this letter's blood observations lies with Leeuwenhoek's sense that his blood cells are "firmer and harder" when he is ill than when he is well, as he was at the time of this letter. He finds the "…globuls of my Blood softer, and more sticking to one another, and my Body in a good state of health." Dr. G. van Rijnberk, a physiology professor at the Municipal University of Amsterdam, comments in the footnotes that this is "once more a remarkable observation," as it alludes to the tendency of red blood cells to "agglutinate more easily in health than in illness" and goes on to praise Leeuwenhoek for noting this, as one might have deduced the opposite (Leeuwenhoek, 1939b, p. 301). Leeuwenhoek wonders whether the illness might be caused by the changes from soft corpuscles to hard. Leeuwenhoek also reasons that the

> sanguineous globuls in a healthy Body must be very flexible and pliant, [if they shall pass through the very small veins (which as I have said, are in the films where the flesh is as it were inwoven, and through which the blood circulates, that is to say passes from the arteries into the veins); and that, in their passage, they change into an oval figure, reassuming their former globosity when they come into a larger room, in accordance with their size, this being owing to their strong and frequent movement].

[21] Inspissating: to make thick or thicker due to evaporation or boiling.

Dr. van Rijnberk adds a comment regarding the hardness of blood corpuscles as being "interesting in itself" and knows of no work that had assessed the relative hardness of blood cells and any impact that property might have on circulation through the arterioles, capillaries, venules, and veins. He also places Leeuwenhoek's comment in the historical context of iatromechanics, which was influential in the 17th century (Musitelli, S.; Bertozzi, 2018, p. 113). Iatromechanics suggested that the muscles, organs, and body all resembled machines; Leeuwenhoek's ruminations about the hardness or pliability of corpuscles might have been influenced by this thinking (Leeuwenhoek, 1939b, p. 301).

Two years later, Leeuwenhoek found a way to focus past the stream of blood he had witnessed in the digestive processes of a louse and observe the "red globules" or red blood cells in "crystalline" fluid. His initial observations may have obscured a more cogent set of observations, or he may have been organizing his thoughts about what he had seen. In either case, he continued his work.

In a 14 May 1677 letter to Oldenburg, Leeuwenhoek made observations of brain tissue in which he differentiated between the "Brain-globuls, especially by the perfect roundness which the blood-globuls [have]." He confirmed that the cells individually have "little or no colour," but he also writes that the "sanguineous vessels, which run through the Brain" have blood that "in these small veins was yet red" (Leeuwenhoek, 1941, pp. 221–223).

In a footnote, Heringa writes, "…L. observed haemolysis *in vitro*, i.e. the phenomenon that the colouring matter leaves the blood-corpuscles so that the blood becomes transparent" (Leeuwenhoek, 1941, p. 225). Based on the English translation, this interpretation is difficult to support but may be more evident in the original Dutch.

In his 5 October 1677 letter, Leeuwenhoek explores a common, although spurious, belief about eel blood. He attempts to understand why the eel blood, when it gets in a fisherman's eye, "causes an incredible pain lasting a whole day." He observes that the blood cells are "thin rods about twice as long as the thickness of a blood-globule," which Schierbeek explains is because Leeuwenhoek observed the blood corpuscles "on their narrow side," rather than as round globules. Schierbeek notes that Leeuwenhoek would not recognize their lenticular nature until letters in 1682 and 1683 (Leeuwenhoek, 1941, p. 243).

In his 14 January 1678 letter, Leeuwenhoek writes to Robert Hooke, author of *Micrographia* (1665) and then secretary to the Royal Society after Oldenburg's death in 1677. Hooke had written Leeuwenhoek on 30 November 1677, but the letter did not arrive until 8 January 1678. Leeuwenhoek started the letter by writing that he has extended his consideration of blood globules from eels to his blood and concludes that he "doubts" the source of the eye pain is caused by eel blood. A blood-borne toxin causes the pain, although this would not be known until much later. Leeuwenhoek engaged in some new techniques to assess the pliability of the globules as he "…bended these Globules before my eyes, that they were three times as long as broad, without breaking [the vesicles which form the surface of the globules]," while "they recovered their former globulosity" (Leeuwenhoek, 1941, p. 307). Heringa adds that the shapes the blood cells take on may refer to "the usual disc shape (when seen on their flat side)," "the form of rods (when seen in profile)," or their biconcave shape "in the form of cups." Leeuwenhoek did not mention this shape among the "many sorts of figures" alluded to in his letter (Leeuwenhoek, 1941, p. 307).

More notably, Leeuwenhoek then describes the shape blood cells adopt while dehydrating. Each blood cell takes on a thorny or rocky appearance, while the globular, rod, or lenticular shape, depending on the viewing angle, is transformed while leaving the

cell intact. Heringa calls these the "thorn-apple" formations. This cell shape can be formed in hypertonic salt solutions. Leeuwenhoek's observation began a series of observations on the "smaller globules" that appear during dehydration; in this case, he sees "six smaller Globules." If nothing else, his comments address the resolving power of his lenses, as the more minor deformations in the healthy blood cell must be approximately 1 micron or less in size (*Figure 7.1.*) (Leeuwenhoek, 1941, p. 309).

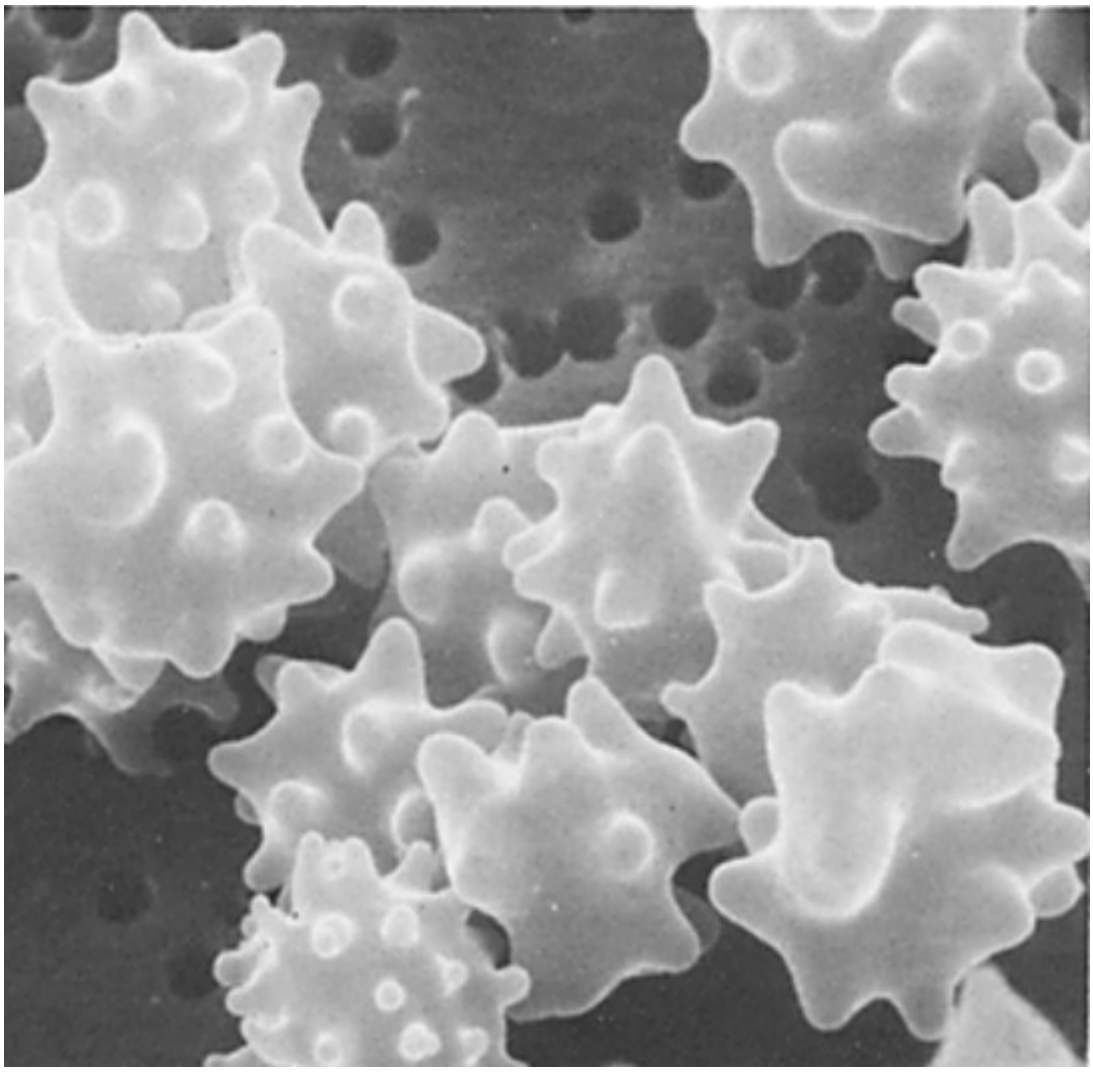


Figure 7.1. *Electron photomicrograph of erythrocytes in a hypertonic solution, an example of the "thorn-apple" configuration of red blood cells (Sheetz et al., 1976, p. 196).*

Interestingly, Leeuwenhoek describes putting "…the greater Globules into so violent a motion, that their Vesicles burst into pieces, and then the lesser Globules appeared plainly to be scattered" (Leeuwenhoek, 1941, p. 311). This description clearly describes hemolysis, while the previously cited Heringa passage is vague.

In a letter addressed to Hooke and dated only "March 1682," Leeuwenhoek seems to have realized that blood globules are "flat, oval particles, thickish, floating in a crystalline water." He also repeats his previous observations regarding their individual and group color ("no colour" and "red colour") (Leeuwenhoek, 1948, p. 405). As these observations are made from the blood of cod and salmon, Leeuwenhoek notes something he has never seen in human blood: "…some of them enclosed in a small space a little, round body or globule," which Heringa believes to be "the first observation of the nucleus which L. discovered in the erythrocytes of fishes." Heringa also notes Leeuwenhoek's comment that "…3, 4, 5, 6, nay as many as 8 globules" is probably due to the heating of the blood sample, which causes the formation of vacuoles around the nucleus (Leeuwenhoek, 1948, p. 407).

In a 22 January 1683 letter to Christopher Wren, who had concluded his tenure as president of the Royal Society in 1682, Leeuwenhoek made some minor comments regarding the effect of mixing his blood with *sal volatile oleosum*. He was interested in comments by an unnamed physician that "many people suffering from fever were entirely cured after having taken *Sal volatile oleosum*." The archaic medication was a mixture of cinnamon, nutmeg, nutmeg flower, lemon tree bark, salts of tartrate and ammonium, and alcohol, with some fermentation and distillation steps included in its preparation. The ratio of the ingredients probably varied from one apothecary to another, but it was supposed to "make the blood very quick and thin." It is interesting to note that Dr. Pieter van der Wielen, formerly a professor of pharmacology at the Municipal University of Amsterdam, states in

the footnotes that *sal volatile oleosum* was probably used as a carminative or a medication that expelled gas from the stomach and bowel. Dr. van der Wielen indicates that around 1952, *Eau des Carmes*, an herbal preparation, or *Spiritus polyaromaticus*, a distillate preparation from balsam, both archaic remedies, could be substituted for *sal volatile oleosum* (Leeuwenhoek, 1952, p. 29). Heringa comments that the effect that Leeuwenhoek witnessed when mixing an equal or double amount of blood with *sal volatile oleosum* was probably hemolysis of the blood cells. Leeuwenhoek also speculates on a correlation between his blood's appearance and the illness he was suffering. This belief originated in the Middle Ages and continued to affect thinking in the 17th Century (Leeuwenhoek, 1952, p. 31).

He wrote Wren again on 16 July 1683. However, it is unclear why Leeuwenhoek wrote to Wren as he had ceded the presidency to John Hoskyns, another founding member of the Society. In this letter, Leeuwenhoek extends his comments regarding the colors of blood corpuscles to include those of the frog but confirms what he has "previously said about the salmon etc" (*Figure 7.2.*). Otherwise, Leeuwenhoek describes "a very light oval-shaped glimmer at the center" of some globules, "while others seemed to be composed of many oval disks, some larger than the others." Heringa suggests (1) that the glimmer was due to the nucleus shimmering "…when the microscope is adjusted to a deep level. If the adjustment is outside the level of the centre of the blood-corpuscle diffraction lines arise, parallel to the circumference, which causes L. to speak of 'many oval disks, one larger than the other,'" and (2) that the "very small globules" are "in all probability particles of broken up thrombocytes," which are the nucleated cells found in amphibians instead of platelets. The thrombocytes aggregate to form clots, much like platelets in mammalian blood (Leeuwenhoek, 1952, pp. 73–75).

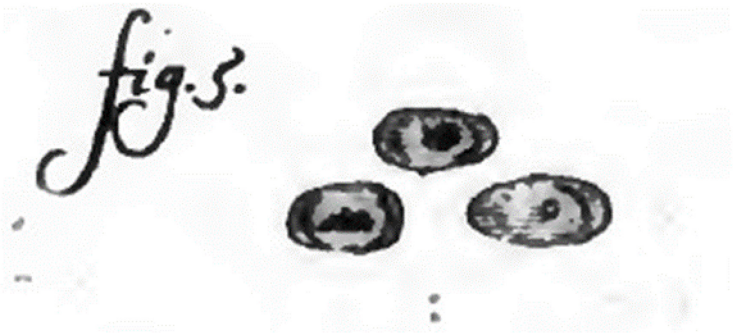


Figure 7.2. *Erythrocytes in a fish's blood (Leeuwenhoek, 1722, p. 51).*

On 14 April 1684, Leeuwenhoek wrote Francis Aston, secretary of the Royal Society from 1681 to 1685, to confirm that there is "no difference in size between the globules in the blood of human beings and the said [ox, sheep, rabbits] animals," and that the blood of fishes, birds, and "all animals that live in water, consists of no other figures than flat, oval particles" (*Figure 7.3.*) (Leeuwenhoek, 1952, pp. 241–243).

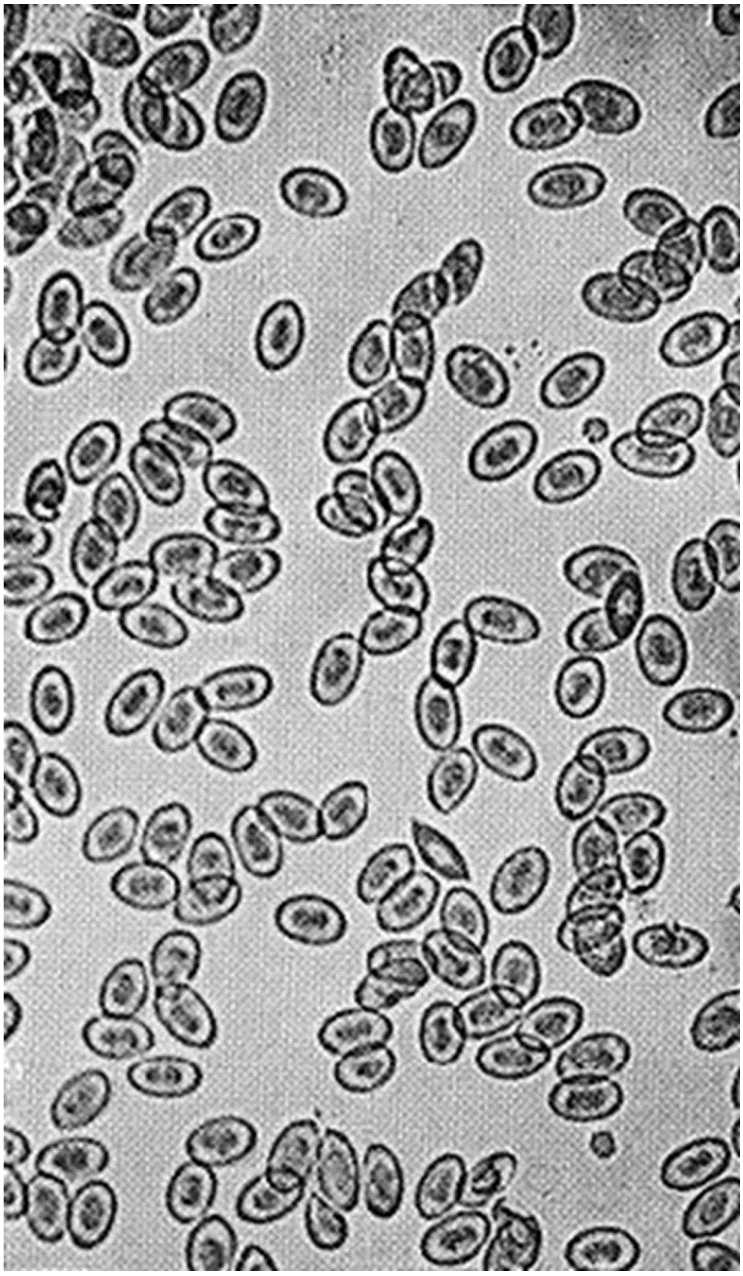


Figure 7.3. *Erythrocytes of a hen (Leeuwenhoek 1952: 405).*

Leeuwenhoek's subsequent twelve letters — from 24 July 1684 to 12 August 1692 — including content relating to blood globules are all addressed to "The Royal Society" rather than to a specific secretary or president of the Society. Leeuwenhoek’s 24 July 1684 letter provides an alternative estimate of the size of a red blood cell, previously estimated in his 1 June 1674 letter:

> I determined that the perfect globules, which colour our blood red, are of such size that if 100 of them were put end to end they would not attain the diameter of a coarse grain of sand, so that a 1.000.000 [1 million] blood-globules are the same size as one coarse grain of sand" (Leeuwenhoek, 1952, p. 267).

After a nearly four-year hiatus from describing blood cells, his 25 May 1688 letter describes mixing oil and water in various proportions with substances, including blood. He seems to revert to describing the globules as having "complete roundness." Given his description of the water and blood mixture, it may be that Leeuwenhoek saw erythrocytes in a hypotonic solution, a condition that would cause the blood cells to take on water and lose their lenticular characteristics. This observation was accompanied by statements that "no globules whatsoever were cohering together, but that each lay separate from the others (which I had never observed)." He goes on to say that "nearly all the globules of blood were composed of several globules, which was a very agreeable sight for me, especially when I made such a large multitude of completely round globules, all of the same size, move about together." This statement remains mysterious, although it may be that he was witnessing the result of blood in hypotonic conditions in the process of hemolysis. The editors do not mention the possibility of hypotonicity, nor does hemolysis of the blood cells lead to "nearly all the globules of blood" being composed of "several globules" (Leeuwenhoek, 1964, pp. 191–193).

Later in the same letter, Leeuwenhoek experiments with adding salt derived from bladder stones to blood and seeing the opposite effect — hypertonic, high salt solutions cause the thorn-apple appearance in red blood cells, as commented by the editors, although Leeuwenhoek also says that the appearance of the globules is "…as if the heat had expelled, or driven out, the moisture from them." He further characterized the possibly hypertonic cells:

> For each globule of blood almost showed its shape; some were flattish, and one could see that they, in their turn, consisted of globules; others resembled dead little animals with legs: in short, I saw so many figures that one cannot give them names (Leeuwenhoek, 1964, p. 203).

Leeuwenhoek might have seen the result of an inhomogeneous salt solution diffusing through the blood cells, resulting in various forms of blood cells; this would probably not have occurred if the solution were homogeneous. In the next paragraph, he describes a phenomenon like hemolysis, although he describes what he sees and does not categorize the phenomenon:

> After this I took the water in which the volatile salt had melted, this water I also mixed with blood, and at once observed that many globules of blood changed or fell apart in such a way that the globules could no longer be recognized, except by giving the closest attention, whereas other globules of blood remained whole for a long time. But this I did not observe unless I had mixed very little blood with much water (Leeuwenhoek, 1964, pp. 203–205).

Leeuwenhoek had become fond of the experiments combining blood, oil, and water with and without salt from accreted stones such as bladder stones and *lapis bezoar*. When he mixes blood with oil, the blood cells "…at once coagulated together so firmly as I had never seen before, and however much I moved the mixture about, the globules of blood invariably stuck together, although I had left the same lying for a whole night". The editors believe that Leeuwenhoek witnessed agglutination here, wherein the cells clump together as their surfaces are more attracted to each other than to the oil in which they are suspended. In further experiments with a solution of oil, water, and a volatile salt, he again witnessed the formation of the irregular shapes characteristic of blood in a hypertonic medium. These

"burst apart, as it were, although they still clung together by their surfaces, and these again to other globules." He observed this process while seeing that "these globules of blood became fewer and fewer, and that the water began to take on a red colour" (Leeuwenhoek, 1964, p. 247). Leeuwenhoek follows these experiments with additional ones that produce hemolysis (*Figure 7.4.*) (Leeuwenhoek, 1964, p. 257).

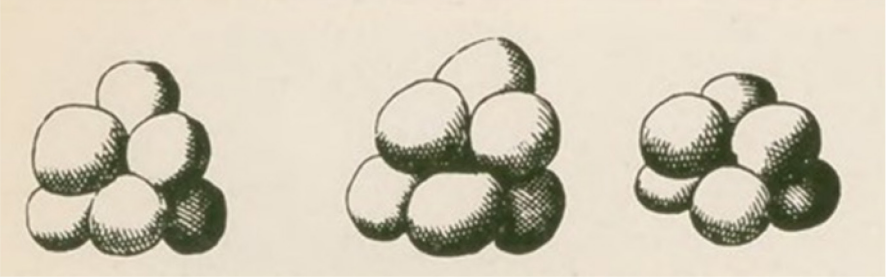


Figure 7.4. *Agglutinated red blood cells as illustrated in Leeuwenhoek's Arcana Naturae Detecta (Haden, 1939, p. 42).*

In his 7 September 1688 letter to the Society, Leeuwenhoek makes some observations regarding the movement of blood globules in tadpoles ("frog-worm"). In some ways, he simply confirms previous statements that "Arteries were not wider than to allow a single particle of blood (which, so to see, looked like globules, while they are nevertheless flat, oval particles," but he also…

> saw that the particles of blood, because of the thinness of the blood-vessel, changed into a long, round shape, and when I took the little Animal out of the water, and it got so far that it began to die, I saw that the blood in the thinnest Arteries sometimes stood still, and when the blood in the same Vessel was again being driven forward, I saw that several particles of blood became stretched quite twice as long as the width of such a particle, and that they then appeared to taper to a point at both ends (*Figure 7.5.*) (Leeuwenhoek, 1967, p. 29).

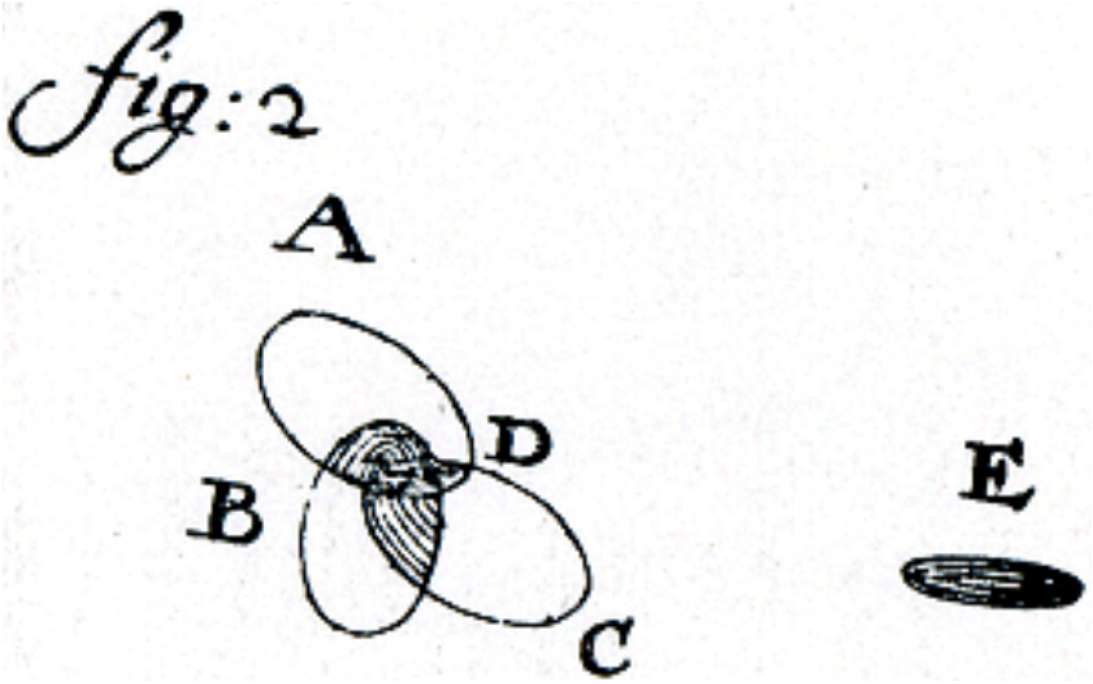


Figure 7.5. *Erythrocytes of a frog (Leeuwenhoek, 1952, p. 390).*

While Leeuwenhoek's numerous comments on blood circulation in various creatures lie beyond the scope of this paper, he also witnesses "Arteries so long as they carry the blood into the furthest parts of the small vessels; and Veins, when they carry the blood back to the Heart" (Leeuwenhoek, 1967, p. 29). The editors state that this and other comments Leeuwenhoek makes in his letters describe the anastomosis of arteries to veins and confirm Malpighi's discovery. However, Leeuwenhoek would have been unaware of Malpighi's work (*Figure 7.6.*) (L. Robertson et al., 2016a, pp. 139–140).

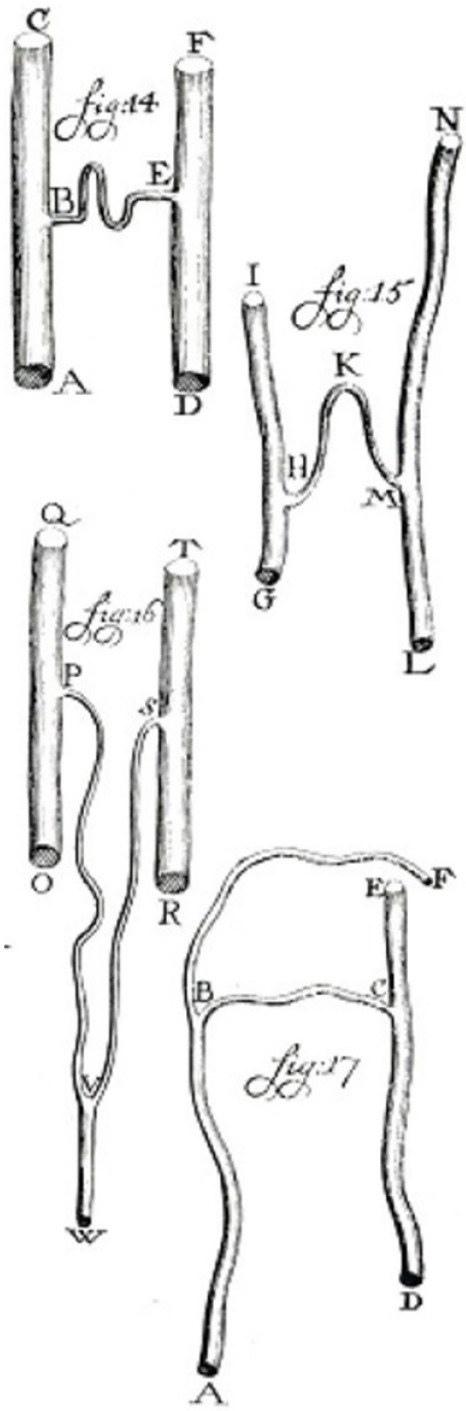


Figure 7.6. *Figures 14, 15, 16, and 17 show the junction of the artery and vein in perch, pike roach, and carp (Leeuwenhoek, 1967, p. 391).*

Observations from letters written on 7 September 1688 and 12 January 1689 principally restate earlier observations regarding the size and shape of blood globules. However, a footnote for the 7 September letter suggests that Leeuwenhoek extended his observation to

frog red blood cells and that the cell diameter would be about 9 microns. This size is consistent with what we currently know about frog red blood cells.

One aspect of Leeuwenhoek's observations that makes them so valuable to the history of science is that they are observations of dynamic processes. He not only sees a globule, round from one perspective and flat from another, but follows it as it moves through the blood medium, through the arteries, capillaries, and veins, as the globules are stretched into long, thin cells on their way through capillary beds, or as they tumble:

> Now the fact that these particles appeared to me in so many different changes of shape, was because these parts many times turned over (so to speak) in their course: For that which I, at one moment, was lying on one side before my eyes, lay after a little progress with its flat side before them: again, another particle of blood was thrown about in its length within the progress of a hair's breadth. In short, I here saw as many overturnings of the flat particles of blood as I could possibly imagine (Leeuwenhoek, 1967, p. 53).

Later in the 12 January letter, Leeuwenhoek starts a series of observations in shrimp and crab — arthropods that do not have red blood cells or hemoglobin — although again, Leeuwenhoek simply accounts for what he sees: "These globules had no colour and were somewhat transparent" (Leeuwenhoek, 1967, p. 111). Leeuwenhoek observed hemolymph and hemocytes, the "blood" and oxygen-carrying fluid in all arthropods – insects, crustaceans, spiders, and many other classes. We now know that the hemocytes carry hemocyanin, a copper-containing protein that facilitates oxygen transport in arthropods. Just as Leeuwenhoek would not have known about hemoglobin, he would not have known about hemocyanin.

His 1 April 1689 letter contains a lengthy set of observations of blood circulation in a bat, which he keeps in a box and feeds for several days. Leeuwenhoek was particularly

interested in congealed or clotted blood in the ears and wings, but the letter does not contain any new information regarding the blood cells (Leeuwenhoek, 1967, pp. 159–169).

In his letter of 24 June 1692, Leeuwenhoek observes the hemolymph of a grasshopper. The "…blood that was in these vessels had a green Colour". The editors correct the notion that the grasshopper had green blood and state that the green coloration is due to chlorophyll circulating in the hemolymph. As is typical for him, Leeuwenhoek pushes his observation as far as possible – down to resolution of a single globule:

> But when the blood formed so thin a layer that I judged it to be only a single globule of blood, it had no colour whatsoever; and then it most nearly resembled a transparent substance. And here it was clear to me that all these green globules were embedded in a thin transparent liquid (Leeuwenhoek, 1976, pp. 51–53).

In his 12 August 1692 letter, Leeuwenhoek goes a bit astray:

> For all the brilliant, beautiful red colour of Arterial blood, in contrast to the blood from the Veins, is only due to the fact that Arterial blood contains more of the substance which is called the Serum of the blood than does the blood in the Veins, and the less there is of the so-called serous substance in the blood, the more the blood tends towards black, as has also been said on a previous occasion (Leeuwenhoek, 1976, p. 121).

The idea that arterial blood is a "brilliant, beautiful red colour" due to the presence of more serum may have made sense from a colorimetric point of view. However, Leeuwenhoek would not have known about the oxidation-reduction cycle of hemoglobin when he made his observations. The oxygen-binding properties would remain a mystery until scientists made discoveries in the early 19th century. Still, his comment underlines his strength as an observer rather than a theoretician.

With his 14 September 1694 letter, Leeuwenhoek resumed writing to individuals within The Royal Society. Richard Waller, appointed secretary of the Society in 1691, received the third of Leeuwenhoek's letters commenting on arthropod "blood," this time focusing on crab hemocytes. Leeuwenhoek's observation is remarkable for presenting a dynamic portrait of the circulating hemocytes. However, because he comments that they are round globules "although they were not red," he captures the truth he observes rather than allowing bias to color his remarks.

> And no sooner had I done this but I saw at once such an inconceivably large number of blood parts, which appeared as round globules to the eye, although they were not red, but only less transparent than the fluid in which they floated, flowing through the blood vessel we call a Vein, and that with such speed and in such large numbers that no Human Being can understand it unless he sees it with his own eyes. Nay, I can compare the said round blood parts moving past my eyes no better than as if we thought we saw through a large tear or the opening of a Window or the like a snowdrift propelled by a strong wind moving past our eye, and I do not know that I ever saw blood being propelled with such speed through its vessels (Leeuwenhoek, 1979, p. 149).

Leeuwenhoek’s 10 April 1695 letter to Antoni Heinsius, then Grand Pensionary of the States of Holland and a friend of Leeuwenhoek’s since the 1660s (Anderson, n.d.), expands on hemocyte observations:

> the Blood globules which were propelled in all the vessels are very few in number in comparison with the globules that are in the blood of the Animals living on the earth or the Animals or Fishes which are in the Waters and whose blood is red. Nay, I even

believe that the blood globules of those which have red blood are at least twenty-five times more numerous than those I perceived in the crab (Leeuwenhoek, 1979, p. 171).

Dr. D. H. Spaargaren, a biologist who was then with the Netherlands Institute for Sea Research at Den Hoorn, comments that there are about 50,000 hemocytes per mm^3 in crabs and 4.5 to 5 million per mm^3 in humans (this varies by gender; normal ranges also are somewhat broader than Dr. Spaargaren states), so Leeuwenhoek's estimate here is four times too low.

A 25 September 1699 letter, again to The Royal Society, incorrectly observes that frog tadpole blood, once clotted, can resume its previous corpuscular form and flow again after a few days. In the footnotes, Dr. A. D. F. Addink, a professor of animal physiology at the State University of Leiden, corrects this by adding that phagocytes deconstruct clots, an irreversible process discovered a few centuries afterward (Leeuwenhoek, 1989, pp. 343–347)

Leeuwenhoek wrote a letter dated 9 July 1700 to Hans Sloane, secretary of The Royal Society since 1693, making minor comments about blood cells in flounder. However, he provides details about blood vessels' size compared to the corpuscles, thus clarifying some circulatory questions in the flounder (Leeuwenhoek, 1993, pp. 135–137).

Further on in the same letter, Leeuwenhoek comments on experiments involving higher magnification lenses. The effect of using the higher magnification was that the blood cells moved past the lens more swiftly. Leeuwenhoek then would pinch the blood vessel, which caused the blood "particles" to be "so far apart that no blood particles, not even those of which six had made up one blood particle, were to be seen in those vessels, but only a homogeneous fluid substance, which was slightly coloured, flowed through the vessels" (Leeuwenhoek, 1993, p. 147). He also confirms the oval shape of the blood cells.

Leeuwenhoek provides some drawings of his observations (Leeuwenhoek, 1993, p. 426). However, he also provides some new details which remain mysterious:

> I made up such a globule as shown in Fig. 5 out of six wax globules, so that I could show the blood globules to those who ask me for an exact description of these globules, adding that I am certain that each of our blood globules is composed at least of thirty-six globules. When the globules so composed are squeezed and moved, they are compressed because they are flexible, and they assume a perfectly spherical form, as shown in Fig. 6. It is easy to understand that through this disposition the globules of our blood and of that of animals acquire their roundness, but we cannot conceive how the said oval blood particles are composed out of six globules (*Figure 7.7.*) (Leeuwenhoek, 1993, pp. 147–149; 426).

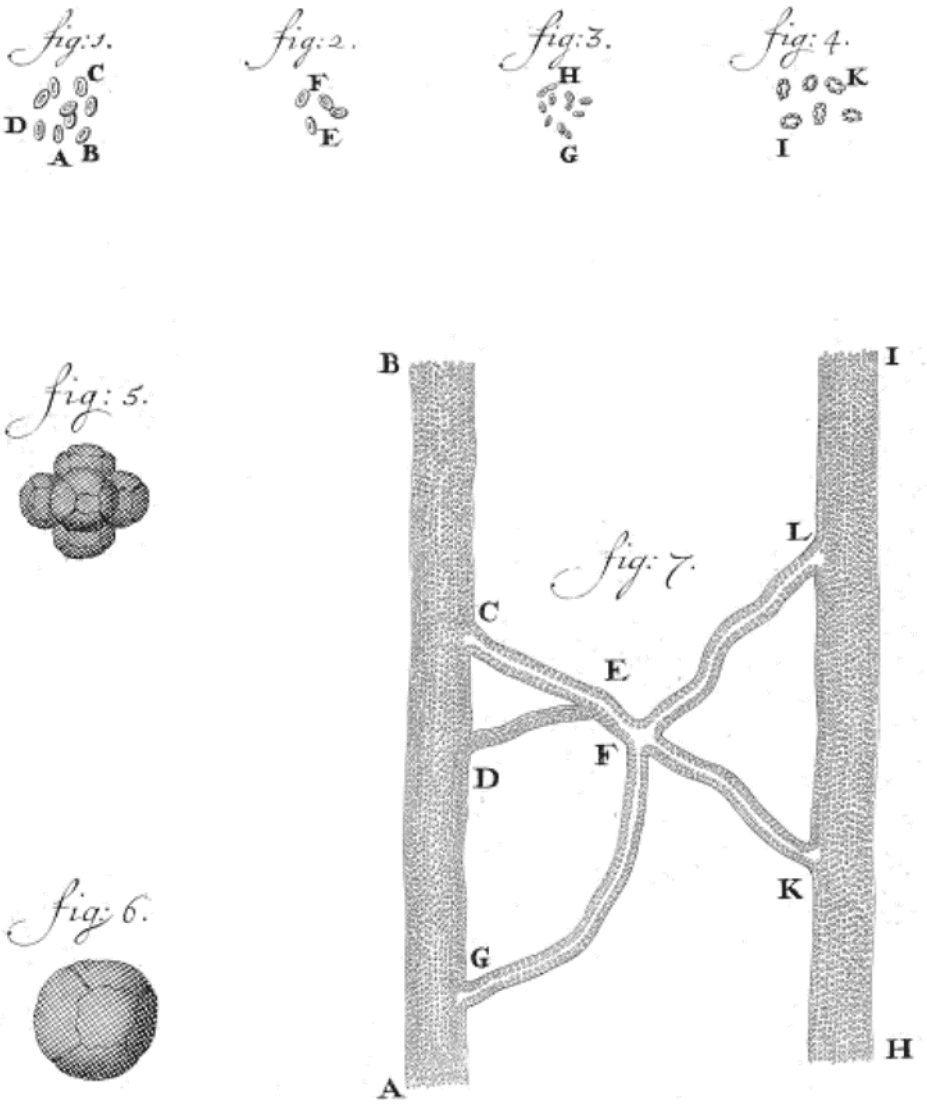


Figure 7.7. *Erythrocytes. fig. 1 salmon; figs 2-4 flounder; figs 5-6 wax models of the structure of erythrocytes, according to Leeuwenhoek (1702) (Leeuwenhoek, 1993, p. 426).*

It may be that the flounder erythrocytes, which are nucleate, show divisions into six subunits. However, it remains unclear what Leeuwenhoek was referring to when he wrote about thirty-six globules. He further describes a vision of the microscopic realm that is very perceptive, although exaggerated:

I learned to my astonishment that there are some people who venture to say that they will describe the origin of some things. For my part, even if I could discover the form

> of particles a thousand million [1 billion] times smaller than a blood globule, I am certain that I should still be far from being able to see their first constituents (Leeuwenhoek, 1993, p. 149).

If Leeuwenhoek could have seen 1,000 times smaller, he could have seen antibodies (~15 nanometers in diameter); if 10,000 times smaller, he could have seen individual water molecules (280 picometers) and carbon atoms; 1 billion times smaller (~7 femtometers) would have allowed him to view a proton – beyond the resolution power of light microscopy. His comment revealed he was not entirely content with what he saw but wanted to see even further into life's tiniest constructs.

In a 21 June 1701 letter to The Royal Society, Leeuwenhoek adds to previous comments on spider blood hemocytes. As in previous notes on hemocytes, he dismisses the "ancient" idea that spiders and other arthropods were bloodless animals. Leeuwenhoek manages to find poetry in the moment:

> Although this animal will have been known among the ancients as a bloodless animal because they have not seen any blood-red substance when they broke up these little animals or killed them, still I saw the particles of blood, which to all appearances were round globules, propelled in a liquid substance, so clearly as if one saw small peas rolling down a slanting board (Leeuwenhoek, 1993, p. 321).

In a letter to Heinsius dated 17 December 1712, the last of the published letters to mention blood corpuscles, Leeuwenhoek returned his attention to shrimp blood, at first confirming what he had reported previously, then commenting on the salts that formed after the hemolymph had been allowed to evaporate:

> after I had cut the shrimp crosswise, I found that as much as a drop of blood emerged from the fish-parts, which I observed, and I saw again the transparent globules of blood

> which floated in the watery fluid, the serum. After these blood-globules had been outside the shrimp for some little time, they floated in the fluid together in greater or smaller numbers, without coagulating, and only touching one another (Leeuwenhoek, 1993, p. 23).

With this comment, Leeuwenhoek ends his observations of red and transparent blood globules, although he continues throughout Volume 17 to add to his notes on circulation. Strangely, his first and last observations of blood were in arthropods. His comments on a louse feeding on blood in 1673 had not noted the hemocytes he would discover in shrimp about sixteen years later.

Recognition of the enormity of his work, which added nuance and detail to revelations provided in earlier letters to his correspondents, has been less than complete. Although this may have been due to incomplete editions of his letters before the publication of *Alle de Brieven*, the recognition has remained slight.

Why are Leeuwenhoek's many observations of blood cells important? Given his consuming curiosity and the resolution of his microscopes, it may have been inevitable that Leeuwenhoek became the first scientist to assess the number of red blood cells to fit in a volume ("250000 times smaller than a grain of sand" (Leeuwenhoek, 1939a, p. 103) or "1.000.000 blood-globules are the same size as one coarse grain of sand" (Leeuwenhoek, 1952, p. 267). He was a keen observer of corpuscle behavior under various conditions (e.g., dehydration of the serum and blood, hemolysis, shape distortion when passing through capillaries). Leeuwenhoek also commented on the absence of color in blood cells when their "thickness [is] not amounting to more than four or five globules" (i.e., cells) (Leeuwenhoek, 1939a, p. 85). Later letters described the blood changing from red to "dark-red or blackish"(Leeuwenhoek, 1939a, p. 95). In addition, he noted that whatever was

circulating in arthropods was not the same as his “red blood globules.” His observations often are lumped together with those of Marcello Malpighi and Jan Swammerdam, although their observations were brief and incomplete, whereas Leeuwenhoek’s observations span much of his life.

In a sense, his single-lens examination of red blood cells could be considered a prelude to the hemometer. This lens-based device would go through several iterations in the mid-19th century. While his observations were far from foreshadowing the invention of hemoglobinometers — colorimeters measuring hemoglobin concentration — his work might have alerted scientific minds in the 18th century that assessing color could be important for evaluating the properties of blood in general and red blood cells specifically.

CHAPTER 8 | Modeling disease from the hematological data of Gabriel Andral

8.1 Introduction

Gabriel Andral had the good fortune to be born into one of the most fecund periods in the development of medicine. It was also a period teeming with controversies, with Andral ultimately representing a new set of beliefs and winning over the minds of the next generation. He was born into a family that had nurtured three generations of doctors before his birth. By 1821, he had obtained his doctorate and was elected an associate member in June of 1823 to the *Académie royale de médecine*, followed by full membership in 1833. In 1843, he became a member of the *Académie des sciences*.

Andral was one of several French physicians and chemists, including Louis-René Le Canu, Jean-Louis Prevost, Jean-Baptiste Dumas, and Alfred Donné, who reignited interest in using microscopes as essential tools in anatomy, physiology, and pathology. As he wrote in his *Essai d'Hematologie pathologique*,

It was natural indeed that a short time after the discovery of Leeuwenhoeck, men should endeavour to discover what became of the globules of the blood in disease…. Physiology and pathology rejected as useless, or dreaded as a source of error, the employment of the microscope; and this instrument was completely abandoned, as chemistry had been before it. The glory of returning by the experimental method, to the microscopic, as it has done to the chemical study of the blood, was reserved for our epoch (Andral, 1844, pp. 26–27)[22].

Starting in 1674 and for thirty-nine years afterward, Leeuwenhoek documented his careful, dynamic, and revelatory investigations and observations of red blood "globules" in

[22] Andral wrote this passage 120 years after Leeuwenhoek's death and roughly 170 years after Leeuwenhoek's first mention of red blood globules. Whether this constitutes a "short time" is left to the reader to assess.

the circulatory systems of humans and other creatures and of hemocytes in the hemolymph of arthropods (I. M. Davis, 2022c, p. 1).

While Andral gave some credit to Leeuwenhoek, he may not have been aware of the interest Leeuwenhoek took in observing his own blood's behavior while he was ill (Leeuwenhoek, 1939a, p. 301). Andral did not reference knowledge of blood corpuscles provided by two other philosophers who worked in the 18th Century. William Hewson, FRS, published several articles, as well as sections of his book, describing his microscopic observations of blood (Hewson, 1771, pp.368-83; 1773, pp. 303-23; 1777, pp.1-44). Hewson also refers us to a little-known theologian, philosopher, and lens crafter from Naples, Father Josephus Maria de Turre[23] (Hewson calls him de la Torré), who created single lens "globules of glass" capable of magnifications of up to 2,560 times, allowing di Torre, as well as Baker, Stiles, and others, to view red blood globules (Baker, 1766;Stiles, 1765).

Hewson had relevance to Andral's research endeavor, which began in 1830 and concluded with his publication of three papers and a book between 1840 and 1843. Hewson sought to correlate the amount of *crassamentum* (fibrin clot) and serum with the subject's health from whom the blood was drawn. In his paper *Experiments on the Blood with some Remarks on its Morbid Appearance*, Hewson provided the results of experiments he conducted that correlated post-draw formation of the *crassamentum* in the presence of heat and at room temperature. He[24] remains solid" (Hewson, 1771, p. 371). More cogently,

[23] F.H.E. Stiles and Henry Baker called him di Torre, although the Philosophical Transactions reprints a letter from de Torre in Latin in which he refers to himself as "inclytae Societati Regiæ Anglicanæ Jo. Maria de Turre D.D.D." or "the renowned Royal Anglican Society" (Stiles, 1765). Whether this was a misprint by the editors or an exact transcript of the letter from de Turre is unclear.

[24]wson notes that "the crassamentum consists of two parts, of which one gives solidity, and is by some called the fibrous part of the blood, or the gluten, but by others with more propriety termed the coagulable lymph; and of another, which gives the red colour to the blood, and is called the red globules" (Hewson, 1771, pp. 370-1), thus preferring the term "coagulable lymph." However, later this would be called the fibrin.

Hewson makes the following general observation regarding the proportions of crassamentum and serum in healthy ("strong") and ill subjects, along with some recommendations for general treatment:

These two parts differ in their proportions in different constitutions: in a strong person, the crassamentum is in greater proportion to the serum than in a weak one and the same difference is found to take place in diseases; thence is deduced the general conclusion, that the less the quantity of serum is in proportion to the crassamentum, bleeding, diluting liquors, and a low diet, are the more necessary: whilst in some dropsies[25] and other diseases where the serum is in a great, and the crassamentum in a small proportion, bleeding and diluting would be highly improper (Hewson, 1771, pp. 368–369).

Further along, Hewson correlates the coagulable lymph or fibrin to a variety of conditions:

It is this coagulable lymph which forms the inflammatory crust, or buff as it is called. It likewise forms polypi of the heart, and sometimes fills up the cavities of aneurisms, and plugs up the extremities of divided arteries. It is supposed, by its becoming solid in the body, to occasion obstructions and inflammations; and even mortifications, from the exposition to cold, have been attributed to its coagulation. In a word, this lymph is supposed to have so great a share in the cause of several diseases, that it would be desirable to ascertain what brings on that coagulation, either in the body or out of it (Hewson, 1773, p. 376).

[25] Dropsy is an archaic term used to describe the build-up of fluid in body tissue (Comrie, 1928).

8.2 Gabriel Andral Studies Changes that the Blood Undergoes in Diseases

In 1829, Gabriel Andral and Jules Gavarret started large studies of human and animal blood. The studies occupied their work at least through the publication of *Essai d'hématologie pathologique* (Andral, 1843), translated into English as *An Essay on the Blood in Disease* (Andral, 1844). Andral intended to develop a basis for the "…refutation of the systematic physiology of irritation and of the exclusive solidism to which this physiology was chained," as Paul Emile Chauffard wrote in *Andral: la médecine française de 1820 à 1830* (Chauffard, 1877, p. 58). Chauffard continued that this "…was the anticipated justification for the anatomo-pathological study of blood, a study to which Andral was soon to devote himself entirely, and of which he was to deliver the fruits, ten years later, in 1840."

The study also reflected both physicians' interest in extending the work of Pierre Louis (1787-1872), a French physician whose interest in establishing numerical rigor in medicine served as a precursor to the fields of epidemiology and the design of clinical trials (Morabia, 1996, 2009). Andral and Gavarret had attended intense debates that had occurred in the *Académie des Sciences* in 1835 and in the *Académie de Médecine* in 1837 and were persuaded by Louis' *la methode numerique*. Gavarret was sufficiently motivated by these arguments that he wrote and published in 1840 *Principes Généraux de Statistique Médicale*, considered the first textbook on the application of statistical analysis to medicine, work that also relied on concepts taught by Siméon Poisson (1781—1840) and Pierre-Simon Laplace (1749—1827) (Huth, 2008).

The first of the Andral-Gavarret blood studies to be published was their human blood study, conducted between October 1839 and July 1840 and involved three hundred sixty bleeds from two hundred patients at the *Hôpital de la Charité de Paris*. The study resulted

in the accumulation and publication of over 1,850 values for components of blood, including blood corpuscles, fibrin, water, organic solids, inorganic solids, and total solids in samples collected from subjects in various states and stages of illness. It is probably the earliest large data set of blood-related data. It may be one of the earliest published large data sets in human health. It is certain that it was influenced by the work of Pierre Louis.

The subjects from which blood samples were drawn had been diagnosed with illnesses including shingles, face tic, jaundice, uterine hemorrhage, metrorrhagia/uterine cancer, rheumatoid arthritis, acute articular rheumatism, subacute and chronic rheumatism, pneumonia, acute capillary bronchitis, chronic bronchitis with pulmonary emphysema, pleurisy, acute peritonitis, tonsilitis, erysipelas, bladder infection, pulmonary tubercules, continuous fevers, simple fevers, fevers, typhoid fever, smallpox, varioloid (mild smallpox), measles, scarlet fever, intermittent fevers, cerebral congestion, cerebral hemorrhage, and beginning and confirmed chlorosis (Andral, Gabriel; Gavarret, 1840a, pp. 225–322).

All samples were treated as identically as their technique allowed. The method was adopted from the 1823 publication by Jean-Louis Prevost and Jean-Baptiste Dumas (Prévost & Dumas, 1823). Dumas, a prominent chemist of his time, provided direct encouragement to Andral and Gavarret. It is important to understand that the study was conducted before methods for red blood cell counts by microscope and micrometer had evolved. In fact, corpuscles were calculated by difference as described in the method summary provided below.

Andral and Gavarret collected each blood sample into two containers (*capsules*) by draining the first and fourth quarters of a bleed into one container and the second and third quarters into a second container. The first and fourth portions were allowed to coagulate,

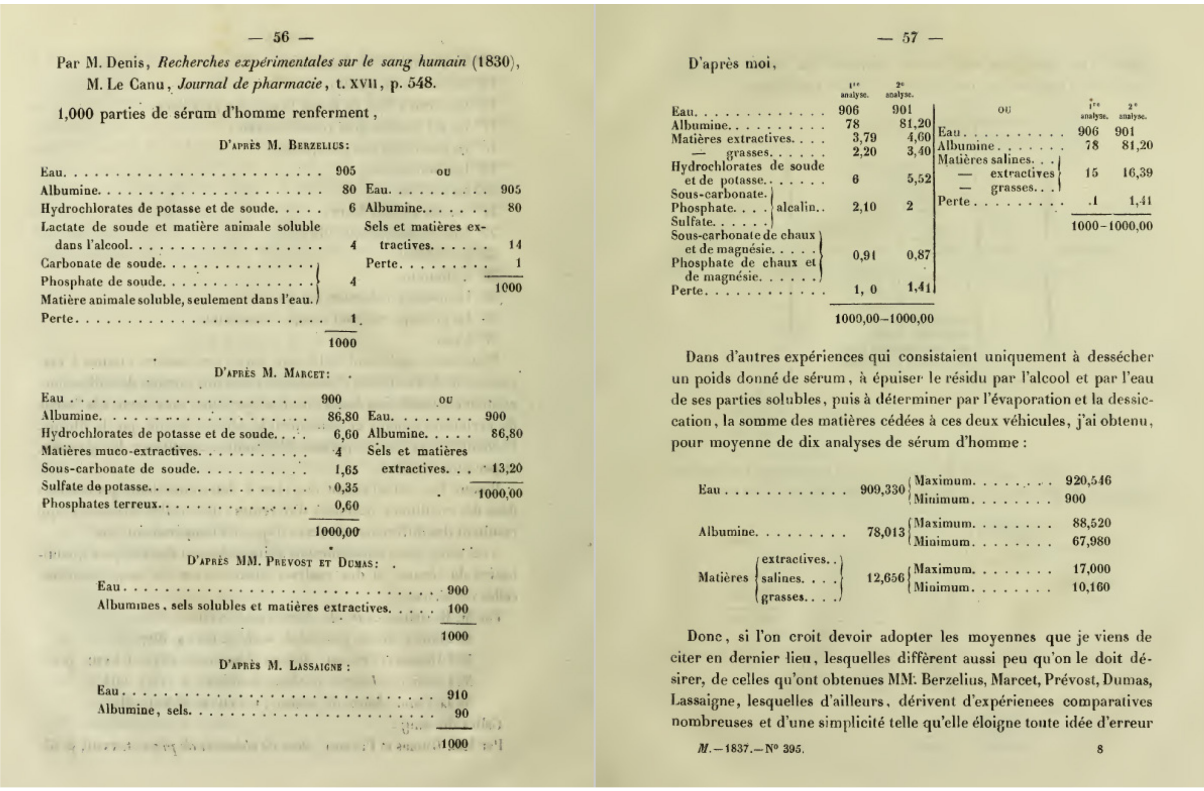
while the second and third portions were beaten to separate the fibrin from the sample, then the fibrin was washed "with care." The sample composed of portions one and four would contain serum, and the "clot," which represented the coagulated blood cells. The serum was "carefully separated from the clot and dried" and "its composition in water and solid materials" was determined. The clot was dried separately from the serum and weighed. Finally, "by subtracting from the weight of the dry clot, the weight of the fibrin, plus the weight of the solid materials of the serum which it contains, and which has been calculated, there remains the weight of the globules which the clot contains." By following this method, Andral and Gavarret were able to determine 1. the weight of the fibrin, 2. the weight of the blood cells, 3. the weight of the serum solid materials, and 4. the weight of the water in the samples. An additional incineration step made it possible to separate the organic (combustible) solids from the inorganic materials (Andral, Gabriel; Gavarret, 1840a, pp. 227–228).

It is not clear from the manuscript what methods were used to ensure equal quarter portions of blood were placed in each container, only that "the two portions of blood thus separated should have the same composition…." It is also unclear what volumes of wash were used on the fibrin fraction. However, the purpose of the wash was to separate the organic and inorganic solids from the fibrinous material and the aqueous fibrin wash was then dried and weighed. The fibrin was dried and, in comparison with portions one and four, the weight of the clot is determined by difference (Andral, Gabriel; Gavarret, 1840a, p. 227).

Andral did not generate a normal range from healthy subjects. Instead, he relied on the work of Louis-Réné Le Canu (1800—1871) as presented in his doctoral thesis *Etudes Chimiques sur le Sang Humain* presented on 23 November 1837 to the *Faculté de médecine*

de Paris (L.-R. Le Canu, 1837). The thesis had earned Le Canu a Prix de Monthyon from *l'Academie des sciences*. Andral indicates that the values Le Canu published were also presented in courses taught by Jean-Baptiste Dumas.

In fact, Le Canu provided a more thorough report of the components of blood than Andral, along with data tables from previous publications by Jakob Berzelius, Alexander Marcet (1770—1822), Jean-Louis Prevost and Dumas, and Jean-Louis Lassaigne (1800—1859). When Le Canu averaged the serum data from all previous investigators for water, albumin, and "*matieres*" (salts, fats, and extractives), he arrived at 904.940 for water, 80.800 for albumin, and 14.260 for materials. Le Canu then evaluated the proportions of serum and globules, reporting the results provided by Prevost and Dumas at 870.8 parts serum and 120.2 parts globules. Le Canu's results were similar, although he specified that his results are the average of ten analyses, with the serum average at 867.5094 parts, globules at 132.4906, along with maximum and minimum value range data (serum max/min: 884.150/851.550; globules max/min: 148.450/115.850). He expanded on his findings, writing that he found the following values for the components of blood: water = 790.3707; materials = 10.9800; albumin = 67.8040; globules = 130.8453. In summary, he defined serum as the total of water, materials, and albumin with a value of 869.1547 and globules at 130.8453 (*Figure 8.1.*). He also published a number for red blood cells (globules) with a value of around 130 parts per thousand. Le Canu's evaluation of globules, albumin, materials, and water was the most relevant data set to compare with Andral's work (L.-R. Le Canu, 1837, pp. 56–59).



— 56 —

Par M. Denis, *Recherches expérimentales sur le sang humain* (1830), M. Le Canu, *Journal de pharmacie*, t. XVII, p. 548.

1,000 parties de sérum d'homme renferment,

D'APRÈS M. BERZELIUS:

		ou	
Eau	905	Eau	905
Albumine	80	Albumine	80
Hydrochlorates de potasse et de soude	6		
Lactate de soude et matière animale soluble dans l'alcool	4	Sels et matières extractives	14
Carbonate de soude, Phosphate de soude, Matière animale soluble, seulement dans l'eau.	4	Perte	1
Perte	1		1000
	1000		

D'APRÈS M. MARCET:

		ou	
Eau	900	Eau	900
Albumine	86,80	Albumine	86,80
Hydrochlorates de potasse et de soude	6,60		
Matières muco-extractives	4	Sels et matières extractives	13,20
Sous-carbonate de soude	1,65		1000,00
Sulfate de potasse	0,35		
Phosphates terreux	0,60		
	1000,00		

D'APRÈS MM. PREVOST ET DUMAS:

Eau	900
Albumines, sels solubles et matières extractives	100
	1000

D'APRÈS M. LASSAIGNE:

Eau	910
Albumine, sels	90
	1000

— 57 —

D'après moi,

	1re analyse.	2e analyse.
Eau	906	901
Albumine	78	81,20
Matières extractives	3,79	4,60
— grasses	2,20	3,40
Hydrochlorates de soude et de potasse	6	5,52
Sous-carbonate, Phosphate, Sulfate alcalin.	2,10	2
Sous-carbonate de chaux et de magnésie, Phosphate de chaux et de magnésie	0,91	0,87
Perte	1, 0	1,41
	1000,00	1000,00

ou	1re analyse.	2e analyse.
Eau	906	901
Albumine	78	81,20
Matières salines	15	16,39
— extractives		
— grasses		
Perte	1	1,41
	1000	1000,00

Dans d'autres expériences qui consistaient uniquement à dessécher un poids donné de sérum, à épuiser le résidu par l'alcool et par l'eau de ses parties solubles, puis à déterminer par l'évaporation et la dessiccation, la somme des matières cédées à ces deux véhicules, j'ai obtenu, pour moyenne de dix analyses de sérum d'homme :

Eau	909,330	Maximum	920,546
		Minimum	900
Albumine	78,013	Maximum	88,520
		Minimum	67,980
Matières extractives, salines, grasses	12,656	Maximum	17,000
		Minimum	10,160

Donc, si l'on croit devoir adopter les moyennes que je viens de citer en dernier lieu, lesquelles diffèrent aussi peu qu'on le doit désirer, de celles qu'ont obtenues MM. Berzelius, Marcet, Prévost, Dumas, Lassaigne, lesquelles d'ailleurs, dérivent d'expériences comparatives nombreuses et d'une simplicité telle qu'elle éloigne toute idée d'erreur

M. — 1837. — N° 395. 8

Figure 8.1. *Normal blood component results as presented in the 1837 thesis presented by Louis-Rene Le Canu (L.-R. Le Canu, 1837, pp. 56-7).*

Still, Andral's study might have been strengthened had he developed a robust normal range using the techniques he and Louis Jules Gavarret implemented, if only to confirm the results other scientists had provided. Given the thorough coverage Le Canu provided in his thesis, it is understandable that Andral believed that existing blood analysis methodology was sufficiently mature to provide a normal baseline against which he might compare blood analyses from ill patients.

8.3 Results of Andral's Blood Analysis from Ill Patients

A principal concern for Andral was assessing any differences between inflammatory and pyretic illnesses. He hoped to discover differences in the measurement of components in blood, particularly in fibrin and blood corpuscle trends in patients who had been diagnosed with an inflammatory or febrile illness. In either inflammation or fever, the other condition

might exist but one of the conditions would dominate. Although the terms would be adopted much later in medical practice, Andral searched for blood-based surrogate endpoints, also known as biomarkers.

Blood sampling in cases of rheumatoid arthritis focused on a disease that was adequately understood in terms of its symptoms. However, different subtypes of rheumatism were identified as acute articular, subacute, and chronic rheumatism. The patients could report their pain and variable fevers, thus presenting a basis for the blood draws. Andral believed that fibrin increased during periods where pain and fever from rheumatism increased and diminished when pain and fever decreased. Changes in fibrin levels were not accompanied by changes in the number of blood corpuscles. His testing sought to confirm or refute his belief.

On the other hand, Andral's categorization of "continuous fevers" came with the explanation that they are one of five types of pyrexia. Three of these pyrexias—continuous, simple, and those that he identified simply as fevers—are associated with a variety of conditions, some of which are prodromal or an early symptom of disease, while typhoid fever, and the "eruptive fevers" smallpox, measles, and scarlet fever, are specific diseases associated with fevers. Andral explains that the pyrexias are characterized by "a general disturbance which is manifested by a febrile movement of variable duration and intensity." Philippe Pinel (1745—1826) divided them into categories of inflammatory and bilious fevers, but Andral sets aside this distinction (Andral, Gabriel; Gavarret, 1840a, p. 273). The underlying illnesses that resulted in "simple fevers" included chronic gastritis, "organic disease of the heart," chlorosis, and colitis. The illnesses that resulted in "fevers" included cases of angina, erysipelas, tonsilitis, bronchitis, and mild meningitis. The overall category is complicated by the variety of underlying conditions and the relative lack of information

other than that a fever of some type is involved. Typhoid fever was a well-characterized illness; Andral developed a large data set to investigate typhoid fibrin and corpuscle trends.

Andral also addressed the illness known as chlorosis, a poorly characterized illness but in Andral's time a "…form of hypochromic anemia possibly associated with gastric ulceration and poor diet" or "…a disorder of psychogenic origin resembling, but not identical to, anorexia nervosa" (Loudon, 1984, p. 27). Strangely, diagnoses of anorexia nervosa started in the 1870s, while chlorosis disappeared in the early 20th century (Loudon, 1984, pp. 32, 34). Loudon notes an interesting correlation between socioeconomic position and the symptoms of chlorosis patients presented, with middle to upper class women presenting symptoms associated more closely with chloro-anorexia, while working women presented symptoms related to chloro-anemia (Loudon, 1984, p. 33). Andral studied variations in blood components related to "beginning chlorosis," confirmed chlorosis, and "chlorosis in a man"— a condition that seems contrary to diagnoses of chlorosis in Andral's time.

CHAPTER 9 | Blood Feud: Carl Schmidt, Karl von Vierordt and the Evolution of Quantitative Blood Methods

9.1 Abstract

This paper will examine the accomplishments and quarrels of two mid-19th-century German physiologists who worked towards characterizing the components of human blood. Between 1848 and 1852, Carl Schmidt and Karl von Vierordt, two accomplished investigators who taught at universities in Tübingen and Dorpat, published research that quantified the organic and inorganic components in human blood (Schmidt) and counted the number of red blood cells in a unit volume of blood (Vierordt). Their work had little overlap except for their mutual interest in improving blood fluid analytical techniques. Schmidt's work attempted to determine how cholera and other diseases affected the amounts of blood components, while Vierordt's goal intended to improve blood cell microscopy methods, a goal that he accomplished.

Schmidt's 1850 publication may have hastened Vierordt's publications in 1852, three of which included criticisms of Carl Schmidt's methods. Schmidt replied to the first two criticisms and left Vierordt's final response unanswered. In reviewing their criticisms, it is difficult to understand the reason for their quarrel. They shared an interest in attempting to manage error in their determinations.

Schmidt and Vierordt might have avoided their public dispute by communicating their misunderstandings via a series of letters or through the colleagues and mentors they had in common. This article provides a brief biography and work summary of each physiologist and a summary of their disagreement.

9.2 Introduction

At the beginning of the 19th century and after a century of use by hobbyists and naturalists, microscopy gained the attention of physiologists and chemists as an essential means of gathering information. Chemistry, particularly the use of chemistry to untangle the questions posed by human and animal physiology, also saw innovations in quantitative methods for determining organic and inorganic components in blood.

It was principally French physiologists and physicians, including Jean-Louis Prévost, Jean-Baptiste Dumas (Prévost & Dumas, 1823), Francois-Vincent Raspail (Raspail, 1830), Alfred Donné (Donné, 1831), Louis-Réné Le Canu (J.-L. Le Canu, 1837), Gabriel Andral, and Louis Gavarret (Andral, Gabriel; Gavarret, 1840b) that recognized the applicability of microscopy to the study of human physiology, and particularly to the analysis of blood. It was a time during which chemistry and physiology were clarifying their separate roles in medicine and when the tools used in each discipline were found to be helpful by other investigators. Raspail, later to be known as the father of histochemistry, poked a little fun at this phenomenon when he wrote in his 1830 paper: "The physiologist was ignorant of the art of using reagents; the chemist did not know that of using the microscope; the botanist was ignorant of both" (Raspail, 1830, pp. 3–4). Vierordt saw his work as an integration of microscopy techniques from physiology and chemistry much in the way that Raspail had inferred should be so.

Influential German physiologist Rudolph Wagner (1805—1864) had published his work titled *Zur vergleichenden Physiologie des Blutes. Untersuchungen über Blutkörperchen, Blutbildung und Blutbahn*[26], a study of the sizes of red blood cells in humans and other

[26] On the comparative physiology of blood. Examinations of blood cells, blood formation, and bloodstream.

species in 1833, possibly igniting interest amongst his colleagues (Wagner, 1833). In the 1840s, following a significant effort by Andral and Gavarret to define illnesses by quantitative assessment of blood components (Andral, Gabriel; Gavarret, 1840b), much of the innovative work in the new field of hematology shifted to investigators in Germany. Two investigators emerged as innovators in the field of blood physiology: Carl Schmidt and Karl von Vierordt.

9.3 A New Generation of Physiologists

9.3.1 Carl Ernst Heinrich Schmidt (1822—1894)

Carl Schmidt was born in Mitau on 13 June 1822 and learned chemistry and pharmacy methods with his father, the local pharmacist. Schmidt studied medicine at the University of Berlin but also studied with chemist Heinrich Rose (1795—1864), author of *Handbuch der analytischen Chemie* (1830) and other foundational texts. Schmidt earned a Ph.D. in 1844 with Justus von Liebig (1803—1873) at Giessen, transferred to Göttingen, worked with chemist Friedrich Wöhler (1800—1882) and physiologist Rudolph Wagner (1805—1864), from whom he earned his first M.D. in 1845. Schmidt then moved to the University of Dorpat in Estonia, where he was awarded a second M.D. in 1846 while working at the military medical academic in St. Petersburg with anatomist and surgeon Nikolai Pirogoff[27] (Koutsouflianiotis et al., 2018), a mentor to whom Schmidt would refer in his 1850 cholera article. At the time, Estonia was administered by the Russian Empire. A second M.D., granted by a Russian institution, was necessary for physicians working in the Empire. He worked as an unpaid lecturer (*Privatdocent*) until 1850, when he became a pharmacy

[27] This is the spelling used by Schmidt; Pirogov is an alternative spelling.

professor and then a chemistry professor in 1851, a position he would retain for the next 40 years (Zaleski, 1894).

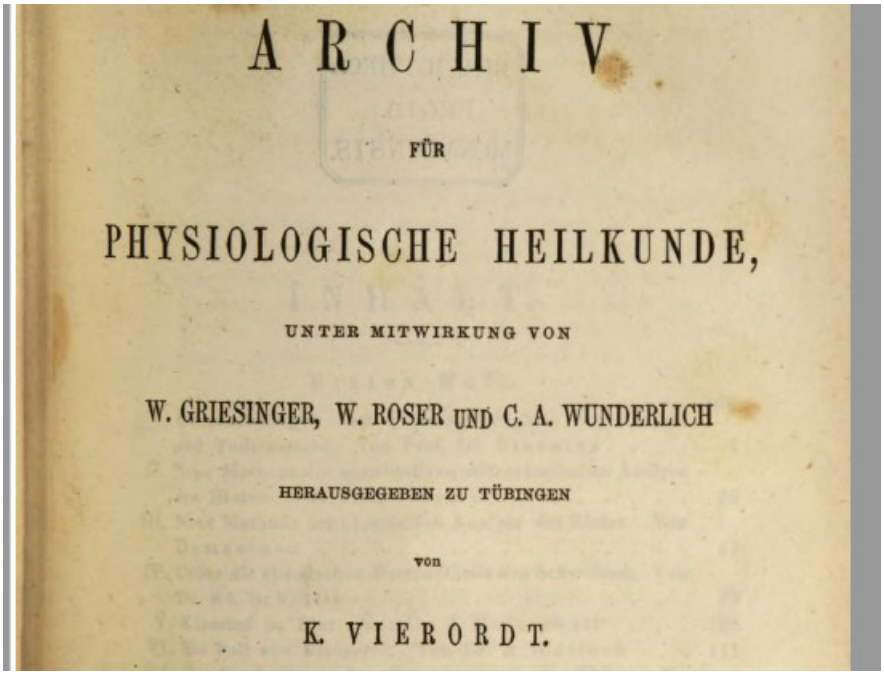
Schmidt had rapidly earned credentials as a physician working with a leading physiologist and an innovating surgeon, and he had also studied with some of the finest minds in chemistry.

9.3.2 Karl von Vierordt (1818—1884)

Karl von Vierordt (1818—1884) was born in Lahr, Baden, to Dr. Carl Friedrich Vierordt, a deacon who became lyceum director. His mother was the daughter of a Lahr merchant. In 1836, Vierordt started studying medicine at the University of Heidelberg. His mentors included anatomist Friedrich Tiedemann (1781-1861), physiologist and biologist Theodor Bischoff (1807—1882), and chemist Leopold Gmelin (1788—1853). Vierordt studied for a year (1838—1839) in Gottingen with anatomist and surgeon Conrad Langenbeck (1776–1851) and chemist Friedrich Wöhler (1800—1882), although he did so five to six years before Schmidt worked with Wöhler. After studying in Berlin, Vierordt returned to Heidelberg and, in May 1841, passed his doctoral examination with a top grade. One of his professors, Franz Nagele (1778—1851), remarked to Vierordt that "*Wir sind mit Ihnen vollständig zufrieden* (We are completely satisfied with you)" (H. Vierordt, 1885, p. III). During the coming decade, Vierordt published on the pathology and therapy of strabismus, respiration physiology, and typhoid anatomy and wrote a general perspective on physiology.

Vierordt became editor of the journal *Archiv für Physiologische Heilkunde* (Archive for Physiological Medicine) in 1850 (*Figure 9.1.*), a position he retained until 1856. With the French physiologists preceding him and Julius Vogel's blood measuring techniques (Vogel,

1841) and Carl Schmidt (Schmidt, 1850a) spurring him on, Karl von Vierordt started documenting his work on blood and red blood cells.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FÜR

PHYSIOLOGISCHE HEILKUNDE,

UNTER MITWIRKUNG VON

W. GRIESINGER, W. ROSER UND C. A. WUNDERLICH

HERAUSGEGEBEN ZU TÜBINGEN

VON

K. VIERORDT.

Figure 9.1. *The title page for Archiv fur Physiologische Heilkunde (1851) is the journal Karl von Vierordt edited from 1850 to 1856 (K. von Vierordt, 1851a, p. Title).*

He published a preemptive issue of *Archiv…* comprised entirely of his article *Mittheilung Zweier Neuen Methoden der Quantitativen mikroskopischen und chemischen Analyse der Blutkörperchen und Blutflüssigkeit*[28] (K. von Vierordt, 1851a). The 1852 issue (Volume XI) contained five articles by Vierordt addressing various aspects of blood analysis, including two articles taken from his 1851 publication (*Neue Methode der quantitativen mikroskopischen Analyse des Blutes*[29], *Neue Methode der chemischen Analyse des Blutes*[30]), along with *Neue Methode der Bestimmung des Rauminhaltes der*

[28] Communication of two new methods of quantitative microscopic and chemical analysis of blood cells and blood fluid.

[29] New method of quantitative microscopic analysis of blood.

[30] New method of chemical analysis of blood.

Blutkörperchen[31], *Zählungen der Blutkörperchen des Menschen*[32], as well as *Untersuchungen über die Fehlerquellen bei der Zählung der Blutkörperchen*[33] (Vierordt, 1852, pp. 855–880).

Vierordt stated that his work was driven principally by a belief that "…science is not in possession of useful counts about the numerical ratios of the blood corpuscles in one given blood volume" (K. von Vierordt, 1851a, p. 1). These articles would be judged much later as being "such an integral part of everyday knowledge that their discoverer tends to be forgotten" (Verso, 1971, p. 61).

His work had important precedents, going back to a 1674 letter Leeuwenhoek wrote Henry Oldenburg at the Royal Society. In it, Leeuwenhoek described the blood cells as "… red Globuls of the Blood I reckon to be 25000 times smaller than a [fine] grain of sand," placing the size of a blood cell at around eight microns. He clarified this further in a 1684 letter, stating that "…the complete globules that make our blood red are so small that 100 of them laid lengthwise would not make up the axis of a coarse sandgrain." Leeuwenhoek's measurements indicated how many blood cells were proportional to a sand grain but did not count the number of blood cells in a fixed volume. He often reported the number of organisms or objects within a non-standard volume, such as a millet seed or a sand grain, which might have provided the earliest information regarding the number of red blood cells suspended in a volume of fresh blood. Leeuwenhoek found blood cells and their circulation so fascinating that they would occupy large portions of his correspondence (I. M. Davis, 2022a;L. Robertson et al., 2016a). Work performed by French physiologists and Carl Schmidt documented the percent of water, blood cells, plasma, and organic and inorganic

[31] New method of determining the volume of blood cells.
[32] Human blood cell counts.
[33] Investigations into the sources of error in the counting of blood cells.

components in blood fluid. However, it did not determine the number of blood cells in precipitated or compressed blood cells.

9.4 Published Research

9.4.1 Carl Schmidt's Blood Publications

Schmidt's blood publications started in 1847, the year in which he published *Ueber das specifische Gewicht des Albumins, Muskelfibrins, der Blutkörperchen und Sehnen*[34] and *Ueber die Zusammensetzung der Blutkörperchen und die Ermittelung der Blutmischung aus dem specifischen Gewicht*[35]. Both were published in *Justus Liebig's Annalen der Chemie* (Schmidt, 1847a, 1847b). These works were followed by *Die Diagnostik verdächtiger Flecke in Criminalfällen*,[36] focused on the application of blood analysis in forensic medicine (Schmidt, 1848). Schmidt described methods of distinguishing human blood from the blood of other species and from a range of other red-colored stains that might be found at the site of criminal activities. The article presented a table that shows the various sizes of blood cells from human males and eleven other species, along with some simple statistics showing averages and range of blood cell sizes. The work does not mention the 1833 work by Rudolph Wagner that also addressed size differences of red blood cells by species but cites a series of works by other scientists working in forensic sciences: Alfred Bequerel[37] (1814—1862) and Alexandre Rodier (1811—?), both French physicians, along with chemists Karl Enderlin (dates unknown) and Wilhelm Henneberg (1825—1890), both Liebig students publishing in *Liebig's Annalen*. A second table shows the effect on blood cells from fewer species when they have dried on wood or various tissues, although the

[34] On the specific gravity of albumin, muscle fibrin, blood cells and tendons.
[35] On the composition of the blood cells and the determination of the blood mixture from the specific weight.
[36] The diagnosis of suspicious spots in criminal cases.
[37] Schmidt spells Dr. Becquerel's name as "Bequerel".

table is silent on what other tissues might have been examined. Schmidt reported that blood cells that had fallen on an absorbent surface, such as wood, were smaller than those viewed on a glass plate, illustrating the effect of serum leaching out of the blood cells. In the process of collecting replicate blood cell size data sets for humans and other animals, Schmidt developed methods for placing the blood cells on microscope slides "in a layer not more than 0.002 to 0.008 millimeters thick." He recommended "a linear magnification of 500 times (Schmidt, 1848, p. 34) and the use of Weber's optical glass micrometer (Schmidt, 1848, p. 4). In this or any of the other papers cited, Schmidt does not indicate that he has counted the number of blood cells in a fixed volume of blood.

While making his case for new blood detection methods, he wrote *Experimentalkritik* sections finding fault with methods described by other scientists developing forensic methods. His comments targeted work by Mathieu Orfila, considered one of the fathers of forensic toxicology and medicine, J. P. Barruel, a chemist with the faculty of medicine in Paris, Alphonse Chevallier, a chemist and professor at the Paris School of Pharmacy, and pathologist Charles-Prosper Olliver d'Angers.

Mentioning these "critical" interactions might seem unnecessary; given the unfortunate exchanges between Schmidt and Vierordt, they become relevant. It is interesting to note that Schmidt was careful to cite other investigators with whom he found fault or favor. His criticisms were reserved for methods described in the papers, not for his colleagues.

The Schmidt paper Vierordt cited as flawed was *Charakteristik der epidemischen cholera gegenüber verwandten Transsudationsanomalieen*[38] (Schmidt, 1850b). Schmidt strived to establish blood analysis methods that could help define the onset and presence of cholera and other illnesses. As such, he attempted to improve on the work done previously

[38] Characteristics of epidemic cholera versus related transudation anomalies.

by Le Canu (L.-R. Le Canu, 1837), who worked towards defining blood-based surrogate markers in his dissertation, and by Andral and Gavarret, who collected over 1,850 data points from blood samples to establish surrogate marker trends for a variety of illnesses (Andral, Gabriel; Gavarret, 1840a).

Schmidt approached the analysis of organic and inorganic blood components stepwise, initially developing a yeast cell model and then moving to the chemical analysis of blood from various animals. Justus von Liebig had foreshadowed the need for this approach in *Chemische Untersuchung über das Fleisch und seine Zubereitung zum Nahrungsmittel*[39]*:*

If one considers with any attention the facts established in animal chemistry, one will be astonished to see how few of them are built on firm conclusions. The reason for this seems to me to be that up to now only a comparatively very small number of professional chemists have seriously concerned themselves with the cultivation of this field and have made it the subject of thorough research (Liebig, 1847, p. 1).

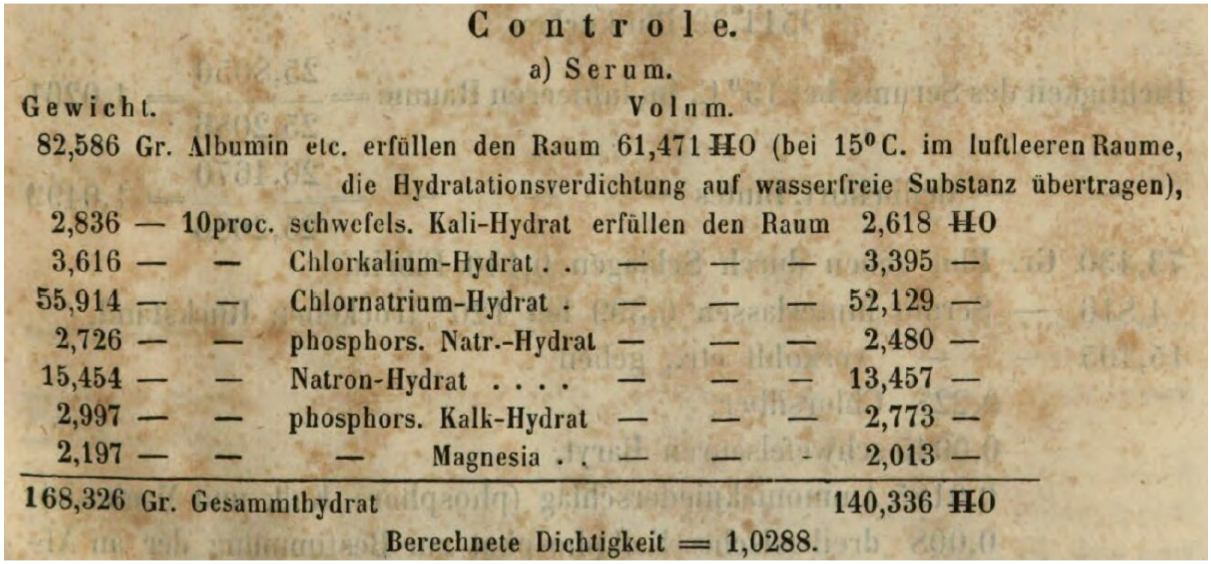
Vierordt seemed to agree with Liebig's perspective when he wrote, "The power of chemistry in examining the blood is greater than is often believed; the inadequacy of the previous procedure does not lie in the actual chemical procedures, but rather in the fact that what was to be analyzed did not properly fall into the hands of the chemist" (K. von Vierordt, 1851a, p. 29). Vierordt was silent on whether he was echoing Liebig's thoughts or addressing Raspail's observation.

Schmidt's method investigations provided time-based data on blood clotting in horses and sheep, all the while measuring density, serum, solid residues, and anhydrous substances. This forms the basis of a method for pressing the serum out of the blood clot

[39] Chemical analysis of meat and its preparation for food.

and for determining the relative amounts of clot and intercellular fluid. He studies blood and intercellular fluid trends with respect to diet: omnivore (humans), carnivore (dog, cat), and herbivore (sheep, goat). The human subjects are a mix of healthy and ill subjects with cholera, albuminuria, dropsy, and diabetes.

At the core of the methodological portion of his monograph, Schmidt reported a series of controlled experiments that measured "non-volatile substances at 120 °C," or the hydrated and anhydrous inorganic materials found in blood cells and serum (*Figure 9.2.*). For the blood cells, he also reports the amount of water, hematin, "blood casein[40]," and chlorine, while for the intercellular fluid, he reported water, fibrin, albumin, and chlorine, along with the density of cells and fluid. Not surprisingly, the cells are found to be denser than the intercellular fluid.



Controle.

a) Serum.

Gewicht.			Volum.
82,586 Gr.	Albumin etc. erfüllen den Raum		61,471 HO (bei 15° C. im luftleeren Raume, die Hydratationsverdichtung auf wasserfreie Substanz übertragen),
2,836 —	10proc. schwefels. Kali-Hydrat	erfüllen den Raum	2,618 HO
3,616 —	— Chlorkalium-Hydrat . .	— — —	3,395 —
55,914 —	— Chlornatrium-Hydrat .	— — —	52,129 —
2,726 —	— phosphors. Natr.-Hydrat	— — —	2,480 —
15,454 —	— Natron-Hydrat	— — —	13,457 —
2,997 —	— phosphors. Kalk-Hydrat	— — —	2,773 —
2,197 —	— — Magnesia . .	— — -	2,013 —
168,326 Gr.	Gesammthydrat		140,336 HO

Berechnete Dichtigkeit = 1,0288.

Figure 9.2. *An example of a data report from the analysis of a blood sample (Schmidt, 1850b).*

[40] Casein is now known as a milk protein. Schmidt was probably using "blood casein" as a stand-in for organic material that did not behave like fibrin or albumin under his analytical conditions. It may have been related to the protein portion of hemoglobin, which had not been identified at the time of Schmidt's work.

The core of his work is in identifying the acidic and basic salts present in the cells and fluids by using various evaporative and precipitative methods available to quantitative chemists of his time. Schmidt was probably following methods he learned when working with Dr. Heinrich Rose. Schmidt identifies potassium, sodium, magnesium, calcium, and ammonia salts in their phosphate, sulfate, bicarbonate, and chloride forms, along with iron oxide. In all cases, he tracks density, sometimes reporting his findings out to four decimal places (e.g., 1.0955 for blood cells and 1.0351 for intercellular fluid).

Schmidt's analyses resulted in two tables, one providing a "predominantly quantitative" list (*Table 9.1.*) of pathognomonic symptoms and the other a "predominantly qualitative" list (*Table 9.2.*).

Table 9.1. *Predominantly quantitative list of pathognomonic symptoms.*

General observation	***Specific condition***	***Specific illness***
1) Increase in total blood volume:	General congestion	Hyperemia
2) Reduction of the same:	General lack of blood	Anemia
3) Increase in all solid substances, reduction in water content:		Cholera, in part
4) Decrease in all solids, increase in water content:		Hydremia
5) Increase in some, decrease in other solid substances, in fact		
α) Reduction of water and salts, increase in albuminates		
β) Decrease in albuminate, increase in water and salts:		Dropsy
γ) Reduction in blood cells and individual albuminates (protein), increase in others (fibrin) in the intercellular fluid in addition to water:		Inflammation
		Dysentery
		Albuminuria
δ) Decrease in blood cells, increase in water:		Chlorosis

Table 9.2. *Predominantly qualitative list of pathognomonic symptoms.*

	Unusual discharge from the circuit:	***Therefore, onward flowing blood is characterized by:***
1)	a) Through the intestinal capillaries, b) From water and salts c) From blood cells, lots of protein, little salt, and water	Increase in protein and blood cell content, relatively significant reduction in salts: 1 a.) Cholera. b) Artificially induced intestinal capillary transudates (laxative effects). Decrease in protein and blood cells, increase in fibrin: 2. Dysentery. Reduction in protein, increase in salt and, under certain circumstances, fibrin content (entry of a diffusion equivalent of salt in place of the existing protein); 3. Albuminuria. Diminution of albumen, increase of salts in the appropriate proportions. 4. Dropsy.
2)	Through the kidneys, a) Of albumen and water	
3)	Through the capillary system of connective substance layers (serous membranes, subcutaneous connective tissue), a) Water and salts in the average ratio of the intercellular fluid (1000: 8 to 9), on the other hand, of albumen in significantly different quantities (4 to 50 p.m.), depending on the organ of excretion.	

It is clear from these tables and from roughly half of Schmidt's monograph that he is more concerned with the description of disease states through the presence, absence, and changes in amounts of various "albuminates, carbohydrates (sugar, gum, starch) and hydrocarbons (fats) into the organism versus the elimination of certain oxidation and decomposition products (carbonic acid, water, urea)… then, as is well known, certain

mixed and functional anomalies result … whose essence we call 'disease'"(Schmidt, 1850b, p. 35).

We will now examine Vierordt's work, which had a different intent.

9.4.2 Vierordt Counts the Number of Red Blood Cells in a Cubic Millimeter of Blood

Vierordt's requirements for the determination of the number of red blood cells in a cubic millimeter of blood fluid were extensive. These started with the specifications of capillary tubes used to draw the blood from a finger prick and expel the blood onto a microscope slide (*Figure 9.3.*). Vierordt's capillary tubes had to have consistent interior diameters for at least the height of the blood column, which should be five to eight millimeters, openings of 0.087 or 0.1805 mm, and a thin open edge so that blood would not adhere to the lip. When the volume of the blood column was calculated, the investigator had to consider the volume of the concave menisci at either end of the column (K. von Vierordt, 1851a, pp. 5–9).

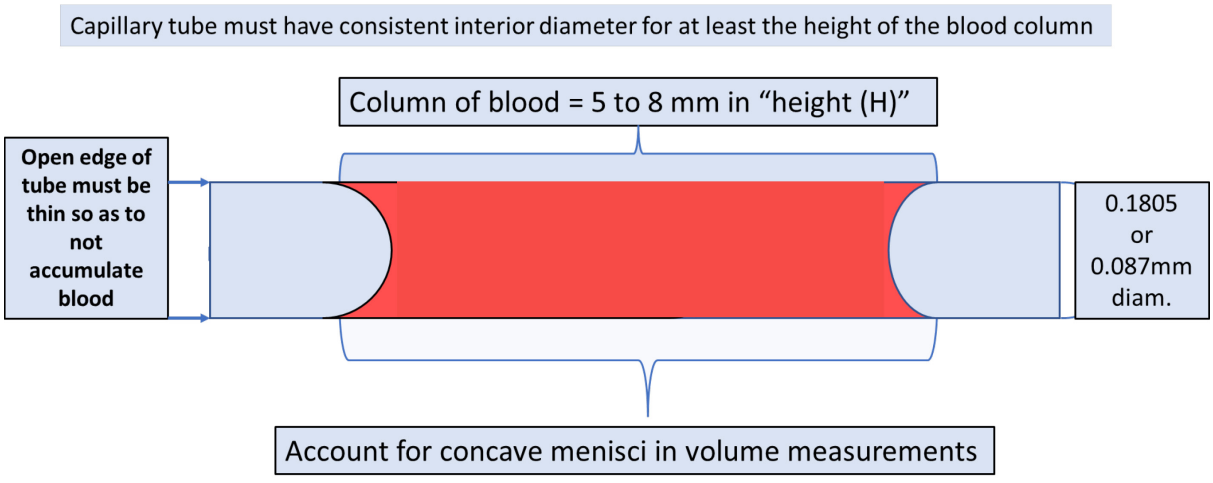


Figure 9.3. *Diagram of Vierordt's capillary tube for collecting blood fluid for blood cell counting.*

Measuring the precise height of the blood column and the menisci required placing the capillary on a micrometer slide under the microscope's objective lens. Vierordt provided equations for calculating the contribution of the menisci volumes and stated that, although there can be differences in menisci measurement techniques, the error was less than 1 percent.

He indicated that one must be careful in using blood too soon from a pinprick, as the initial blood droplet might be diluted by parenchymal fluid that he called *liquor 220nutritius*[41].

Application of the blood to a slide must be made in a "menstruum[42]" coat that acts as a sample preservative and stabilizer. The blood was best measured when smeared onto the slide like the "spokes of a wheel." Vierordt initially used albumin, then switched to gum Arabic, then to pure egg white (Vierordt, 1852, pp. 45–46). Later in the same article, he determined that "…pure, highly concentrated rubber solution" is the preferred diluent.

He does not recommend cell counting to those without experience, as he characterized it as a "…true torment of Tantalus." In Greek mythology, Tantalus was a god cursed to spend eternity in a pond under a fruit tree, doomed never to reach the low-hanging fruit or drink from the elusive pond waters. Vierordt then says that it is "…nowhere near as frightening as one might think at first sight. It is of course not at all suitable for imprecise and unscrupulous workers."

Germany was on the cusp of working within the metric system developed in the late 18th Century in France. While Schmidt seemed to have adopted the metric system for his measurements, Vierordt still used both localized and metric measurements, including the

[41] A term used to describe lymph.
[42] A substance that dissolves a solid or holds it in suspension.

German "*linie*," "*zoll*," and "*fuss*. A *fuss* was about 11.26 present-day inches (a *zoll* in 1850s Tübingen) or 286 millimeters. A *linie* would have been either 0.094 or 0.078 of a *fuss;* Tübingen's localized measurement systems in Vierordt's time placed each *linie* at either $1/10^{th}$ or $1/12^{th}$ of an inch. There were roughly 11.26 inches or 286 millimeters in a *fuss* or foot in the Baden-Württemberg region (Niemann, 1830; Cardarelli, 2003). A simplified table of these conversions can be found in *Table 9.3.*. If we consider the divisions into millimeters, the *linie* would have been 2.38 millimeters or 1.99 millimeters. When Vierordt reported his blood corpuscles counts, he used millimeters and cubic millimeters. Given that his micrometer was scored in *linie*, given that the metric system did not become the obligatory measurement system in Germany until 1872, one year after the unification of German states, twenty years after Vierordt wrote these papers, it is unclear whether Vierordt's millimeters were based on new or old standards. Vierordt might have used the metric system or a hybrid system that relied on the localized system for a determination of metric measurements.

Table 9.3. *Archaic 19th C. German Length Measurements (Cardarelli, 2003).*

Old German Units of Length – Old German units were employed under the Prussian system and had many localized variations [1 fuss (Rheinlandischer) = 0.313857 meters]			
Elle	Fuss (foot	Zoll	Line
= 96/17 (= 5.6471)	= 12	= 144	= 1728
1	= 17/8 (= 2.125)	= 51/2 (= 25.5)	= 306
	1	= 12	= 144
		1	= 12

Vierordt used two micrometers for his corpuscle counting procedures. The objective lens micrometer divided a *linie* into 30 parts, each of the drawn lines nearly nine *linie* in length. Perpendicular to these lines were drawn eighty marks (*Striche* or strokes), each

equally placed and each slightly over one *linie* in length, with the 5th and 10th drawn out longer to make the observer's work easier. The result was that the corpuscles were counted with the objective lens micrometer using subdivisions of nine square *linie*. While Vierordt was very clear about the objective lens micrometer's design, manufacturer, and cost, he did not reveal the same information about the ocular micrometer. He only comments that it was of superior refinement to the "large micrometer."

Unlike Schmidt, who had adopted the Celsius scale for his temperature measurements, Vierordt recorded the ambient temperature of his laboratory during blood cell counts using the Réaumur (°R) temperature scale. This scale was calibrated to read the boiling point of water at 80 °R, but the reading could vary based on thermometer manufacturer and what liquid was enclosed in the thermometer (Middleton, 1966, pp. 79–80; Gauvin, 2012). It is improbable that current researchers could reproduce Vierordt's temperature conditions given his use of the Réaumur system without having access to the specific thermometers he used and cross-calibrating them with current thermometric devices.

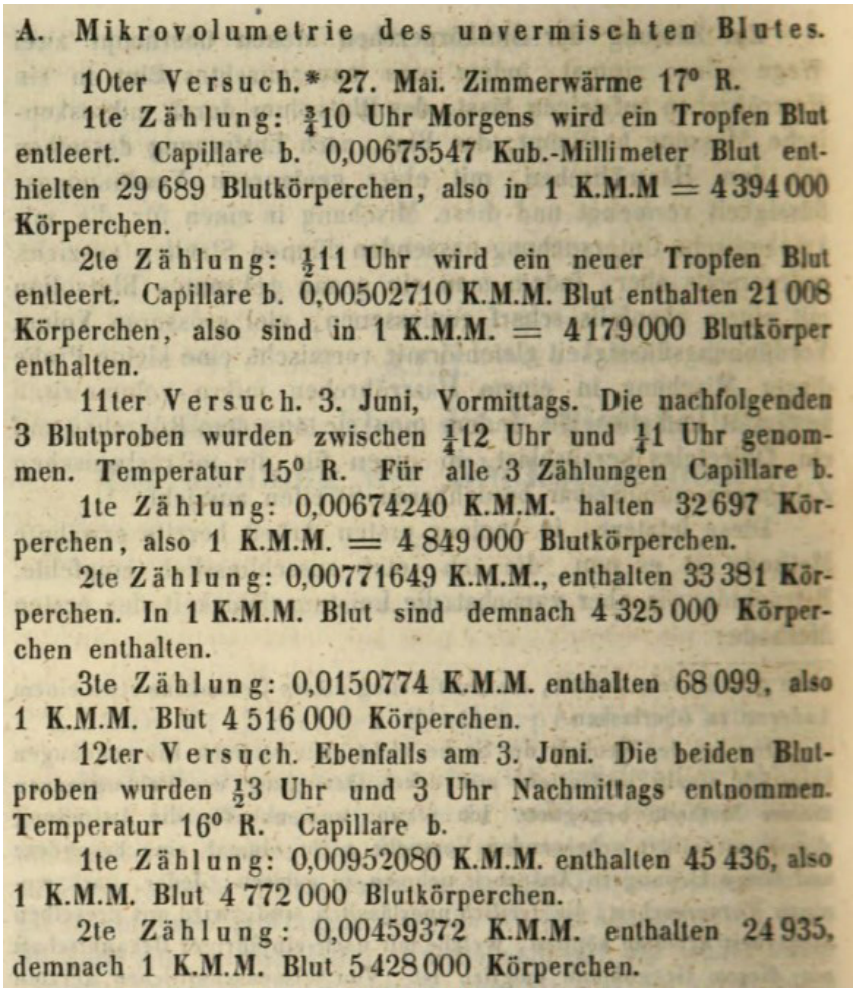
When it came to reporting blood corpuscle concentrations, Vierordt reported his raw measurements — the number of corpuscles and a specific blood column volume. He then rounded all calculated counts to thousands (*Table 9.4.*). In three of these instances (displayed in red text), current rounding practices would have rounded the raw calculation up rather than down.

Table 9.4. *Vierordt's Initial Corpuscle (red blood cell (RBC)) Counts; Vierordt lists these as "10ter Versuch, 1te Zählung , 2te ...," "11ter Versuch, 1te Zählung, ..."etc., where they have been abbreviated above as 10.1, 10.2, 11.1, etc.*

Attempt & Count #	**# RBCs/Blood Volume**	**# RBCs (calculated)**	**Vierordt's Rounded #**	**Rounded (down/up)**
10.1	29,689 cells/0.00675547 **mm^3**	**4,394,809**	**4,394,000**	down
10.2	21,008 cells/0.00502710 **mm^3**	**4,178,950**	**4,179,000**	up
11.1	32,697 cells/0.00674240 **mm^3**	**4,849,460**	**4,849,000**	down
11.2	33,381 cells/0.00771649 **mm^3**	**4,325,931**	**4,325,000**	down
11.3	68,099 cells/0.0150774 **mm^3**	**4,516,628**	**4,516,000**	down
12.1	45,436 cells/0.00952080 **mm^3**	**4,772,288**	**4,772,000**	down
12.2	24,935 cells/0.00459372 **mm^3**	**5,428,062**	**5,428,000**	down
	Mean Population standard deviation % Coefficient of Variation	**4,638,018** **390,775** **8.4 %**		

In the following excerpt from an initial data set reported in *Untersuchungen uber die Fehlerquellen bei der Zählung der Blutkörperchen* (Vierordt, 1852, pp. 854–880), we see the blood draw number, date, time, time of day, temperature (°R), capillary label (e.g., *Capillare b.,* although he had several others in different sizes), size of the capillary, size of the blood column measured in cubic millimeters, and the number of corpuscles counted, along with a rounded calculation of the count (*Figure 9.4.*) (Vierordt, 1852, p. 856). While

he was displeased by the variation in counts, they are statistically like one another with a mean of 4.64 million cells, a population standard deviation of 390,775, and a percent coefficient of variation (%CV) of 8.4 %. The standard deviation would not debut until its introduction by Karl Pearson in 1893 (Katz, 2009, p. 826) but is used here to help evaluate Vierordt's work.



A. Mikrovolumetrie des unvermischten Blutes.

10ter Versuch.* 27. Mai. Zimmerwärme 17° R.

1te Zählung: ¾10 Uhr Morgens wird ein Tropfen Blut entleert. Capillare b. 0,00675547 Kub.-Millimeter Blut enthielten 29 689 Blutkörperchen, also in 1 K.M.M = 4 394 000 Körperchen.

2te Zählung: ½11 Uhr wird ein neuer Tropfen Blut entleert. Capillare b. 0,00502710 K.M.M. Blut enthalten 21 008 Körperchen, also sind in 1 K.M.M. = 4 179 000 Blutkörper enthalten.

11ter Versuch. 3. Juni, Vormittags. Die nachfolgenden 3 Blutproben wurden zwischen ¼12 Uhr und ¼1 Uhr genommen. Temperatur 15° R. Für alle 3 Zählungen Capillare b.

1te Zählung: 0,00674240 K.M.M. halten 32 697 Körperchen, also 1 K.M.M. = 4 849 000 Blutkörperchen.

2te Zählung: 0,00771649 K.M.M., enthalten 33 381 Körperchen. In 1 K.M.M. Blut sind demnach 4 325 000 Körperchen enthalten.

3te Zählung: 0,0150774 K.M.M. enthalten 68 099, also 1 K.M.M. Blut 4 516 000 Körperchen.

12ter Versuch. Ebenfalls am 3. Juni. Die beiden Blutproben wurden ½3 Uhr und 3 Uhr Nachmittags entnommen. Temperatur 16° R. Capillare b.

1te Zählung: 0,00952080 K.M.M. enthalten 45 436, also 1 K.M.M. Blut 4 772 000 Blutkörperchen.

2te Zählung: 0,00459372 K.M.M. enthalten 24 935, demnach 1 K.M.M. Blut 5 428 000 Körperchen.

Figure 9.4. *Excerpt from Vierordt's initial reporting of blood corpuscle counts listing related information (Vierordt, 1852, p. 856).*

For a more comprehensive set of experiments, Vierordt explained that each attempt was counted two or three times as he worked through the best practices for dilution with protein

and gum Arabic menstruum. He mentions dilutions of 45.35 and 74.120 times before deciding that blood diluted 126.82 times gave him the best results. The specificity of these numbers gives an idea of how deeply Vierordt had committed to precision in reporting the details of his technique, mainly as this paper addresses possible sources of error. In his paper, he restates the optimal dilution amount as "…approximately 130 times," sowing some doubt into whether a dilution of exactly 126.82 is necessary. Why Vierordt was so exacting in some parts of the publication and then rounds his counts to the thousands place is unclear.

Vierordt registered an essential qualifier to his counts. He recommended using an objective lens with 170 times magnification, although 300 times magnification gave more exact results but required more time. He did not mention suggested sources or manufacturers for these lenses.

His raw data allows simple statistical analysis (*Table 9.5.*). Often, the number calculated from his raw data is higher than the rounded number he reported, although, in eight instances, he rounded up. Likewise, in most instances, the raw mean value calculated in *Table 9.5.* is higher than the mean value he reported, although in two instances, they are not. For his repeated observations using specific capillary tubes, his percent coefficients of variation ((population standard deviation/mean) x 100) were very precise, ranging from 0.5 to 4 percent for each group.

The results show a blood corpuscle mean of 5,101,933 using Vierordt's rounded data and 5,104,308 using data calculated from his raw blood cell and capillary blood volume measurements. However, the results showed a consistent upward trend across measurements with variations around the slope (*Figure 9.5.*).

Table 9.5. *Table of Vierordt's corpuscle counting results (column G) as (1) calculated from counted corpuscles divided by blood volume (column F/column E) and (2) as rounded to the thousands place by Vierordt (column H). Simple statistics for several key*

	B	C	D	E	F	G	H	I	J	K	L	M	N
	Capillary letter (e, g, or h)	Corpuscle Read Attempt Number	Capillary Diameter (mm^3)	Blood volume in Capillary (mm^3)	Number of corpuscles in capillary	Raw Calculation: Blood cells in one mm^3 blood	Vierordt Rounded Calculation of Blood cells in one (1) mm^3 blood	Raw Mean Value	Number of blood cells in one (1) mm^3 of blood (as a mean of individual counts)	Difference from the analytical mean expressed in % of the latter	Difference of the 2 strongest deviations in % of the analysis mean	Population Standard Deviation (Rounded Data)	% Coefficient of Variation (Rounded Data)
1	g	13	0.0320944	0.00131539	5,980	4,546,180	4,546,000	4,654,082	4,654,000	-2.3	5.2	89,572	1.9246
2				0.00076685	3,579	4,667,145	4,667,000			0.3			
3				0.00059262	2,839	4,790,591	4,790,000			2.9			
4				0.00082278	3,795	4,612,412	4,612,000			-0.9			
5	g	14	0.0320944	0.00169325	7,119	4,204,341	4,204,000	4,180,112	4,180,000	0.6	1.1	24,000	0.5742
6				0.00059121	2,457	4,155,884	4,156,000			-0.6			
7	e	15	0.0406492	0.00148619	7,368	4,957,643	4,958,000	5,004,680	5,005,000	-0.9	1.8	47,000	0.9391
8				0.00181859	9,187	5,051,716	5,052,000			0.9			
9	e	17	0.0406492	0.00116197	6,402	5,509,609	5,509,000	5,424,333	5,424,000	1.5	3.7	82,175	1.5150
10				0.00126277	6,939	5,495,062	5,495,000			1.3			
11				0.00152433	8,208	5,384,661	5,385,000			-0.7			
12				0.00120422	6,392	5,308,000	5,309,000			-2.1			
13	e	18	0.0406492	0.00108025	5,443	5,038,648	5,039,000	5,129,945	5,130,000	-1.8	5	118,246	2.3050
14				0.00136086	7,208	5,296,651	5,297,000			3.2			
15				0.0013936	7,044	5,054,535	5,054,000			-1.4			
16	e	19	0.0406492	0.00162241	8,917	5,496,145	5,496,000	5,498,243	5,498,000	-0.04	1.8	26,500	0.4820
17				0.00142352	7,900	5,549,623	5,549,000			0.9			
18				0.0012696	6,918	5,448,960	5,449,000			-0.9			
19	e	20	0.0406492	0.00136086	6,703	4,925,562	4,925,000	4,813,312	4,800,000	2.4	4.6	83,968	1.7493
20				0.00158972	7,489	4,710,892	4,710,000			-2.2			
21	h		0.0194828	0.00072145	3,433	4,758,473	4,758,000			-1.2			
22				0.00076652	3,724	4,858,321	4,858,000			1			
23	e	21	0.0406492	0.00106662	5,695	5,339,296	5,339,000	5,259,654	5,259,000	1.5	5.2	113,950	2.1668
24				0.00128457	6,580	5,122,337	5,122,000			-2.6			
25	h		0.0194828	0.00053147	2,870	5,400,117	5,400,000			2.6			
26				0.0005654	2,927	5,176,866	5,176,000			-1.5			
27	e	22	0.0406492	0.00110069	6,226	5,656,452	5,656,000	5,566,643	5,551,000	1.9	10.5	217,197	3.9128
28				0.00161424	8,543	5,292,274	5,230,000			-5.7			
29	h		0.0194828	0.00072995	4,248	5,819,577	5,819,000			4.8			
30				0.0006653	3,658	5,498,271	5,498,000			-1			
					average	5,104,208	5,101,933		5,055,667				
					stdev.p	410,298	409,514.67		424,213.52				
					%CV	8.04	8.03		8.390852258				
					minus 1 stdev	4,693,910							
					plus 1 stdev	5,514,506							

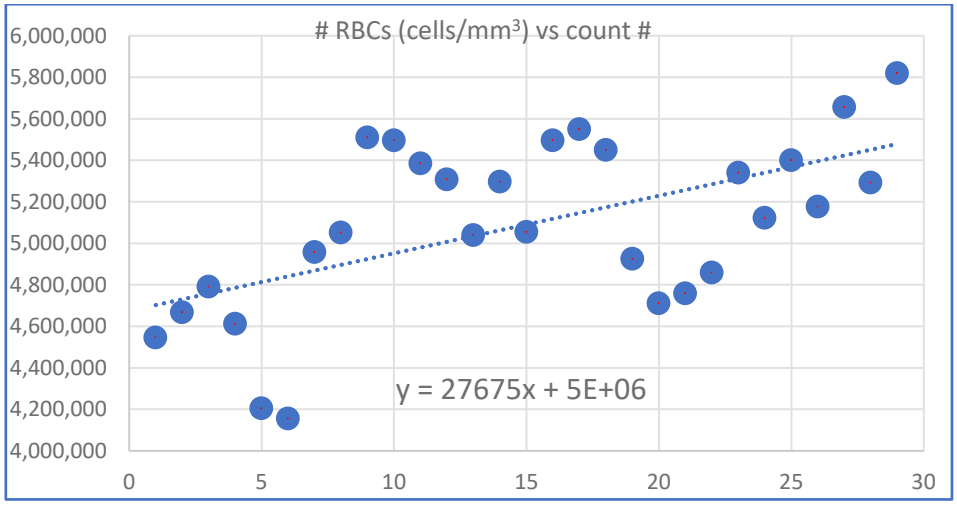


Figure 9.5. *# of RBCs vs. count # (a persistent upward trend with some distribution around the mean).*

Following Vierordt's explanation of experiments he continued to conduct on diluent type (i.e., protein and various amounts of gum Arabic), he might have omitted attempts 13 and 14 from the data and focused his evaluation on attempts 15 through 22. Counts 15

through 22 were diluted consistently using a gum Arabic preparation. Graphing these data would have shown the variation between diluent experiments (*Figure 9.6.*) and demonstrated less variation in the corpuscle counts for samples 15 through 22 (*Figure 9.7.*).

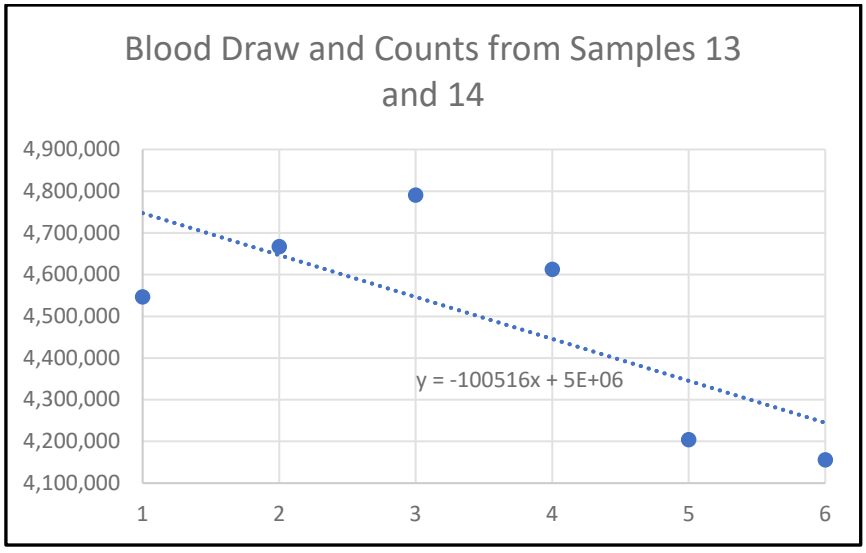


Figure 9.6. *Blood draw and corpuscle counts from Samples 13 and 14 (4 count attempts for sample 13 and 2 count attempts for sample 14).*

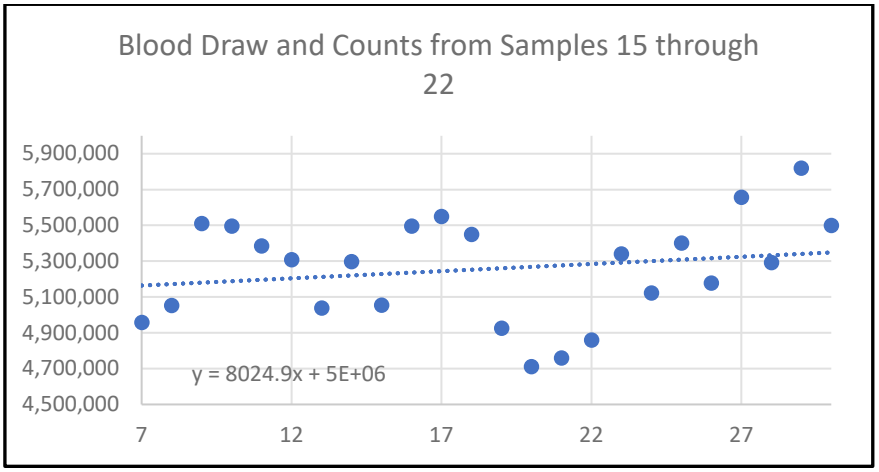


Figure 9.7. *Blood draw and corpuscle counts from Samples 15 through 22.*

Vierordt ascribed some variation in samples 15 through 22 to sampling his blood during and two hours after lunch. However, he also wrote that the lower values did not consistently

correlate with mealtime or digestion. The omission of counts from samples 13 and 14 raise the mean number of blood corpuscles in samples 15 through 22 to 5,256,237 with a %CV of 5.41 %. Vierordt omitted data from sample 16 due to damage caused by the errant placement of the objective lens micrometer.

To summarize, the %CV derived from counts 13 through 22 was precise at eight percent using the raw data he provided and Vierordt's rounded data. While current counts using highly automated cell counters would show virtually no variation between repeat measurements, the precision of his manual method is remarkable. Vierordt's reported mean value for blood corpuscles in a cubic millimeter was 5.1 million, slightly lower than the value calculated from raw data. If he had limited his analysis to samples counted after he had selected gum Arabic as his diluent for samples 15 through 22, he would have reported 5.3 million with improved precision. Vierordt's mean reported value for red blood cells is within the current reference interval for blood corpuscle counts in males (L. Dean. (Bethesda (M.D.): National Center for Biotechnology Information (U.S.)), 2005).

This mean changed slightly in Vierordt's summary, which reported a mean of means, rather than a mean of all reported values. As such, he reported 5.06 million blood corpuscles per cubic millimeter rather than 5.10 million, the average from his rounded data rather than the mean of means. It is interesting that Vierordt does report deviation of individual values from the mean values he calculated, although both mean and individual values are based on the numbers he rounded, thus adding a source of error.

As far as the accuracy of his counts go, Vierordt counted what he saw using the method he had established. He would not have any absolute sense of whether he was correct or not but the precision of his determinations must have given him a sense that he had accomplished his goal.

9.5 Vierordt and Schmidt Disagree

With the publication of *Mittheilung…,* Vierordt initiated criticism of Schmidt's 1848 and 1850 publications on the chemistry and characterization of blood and its components. The arguments Vierordt tendered, and Schmidt countered may best be characterized as misinterpretations of each other's work. Whatever their intentions, their published interactions were heated and probably unnecessary.

9.5.1 Vierordt Criticizes Schmidt

In the second half of the 1851 blood paper, Vierordt proposes new methods for the chemical analysis of blood. He characterizes his method as "a means, hitherto not even remotely suspected, of carrying out the analysis in such a way that the results can be regarded as an approximate expression of the true chemical composition of the blood corpuscles and the blood fluid." (K. von Vierordt, 1851a, p. 31). His method was intended to update the method described twenty-eight years earlier by Prévost and Dumas (Prevost, J.-L., Dumas, 1823) while addressing the problem stated in Lehmann's second edition of Physiological Chemistry: "The more exact determination of the magnitude of this serum content is precisely where the efforts of the most diligent researchers have failed" (Lehmann, 1850, p. 204).

In part, Vierordt addressed the subject by criticizing the methods published by Carl Schmidt in *Charakteristik der epidemischen cholera gegenüber verwandten Transsudationsanomalieen*[43] (Schmidt, 1850a). As shown in the biographical sections, Vierordt took on in Dr. Schmidt an adversary who had earned distinction with some of Europe's most honored investigators in chemical and physiological research.

[43] Characteristics of epidemic cholera versus related transudation anomalies.

Starting on page 23 of his 1851 publication, Vierordt presented several pages of objections with the table of contents heading “Inadmissibility of Schmidt’s Method.” This elicited an 1852 response in *Zeitschrift für Rationelle Medicin* from Schmidt entitled *Ueber Vierordt's Methode der Blutanalyse* published in Henle and Pfeufer’s *Zeitschrift fur Rationelle Medicin* (Schmidt, 1852a, pp. 293–298). Vierordt responded to Schmidt’s critique in *Untersuchungen uber die Fehlerquellen bei der Zahlung der Blutkorperchen*.

Vierordt’s first note to Schmidt addresses a statement from *Charakteristik*… that 95 to 98 % of the blood corpuscles have the same diameter, but Vierordt indicates that this matter requires "renewed attack." It is an odd note as he does not address the question raised except to suggest that it may not be valid under "different normal conditions." Vierordt then wrote about the difficulties of determining a consistent volume for blood corpuscles, suggesting that work would be required to take measurements under "various normal and pathological conditions." This last issue is a matter that concerns Vierordt but is not a criticism leveled directly at Schmidt. Again, it is out of place for Vierordt to criticize Schmidt's work on this topic, as Schmidt's 1850 work was dedicated to analyzing blood samples from patients under pathological conditions.

Vierordt’s principal objection was that Schmidt used a constant to determine the proportion of dried blood corpuscles to their water content. Vierordt dismisses the choice and derivation of the constant 4 (i.e., 4 times the amount of dried blood corpuscles to their water content), and the use of hypothetical quantities in both the multiplicator and multiplicand.

9.5.2 Schmidt Responds

In his 1852 article *Ueber Vierordt’s Methode der Blutanalyse*, Schmidt responded by dissecting Vierordt’s argument. Schmidt has some criticism of Vierordt’s method.

However, he leavens it with a compliment: "Let us turn first to the only positive part of the work, the numerical determination of the blood cells, we encounter a number of sources of error which in and of themselves make the author's further deductions untenable"(Schmidt, 1852b, 293-4). Schmidt goes on to denigrate Vierordt's vague statement about water evaporation error correction, blood that is left on the capillary walls as being too variable to be corrected by calculation, the challenges associated with measuring the length of the blood column given the need for rapid sample handling, and Vierordt's calculation of the meniscus volume. Schmidt estimates that instead of Vierordt's statement that the total error associated with this method may be on the order of $1/200^{th}$ of the measurement, error may be as high as $1/50^{th}$ to $1/20^{th}$ of the measurement, an error estimate 5 to 10 times as great.

Schmidt then examined Vierordt's criticisms of *Charakteristik…* by supposing that Vierordt had read an excerpt from his article rather than Schmidt's entire monograph, which runs to 165 pages. Schmidt first stated that his constant is based on "a series of parallel investigations with types of blood" and that the constant ratio he derived addressed the number of solid constituents so that "the richer the serum in the latter, the more concentrated the blood cake, and vice versa." When he used the Prévost and Dumas method, Schmidt wrote that the dried blood cells are in a "constant ratio" to the "true content of fresh blood cells in the blood." Schmidt based his statements not on "airy *a priori* reasoning," as Vierordt has suggested, but on "bare fact, clear results of direct weighing and measurement; not as absolute truth, but with the natural scientist's modesty, as given the most approximate possible value according to the present state of our knowledge." Schmidt refuted as "undoubted" Vierordt's statement that "it is *a priori* impossible that the solid constituents of the blood corpuscles are in a constant ratio to the water content of the same" and provides a table from his research showing proof that Schmidt agrees with Vierordt and that Vierordt has misunderstood Schmidt's work. Schmidt provided the *coup*

de grace in the following statement, proving that Vierordt has misrepresented the use of the constant 4: "Determined according to the method of limits from several analyses, this coefficient results in almost = 4; a value 0.3 higher or lower is no longer sufficient for all the condition equations."

Arguably, the most unsupportable aspect of Vierordt's criticism of Schmidt's blood component analysis is that Vierordt does not divulge any new chemical method for analyzing blood organic and inorganic components, as Schmidt had done in exhaustive detail. Vierordt refers to the Prevost and Dumas method from 1823 as the basis for his analysis and refers to unspecified work by Louis Figuier. Much research had been published between 1823 and Vierordt's 1851 monograph. Louis-Réné Le Canu summarized blood component research in his Prix de Montyon-winning 1837 thesis (L.-R. Le Canu, 1837). This thesis can be appreciated for its summaries of blood and serum analyses and results from Jacob Berzelius, Alexander Marcet, Prevost and Dumas, Jean Louis Lassaigne, and Prosper Sylvain Denis, but also Le Canu's quest for a definition of blood-based indicators for various diseases, work that Gabriel Andral and Schmidt extended. Schmidt closed his response with the "wish that in future Mr. Vierordt would consider the "*nonum premature in annum*[44]" instead of hurrying up with immature plans to endanger his reputation."

Schmidt's *Ueber…* received a published response in Vierordt's 1852 article *Untersuchungen uber die Fehlerquellen bei der Zahlung der Blutkorperchen*, which begins with a footnote stating, "I firmly reject [Schmidt's] badly placed rebuke" (K. von Vierordt, 1852). Vierordt takes offense to each of Schmidt's comments and explains away Schmidt's

[44] "Let your first draft be kept back from publication until the ninth year" from Horace's advice to Piso's elder son in *Ars Poetica*.

statements as merely misunderstandings, based primarily on Schmidt not attempting the techniques Vierordt has described.

The published dialogue, which concluded with Vierordt's *Untersuchungen…* response, was an unfortunate example of two scientists of estimable reputations, talking past each other when a private correspondence or meeting could have resolved their misunderstandings in a respectful manner. Both men had published significant work in their fields. Both had innovated and improved on blood analysis techniques that had been used before them. While Vierordt's contribution to blood cell counting probably had a more lasting impact in his time, Schmidt's application of quantitative chemical analysis to the organic and inorganic components of blood cells and intercellular fluid, particularly in the range of diseases he studied, was at least as remarkable. Electrochemical and colorimetric methods would supplant his techniques for analysis of these materials; Vierordt's techniques would be supplanted by cell counters, such as the hemocytometers developed in the 1870s, as well as by more sophisticated cell counters established in the late 1940s and early 1950s through Wallace Coulter's research (Robinson, 2013).

It may have been that their geographical distance from each other discouraged a more amicable resolution to their quarrel; the direct distance from Dorpat (Tartu) to Tübingen is about 1600 kilometers and would have been a daunting journey in the 1850s. The tenor of the argument that ensued was due to choices made by Vierordt. While Carl Schmidt published material with which Vierordt disagreed, it was Vierordt that chose to publish criticism in the journal he edited.

The criticism was unfortunate and unnecessary for several reasons:

Schmidt's principal focus was determining the organic (e.g., albumin, "casein," fibrin, urea, etc.) and inorganic (e.g., water, acid, alkali, iron, sodium, potassium, calcium, and

magnesium salts, phosphates, sulfates, bicarbonates, etc.) contents of blood corpuscles and "intercellular fluid" (plasma) or serum. He intended to monitor these components in subjects who had contracted cholera; he determined the quantities of components in the presence of albuminuria, dysentery, dropsy, and other conditions. Vierordt's work primarily focused on presenting an accurate and precise method for counting blood cells in samples of his blood. His work did not address fluctuations in blood cell counts in the presence of disease. Neither encroached on the other's published work.

Schmidt's monograph is full of detailed analytical methods and includes comments on the magnification power of his objective lens and the source of his optical micrometer. Vierordt only divulged the specifications for his objective micrometer and his preferred linear magnification. Furthermore, Schmidt cites his sources and influences, often writing lengthy footnotes to render the cited material relevant to his work. Vierordt does not tend to cite others, although, by 1852, much work had been published on blood analysis and its components.

1848 through 1852 established significant gains in blood analysis and its components. The work started by Prévost and Dumas and developed by other noteworthy French and German physiologists started to define the field of hematology. With the development of hematology came new insights into how blood components might be examined to determine the effects of a disease. Two distinct and complementary approaches were defined by Carl Schmidt's 1850 publication and the cascade of publications by Karl von Vierordt two years later. Although it specifies the microscopy equipment he used, Schmidt's work employed a series of thorough analytical chemistry determinations to understand the impact of various diseases on blood components. Vierordt's work, although he proposes a few equations for determining the amounts of blood components, is primarily

a work of microscopy and is not entirely transparent regarding his microscopy equipment. Vierordt provides no quantitative analytical results for organic and inorganic components of blood.

Despite working towards different goals, Vierordt published his critique of Schmidt's methods. The critique resulted in Schmidt's repudiation of Vierordt's methods and Vierordt's response to that criticism. The criticisms did nothing to diminish the quality of the other's work but may have tarnished their reputations amongst colleagues. Vierordt used rhetorical flourishes in his critiques, while Schmidt was to the point. Schmidt believed that Vierordt must not have read his paper in its entirety as Vierordt's criticism seemed off the mark.

Both physiologists had emerged unscathed following this disputatious encounter. Neither followed these works up with other publications centered on blood chemistry, although both were published extensively during the remainder of their careers. Schmidt would focus on agricultural chemistry, mineralogy, and botany. He traveled to Britain twice to assess their industrial methods to benefit Estonian commercial development (Ross, 2005). Vierordt developed several additional approaches to assessing human health. His studies of the pulse and blood pressure led to the development of a rudimentary sphygmograph, almost immediately seen as a worthy addition to healthcare technology and the basis of blood pressure assessments thereon. His extension of the work of Joseph von Fraunhofer, Robert Bunsen, Gustav Kirchhoff, and Felix Hoppe-Seyler led to the publication of the spectrum of hemoglobin and the development of hemoglobinometers by Louis-Charles Malassez, Georges Hayem, and William Gowers. Vierordt also wrote an outline of physiology that went through five editions between 1860 and 1877.

Disagreeable interactions between erstwhile colleagues pockmark the history of science. While the dispute between Vierordt and Schmidt had no lasting impact on either of their careers, it is one example of a quarrel that could have been defused quickly and privately had Vierordt not fired the first shot.

Postscript: This work required translating many original German and some French articles. I used Google Translate for most of the translations, with disambiguation help from Linguee. I owe a great deal to the excellent national libraries in Germany and France, which had the foresight to make these documents available in Portable Document Format (pdf).

CHAPTER 10 | The Clinical Chemistry Method as a Metastructural History

10.1 A Brief History of a Single Clinical Chemistry Test

The clinical chemistry test for alkaline phosphatase (ALP) measures the amount of the enzyme in human serum. In 1981, Posen and Doherty published a review of alkaline phosphatase methods (Posen & Doherty, 1981). They generally stated that "… large numbers of assay methods differing from one another in types of substrates employed, substrate concentrations, buffer types, buffer strengths, pH values, and temperatures of incubation" (Posen & Doherty, 1981, p. 167). In it, they referred to a recent book called *Alkaline Phosphatase,* coauthored by Posen (McComb et al., 1979), along with a couple of earlier papers that describe recommendations of German and Scandinavian committees for practices within clinical laboratories governed by the respective national institutes (German Society for Clinical Chemistry, 1972; Keiding et al., 1974). The Scandinavian paper states that they have "…ample evidence to stress that even minor deviations from meticulous precision in these procedures may have a marked influence on the analytical result" and recommend it for "routine methods and as a basis for reference" (Keiding et al., 1974, p. 292). In other words, following their method recommendation for any ALP analysis is appropriate. The German Society paper is preceded by a 1970 paper (German Society for Clinical Chemistry, 1970).

Posen et al. also published a helpful table of previous methods showing reference intervals (normal ranges), principal author, and reference numbers. The German and Scandinvian papers are cited, along with eight others shown in the table below (*Table 10.1.*). The table caption is taken directly from the review. Publication dates have been added following the reference number to provide timeline information; this was not provided in the original review paper.

Table 10.1. *(a) Values are approximate, obtained in adults by different methods, and expressed in different units. Different ranges are obtained if males and females are treated separately (G4). (b) These methods differ from one another by reaction conditions (buffer, temperatures) so that different values are obtained even though all results are expressed in "International Units" (17). (c) Abbreviation for Society for Clinical Chemistry (Posen, 1981, p. 167).*

Normal Ranges for Alkaline Phosphatase[a]		
Method (from Posen 1981)	**Unit**	**Normal Ranges**
Bodansky (1933)	Bodansky	1.5-4.0 units/deciliter
King & Armstrong (1934)	King-Armstrong	3.5-13 units/deciliter
Buch & Buch (1939)	Buch & Buch	2.2-6.6 units/deciliter
Shinowara et *al.* (1942)	Shinowara Jones-Reinhart	2.2-8.6 units/deciliter
Bessey et al. (1946)	Bessey-Lowry	0.7-2.7 units/milliliter
Babson et *al.* (1966)	Babson	11-44 units/liter
Sigma (1971)	Sigma	0.8-3.0 units/milliliter
Bowers & McComb (1966)	International[b]	6-110 units/liter
German SCC[c] (1972)	International[b]	30-170 units/liter
Scandinavian SCC[c] (1974)	International[b]	70-390 units/liter

From this tabular presentation of methods, one can assume that the history of ALP clinical methods started in 1933. That assumption is incorrect. The following qualifiers are provided to improve our understanding of the history of ALP methods:

1. An examination of the Bodansky publication shows that it was "received for publication" on 24 September 1932.

2. A footnote on the first page indicates that the method was demonstrated at a meeting in Philadelphia in April 1932.

3. The Bodansky method was not developed and perfected on dates in April 1932, but the work preceded the April presentation by an unstated number of weeks, months, and perhaps years.

4. The first line of Bodansky's paper alludes to the "Benedict-Theis method for the determination of serum 'inorganic phosphate'"; however, Bodansky does not mention this method again. The second line explains why the Benedict-Theis method is inappropriate for measuring organic phosphates such as glycerophosphate, Bodansky's analytical target.

5. Bodansky references methods by Kuttner and Cohen, Kuttner and Lichtenstein, and Raymond and Levene (Raymond & Levene, 1928) published in 1927, 1930, and 1928, respectively. Bodansky's paper incorrectly cites the Raymond/Levene article page. The article encompasses pages 621 through 635.

6. The first paragraph of the Bodansky paper provides details regarding why the methods he has cited are unsuitable for his work.

Another insight from Posen's table is that the ranges for alkaline phosphatase reported by the ten listed methods vary substantially, even for the German and Scandinavian reference methods recommended within two years of each other in 1972 and 1974. The units require explanation. The reported numbers all use the numerator "units," "U," "international units of enzyme activity," or "IU." A unit of enzyme activity is defined as "…that amount which will catalyse the transformation of 1 micromole of the substrate per minute under standard conditions". In the same publication, a new unit of activity was defined to align it with SI units. It was named the katal (kat or z) and is defined as "…catalytic activity that will raise the rate of reaction' by one mole per second in a specified assay system.". The katal has units of mole per second (mol/sec or $mol*s^{-1}$). It is related to U as follows:

1 U catalyses a rate of 1 μmol/min = 1/60 pmol/s ≈ 16.67 nmol/s; 16.67 nkat catalyse a rate of 16.67 nmol/s. Therefore, 1 U corresponds to 16.67 nkat ("Units of Enzyme Activity. Recommendations 1978," 1979).

In less mysterious terms, the gravimetric amount of an enzyme is not used as the numerator for many clinical chemistry tests, nor is the amount of reactant or product formed. Sometimes, the gravimetric equivalent can be calculated. Enzymes are proteins that change a reactant or substrate to a product, often in the presence of cofactors, such as small molecules and ions that provide or subtract electrons or protons and reduce or oxidize the reactant. In an ideal enzyme solution, each protein, which has a molecular weight defined by its component amino acids and other moieties (e.g., porphyrins in hemoglobin), would have identical activity. One mole of the enzyme ($6.02x10^{23}$ molecules) paired with one mole of reactant would result in one mole of product. This is not the case; some of the enzyme entities in the solution are inactive for one or more reasons (i.e., inhibited, denatured). Many enzymes exist in several forms called isoenzymes or isozymes that differ in some amino acids and some substituents but generally do the same catalytic work. They may do the same work but may work better on some reactants than others (e.g., isozymes of cytochrome p450). They may differ in how rapidly they do the work or under what conditions (e.g., temperature, pH, cofactors) they perform optimal catalysis. Instead, the observable fact that reactant is converted to product by some number of the enzymes under specific conditions forms the basis of the numerator "U." Clearly, the origin and evolution of the terms have a history, summarized authoritatively in Compendium of Terminology and Nomenclature of Properties in Clinical Laboratory Sciences (Férard et al., 2016).

Each of the cited methods uses a variety of reactants and reagents in solutions that provide controlled conditions for the conduct of the experiments elaborated upon within

the text. Returning to Bodanksy, the method refers to: sulfuric acid (10N, an allusion to the history of aqueous solutions and to our understanding of atoms, molecules, and their properties); 7.5 % sodium molybdate solution (weight-weight, weight-volume, and volume-volume mixtures, however they were characterized at the time, were probably the earliest types of purposeful mixtures), the molybdic acid from a manufacturer that meets a specification for the molybdic acid being ammonia and phosphate-free, and solubilized in 5N sodium hydroxide, no manufacturer given, in specific glassware (glass as various types of laboratory-ware has a long history), titrated to a final solution that is "faintly alkaline to phenolphthalein," a common pH indicator (without source information given) that turns pink at an alkaline pH of 8.2; 60 % stannous chloride solution, with no other source specification in this case, but solubilized in concentrated hydrochloric acid (usually around 12N, the maximum concentration at which hydrogen chloride gas is retained by water, but unspecified here); potassium acid phosphate purchased from a manufacturer that labels their product as "buffer grade," whatever that meant in the years leading up to 1932 publication of Bodansky's paper; acidified with concentrated sulfuric acid (36.8N or 18.4M, but left unspecified and with no source cited), and diluted with water, so common a material that it barely registers among the ingredients used, but our understanding of water has a history of its own; a 5 % solution of trichloroacetic acid, though we are not told the source of the acid; a solution of glycerophosphate, although we are not told the source, purity, solution concentration, solvent, or other information; and a "drop" (now understood to be approximately 50 microliters, although Bodansky's drop might have been different) of toluene, source not mentioned.

It might seem odd to say that chemical reagents such as molybdic acid, stannous chloride, and potassium acid phosphate have histories. However, they do, as certainly as their manufacturer and the laboratories in which they were used have histories. Neither was

known to humankind, and then they were teased out of the background noise of the earth's crust, atmosphere, and oceans to be given special duties in human affairs. At certain times, tin- and molybdenum-containing minerals were discovered and used before their pure forms were refined—in the bronze age (around 3,000 BCE) for tin and in 1778 by Carl Scheele for pure molybdenum. However, its mineral forms were known before then. The manufacturers Bodansky cites are Eimer and Amend[45] for the molybdic acid (H_2MoO_4) and stannous chloride ($SnCl_2$), and Lamotte[46] for potassium acid phosphate (K2HPO4). A chemist working today to reproduce exactly Bodansky's method would face several dead ends. Without manufacturers cited for the sulfuric or hydrochloric acid, without their normality (N) or molarity (M) verified, without certificates of analyses, and without a sense of how the water was treated (e.g., whether it was distilled, perhaps multiple times, deionized by treatment with an ion exchange resin, or by other means is lost to time or buried in the laboratory notebooks from Bodansky's lab[47]), the chemist would be left to make a series of assumptions that might or might not result in replicating Bodansky's work in the modern clinical laboratory. More information needs to be provided on the purity of the reagents used, which may or may not be significant and may or may not be understood to mean that the purity of these reagents was sufficient for the method described. However, we need explicit guidance on this issue. When information is missing in science, specifically clinical chemistry methods, the result is that new rounds of experimentation

[45] Eimer and Amend was founded in 1851 by two brothers from Darmstadt, Germany. In 1940, it was acquired by Fisher Scientific, destined to become one of the largest laboratory suppliers in the world, and is now part of ThermoFisher Scientific (*Company-Histories.Com*, n.d.).

[46] Lamotte, founded in 1919, is now a supplier to water treatment and analysis industries. The company manufactures analytical reagents, laboratory apparatus, electronic instrumentation, and complete portable test kits for chemical analysis in hundreds of applications (*Lamotte.Com*, 2023)

[47] New York University Medical Archives were contacted, and Glenda S. Barahona, the reference archivist, responded by indicating that neither they nor the branch manager for the Herman Robbins Medical Library/NYU Langone Orthopedic Hospital have any papers from Dr. Aaron Bodansky's laboratory operation in their possession. Emails documenting this exchange are available upon request.

are required to determine what might have been true and what might work with the materials available to the current investigators. New experimentation with new equipment, materials, reagents, and solvents is more likely to create new results than replicating previous ones.

Bodansky's list of reagents, their concentrations, purities, manufacturers, formulation, expiry dates, and conditions governing their use implies a history of thinking about the documentation of science, of experimentation, and of the creation of reproducible, replicable methods for the analysis of materials, in this case, the analysis of a physiologically important enzyme in human serum. It needs to be a complete history, as documented in his 1932 paper. A thorough review of his documentation practices would have to involve examination of the laboratory notebooks, reagent orders, chemical manufacturer's catalogs from which materials were ordered (the most likely source of missing information regarding purities), and even files from the manufacturers themselves, who may have kept records regarding lot and batch purity, records of chemical analyses, manufacturing and purification methods, their sources of raw materials, information regarding the possible stabilities of materials used and produced, as well as some storage information (e.g., in a desiccator, away from light, away from oxidizers, or other critical storage conditions).

10.2 The Complex History of Colorimetry in Bodansky's Method

The various solutions are mixed, as the paper indicates, although with fewer details than one would want, and the results of the alkaline phosphatase reaction are measured by colorimeter. Early in this document and sporadically in the experimental section, allusions were made to Beer's law, also known as Bouguer-Beer's law, the Beer-Lambert law, or most inclusively as the unwieldy Bouguer-Lambert-Beer law. These names refer to various

refinements in analyzing the transmission or absorption of light by a solution. Each law and the portmanteau law with its triumvirate of names has a history. Bodansky's paper mentions that all measurements are done with a colorimeter but does not indicate its manufacturer or type at any point in the paper. Instead, he cites an earlier paper by Kuttner and Lichtenstein (Kuttner & Lichtenstein, 1930) that refers in turn to Kuttner and Cohen (Kuttner & Cohen, 1927) that warns against using a Duboscq colorimeter and favors “a new pocket colorimeter” introduced by Kuttner in the Journal of the American Medical Association (Kuttner, 1915). Kuttner’s brief introduction to his colorimeter design (with a note stating that it was being made by E. Leitz, New York) refers his readers to the Sahli-Gower[48] hemoglobinometer, which represented Swiss internist Hermann Sahli’s (1856—1933) modification (Sahli, 1902, pp. 616-8) of neurologist Sir William Gowers’ (1845—1915) “apparatus for the clinical estimation of hæmoglobin” (Gowers, 1879). Sahli’s 1902 nine hundred-page textbook titled *Lehrbuch der klinischen Untersuchungs-Methoden: für Studierende und praktische Ärzte*[49] established many standard practices in its time. Sahli was a Swiss internist who focused significant attention on hemodynamics. The 1902 publication was an update of his 1894 text. Gowers mentions that the only "exact method is the chemical estimation of the amount of iron in the blood." However, he also states that the chemical estimation method is "unavailable for clinical work" while offering no reason why it should not be. He states that using a spectroscope is "difficult in practice and is scarcely a clinical method." Other methods, which compare the "colour of a given dilution with that of a series of coloured standards," are “more or less unsatisfactory” (Gowers, 1879, p. 65). In effect, Gowers introduced, along with the instrument manufacturer Hawksley, a colorimeter designed to measure hemoglobin. His instrument was the

[48] Interestingly, Kuttner used the name "Gower" in his 1915 description of the Sahli-modified apparatus, as the physician's last name was Gowers.

[49] Textbook of clinical examination methods: for students and general practitioners.

hemoglobinometer of choice for the next twenty years until the modification of the technique, first by J.S. Haldane (1860—1936) and then by Hermann Sahli.

Before Gowers, though, an interesting bifurcation had developed in the history of blood-focused quantitative methods. In this bifurcation, the colorimetric (hemoglobinometer) and cell-counting (hemocytometer) technologies, which owed precedence to work done by Vierordt, had separate paths towards separate futures.

Vierordt first established a method for the precise and accurate counting of blood in articles published in 1851 and 1852 (K. Vierordt, 1852d, 1852b, 1852e, 1852a, 1852c; K. von Vierordt, 1851b). These were followed by suggestions for improvements published by Hermann Welcker (1822—1897) in 1853 and 1854 (Welcker, 1853, 1854). Antonie Cramer (1822—1855) published an article suggesting improvements in 1855; it contained a postscript from the journal editor noting that Dr. Cramer had died on 1 January 1855 (Cramer, 1855). In addition to his suggestions for improving blood cell counting techniques, which relied on the microscope, Cramer had made a considerable contribution to ophthalmological science. He invented the ophthalmoscope, which used optics then associated with microscopes, and had one manufactured by Leonard Deutgen (1814 - 1896) in Groningen. With the instrument, he could identify that the eye's accommodation to focus on objects is accomplished when the lens changes curvature. This finding gave Cramer priority of discovery over Hermann von Helmholtz, who also created an ophthalmoscope and was working on the accommodation problem; Franciscus Cornelis Donders (1818—1889), Cramer’s mentor, had suggested the accommodation problem to Cramer and affirmed his priority, although Helmholtz is widely given credit as well (Den Tonkelaar et al., 1990).

The Gowers haemacytometer was the first method to employ a specialized microscope to measure the number of red blood cells in a blood sample. However, the medical literature soon added numerous papers that suggested improvements to Gowers' instrument (Gowers, 1877). The evolution of the hæmometer, also known as the hemometer, hematimitre, haemacytometer, and hemocytometer, was preceded by the work of Louis Malassez (1842—1909), who published *De la Numeration des Globules Rouges du Sang*[50] in 1873 and Georges Hayem (1841—1933), who published *Recherches sur l'anatomie normale et pathologique du sang*[51] in 1878. Gowers, Hayem, and Malassez each cite Vierordt's 1852 blood cell method and mention that Antonj Cramer and Hermann Welcker improved upon Vierordt's cell counting methods.

Hoppe-Seyler, who defined the spectrum of hemoglobin in terms of spectrum absorption bands Wollaston, Fraunhofer, Kirchoff, and Bunsen had defined, in turn, inspired Vierordt to use the early spectroscopic techniques to measure the amount of hemoglobin in blood samples (K. Vierordt, 1881; K. von Vierordt, 1869, 1871, 1873; Welcker, 1853, 1854). Ångström was also responsible for the introduction of the concept of wavelengths as continuous rather than dependent upon solar dark lines, absorption lines, or emission lines, as was true for the systems introduced by Fraunhofer, Kirchhoff, and Bunsen, but some writers believe Ångström's contribution to these developments in spectroscopy were minimized by Kirchhoff and Bunsen (Fraunhofer, 1817; Jackson, 2014; Kirchhoff & Bunsen, 1861; Reif-Acherman, 2014; N. C. Thomas, 1991; Wollaston, 1802).

[50] The counting of red blood cells.
[51] Research on the normal and pathological anatomy of the blood.

10.3 The Benefits of Following a Single Thread

A complicated history was revealed by choosing one clinical assay and researching its roots. This history led to Bodanky's method development efforts, the gestation of alkaline phosphatase methods in the methods for inorganic phosphate- relevant to farmers and land use improvements- back further to the creation of various colorimeters, cell counting, and uses for microscopes, and to research that was initiated through a telescope at the Royal Greenwich Observatory by William Hyde Wollaston.

The history of alkaline phosphatase methods, as described in 1979 and 1981 by Solomon Posen and others, also had a relatively unknown and well-documented future. Alkaline phosphatase methods, based on Aaron Bodansky's published literature or others, were modified in clinical and research laboratories worldwide. The methods may or may not have been adequately documented in laboratory notebooks, and the results obtained may or may not have helped answer questions posed by the researchers in those labs. These methods, documented or not, calibrated or not against reference laboratory values obtained using state-of-the-art reagents, instruments, scientists, and methods, are, in sum, known as laboratory-developed tests (LDTs). The lack of state, national, or global standards for LDTs poses problems when the results may have international value to research teams elsewhere. The tests may also have implications for individual patients and their families, who may rely on insufficiently validated and calibrated LDTs performed in a clinical or hospital laboratory to assess patient health. In the United States alone, efforts by regulatory agencies to establish uniform testing standards have failed (Genzen et al., 2017). The European Union is establishing regulations known in total as in vitro diagnostics regulation (IVDR) (Lubbers et al., 2021). Implementing and harmonizing any set of regulations is a labor of years and sometimes decades. The most challenging element in achieving harmonization is

the willingness of administrators, scientists, and technicians to take all necessary, specific actions to implement the changes. While a harmonized and regulated laboratory environment benefits the physicians and patients who rely on test results being consistent wherever the test is performed, individual laboratories can forget the importance of the goal in the torrent of changes that must take place.

This single paper and many others published throughout scientific research show the basis for good laboratory practices (GLP), good clinical laboratory practices (GCLP), and other regulatory guidelines. While it would be decades between Bodansky's publications and the initial publication of GLP or GCLP regulations became a basis for auditing laboratories or evaluating analytical methods, either proposed or practiced, and several more decades before bold regulators started to wrestle with the complexities of harmonizing LDTs, it should be clear that an unregulated environment poses significant risks to rendering best-in-class patient-focused diagnostics, (Baldeshwiler, 2003; Ezzelle et al., 2008; Todd et al., 2014).

It is to state that Aaron Bodansky and the people working in his laboratory in the 1930s probably achieved excellent results for the time. The methods probably helped many other labs evaluate alkaline phosphatase levels in patients and led to significant overall progress in implementing alkaline phosphatase as a critical clinical laboratory test. It is also worth noting what is written above—there are enough unanswered questions raised by Bodansky's papers that implementation at other laboratories was probably not straightforward. The goal, after all, is that published science should assist the entire scientific community in moving forward without obstructions placed in the way. A method should be reproducible with similar if not identical, precision, accuracy, and regression

functions as the published method. If these and other criteria are not facilitated, scientific progress is hampered when it could have been helped.

CHAPTER 11 | Cholera comes to Portugal: Myth and science during the 1833-1834 epidemic and beyond

11.1 Abstract

In 1833, William Lardner was a surgeon on the ship *Rainha de Portugal* stationed off Porto, and in 1834, a surgeon at the Royal Marine Hospital in Lisboa. Lardner wrote two letters to the prestigious British medical journal *The Lancet* (Lardner, 1833, 1834). He described the onset of cholera, first in Vigo, then in Porto during skirmishes between Liberals and the *Miguelistas*, then in Aveiro, and eventually in Lisboa (S. Thomas, 2006). Lardner states that neither Spain nor Portugal had experienced cholera until the *London Merchant*, sailing from Dover and Falmouth, made port in Vigo in late December 1832. Lardner's ship, the *Rainha*, was berthed in Vigo then and proceeded to Porto, arriving on 2 January 1833. Lardner believed that the troops under General Jean-Baptiste Solignac had contracted cholera from persons on the London Merchant and transported the disease to Porto. Cholera progressed down the coast to Aveiro by 3 February 1833 and then to Lisboa in mid-June 1833.

Lardner's letters to *The Lancet* provide a wealth of information on the progress, diagnosis, and treatment of cholera in Portugal, as well as many scurrilous comments regarding the practice of medicine in that country at that time. In this paper, we will examine elements from his letters and additional context from other sources and consider how these elements might relate to the Covid-19 pandemic.

Keywords: Cholera, Portugal, epidemic, Porto, Lisboa, Aveiro, William Lardner

11.2 Introduction

As the controversy over the origin of the SARS-CoV-2 virus continues, when contact and infection tracking has been overwhelmed by (1) the rapid spread of the virus and (2) political and individual resistance to sharing private information with health-focused and government agencies, it is remarkable to read two letters written in 1833 and 1834 and published in the British medical journal *The Lancet* that provide a first-hand account of when and how cholera arrived in Portugal (Lardner, 1833, 1834). William Lardner[52] wrote the letters, previously in charge in 1831 of the Bagatelle Hospital in Warsaw, at which he was responsible for "upwards of three hundred patients …" sickened by cholera. In his 1833 letter, Lardner also stated that he ascribes his interest in cholera to Charles Searle ("Mr. Searle;" 1792—1868), who had published his views on cholera in 1828 in Madras, India (Searle, 1828) and updated his thoughts in a book published in 1830 by London publishers. Although Searle had treated many cholera cases during his services in India, a sufficient reason for Lardner's interest in Searles's work, Searle believed that cholera resulted from malaria infection (Searle, 1830).

Following his Warsaw posting, Lardner had become a ship's surgeon initially stationed aboard the *Rainha de Portugal* (previously a British vessel named *Congress* (Bent, 2019, p. 637) before transferring to *The London Merchant* in Vigo, Spain (Lardner, 1833). The *Merchant*, which had made at least two previous deliveries in 1832 of infantry, cavalry, and supplies (Bent, 2019, p. 639), carried about 400 soldiers who had departed Ostende, Belgium, traveling to Vigo, Spain by way of Dover and Falmouth to assist the Portuguese forces fighting for Dom Pedro and loyal to Dona Maria II (1819—1853) (the liberalist,

[52] After an extensive review, it must be admitted that William Lardner's birth and death dates remain elusive. Librarians at the Royal College of Surgeons for England and Edinburgh and The National Archives in Kew have yielded no information. The Virtual International Authority File (viaf.org) has no entries for Dr. Lardner.

constitutionalist or loyalist forces) in the civil war against the *Miguelista* (absolutist) usurpers.

This paper assembles information related to the arrival of cholera in Portugal and how it was perceived by physicians, officers, journalists, and the public. It also reviews attempted treatments, some of which were successful and some of which were little more than wishful thinking.

The paper will explore the following topics:

1. What is cholera?
2. 1832-1834 disease impact in Portugal
3. Cholera timeline
4. Causes of cholera – who/what/where to blame?
5. Treatments
6. *Cholera morbus* in the press

11.3 What is Cholera?

Although its cause was unknown in the 1830s, cholera resulted from infection by a water-borne bacterium – *Vibrio cholerae*. This bacillus thrives in warm water and is passed to others in the excreta of victims and carriers, entering the body through the mouth and digestive system. It was also referred to as malignant cholera, *cholera morbus, cholera asiatica, cholera indica, cholera spasmodica*, and blue cholera (McGrew, 1960, p. 61); the term cholera morbus was coined by Thomas Sydenham (1624—1689), who was differentiating the disease from a state of anger, i.e., a choleric temper (Barua, 1992, p. 1).

Cholera presents specific symptoms, the most dramatic being copious acute diarrhea described as whey-like or rice-water-like, severe dehydration, rapid pulse, fatigue, a comatose, apathetic state, nausea, massive vomiting, decreased body temperature, spasms, delirium, sunken eyes, and abdominal cramps. Sometimes, the patient's skin turned pale blue or blue-grey, giving the disease its odd nickname (Evans, 1988, p. 127). While the European cholera epidemics in the 1830s inspired several physiologists to search for blood-based indicators that cholera had afflicted their patients, the rapid onset of dehydrating diarrhea was an unmistakable indicator that a patient had become infected (I. M. Davis, 2022d; L. R. Le Canu, 1831; L.-R. Le Canu, 1833).

Various authors believed that cholera, or the "true cholera," was well known to people in the Bengal region of India long before Europeans initiated trade routes; the 19th century Bengal region is now known as the nation of Bangladesh and East Bengal, India. Reviews written by Robert Pollitzer (1885—1968) for the World Health Organization suggest that cholera was known to Hippocrates as "bilious diarrhea." Gaspar Corrêa (1495—1561), Portuguese author of *Lendas da India (1858-1866)*, documented outbreaks in 1503 and 1543 of an illness that resembles cholera, "…which struck with pain in the belly, so that a man did not last out eight hour's time'" and of an illness the locals called "moryxy" "…the fatality rate of which was so high that it was difficult to bury the dead" (Pollitzer, 1954, pp. 422-3).

In the 19th Century, cholera spread in pandemic waves. The first wave started in 1817 and originated from "…the hinterland of Bengal between the Ganges and the Brahmaputra [rivers, reaching] Calcutta early in August" (Pollitzer, 1954, p. 428). Evans states that the cause of the initial breakout was that the Marquis of Hastings fought battles against Maratha warriors; Hastings lost thirty percent of his ten thousand troops to cholera during these

battles (Evans, 1988, p. 134), although other accounts give higher numbers of troops, followers, and dead (Hawthorne, 1834. p. 5). Cholera moved on to strike Sri Lanka (Ceylon), Myanmar (Burma), and Thailand (Siam) before reaching Penang, Singapore, Java, Borneo, other Indonesian islands, and the Philippines before or while spreading to China, Japan, Madagascar, and the east African coast opposite Zanzibar (Evans, 1988). By 1821, cholera had been exported with a British expeditionary force to Oman, then to Bahrain, and into parts of present-day Iran, including Shiraz, Tehran, and Resht, which sits on the shores of the Caspian Sea. Pollitzer writes that it was inevitable that in 1822, once winter had subsided, cholera would infest Iraq, starting at the Persian Gulf port of Basra and heading up to Baghdad. The Persian army engaged in battles that defeated the Turks in Yerevan and retreated to Khoi in Iran, spreading the disease as they traveled onward to Tbilisi and Astrakhan in late 1822 or early 1823, where it may have been halted by a severe winter (Evans, 1988, p. 125; Pollitzer, 1954, p. 424-30).

The second 19th-century cholera pandemic, thought to have been transmitted to Europe in 1829, started from Bengal in 1826 "…westward along the Ganges and Jumna rivers and in 1827 by an invasion of the Punjab" (Pollitzer, 1954, p. 432). While little is known about what areas cholera afflicted in 1828, it is known that Afghanistan and Persia saw outbreaks, as did the Silk Road cities of Khiva (Chiva) and Bukhara (Bokhara) in Uzbekistan. These instances led to Orenburg in southeast Russia, as cholera was spread presumably by caravan. Charles Macnamara (1832—1918) quotes *Commercial Statistics* that states that in 1833 alone, "…fourteen caravans of 2,547 camels, exclusive of horses; and thirteen caravans of 4,769 camels, and 264 horses, departed, laden with goods, for various parts of Asia," along with undocumented numbers of merchants, assistants, and fellow travelers (Macnamara, 1876, p. 88). If any man or beast carried cholera-contaminated water, pandemic dissemination was almost guaranteed.

Macnamara, quoting Dr. R. J. Graves (1796—1853) from *Clinical Lectures on the Practice of Medicine* in *A History of Asiatic Cholera,* states that a second "stream of cholera, which entered Russia from the northern provinces of Persia, formed a junction with that which flowed through Orenburg" (Macnamara, 1876, p.89).

After a pause in Orenburg, cholera spread rapidly in the spring of 1830 to most of Europe, "…large parts of the Americas, as well as of Arabia and East and North Africa" (Pollitzer, 1954, p. 432). The northwestern progress of cholera from Orenburg started affecting Moscow, St. Petersburg, and Archangel after a winter lull bridging from 1830 to 831, then moved northward into Finland and westward into the Baltic provinces and Poland, where Russian and Polish troops were engaged in the November Uprising or Cadet Revolution starting 29 November 1830. Cholera among these combatants facilitated its spread to the Austrian province known as Galicia, and to Vienna in August 1831 and other parts of Austria in 1832. Hungary had seen its first cases by June 1831. The Polish and Baltic state infections moved westward through Prussia into Berlin and Hamburg in the spring and summer of 1832. The Baltic and German port cities seem to have been the source of cholera's spread to the east coast of England by June 1831 and spreading to many cities in England, Scotland, and Ireland through the rest of 1831 and into 1832. By March 1832, cholera appeared in Calais and Paris; it eventually affected fifty-one of the eighty-six French departments, sparing the southern and eastern mountainous regions. At roughly the same time cholera spread through France, it spread into Belgium. Whether it was due to the general pandemic in France and Belgium or whether it was due to the peripatetic nature of soldiers who may have been infected at any point in their travels, cholera made its way to Vigo, Spain, and into Porto via a military vessel leaving Ostende, Belgium.

11.4 1833-1834 Cholera Impact in Portugal

According to Lardner, cholera arrived in Vigo on 31 December 1832 on *The London Merchant*, along with European soldiers under Marshal Jean-Baptiste Solignac (1773—1850). On 2 January 1833, Lardner disembarked with the soldiers at Foz, a small cape just west of Porto at the mouth of the Douro River. From there, he ventured to the Foz military hospital nearby to observe the treatment of soldiers, some of whom had developed symptoms of cholera.

Cholera had a substantial impact on Porto's citizens, spreading from military hospitals to homes, then to Porto's eastern sector, where people gathered to avoid the consequences of *Miguelista* bombardment. However, the disease had an outsized impact on hospital and laundry workers. Bernardino Gomes (1806—1877) summarized the Porto infection in an 1866 review of cholera for the International Sanitary Conference:

Many of these conditions were, of course, much changed during the siege. Dirty streets and dwellings, dead animals rotting in ditches, the cold and dampness of a severe winter, boredom, the privations of a long siege, all came together and conspired to engender disease or to increase the disposition to contract them. Also, bronchitis, pneumonia, intestinal affections, rheumatism, fevers, and especially typhus, that inseparable companion of long campaigns, were not lacking. True epidemics, however, did not exist in Porto until the end of December 1832. It was under such circumstances that cholera broke out in the city the following month, January 1833 (Gomes, 1866, p. III).

Cholera peaked in Porto in February but continued until it disappeared in August, having killed 3,624 people out of the 7,356 total dead from January to August. It spread to Aljuba prison, where prisoners of war were held. Of the two hundred prisoners, 119 contracted cholera, and 79 died.

By 3 February, cholera was present in Aveiro, although Lardner wrote that he was unsure how it traveled sixty kilometers south from Porto. *Miguelistas* held Aveiro so that cholera may have been introduced by soldiers falling back from the banks of the Douro River and infecting water sources there.

Lardner documented cholera's arrival in Lisboa in mid-June, where he became the surgeon in charge of the Royal Marine Hospital.

Lardner includes passages that he ascribes to Mr. Clay, "a reporter for one of the morning papers." Although it is difficult to tell what Lardner used from Mr. Clay's work, as Lardner does not use quotation marks or other attribution methods, Lardner wrote that cholera entered "on the north margin of the Tagus, at Belem…." He followed this observation—perhaps from Mr. Clay—with a section worthy of Edgard Allan Poe:

"A thick, dense, dry mist approached Lisbon from the entrance of the Tagus, and overspread the whole river and its adjoining mountains, as thick and as black as a London fog in December, but not moist. It was a dry, deadly-looking mist, and all vegetation apparently shrunk beneath its influence. The large leaves of the fig trees hung drooping, evidently in a state of disease, several of them being within a few yards of my residence; and I was told by my friends that along the banks of the Tagus the same effect was produced on the vegetation generally" (Lardner, 1834).

Darkly poetic descriptions like the Lardner material were entered in other accounts, as Dr. George Stuart Hawthorne (~1793—1858) quoted Psalm 91:6:

"… the pestilence that walketh in darkness" (Hawthorne, 1834, p. 8),

although it is difficult to see how Hawthorne's item does not apply to every epidemic disease in human history.

Cholera opened a second front in the south, in the Algarve, when Dom Antonio de Noronha landed his troops at Cacela Velha (Cacella, from (Gomes, 1866, p. VI)) and marched them through the Algarve and Alentejo regions to Lisboa. This movement freed Lisboa troops to deploy north through Torres-Vedras, Leiria, Coimbra, and Caldas areas, spreading cholera as the soldiers marched. Eventually, the *Miguelistas* contracted the disease, although they had been mainly spared from cholera except in Aveiro,

By the time it died out, the 1832-1834 cholera epidemic in Portugal had claimed around 40,000 lives, more than that caused by the Portuguese Civil War.

11.5 Causes of Cholera: Who and What was to Blame? When did it Arrive and Where?

Without understanding its etiology, cholera was blamed generally on “certain high-risk groups” and conditions. It was vigorously debated whether it was a contagious disease or non-contagious.

Cholera is not contagious but is communicable by swallowing a portion of the poisonous discharge from a person labouring under the disease. This poison when dry is innocuous, but will retain its properties for an indefinite time, until, when dissolved in water, it becomes at once violently active for a limited time until decomposition is affected (Macnamara, 1876, p. vii).

While Macnamara literally takes the non-contagious position, non-contagion or anti-contagion arguments also focus on the notion that miasmatic forces spread cholera. Possible and improbable causes for the epidemic arose as often as people could write down their

thoughts or communicate them to someone who might take them seriously. Some thought growing crops where forests once stood was a cause (de Almeida, 2012, p. 49). Reginald Orton (1810—1862), writing about his experiences with cholera in India, provided examples that correlate periods of heavy rainfall as witnessed in Bengal with "extensive ravages" of cholera. Orton also believes that disease-causing periods can be brought on "very heavy showers, attended with strong gusts of wind… leaving long intervals of cloudy and fair, and very frequently of the most serene weather, which continue until the new or full moon, or other causes, bring returns of the rains." He devotes a chapter to "meteorological occurrences attending the epidemic," quoting other writers as to the importance of understanding the effects of "epidemic meteoration" which "probably arises principally from variations in the quantity of [atmospheric] electricity" (Orton, 1831, p. 170; Orton, 1831, p. 381). Causes that fall into the category Orton describes are generally known as morbific influences. George Roupell (1797—1854), in his Croonian Lectures to the Royal College of Physicians, took time to dismiss morbific causes as baseless (Roupell, 1833, p. 23). On the other hand, Charles Searle, Lardner's mentor during his service in Warsaw, believed that morbific influences brought on bouts of cholera (Searle, 1828, pp. 33-4).

Roupell came up with a simple approach to characterizing disease as being based on three principles:

When the combination of circumstances exists, in the first place of the atmosphere to permit infection, in the second, of the person to receive it, and the actual presence in the third place of the germ of the disease itself; When all these essential circumstances are present, any infection will probably spread (Roupell, 1833, p. 37).

Roupell was writing from the perspective of early germ theory here, presenting an alternative to the prevalent miasma theories.

On the other hand, much later in the century, Max von Pettenkofer (1818—1901) wrote that three primary conditions and individual disposition to cholera must exist for it to infect a person or population.

The occurrence of Cholera, and its frequency, depend therefore essentially on the simultaneous co-operation of several, but chiefly of three, causes, viz., the traffic, the local and the temporal disposition, and the individual disposition. If one of those factors be wanting, no matter which, there can be no outbreak of Cholera (Pettenkofer & Hime, 1883).

Pettenkofer also opposes quarantines, stating they "…would be a far greater calamity than the disease itself…" (Pettenkofer & Hime, 1883, p. 27).

He also warns about dirty linen and other “wearing apparel, especially if moist and soiled," but then qualifies his remark by saying that the linens or clothing should not cause concern if they come from a cholera patient but from a cholera district, resulting in the new district into which the materials have been transferred becoming a new cholera district. He believes eating "damp, very watery, and slimy articles of food which have been in a Cholera-house" may serve as an invitation to cholera, rationalizing this statement by referring to the Swiss observation that boiled cow's heel and Indian rice cakes may infect consumers if not "thoroughly purified or recooked." Continuing his catalog of possible causes, Pettenkofer states, "fresh excretions of Cholera-patients are not dangerous, but only such as have become decomposed." Ultimately, while twenty-one governments in attendance at the 1874 international sanitary conference voted unanimously that "ambient

air is the principal vehicle of the generative agent of cholera," Pettenkofer believed cholera originated in groundwater (Howard-Jones, 1984, p. 380).

Pettenkofer (1818—1901) contributed substantially to advancing modern hygiene and public health. Nevertheless, he also advocated for the miasma theory of disease, eventually disproven, rather than the spread of disease by specific microbiological entities. This position placed him in opposition to Robert Koch (1843—1910). Koch identified the bacterial cause of cholera following extensive field research in Egypt and Calcutta starting in 1883, concluding in 1884, a year after Pettenkofer's book on cholera and about thirty years after John Snow (1813—1858) identified the epidemiological basis for transmission through infected water sources. Ironically, Koch's research concluded thirty years after Filippo Pacini's (1812—1883) initial discovery of what he called vibrions. Although Pacini's work was published in six articles and is documented step-by-step in unpublished memoirs held at the Central National Library of Florence, there is no evidence that Koch was aware of the work. It is thought that physicians following the miasmatic theory ignored Pacini's work into temporary oblivion (Lippi & Gotuzzo, 2014).

Lardner provides his initial published thoughts in his 1883 letter on cholera's real and imagined causes. He believed the conditions in the fore cabin of *The London Merchant*, which he describes as crowded and filthy, "with no means of removing the foul discharges which the body throws off from all its openings," caused the illness. Lardner also wrote that breathing the exudations of soldiers' bodies in a state of decomposition and earthly emanations caused the disease. Lardner's observations are consistent with miasmatic theory but further on he states that he finds "as many facts bearing in favour of contagion as against it" (Lardner, 1833, p. 301).

Lardner writes that Admiral George Sartorius (1790—1885), who presided over the Portuguese navy in the service of Dom Pedro IV (1798—1834), blamed cholera on "an express visitation of Providence." Sartorius also believed that the "poison" was sold to the crew by "bomb-boats from Vigo, through the medium of oysters." The admiral contracted cholera but recovered due to the ministrations of his surgeon. Another surgeon on the ship related a story that seems to blame illness on the ship on a raccoon that was brought on board healthy and died within a half-hour, "instantly seized with cramps and vomiting." The raccoon was placed in the vessel's hold to kill rats. The hold contained earth floors and flat surfaces "near the source of the poison" (a leap in logic since the "poison" was unknown to anyone at the time). Lardner reminds his readers of the miasmatic "axiom" that "those persons who sleep on the ground floors, or nearest to the earth, are the most subject to be attacked." Cholera stopped infecting the crew once the hold was cleared out and "made pure," as it was in an "extremely foul state." Lardner's supposition that the raccoon and the ship's hold were to blame was proven true by the raccoon's death and hold clearing. Future scientists would find that many animal species are immune to *Vibrio cholerae*.

In his 1834 letter to *The Lancet*, Lardner indulges in another round of prosody, a renewed attempt to explain the origin of cholera:

Its mysterious marches, its means of conveyance, its sudden and unexpected invasions, are worthy of great attention. Does it flow in the wind like a wave, the greatest convexity of which catches one place, and then another? Or is it borne along by an aquatic or an electric medium? The whole mass of atmosphere in so deranged a state, that the disease is created by ever-occurring events? I am disposed to answer the latter query in the affirmative (Lardner, 1834, p. 315).

He was brought to this sense of the disease by his observation that the *Rainha* had many cases on board. Still, the *Dona Maria*, berthed next to the *Rainha* and with which the *Rainha* was in free and constant communication, did not develop cholera cases.

Lardner also blamed the cholera epidemic on Portuguese physicians and public hygiene, railing against "the mean, knavish, jesuitical [*sic*] character of their medical men, who… gull the public to a far greater degree even than the priests.

These men mingle with their practice a species of alchemistical mystery, to rob the ignorant. A mustard cataplasm on the soles of the feet to cure a toothach [*sic*],—*three* leeches applied to *three* different parts of the spine to cure the belly-ach [*sic*],— and bleeding in the great-toe for tic douloureux of the forehead,—are by no means uncommon or extraordinary methods of extortion used by this enlightened body (Lardner, 1834, p. 318)

Lardner follows this by describing the mass burial procedures distant from “filthy Lisbon.”

While all this caution is being observed about dead bodies, filthy Lisbon remains neglected; the dirt and abomination of every house and its numerous inhabitants is thrown out of the windows and is only removed from the streets by the myriads of dogs who loiter about, looking out for such window falls (Lardner, 1834, p. 318).

Arguably, the most unfortunate aspect of the disease coming to Portugal was the increased moral judgments about various portions of the Portuguese population. We see this captured in a passage found in an article by Dra. Pires de Almeida.

The disease was spread by certain high-risk groups, such as soldiers, sailors, merchants, and beggars, and its effects were heightened by poor hygiene in houses and streets, the use

of polluted water, the concentration of patients in confined spaces in hospitals, and food of poor quality (de Almeida, 2012, p. 44).

As if abandoned girls did not have enough going against them in the early 19th C., they were blamed for the infection “because of the indiscretions of some of the recluses” (de Almeida, 2012, p. 49). Dra. de Almeida summarizes the possible causes of the disease, some of which were at odds with each other. Some situations to blame included eating fruit and other raw food, or quite contrarily, heavy meals at supper. Cucumbers and salads, as well, were leading causes, along with degeneration, perversion, excessive consumption, dirtiness, and unruliness. If you ate a salad – you might cause cholera; if you overate, you might contract or cause cholera. If you ate in moderation but were degenerate, perverse, dirty, or unruly, cholera might be your punishment for these transgressions.

11.6 Treatments for Cholera

Treatment methods were suggested by nearly anyone and in a bewildering range. Remedies might be natural—exposure to sunlight and fresh air, dangerous—preparations containing mercury, such as calomel and *hydrargyrum cum Creta*, and might involve alcohol fermented from various flora. Some writers recommended a series of remedies staged one after another to form a treatment plan. James Keir (1735—1820), writing in 1832, summarizes the complexity of approaches as follows: "The treatment of the disease must vary according to the circumstances of the individual case; but our success will depend not only on the remedies employed, but also on their timely employment" (Keir, 1832, p. 109).

Orton quotes Thomas Sydenham’s recommendation that a single dose of opium “given at the commencement of the disease… puts an effectual check to its progress.” Orton then immediately warns against abusing opium as a treatment (Orton, 1831, p. 304). A surgeon

writing to *The Lancet* from India after fifteen years of treating cholera patients recommends treatment with opium or laudanum and then, later in the same letter, states that much harm has been done by the employment of opium ("The Malignant Cholera," 1833, p. 302).

Bloodletting is often cited as a cure and as a treatment to avoid. Some bloodletting recommendations depend on when the bloodletting is performed during disease progression.

Searle provides examples of treatments from several of the leading physicians of his time. He goes through the details of a case of cholera he contracted, treated, and overcame. The treatment involved numerous enemas, imbibing various salt solutions, including those composed of "Cheltenham salts" (presumably, iron-rich salts from the Cheltenham spa), antimony, magnesium, sulfate, and ammonium in various proportions, accompanied by application of warm water compresses. Searle works his way to a remedy and details the various diarrheal and emetic discharges accompanying the treatment (Searle, 1828, pp. 34-9).

William Stevens (1786—1868), writing in 1832, had a cynical view of the proposed treatments:

When Cholera first appeared on the isolated shores of this island, white wine whey with spice, hot brandy, and water, cajeput oil, peppermint, laudanum, &c. were officially recommended to the public by one Board of Health; whilst another pressed into the service all the remedies which had ever been thought of and recommended the whole to the profession in a confused mass. They approved of red-hot irons to the spine, and bleeding, together with the internal use of opium and emetics, James's powders, calomel, Cayenne pepper, chalk and brandy, ice, quinine, *salts, acids*, &c., &c…. There were, however, some

exceptions to this rule; and from what I have now seen, my conviction is, that when Cholera is taken in time, and properly treated, it is, in most cases, almost as easily cured as either the common typhus or marsh fever (Stevens, 1832, p. 430-1).

Dra. de Almeida lists many treatments published in Portuguese papers during the first and second cholera epidemics. These include spirit of camphor, which was provided without charge to people experiencing poverty and led to overuse; ginger liqueur; "hot water, tea, chicken broth, gum Arabic syrup, egg and laudanum;" spearmint; iodine; rhatany extract; and cider peer syrup. Dra. de Almeida includes a passage recommending two-fifths absinth, one-fifth elder tree flowers, one-fifth mint leaves, and one-fifth liquorices, "all to be boiled" (de Almeida, 2012, p. 50). She also references a mass cure enabled by walking herds of cows through the streets because their breath would purify the air. While this seems absurd, Portuguese physician Garcia da Orta (1490—1568) wrote that bezoars, “tightly packed, partly digested agglomerations of hair or vegetable matter,” were the “best treatment of a very serious disease, *Mordexi Seco* (Cholera morbus).” Orta described the bezoars with which he was familiar as “onion-like, layered formation around a small straw in the paunch of a male goat from Khorasan and Persia” (Do Sameiro Barroso, 2014, pp. 82–3).

In the final analysis, near-constant communication between the physician and the patient and the provision of various salt solutions were as likely to bring the patient back to health as any specific therapy.

11.7 Cholera Morbus in the Press

Much as is true now, journalists and the press served two purposes: one, to inform, and two, to misinform the public. The press advertised outlandish reasons for cholera to be published alongside quack remedies. To be fair, no antibiotics were available then, and no

one knew the cause was bacterial. The press helped spread the idea that immorality and filth caused cholera, ideas that were not helpful if you were poor or lived in a poor area. To make matters worse, wealthy people who contracted cholera were less likely to have their misfortune reported.

Papers also published stories that should remind us of present-day misinformation campaigns propagated through reputable sources and internet blogs alike. Papers claimed cholera was not contagious and spread due to miasmatic forces, although the etiology was debated. Papers stated that large gatherings of people heightened the risk of transmission. It was said that the economic necessities of the population were more critical than managing disease risk. Some papers published articles stating that quarantines or "sanitary cordons" were useless, a position held by Max von Pettenkofer and others far earlier in the 19th Century. Some papers published stories condemning attempts to control the cholera epidemic as controls placed “the freedom of the individual and commerce against the shackles of despotism and reaction” (de Almeida, 2012, p. 46). Other journalists echoed works by physicians who had worked with epidemics in India and elsewhere; Orton quotes Monsieur Labal: "…cholera commits less ravages than the typhus in our army" (Orton, 1831, p. 468). Asa Briggs (1921—2016) quotes John Snow from an 1855 publication: “It is possible that seven times as many deaths have taken place from typhus as from cholera since the latter disease first visited England in 1831” (Briggs, 1961, p. 91). While these morbid assessments were made by physicians and published in the press, it is difficult to understand why it was appropriate to diminish the mortality due to cholera compared to other epidemic diseases. The number of dead accumulated from cholera and typhus (and any other mortal illness present), rather than one supplanting the other.

Surprising similarities exist between the reception that greeted the cholera epidemic in Portugal in the 1830s and the medical, journalistic, and popular reception Covid-19 has received in our current era. Diagnostic and therapeutic medicine has moved far beyond the 19th century, but blatant misinformation, fraud, and quackery persist despite enormous advances, a situation termed an "infodemic' by the Director-General of the World Health Organization (Bin Naeem & Kamel Boulos, 2021; Roozenbeek et al., 2020). Portions of the global population seem stuck in the 19th century or earlier. It is relatively easy to imagine people in Sumer reacting to the coronavirus pandemic similarly to how some people reacted in Seattle or Spain. Large portions of the global population have access to the internet, a supercharged version of all the best information dissemination and the worst aspects of rumor-mongering and gossip from past eras. Even those who do not have access may hear from a friend of a friend who has read something somewhere about Covid-19 or other diseases. People with impressive, moderate, and nonexistent backgrounds in epidemiology, virology, pharmacology, public health, or general science—politicians, religious leaders, lettered physicians—have proposed cures and behaviors to prevent the virus. While some of what has been written about Covid-19 is accurate or at least recommended caution until additional information could be developed, some information has been as fraudulent and full of misinformation as anything published in newspapers and scientific journals in the 19th century.

One global challenge going forward, as the human population deals with new Covid mutations and new viral (V. J. Lee et al., 2020), bacterial (Laxminarayan, 2022), and fungal (Bongomin et al., 2017) threats, is to create simple information that people will understand and follow with greater trust than was true for the current pandemic. It is a challenging goal as simple information will be rearranged and restated in ways that complicate the spread, diagnosis, and treatment of each emerging threat.

11.8 Acknowledgment

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CHAPTER 12 | SARS-CoV and COVID-19: Lessons learned; opportunities missed

12.1 Abstract

SARS-CoV-2 and Covid-19 have made a retrospective analysis of other coronavirus diseases important, so this article reviews the history of the SARS-CoV viral disease from 2003. Standard and clinical chemistry diagnostics were developed in response to the outbreak. The response to SARS is examined to determine if lessons were learned before it disappeared in June and July 2003. Various diagnostic approaches were developed and implemented to assist in the rapid identification of patients and treatment of their illness, yet many of the approaches required days or weeks from the onset of fever to show statistical significance. Most therapeutic methods used during the outbreak relied on treating symptoms of the underlying illness, such as lower respiratory infections and systemic infection, rather than effectively suppressing or curtailing the replication of the virus. Retrospective studies are examined to determine how the SARS outbreak was viewed ten years later and what the authors hoped would be instructive patterns for future pandemics. Implementing some of these recommendations might have helped ease the current pandemic but were overlooked for budgetary reasons that seem short-sighted now.

12.2 Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the resulting coronavirus disease 2019 (COVID-19) pandemic have catalyzed a global effort to diagnose, treat, and cure increasing numbers of patients presenting with symptoms in their homes, communities, doctors' offices, and hospitals (World Health Organization, 2020b). As of this writing, infected patients surpass ten million, while deaths exceed 500,000 (Johns Hopkins University & Medicine, 2020). While these numbers are reported from one of several official global reporting resources, it is assumed by some epidemiologists that the numbers underestimate the actual extent of the disease due to limited or inadequate testing, lack of diagnosis, or deaths due to other factors while COVID-19 is a comorbidity, thus allowing an alternative cause of death to be reported (Hall et al., 2020; Krantz & Rao, 2020; Weinberger et al., 2020). It is impossible to know how many people will be infected, how many will die, and how many will recover. It is impossible to know for how long the pandemic will be active or how significant its global financial impact will be. It is unknown whether those infected can be re-infected, which will make it possible for them to infect naïve subjects who thought their families, friends, coworkers, or collocated persons had recovered.

While there are many differences between the severe acute respiratory syndrome (SARS) outbreak of 2003 and the current COVID-19 pandemic caused by SARS-COV-2, it is worthwhile to examine the past SARS epidemic to understand what was learned and what might have been done better in the intervening years. Both, for instance, are coronaviruses. Both seem to fit the definition of zoonotic diseases. Both present with an initial fever, followed by other symptoms resulting in lower respiratory infection or acute

respiratory distress syndrome (ARDS). Both have required a massive global effort to define, treat, and cure those who have become ill.

Among the purposes of history should be counted its capacity to inform us of how events have transpired and guide us through similar events in the present and future. This is true for histories of disease, diagnosis, treatment, and recovery, as well as scientific research and application of findings. The following is a condensed history of the brief and deadly SARS pandemic, along with some possible lessons to be learned for current and future viral pandemics.

12.3 A new disease starts to spread

As the SARS coronavirus appeared and started spreading in humans in February 2003, global, governmental, and local health organizations worldwide started to come to terms with this new viral threat. It was quickly thought that a case of atypical pneumonia that presented in November 2002 may have been the first harbinger of the virus. However, it was not until February 10, 2003, that the World Health Organization (WHO) was alerted to a disease that had "already left more than 100 people dead" (World Health Organization, 2003e). The Chinese Ministry of Health reported on February 11 that there were 305 cases in Guangdong, with five fatal cases (Centers for Disease Control and Prevention, 2003). It would take until March 12 for the WHO to issue a global alert. Two days later, the Centers for Disease Control and Prevention (CDC) activated its emergency operations center to support the WHO.

12.4 Defining a diagnosis

Examination of the report issued by Morbidity and Mortality Weekly on March 21, 2003, shows that evidence was accumulating for a more dire outbreak than had previously

been assessed. The WHO had reported 246 cases in eleven countries, with the preponderance of the cases in Hong Kong, Singapore, Vietnam, the U.S., and Canada (World Health Organization, 2003a). The illness was characterized by "rapid onset of high fever, myalgia, chills, rigor, and sore throat, followed by shortness of breath, cough, and radiographic evidence of pneumonia," along with low platelet (cells that aid in clotting), lymphocyte (natural killer (NK), T, and B cells), and leukocyte (white blood cell) counts. The WHO reported that the cell counts initially might present as normal and take three to four days from fever onset to show a significant decrease (WHO, 2003). An additional criterion is that a suspected patient must have been in "close contact within 10 days of onset of symptoms," which is "defined as having cared for, having lived with, or having had direct contact with respiratory secretions or bodily fluids of a person suspected of having SARS" within ten days of symptom onset (Peiris et al., 2003).

Diagnostic problems frustrated the identification of the infection. Initial diagnosis relied substantially on self-reported symptoms (e.g., fever, myalgia, chills, sore throat, shortness of breath). Consultation with a medical professional and return of hematology laboratory results and chest radiography also could indicate a range of causes. Reporting of proximity to an infected person also required prompt, honest self-reporting. It may have been complicated by a newly exposed person not knowing whether they were in the presence of a SARS-affected patient within the previous ten days. However, even with this battery of possible symptoms, only fever presented in 100% of the cases identified later as SARS-positive. Other symptoms occurred in between 10% and 74% of patients, further confusing diagnosis (Centers for Disease Control and Prevention, 2003a).

12.5 Determining the cause

CDC reported that some initial data indicating that “paramyxovirus-like particles” were being reported, a misleading clue (Centers for Disease Control and Prevention, 2003). The CDC report was remedied in the March 28 issue, which placed blame on a "previously unrecognized coronavirus” (Centers for Disease Control and Prevention, 2003). A new coronavirus—SARS-CoV—was identified as one of a family of RNA viruses that cause respiratory illnesses, including a coronavirus that causes some cases of the common cold (Gerberding, 2003). Identification of the virus led to its isolation and genome sequencing on April 14, 2003, through a collaboration involving laboratories in the U.S., Canada, The Netherlands, and Germany (Marra et al., 2003; Rota et al., 2003). Genome sequencing allowed the identification of sequences that allowed the development of diagnostic tests, particularly by reverse-transcription polymerase chain reaction (RT-PCR) assays (Marra et al., 2003), but also identified structural and nonstructural proteins as possible targets.

By March 28, the Weekly Epidemiological Record reported that probable cases had reached 1,323 with 49 deaths (World Health Organization, 2003c). The April 4 issue dedicated over half of its twenty-four pages to issues surrounding the spread of SARS, travel precautions, and reporting protocols (World Health Organization, 2003d).

12.6 Searching for Biomarkers

Starting in March 2003, a group at the Chinese University of Hong Kong started using an indirect immunofluorescence (IFA) method to detect antibodies to SARS-CoV. Their work involved a control group of 635 naïve subjects and 103 SARS patients who screened positive for the virus by RT-PCR and the WHO criteria for SARS. The test helped identify and differentiate all SARS-positive patients from the control group. It also showed that the earliest seroconversion—the appearance of SARS-CoV antibodies in the patient—was seen

six days after fever onset, although samples collected 5-10, 11-15, and 16-20 days after fever onset were increasingly likely to test as antibody-positive (34.3%, 78.3%, and 97.7%, respectively) (Chan et al., 2004). The paper stated that the method was labor-intensive and required experienced technicians. Given the delay in obtaining confirmatory results between six- to twenty days post-fever onset and the increasing likelihood of confirmation during the later period, it could have been of little help in determining patient treatment protocols. In the first 1 to 10 days following fever onset, up to 66% of samples would have tested negative. While their method may have been shared between clinical sites, the paper was not published until March 2004. Whether their work was formally cross-validated into other laboratories remains a question.

In the April 19, 2003 issue of The Lancet, Peiris et al. published results from their work with fifty patients who met the criteria for SARS infection. Peiris et al. screened nasopharyngeal aspirates using IFA antigen detection for "…influenza A and B, parainfluenza types 1, 2, and 3, respiratory syncytial virus and adenovirus," along with cell culture-based assays for "conventional respiratory pathogens" including human-metapneumovirus (Peiris et al., 2003). None of the IFA results were positive for these well-characterized viral agents. A year later, Bermingham et al. wrote that eliminating SARS-CoV from consideration should incorporate screening for *Mycoplasma*, *pneumoniae*, *Legionella pneumophila*, and human metapneumovirus, “particularly in returning travelers from countries where SARS is considered likely to re-emerge from an animal reservoir”(Bermingham et al., 2004). The Peiris group also reported mixed signals for hematology and enzyme biomarkers:

> …lymphopenia was present in 68%, leucopenia in 26%, thrombocytopenia in 40%, and anaemia in 18%. Alanine aminotransferase (45–350 U/L) and creatinine kinase (141–1379 U/L) were raised by 34% and 26%, respectively (Peiris et al., 2003).

Some investigators found potentially significant changes in some cytokines. Samples were collected within 3 to 7 days of onset of symptoms. Increases in IL-6 and INF-γ and decreases in IL-8 and TGF-β seemed to hold some promise for diagnostic purposes. However, other investigators found that IL-13, IL-16, TGF-β, and TNF-α at high levels during the illness's initial phases (3 to 7 days) (Pang et al., 2003; Zhang et al., 2004). A study published by Huang et al. showed that "…IL-10 and TGF-β were continuously overproduced for the entire course of SARS infection," in direct conflict with Zhang et al.(World Health Organization, 2003b).

12.7 Attempting treatment

While the definition of biomarkers was being pursued, pharmacotherapy was being tested in a scattershot approach (Peiris et al., 2003) using antibiotics (levofloxacin, amoxicillin, clarithromycin, ceftriaxone, and azithromycin) and anti-viral drugs (oseltamivir, ribavirin, and amantadine). The antibiotics were given to address the incidence of bacterial lung sequelae to the initial viral infection and immunocompromised state caused by SARS-CoV. The glucocorticoids hydrocortisone and methylprednisolone were given intravenously for 2–3 weeks in gradually decreasing doses and showed mixed results. Peiris concluded that early use of anti-virals and steroids might be helpful, but the approach lacked consistency or large numbers of patients at publication (Peiris et al., 2003). It was shown that early, accurate detection is the mainstay of determining the underlying illness and prescribing an appropriate therapeutic response.

12.8 An unexpected end

After a substantial rise in global cases of SARS in late March and April, the illness tapered off on July 22, 2003 (Skowronski et al., 2005). As of June 11, 2003, the WHO reported a cumulative total in 29 countries of 8435 probable cases with 789 deaths (World Health Organization, 2003b). On August 29, 2003, the WHO issued a tabular report summarizing all known cases of SARS. Out of 8422 cases, 64 patients were still hospitalized, 7442 patients recovered, 916 patients died, and the case fatality rate was 11%. The overall patient count included 1726 healthcare workers—about 20% of affected patients (WHO, 2003). While the healthcare workers probably understood the use of personal protective equipment (PPE) better than their patients, many still contracted the virus. Was the appropriate PPE used? Did they use PPE properly in every instance? Was enough PPE available to all healthcare workers? Was PPE reuse necessary? Addressing these questions at the time could have helped inform responses to the current pandemic.

Aside from an afflicted group of virology researchers in early 2004, SARS infections seemed to disappear from the global population. Thus, SARS and the coronavirus that caused the outbreak resolved as quickly as they had occurred by applying time-honored isolation techniques.

12.9 Summarizing SARS

The single, highly predictive diagnostic for SARS seemed to be the onset of fever caused by the induction of endogenous pyrogens, such as IL-1, TNF-α, IL-6, or other cytokines (Bush, 2018). SARS showed mixed results for common cytokine biomarkers even when measured several days after the onset of fever.

All clinical tests, however simple or complicated, either 1) required several days or weeks for a biomarker to show a statistically significant difference from the control group, 2) were present in varying percentages of patients tested, 3) were ambiguous when examined across studies, or 4) all the above. In 2004, Oxford et al. published a study on recommendations for using neuraminidase inhibitors (NAIs) to treat SARS and other viral respiratory illnesses. The paper covered matrix-2 (M2) protein blockers (amantadine, rimantadine), broad-spectrum anti-virals (ribavirin, cidofovir), and the NAIs oseltamivir and zanamivir. It suggested that, while it is crucial to avoid wasting anti-virals in non-influenza respiratory disease, it could be essential to develop strategies to use available anti-virals that hold the promise of efficacy within hours of fever onset when an outbreak of viral respiratory disease is present in a community or region (Oxford et al., 2004). While medical costs present barriers to the early and widespread use of anti-virals, preventing hospitalizations, lower respiratory tract infections, and effects resulting from cytokine storm should take priority over the cost of pharmacotherapy (Ye et al., 2020).

In a review article, Cinatl et al. provided a list of anti-virals studied for SAR-CoV that have demonstrated mixed efficacy, usually in combination pharmacotherapy. Classes of anti-virals studied include ribavirin and analogs, interferons, HIV-1 protease inhibitors, nitric oxide and donor molecules, calpain inhibitors, glycyrrhizin and derivatives, SARS-CoV main proteinase inhibitors, SARS-CoV entry inhibitors, anti-viral antibodies, and others. The paper states that research was inconclusive, leading to mixed results between laboratories, and calls for “predictive correlation between in vitro activity and anti-viral effects in relevant animal models that reflect the situation of SARS-CoV-infected humans” (Cinatl et al., 2005). In short, the conclusion was drawn that if SARS returns or a similar viral disease occurs, much work remains to be done for an effective response.

Effective early anti-viral therapies are vital, as are ultra-sensitive immunoassay, RT-PCR, and cell counting (e.g., flow cytometry) methods that can determine cell type and cytokine levels, blood cell (platelet, leukocyte, lymphocyte) trends, along with any other biomarkers indicative of the onset of viral disease. These may reduce the number of patients who become immunocompromised or have accelerating lower respiratory infections. It is insufficient to have these techniques and technologies available at a few sites. The techniques must be implemented globally within weeks of an outbreak if they are not already in place, with best-in-class methods validated and cross-validated to equivalency so that all investigators and healthcare workers evaluate their patients in as identical a manner as possible. Collaborative international best practices committees focused on achieving consensus on international excellence in testing should be formed and maintained continuously. Much work that went into defining the structure and genetics of the SARS-CoV virus was intended for use in vaccine development and pharmacotherapy to mount a response to that specific coronavirus. Given the brevity of the outbreak, some of the work might have been less useful than efforts focused on diagnostic technologies and biomarker definition—although greater knowledge of any virus helps with diagnostics as well.

Importantly, SARS-CoV and the illnesses and deaths it caused allowed scientists worldwide to access new information about the nature of viral disease, genetics, protein chemistry, diagnosis, treatment, recovery, and mortality for coronaviruses. The rapid global response to SARS-CoV resulted in 28 vaccine candidates entering preclinical testing, two entering phase 1 clinical trials, and two entering phase 3 trials (World Health Organization, 2020a). The coronavirus research effort then faded between 2003 and the present.

In their 2013 paper, Cheng et al. cited a series of improvements that were realized since the SARS outbreak. They state the need to implement “proactive infection control

measures," particularly as "pathogens may emerge from wild animals as a result of their close interaction with humans in markets and restaurants." They allude to "the advancement of laboratory techniques," indicating they should be implemented into "proactive infection control measures against various bacteria and viruses," adding that "sophisticated molecular and sequencing techniques… also facilitated our investigation of outbreaks and pseudo-outbreaks." Several measures that should assist healthcare workers are also proposed due to the "large number of healthcare workers with fatalities affected by SARS." Lastly, they state that "the concept of extensive contact tracing … has been harnessed for the control of multiple drug-resistant organisms" (Cheng et al., 2013). In the same issue of Anti-viral Research, published on the 10th anniversary of the SARS outbreak, Hilgenfeld and Peiris cite tremendous progress in "the elucidation of structures and functions of SARS-CoV" and vaccine development. They also write

> , "After 2005-2006, it became difficult to obtain funding for research on SARS-CoV in many countries, especially for efforts to find new anti-viral therapies.
>
> Similarly, there was no incentive to develop SARS-CoV vaccines further without an overt threat to human health. Funding agencies and peer reviewers were probably short-sighted in this respect. However, many virologists also failed to take seriously the threat of the re-emergence of SARS or a SARS-like virus (Hilgenfeld & Peiris, 2013)

We may have learned much from the SARS outbreak, but we did not implement the massive local and global controls that were suggested. Fast forward to 2020. Healthcare systems the world over have been overwhelmed. Healthcare workers, again are exposed, are suffering, and dying due to the lack of available PPE. It is, of course, not the fault of these investigators that they did not explicitly foresee the disastrous scope of Covid-19. It is fair to say that some virologists and epidemiologists knew it was possible but could not

convince governments and institutions worldwide to take the exhaustive, pervasive, and persistent measures necessary to adequately control a massive global pandemic. Humanity remained ill-prepared.

SARS-CoV-2 is wreaking devastation worldwide, but some lessons that might have been learned during SARS-CoV are not making the broad and deep impact necessary to truncate the pandemic. Scores of drug therapy candidates and over 160 vaccines are progressing through clinical trials. It will take months, if not years, to bring these therapies to global patients. The novel anti-viral remdesivir has been approved through an emergency use authorization (EUA) that allows the unapproved drug to be used "in adults and children hospitalized with severe disease" (U.S. Food and Drug Administration, 2020). Dexamethasone and interferon-beta have demonstrated efficacy in randomized clinical trials (Hung et al., 2020; Wise & Coombes, 2020). Some monoclonal antibody therapies known as interleukin-6 inhibitors have shown efficacy in some trials (National Institutes of Health, 2020). We are told that any vaccine is a few months away to as many as five years away (Weintraub, 2020). As of July 21, 2020, the WHO lists 24 vaccines being tested in 40 phase 1, 2, or 3 clinical trials and 142 vaccines in preclinical evaluation (World Health Organization, n.d.). Patient testing for SARS-COV-2 or Covid-19 is a patchwork across the globe and generally is seen as incapable of meeting the testing needs of various populations. Any therapies and ultrasensitive methods must be available to all healthcare workers and patients within one to three days of fever onset rather than later. Identifying crucial eicosanoid biomarkers implicated in fever and inflammation onset might aid in rapidly identifying an underlying illness (Yan et al., 2019). The more time that elapses from fever onset, the less likely the outcome will favor patient recovery.

The humanitarian and economic impact of a global pandemic is mind-boggling. It is unknown what the eventual cost of the outbreak will be—in lives lost and diminished, emergency budgets allocated, businesses closed, supply chains interrupted, depressed gross domestic product, and other health and economic metrics. Those costs might have been lessened considerably if a less parsimonious approach had been taken to preparing a broad range of anti-virals and related drug therapies, researching shared weaknesses amongst viruses and bacteria, driving testing technologies to new sensitivities and early disease biomarkers, developing flexible capacity in hospital beds capable of responding to pandemics, and warehousing ready-to-use PPE to prepare for a worst-case scenario. Research into finding universally applicable, genuinely effective anti-viral therapies is not an easy or inexpensive task. However, research groups across the globe should join it. It is a task being modeled by the work done by the Coalition for Epidemic Preparedness Innovations (CEPI) (Coalition for Epidemic Preparedness, n.d.). If remedies are found, they should be made available to any affected person in any country at virtually no cost to the patient. Therapies that benefit the economically gifted while leaving most patients to suffer provide little benefit to the population at large and may result in pandemic flare-ups.

Most fundamentally, a dire need exists for discovering and developing an anti-viral that arrests viral respiratory diseases without causing any significant side effects in a broadly defined target population, that is, everyone, regardless of comorbidities. Such an anti-viral could be dosed in response to illnesses ranging from the common cold to influenza, viral pneumonia, ARDS, and SARS-like illnesses. Developing a safe and effective anti-viral for use in a wide range of viral diseases is not an easy target to achieve and might be impossible; identification of efficacious and safe drugs is one of humankind's more challenging enterprises. The benefits of achieving the target, however, are overwhelmingly positive. The common cold might be a matter of a day's inconvenience rather than days out

of work with their concomitant economic losses. The global population could live with a sense of calm when other viral outbreaks occur, knowing that securing the well-studied, safe, and efficacious anti-viral remedy is as close as a trip to the local pharmacy or physician's office. Achieving this kind of drug development goal is critical; it simply needs the funding and commitment of the global healthcare community.

COVID-19 proves that we must take the lessons of medical history as prescriptive for the future. If we do not heed this lesson, humanity may suffer similar tragedies. We must learn from what has happened and prepare for the possible (Griffiths, 2020).

CHAPTER 13 | Conclusions

The technologies associated with clinical chemistry—with quantitative diagnostic medicine—were technologies enabled by humanity's harnessing of fire to mold raw materials into various metals and glasses. At first, the products were barely improved over the raw substances from which the purified materials were extracted. Over centuries and millennia, lumps of copper, iron, and tin were blended with other materials. They became refined alloys with physical and chemical properties unique to the materials created by our species. Glazes and vitrified, shapeless lumps of glass were refined into objects that were traded between ancient kingdoms and prized in the same way gems were prized, then were refined further into objects that would hold water and could focus the sun's rays into blistering points of light, into surfaces that reflected light and became mirrors. Eventually, the glasses became so pure that lenses were created that helped improve eyesight, and then into lenses that extended sight into the skies and the crevices formed by minerals, flora, and fauna, and then into the realm of previously unimaginable creatures. When the sun's light was teased apart into a spectrum of colors by prismatic glass and lenses were refined to the point that natural colors could be compared objectively instead of subjectively, when Wollaston and Fraunhofer noticed that the sun's light was obscured in various parts of the spectrum, colorimetry, and spectroscopy became possible ways to understand our universe and understand, eventually, human health. When blood was understood to be far more vital than simply one of four humors, simply a substance to bleed out when humans became ill, became a vital fluid composed of a variable mixture of chemical components, blood analysis became a possible way to differentiate one human illness from another through analysis of the fluctuations in the components.

While examining the evolution of quantitative diagnostic medicine, it became clear to me that some of the pivotal research achieved by a variety of natural philosophers and physiologists over the past few centuries had gone unexamined. In particular, the

methodological details of their work had been ignored almost entirely—and it is improvements in methodology and technology that move our understanding of human health and general science forward. How could the nuanced use of micrometric comparisons that Leeuwenhoek relied on in his voluminous correspondence have gone unremarked? How was it possible that Leeuwenhoek's many observations of red blood cells and hemocytes had received only the most cursory mention among historians of hematology? Why was it that the work done by Gabriel Andral and Jules Gavarret, although appreciated in its time, has been unexamined for its enormity and for its diligent attempt to establish biomarker patterns for numerous illnesses? How could there be so little secondary history of medicine literature about Carl Schmidt and Karl von Vierordt's argument about the relative merits of each other's work when both were trained in some of the best research groups of their time?

By asking these questions and providing some answers, this thesis has added to the history of clinical chemistry literature and, thus, medicine and science literature. It has established a broad context in which the innovations and observations of scientists from the 17th through 21st centuries affect the definition of health and the diagnosis of illness, even addressing the fact that medical science is far from finished accumulating an understanding of human health and possible therapies for curing those who have fallen ill.

Among the critical lessons that the Covid-19 pandemic might have imparted to humanity is that research on infectious diseases, disease biomarkers, and pandemic response should always occupy a central place in the research budgets of national and transnational entities. Among those of us who have worked in healthcare-related fields, the level of distrust of scientists and physicians and the willful communication of inaccurate information was more reminiscent of archaic responses to plagues, such as the cholera epidemics in Europe

and Portugal. Communications almost identical to those propagated by the internet, politicians, and other individuals with access to mass communication channels resembled communications by newspapers and physicians during the 1833 pandemic in Portugal. Surely, almost two hundred years of human experience should have made public health communications in 2020 more explicit and less laden with propaganda and misinformation. Surely, humans would understand that even an idealized response to a new health threat would take weeks or months to come into being. A response based on caution and respect for the unknown was preferable to denial and panic. Nevertheless, with all that our scientists and physicians have learned over the ages, humanity often reverts to irrational behaviors when a pandemic arises.

While conducting the research that became this thesis, it became clear to me that there are many more chapters to be written in the history of clinical chemistry. The chapters herein that remain unfinished will be finished, and new chapters will be started. History needs our attention, and so does scientific methodology. We cannot forget the past. While we may be doomed to repeat it, it is better to understand what has happened before to avoid recurrent pitfalls.

CHAPTER 14 | References

A large amount of literature was reviewed during the research required to write this thesis. Probably seventy to eighty percent of what was found was determined to be too superficial or too tangential to the topic for use. The intent of the thesis was to find topics in the history of medicine and of clinical chemistry where the literature provided an inadequate or incomplete account from science history. On the other hand, the introductory sections of the thesis provide some context for the historical elements that constitute the bulk of the work. Applicable literature references are provided when this is the case.

Sections on Auenbrugger, Andral, Schmidt, and Vierordt required several hundred pages to be translated from the original articles in German and French published between the mid-18th and mid-19th centuries. Some biographical material was also translated. Translations are available upon request.

A good example of the resources I found but did not cite was a website maintained by a flow cytometry company called Nexcelom (*The Historical Development of the Hemacytometer*, n.d.). The material posted there pointed me to a thesis written by Jack David Davis (no relation) and I ordered a copy of the thesis, but it turned out to be more superficial than I had hoped (J. D. Davis, 1994).

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