

Technical Note

Historical Perspective on Blood and Phlebotomy

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Abstract:

As we continue to streamline the process of blood collection, let's briefly consider the history of blood as a tool for diagnosis and a route for delivering drugs to their targets.

Ancient Bloodletting

Hippocrates (460 BC) was one of several Greek scholars who advocated deliberate bleeding as a means of rectifying an imbalance of "humors" in the human body. Hippocrates concluded that menstruation in women accomplished the same thing and proposed bloodletting as a substitute process in males (1). The innovative Roman physician Galen, who had invented syringes and surgical instruments, continued the practice in 200 AD and influenced others who relied on bloodletting for almost 2000 years (1) not as a means of diagnosis, but as a conduit to remove "bad blood" from the patient. Many ancient cultures bled the living, but a true appreciation of blood's purpose and the means for its characterization was much more recent.



Understanding Blood

While reading about William Harvey (1578-1657) and his controversial theories about the circulatory system I was drawn to his thoughts about the role of the heart and the pipes going in and out. The notion that blood is pumped in a circular fashion took serious thinking and clever experiments when there were no ultrasound and MRI imaging tools and no transducers for blood pressure or blood flow. Oxygen was also unknown at the time and awaited discovery by Priestley on August 1, 1774. Modern science and technology is spoiled by the frequency of its leaps. Advances came to us less often back then, although they were sometimes huge when they arrived.

By the grace of hyperlinks, these musings lead to Stephen Hale (1677-1761) measuring the blood pressure of a horse in 1727. Many of us are familiar with the famous sketch of the horse's blood rising in a cylinder to a height of a couple of meters while the horse rested calmly beneath a tree. Without photography, the actual event could not be recorded in real time and likely was a bit less tidy than depicted by the artist. The function of blood was the subject of much speculation back then, but actual measurements took another century or two. Truth be told, we still have much to learn. It has only been the last few decades (!) where some of the many very dilute organic components of blood could be both identified and quantified. This remains a very challenging and costly task. The oxygen-carrying protein hemoglobin (a very major constituent) was discovered by Hünefeld (2) in 1840. Then in 1851, Otto Funke (2) isolated hemoglobin crystals from hemolyzed red blood cells. Hemoglobin's reversible oxygenation was described a few years later by Felix Hoppe-Seyler (3), the first to note its visible absorption spectrum and two characteristic absorption bands. He established that the substance could bind oxygen and also that it contained iron. It was not until 1959, 300 years after Harvey, when Max Perutz (4) used X-ray crystallography to sort out the molecular structure of hemoglobin. This work resulted in his sharing the 1962 Nobel Prize in Chemistry (5) with John Kendrew (6) "for their studies of the structures of globular proteins."

The importance of hemoglobin and the Perutz work are evidenced by how rapidly the Nobel Prize was granted. Needless to say, progress in sorting out the constituents of blood has gone very slowly. It's only since the 1950s that automated clinical chemistry instruments came into common practice.

Early in the 20th century there was very little one could do with a blood sample that would impact a diagnosis. By what practice was blood collected then? The following century-old text is available from several sources with free access online:

James Campbell Todd, Ph.D., M.D. "Clinical Diagnosis - A manual of laboratory methods", 2nd Ed., W. B. Saunders, Philadelphia, 1912.

The following is an excerpt from page 183 of that book.

"For most clinical examinations only one drop of blood is required¹. This may be obtained from the lobe of the ear, the palmer surface of the tip of the finger, or, in the case of infants, the plantar surface of the great toe. In general, the finger will be found most convenient. With nervous children the lobe of the ear is preferable, as it prevents their seeing what is being done. An edematous or congested part should be avoided. The site should be well rubbed with alcohol to remove dirt and epithelial debris and increase the amount of blood in the part. After allowing sufficient time for the circulation to equalize, the skin is punctured with a blood lancet...or a pen with one of its nibs broken off². Nothing is more unsatisfactory than an ordinary sewing needle. The lancet should be cleaned with alcohol before and after using, but need not be sterilized. It must be very sharp. If the puncture is made with a firm, quick, rebounding stroke, it is practically painless³. The first drop of blood...should be wiped away, and the second used in examination... When a larger amount of blood is required, it may be obtained with a sterile hypodermic syringe from one of the veins at the elbow."

The familiar Vacutainer®⁴ did not become popular until the second half of the 20th century. Finger prick blood as a self-sampling method accelerated in the 1980s in response to home glucose testing and has been characterized by ever sharper (and smaller) lancets, spring loaded lancets and tests that required lower test volumes, now even below 1 µL (1/25th of a drop). Lateral flow qualitative diagnostics for pregnancy and later for infectious disease also became a driver for processing small volumes of blood (and other fluids) for selected diagnostic purposes. Combined, these tests include over one billion measurements each year.

Blood Banks

The first blood bank in the United States was established in 1936 at Chicago's Cook County Hospital. This too implies blood collection, but in larger volumes. In some respects, Phlebotics, Inc. offers a miniature blood bank, where the objective is to collect viable blood containing sterile and intact cells, preserved by cooling and protected from hemolysis or mechanical shearing during collection. During a modern "blood drive" automated instruments can remove specific components from blood and return the rest to the donor. Apheresis, a method making this possible, was first demonstrated in 1972. A leading company in this field is Haemonetics, Inc. (www.haemonetics.com), founded in

¹ This tiny volume was deemed sufficient by Dr. Todd not because the instruments a century ago were great, but rather because there were so few instruments at all. The microscope was available, but virtually nothing to monitor *chemistry* in a drop was available until ~50 years later and even today, not often in a physician's office. In 1912 there were no practical means to routinely monitor electrolytes, blood gases, pH, or glucose. Analytical chemistry instruments for blood were not routinely practical until the 1950s. Once they became available, blood draws of 10 cc (400 drops) were more typical and remain so. Aspirin was only a decade old in 1912 and "monitoring" therapeutic drugs made little sense since there were so few available and virtually nothing was known about pharmacokinetics or bioavailability. Very little was known about blood typing either, but progress had been made by Karl Landsteiner and colleagues in 1901. We are only today heading back to the requirement of one drop (25 µL) for some measurements, with less required in just a few cases.

² Don't try this at home!

³ We were a tougher species back then.

⁴ Vacutainer is a registered trademark of Becton-Dickinson, Inc.

Massachusetts at about that time and operated today globally. There is much to say about blood transfusions, but it is only quite recently that we've gotten very good at it. Many will remember that transfusions were a major transmission mechanism for HIV in the 1980s. There continues to be work aimed at "artificial blood" for use in emergency care, including battlefield trauma. There has been insufficient success with preparing a stable, safe and effective blood substitute, so far.

Hemodialysis

This widely appreciated method extends the lives of patients with kidney failure and is the invention of Willem J. Kolff, a Dutch-born doctor who also mentored pioneers in artificial heart development. Kolff began his work in occupied Holland during WWII, famously using parts scrounged from other devices. I mention this here only as another example of where large volumes of blood are removed from a patient, in this case, cleaned of some toxins and then returned. This advance is costly, but no doubt has extended both life and the quality of life for many, in some cases while waiting for the availability of a viable kidney for a transplant.

Programmed Blood Collection

The first commercial device for the programmed collection of serial blood samples was originally developed by a research team led by Candice B. Kissinger at Bioanalytical Systems Inc. in 2000 (7). The purpose of the device (Culex®) was to facilitate *in vivo* pharmacokinetics studies in conscious and freely-moving rodents. Beginning with rats, the technique was rapidly extended to larger rodents (guinea pigs) and smaller (mice), and then larger animals (dog, monkey, pig). Serial blood samples were programmed relative to dose, which was also fully automated via intravenous, intragastric or duodenal catheters. An unprecedented degree of detail and reproducibility in pharmacokinetics became possible while also improving the quality of the experiment. Sampling blood from animals became painless, taking place without restraints. Studies of blood from sleeping animals were demonstrated routinely since there was no need to disturb the subject while blood was collected. More importantly, collections from nocturnal rodents could be practiced at night when they (and their metabolism) were most active, without scheduling a second shift of animal technicians to do the work. This device revolutionized practice in the vivarium at pharmaceutical research companies worldwide. Manual venous blood collection (jugular, femoral, saphenous, tail, suborbital) was dropped wholesale in favor of automation and the multiple benefits it fostered.

It's About Time...

Among those substances that we quantify in blood, very few are examined with respect to rate of change in more than a casual way. The oxygenation of blood is routinely determined in hospital settings using the noninvasive technique of pulse oximetry (now readily available for home use by clipping a simple device on a finger). Glucose meters allow for discrete measurements several times a day. Diabetic patient compliance is highly variable. Only in this millennium has it finally become possible to update *in vivo* glucose data using implanted transducers (aka sensors) that are just entering the market. These are operable for the better part of a week. A determination every 5 minutes or so allows the diabetic to not only record the concentration but also see the *rate of change*. The tradition in clinical chemistry is to compare data with a reference range established from measurements using samples from many individuals, both in sickness and in health. That is like knowing the speed of a car, or the price of a stock, without knowing whether they are accelerating, decelerating or at steady-state. Trends matter! There remain more molecules in blood that we can't determine with any routine accuracy and precision. Until we can, we won't know which of them are valuable indicators of patient status.

A common technique used by both engineers and scientists to learn more about a complex system is to probe with a stimulus and observe whether it returns over time to the original or a new condition. Is the system resilient, or does it bend or break? The glucose tolerance test is a good example. A large amount of glucose is added to the system as an impulse function and we watch the time

dependence of the relaxation of circulating glucose back to homeostasis. That provides the physician with information not available from a single glucose concentration measurement. Heart attacks are events we can trigger in animal models and then follow various parameters vs. time. When a patient experiences such an event, a number of markers in blood change over the subsequent hours. We can further stress patients with exercise and measure both physiological and biochemical responses ramping up and then down. Astronauts are stressed with G-forces and a lack thereof. Whenever a new patient is first dosed with a drug, we are stimulating the system. How will it respond? We are on the cusp of knowing much more than we routinely know today. Knowing both the **rate** and **direction** of change will help to achieve better care.

Personalized (Precision, Individualized) medicine

Every person on Earth is different. There are limitations in using averages to reach diagnostic conclusions for any individual. We have our own internal reference ranges that describe our health. When we find a tumor, it is not like all other tumors and proceeds at its individual pace. Thus we now see diseases such as leukemia, breast, brain and prostate cancers as a family of many diseases where treatments must be adjusted. Drug pharmacology (good) and toxicology (bad) is also highly individualized due to different drug receptors, different drug protein transporters and different drug metabolizing enzymes. These hundreds of factors work together to result in an individualized response. We have tended toward the idea that one (or just a few) dose sizes fit all. That's because the science and the technology have not aligned economically to do otherwise.

Now we are much closer to a more individualized pharmacology. It's working well with certain cancers where the cost is justified, but we are just beginning. The language is imprecise. Personalized medicine at this stage means breaking the population down into a few categories (sub-populations). In order to achieve a precise *individualized* therapy we must make measurements! Drugs are best optimized by the circulating "bioavailable" concentration, not by the dose we swallow. After all, we vary a lot from a neonate to a grandmother. Weight is one factor. We also vary in our metabolizing enzymes and transporters, not only as a function of our genes, but also over time with our development to adulthood and beyond. These features determine where a drug goes and how long it lasts. Does it hit the target? Is there collateral damage from off target effects? Do we need to administer the drug continuously or is once a week enough? *The best way to get the answers is to make measurements one patient at a time.* Economics will always limit how much of this we do, but so will the nature of the disease and the drug. Some drugs, like some foods, have a large overhead with respect to safety, others do not. Among the latter, anticoagulants, antirejection (immunosuppressing) drugs, and cancer drugs are examples of where there is opportunity for improved therapy based on more data from individuals. To get that data, we must sample blood and do so as a function of time in response to a dose.

The tools of modern bioanalytical chemistry including mass spectrometry, flow cytometry, lateral flow diagnostics, point-of-care meters, rapid immunodiagnostics, microfluidics and others are evolving very rapidly and improving in specificity, cost and applicability by non-experts⁵. Likewise, "informatics" is improving to help the caregiver to both keep records and interpret their meaning. The Phlebot™ makes all of these tools better by providing a higher quality sample. Diagnostic decisions based on chemical data are only as good as the sample obtained and the instrumentation used to process it quickly. While history shows how long it has taken to get clinical evidence from blood, we

⁵ There will always be debate about where measurements are to be made and by whom. There are multiple choices including at the bedside, in the ICU or ED, or in the central laboratory. The former is best for speed to a decision. The latter is best for the quality of the number. The numbers must be trustworthy. That is much harder than it sounds and depends very much on the specific test and the skill of the staff.

now have the tools to learn much more as a function of time within a single patient. Doing so has the potential to improve outcomes and reduce cost. It's now a matter of time.

I'm fond of the *Lemma of New Technology* expressed by physics Nobelist Herbert Kroemer in 1995 and mentioned again in his Nobel lecture of December 8, 2000 (8):

“The principal applications of any sufficiently new and innovative technology always have been – and will continue to be – applications created by that technology.”

While we have compared the automation of blood sampling with existing manual methods, the new dimension added is likely to carry us in new directions because it enables the previously impossible. This is opportunistic in the same way that telephones evolved to take pictures, play music and send text in ways that were not anticipated by Alexander Graham Bell. In the next decade, many advances in chemical instrumentation that are now in development will be realized in the clinic. These will require smaller sample volumes and be easier to use. Advances in separations science, microfluidics, mass spectrometry and informatics show great promise.

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