3.
Elephants in the Dark
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Introduction

Man has always regarded blood as a living substance, as the essence of life. Millennia ago, primitive peoples recognised blood as a vital element necessary for survival. When enough blood was lost, life stopped. Blood was thought to be related to the heart, that mysterious organ whose motion ceased as life ended. Poets have written of thick blood and thin blood, pale blood, red and blue blood, Royal blood and so on. It was an English poet of the 17th century, John Donne, who spoke of blood as 'pure and eloquent' and this suggested a title to Maxwell Wintrobe for his extraordinary book on the history of haematology. The study of blood and its disorders has led to discoveries of great practical importance including the science of molecular biology. This should come as no surprise since blood is so easily obtained by venepuncture or skin puncture and really resembles a computer disk in that it carries in its cells a myriad coded messages.

Just like elephants in a darkened shop full of small fragile objects, invisible to the eye, human philosophers and scientists have attempted to interpret the real nature of the invisible objects in blood and attribute meaning to their function. But the elephant's trunk is not so insensitive as may first appear. There are many examples of the skilful use of the elephant's trunk. Likewise the earlier human philosopher and scientist, although his tools may have been crude by today's standards, pursued his experiments and studies with a surprisingly delicate sensitivity. In parallel with the development of knowledge of blood and its disorders, there has been the development of more and more sophisticated technologies for its exploration.

All ancient civilizations recognized the importance of blood as a life-giving substance, believing it to hold the body's vital force. The Bible uses the term to 'shed blood' in the sense of to 'kill'. The Egyptians, as judged by an old papyrus from Thebes, thought that food in the stomach was turned into blood by the heart. According to ancient Hebrew thought, blood was the seat of the soul and the drinking of blood was prohibited. Mosaic law demanded that blood be drained before an animal was prepared as food (a practice still followed by Orthodox Jews today). The Romans drank the blood of their enemies, thinking it would confer on them the courage of their vanquished foes.

For many years, indeed centuries, the pervading biological and philosophical concepts were clearly based on the theory of the Four Humours. The idea of the Four Humours became popular in Ancient Greece from about 400BC but it may have originated in Indian medicine and subsequently been picked up by travellers from Greece. In any event it was probably developed by Hippocrates. At that time Greek thinkers were seeking natural rather than supernatural explanations to explain the events in the world around them. These ideas were then applied to medicine. The fundamental belief was that all things were made of four elements: air, water, fire and earth. On this basis Greek doctors therefore believed that in the same way the body contained four humours or fluids: blood, phlegm, black bile

and yellow bile, and if these humours lost their natural balance, illness would result. This was an important step forward because it encouraged the search for natural causes of disease and the provision of natural treatments. The work of Hippocrates can be taken as representing the foundation of Greek medicine. Hippocrates developed a new approach to medicine by refusing to use gods to explain illnesses and disease. This meant that medicine started to become a science rather than a religion. Hippocrates stressed the importance of observation, diagnosis and treatment. However Hippocrates' most important contributions to medicine were in the development of the medical profession and in a code of conduct for doctors.

This foundation laid by Hippocrates became systematised into a complex pattern by Galen some 500 years later. Claudius Galen, born in Pergamon in AD 129, crystallised all the best work of the Greek medical schools which had preceded his own time. Galen was profoundly influenced by Hippocrates' idea of the Four Humours. He developed this by introducing the idea of using opposites to treat illnesses. Galen was a brilliant observer and a wise physician but the doctrine of the humours led him to develop a philosophical system which was not free from contradictions and unfortunately much was in error. It is essentially in the form of Galenical physiology and Galenism that Greek medicine was transmitted to the Renaissance scholars. After his death, serious anatomical and physiological research ground to a halt, because it was assumed that everything that was to be said on the subject had been said by Galen. Galen's reputation was so high that for more than 14 centuries, physicians and scientists rarely questioned his authority. An interesting suggestion made in 1921 by the Swedish scientist Fahraeus, the inventor of the erythrocyte sedimentation rate test, was that the Four Humours could be observed by studying blood clots in a container. After clot retraction, at the bottom of the vessel a deep red, almost black jellylike material collects which consists of the deoxygenated red cells. This corresponds to the 'Black Bile'. Above this there is a thinner layer of red blood containing oxygenated red cells and corresponding to 'Blood'. The next layer above is the buffy coat consisting of leukocytes and platelets corresponding to the 'Phlegm' and finally there is the clear serum corresponding to 'Yellow Bile'.

Galen's books were used as training manuals for doctors until the Renaissance. Even though some doctors questioned his theories, the Catholic Church prevented Galen being challenged during this period, since his theories of anatomy fitted in with their belief in a system ordained by nature. In spite of Galen's mistakes and misconceptions, the wealth of accurate detail in his writings is astonishing. Galen's mistakes did, however, perpetuate fundamental errors until Andreas Vesalius, the sixteenth century Italian anatomist, although regarding his predecessor with esteem, began to dispel Galen's authority. Galen had studied the anatomy of the cardio-respiratory system, but he did not discover the circulation of the blood throughout the body, and believed that blood passed from one side of the heart to the other through invisible pores in the dividing wall. Galen was convinced that the venous and arterial systems were each sealed and separate from

each other. William Harvey (1578-1657), discoverer of the circulation of the blood, wondered how Galen, having got so close to the answer, did not himself arrive at the concept of the circulation. While today the concept of the circulation of the blood seems obvious, it was not until the relatively recent era of the seventeenth century that William Harvey convincingly described the circulation of the blood. He emphasised that both arteries and veins contained only one substance, the blood, but could not show how arteries and veins were connected. This was very soon explained by Marcello Malpighi (1661) who, using a microscope, described the capillaries. Furthermore, Harvey could not explain why venous and arterial blood were of different colours. The explanation of this had to await the discovery of the process of oxidation and the recognition of haemoglobin and its properties of taking up and releasing oxygen.

It is amazing, considering such discoveries, that the very old practice of blood-letting coming from the earliest days of medicine, was still actively practised and continued well into the 19th century. This was strongly advocated by the famous American physician and signatory to the Declaration of Independence, Benjamin Rush, and universally practised together with purging in the treatment of the victims of the epidemic of yellow fever in Philadelphia in 1796 and which probably contributed to the high mortality rate. It is not difficult to make serious errors of interpretation, sometimes with tremendous consequences, when one very strongly concentrates on a small isolated part of the problem especially with preconceived ideas.

The Microscope

The development and refinement of our elephant's trunk in the process of discovering blood cells and their functions are strictly linked to technological advance. The scientific study of blood had to await the invention of the microscope. Before the invention of the microscope, doctors and scientists could only observe what they could see with the naked eye. Early microscopes used drops of water captured in a small hole to create magnification. Magnifying lenses were described by the natural historian Roger Bacon in the 13th century but lenses of sufficient quality for scientific use were not available for another three centuries. The first microscopes as instruments of magnification for minute objects made by combining glass lenses in a tube were described early in the 17th century and were used for the examination of insects and plants by amateur naturalists in the courts of nobles and kings. No one thought to use microscopes for the study of blood until the noted Dutch naturalist Jan Swammerdam (1637-80), described what he called “ruddy globules,” which were presumably red blood cells.

The first detailed description of red cells was produced by the famous (also Dutch) microscopist Antonj van Leeuwenhoek (1632-1723) who was employed in a drapery shop in Delft. He examined his own blood and found it composed of exceedingly small red particles swimming in a liquid. These particles were described, as being so minute that 100 of them placed side by side would not equal the

diameter of a grain of sand. Neither Swammerdam nor van Leeuwenhoek were medical researchers and therefore did not appreciate the importance of their observations. Detailed description of the red blood cell is attributed to William Hewson, the English anatomist and medical researcher in 1773. This work earned Hewson the title 'father of haematology'. Gulliver who differentiated lymphocytes from granulocytes on the basis of size described the colourless leukocytes about one century later. Platelets were first described by Donne in 1842 but it was not until 1875 that improvements in microscope design and the development of counting chambers permitted their enumeration. Bizzozero described their role in blood coagulation and thrombosis in 1882.

In spite of these early observations, development in the knowledge of blood cells was slow. Not only were technological advances gradual, but for more than two centuries following the first description of blood cells there were reactions to and difficulties in accepting them. Microscopes, even great minds stated, 'deceive us by representing objects differently from what they really are'. In 1829, Goethe wrote "Microscopes and telescopes confuse in reality, the pure human judgement". However notwithstanding this atmosphere of scepticism and resistance to new ideas, by the middle of the 19th century, studies were initiated by a number of scientists that would profoundly influence the future course of haematology. These activities started the era of morphologic haematology.

Haematological morphology

Yet again, microscopy was endorsed as the important technological advance. The science of haematology was given a tremendous boost in the late 19th century, when it became possible to examine the microscopic details of blood cells. This was due in large measure to the developing chemical industry, mainly in Germany, which had just discovered the aniline dyes. At the microscopic level virtually all cells are transparent. Most are completely colorless and accordingly few cellular details can be distinguished by looking at unstained specimens even with the best microscopes. It is therefore necessary to stain cells to obtain much useful information from them.

The pre-eminent figure in the world of biological stains was Paul Ehrlich. Ehrlich's contribution to routine haematology was his development of the triacid stain in 1877 while still a medical student. This allowed him to classify white blood cells into a scheme similar to the one in use today. In 1891, the triacid stain was replaced by the eosin methylene blue stain created by Romanowsky. The "Romanowsky stain" was further modified by Richard May of Munich in 1902, Gustav Giemsa of Hamburg in 1905, and J. H. Wright of Boston in 1906. All of these modifications directly descended from Ehrlich's original ideas. These dyes, together with the greatly improved compound microscopes by then available allowed the routine differentiation of white cells in the blood in health and in disease. Almost one hundred years later, two of these, the May-Grünwald-Giemsa stain and the Wright stain, are in use for the examination of the millions of blood smears prepared in clinical laboratories every day.

Haematological quantitation

The next important milestone in the study of blood was the introduction of quantification as a supplement to observation. During the first half of the 19th century, the word "anaemia" was a clinical term referring to pallor of the skin and mucous membranes. The word itself is composed of two Greek roots together meaning "without blood". When Gabriel Andral, the French physician, published his textbook of haematology in 1843, there was no appreciation of the basic concept held today that clinical anaemia is due to inadequate numbers of red blood cells. A method of cell counting first had to be developed. Karl Vierordt performed the first blood cell counts in 1852 in Europe but his technique was too tedious to gain widespread use. The first methods were ingenious, but time-consuming and painstaking. It required one to two hours to complete one sample count. Modifications developed thick and fast. The general microscopic technique of counting the number of cells in a diluted specimen in a transparent chamber of standard dimensions developed, culminating in the basic Bürker chamber in 1905.

Early attempts to measure haemoglobin concentration which included the visual matching of dilutions of whole blood to a liquid colour reference, were introduced late in the 19th century by Gowers, Hoppe-Seyler, Sahli and Haldane. Anaemia classification at that time was based on 'colour index' which expressed the average amount of haemoglobin in the red cell in relation to arbitrarily chosen normal values for haemoglobin and red cell count. The normal colour index was 1.0. It is surprising that reference to the colour index still figures on a prestigious US website today.

The Beginning of Modern Haematology

Towards the end of the 19th and at the beginning of the 20th centuries, a mysterious and fatal form of anaemia was recognised with increasing ease and frequency in hospitals of North America and Western Europe, thanks to the new investigative procedures. It was called pernicious anaemia since survival from the onset was usually no longer than two to three years and spontaneous recovery was extremely uncommon. Scientists such as Thomas Addison and Anton Biermer had exploited the newly developed haematological tools to determine the number, size and shape of the red cells and the amount of haemoglobin in a drop of blood. In 1880, Paul Ehrlich was first to describe in the peripheral blood of certain pernicious anaemia patients, occasional large nucleated red cells that contained much cytoplasm and dispersed lace-like nuclear chromatin. He called these cells megaloblasts or gigantoblasts, the hallmark of pernicious anaemia. A few years later, Paul Erlich, using the dye methylene blue to stain samples of fresh blood, described bluish particles and granules visible in a small percentage of apparently normal red blood cells. Twenty years later these were recognised as young red cells and called reticulocytes. The era of modern haematology is considered to have begun in 1926 when Minot and Murphy at the Harvard Medical School re-

ported that patients who suffered from pernicious anemia could be successfully treated with large quantities of raw liver in their diets. Minot and Murphy shared the 1934 Nobel Prize for this discovery. Minot and Murphy reported at the Congress of the Association of American Physicians, the consistent clinical improvement and increase in red blood cell count and haemoglobin concentration in a series of 45 pernicious anaemia patients treated with a special diet rich in beef liver. Convincing evidence of the therapeutic efficacy of this liver diet and later, liver extract, came from the prompt increase in reticulocytes, that consistently preceded the rise in the red cell count. Reticulocytes became a reliable indicator of the ability of bone marrow to produce red cells. After World War II, the search for the substance(s) in purified liver extracts led to the isolation of haematinic substances called vitamin B12 and folic acid, and the recognition of their fundamental importance in cellular proliferation and metabolism.

During the past two decades, the introduction of flow cytometry has permitted the automation of reticulocyte counting with greatly improved precision and accuracy. Additional reticulocyte measurements are now available such as the immature reticulocyte fraction (IRF) with interesting and innovative clinical applications including the follow up of bone marrow recovery after bone marrow or peripheral blood stem cell transplantation.

Starting from the 1920s, haematology has changed from a descriptive discipline of blood cell morphology into a quantitative science employing refined physiological and biochemical methods. This route has and will lead to further mammoth technological achievements that even the most imaginative scientists of the last century could not have conceived, such as molecular biology, genetic diagnosis and therapy and so on. From slow beginnings, the rate of change has inexorably and now rapidly increased.

Disappearing diseases: Changing diseases

So, physicians armed with more accurate quantitative measurements began to think about disorders of blood in physiological and quantitative terms, but the way was still long and rough. However, preconceived ideas mixed with inappropriate perception tools and limited perspectives can very readily distort the true picture. There is a well known Indian parable, which dates back at least 2000 years, involving six blind men and an elephant. This parable illustrates just how speculation on the whole from too few facts can lead to very large errors of judgement.

In this story, six blind men encounter an elephant, for the first time in their lives. Each gives his analysis of the elephant, and their interpretations are based on the particular part of the elephant they happen to touch. The first blind man touched the stubby side and declared the elephant was a wall. The second man felt the elephant's sharp tusk and declared the elephant was a great spear. The third man grasped the trunk and announced that the elephant was certainly like a snake.

The fourth man, touching along the knee, said that clearly the elephant was a tree. The fifth man examined the elephant's waving ear and was convinced that the elephant was some sort of fan. And the last, grabbing at the elephant's tail, declared that the elephant was a rope. Of course, each was partly correct, since each had only encountered one parameter. However, they were all wrong, because, in their blindness, they had failed to comprehend the system as a whole. This happens in science, and in haematological disorders too. We have seen how technology, in the form of tools to study and manipulate blood, has played a large part in the story of the formation of our speciality of haematology. As the medical historian, Keith Wailoo recalls in his important book "Drawing Blood", these technical advances have had a very significant impact on the social and moral condition besides their effect on health. This book is an excellent example of how many threads can be spun together to create a compelling narrative. It interweaves histories of disease over the past century, of technology, of haematology, and of medicine in the broadest sense. He shows how things like race, gender, and lifestyle influenced the way in which physicians defined and responded to the very diseases that were called into existence by the new technologies they employed.

An excellent example of the impact of physical, social and moral influences on health is afforded by consideration of the disorder called chlorosis. The first description of the disorder is attributed to Johannes Lange, the Heidelberg physician (1485–1565) who called it 'De morbo virgineo'. The term chlorosis is derived from the Greek word meaning green and started to be used in the early 17th century to decribe this disorder. The disease became well known both in medical and in lay circles, the latter calling it the 'green sickness'. Thomas Sydenham (1624–1689), the English physician, is credited with the first use of iron salts for its treatment. Chlorosis occurred almost exclusively in adolescent girls aged 14 to 17 years and the most prominent manifestation was a greenish pallor of the skin. This disease was very familiar to physicians of the 1890s. The girls were pathetic and enigmatic figures presenting a vague complex of symptoms including poor appetite, gastric disturbances, lethargy, and inability to do household or any other physical labour. Their menstrual periods ceased, and their character was nervous and capricious. It was widely believed to result from delay in marriage, a disease of virgins and women who had failed to adopt appropriate family and social roles. From such a standpoint, marriage and cohabitation were considered the best treatment. Between 1890 and 1910, which was the golden era of this disease, or pseudo-disease, there was general agreement that this was an illness originating from the female body's rebellion against economic, social and cultural changes such as high-stress labour for working class and immigrant women, high-pressure education and family relationships for the upper class. The aetiology and therapy devised by doctors were clearly determined by touching only the tail or the nose of the elephant. Since it was believed that the disease was essentially a moral matter, then physicians should act as moral supervisors, and become surrogate parents to the affected girls. Such moral management consisted of removing girls from home and prescribing a controlled regimen of bed rest, regulated bowel movements, exercise, oxygen inhalation and iron pills.

This common disease disappeared suddenly in the early 20th century as a result firstly of improvements in diagnostic haematology, and, secondly, advances in iron therapy. Ideological changes could have had a role, such as women's emancipation and cultural liberation and the disappearance of Victorian attitudes to women, their bodies, and their role in society. Maybe also the abandonment of corsets and bloodletting as a treatment were factors.

But really it was the technological innovations that focused medical attention so intensely on the blood in that era that deserve credit for the disappearance of chlorosis. The invention of haemocytometers and haemoglobinometers suggested that symptoms like pallor and fatigue were due to too few corpuscles in the blood, or too little haemoglobin. These blood parameters were shown clearly to improve in cases that responded to treatment. The emergence of laboratory skills with improved diagnostic techniques demonstrated that chlorosis was, in the majority of cases, a haemoglobin deficiency and that it could be successfully treated with iron pills. However, there was still a conflict between traditional doctors attached to the concept of chlorosis, and new specialists who were reducing everything to hypochromic anaemia.

Still like blind men touching different parts of the elephant, haematologists and physicians re-ordered and reclassified what had been chlorosis into separate disease entities recognised by improved diagnostic procedures as early tuberculosis, anorexia nervosa, chronic iron deficiency anaemia and many others. However, chlorosis disappeared from humans and now it is only a disease of trees caused by insects that make their beautiful leaves lose colour and turn to white. Progress was taking place and with the adoption of the Wintrobe indices a rational classification of anaemia was created. This was further improved by the introduction of automated cell counters with their great improvements in precision, in accuracy and in speed.

Sickle cell disease: The modern model

The history of sickle cell anaemia begins in 1910, when James Herrick reported peculiar elongated, sickle-shaped red blood corpuscles in an American Negro patient with severe anaemia. He attributed this phenomenon to a peculiar and unrecognised chemical and/or physical condition. In 1917 Emmel observed the transformation of the biconcave RBC to the sickle form in vitro. He also noted that the sickling phenomenon occurred both in persons who were severely anaemic and in persons who were apparently healthy, thus recognising the anaemia and the trait. A few years later, this anaemia became regarded as a hereditary disorder occurring only in Negroes. This had important racial effects in the USA during the 1920s. The Mendelian dominant theory meant that interracial marriages would probably spread the disease outward from the black population into whites. This view of sickle-cell anaemia was developed and promoted by physicians from 1910 through the 1940s in spite of the fact that the sickling test revealed cases amongst the white population. The gene for HbS occurs with varying fre-

quency in sub-Saharan Africa, the Mediterranean countries and India and in the descendents of people who emigrated from these regions.

From the introduction of the sickling test, sickle cell disease became less a clinical entity than a technological entity. It was a haematological test for a latent disorder in a person who appeared to be healthy. The power of haematological technology was the power to see disease before doctors, the patient or society could experience or observe it. The sickling test was used to endorse racial segregation, the fundamental biological separateness of black and white races. Cases of sickle-cell disease began to be found in some white patients and white families. Since they were especially common in immigrants of Mediterranean origin, they were regarded as a result of ancient admixtures with African blood. The test became a tool in the search for black ancestry and the detection of Negro blood. The occurrence of the disease in the white race was explained by many on the basis of the admixture of African blood in the family at some time in the distant past, and many pointed to Hannibal's invasion of Spain and Italy, or the Moorish occupation of southern Spain, or the slave trade, as the process which permitted the spread of black blood and transmitted the disease. Even in 1947, the Journal of the American Medical Association stated that sickle cell anaemia depended entirely on the presence of Negro blood, even in extremely small amounts. While race is a strong aetiological factor, haemoglobin studies at a molecular level, suggested that haemoglobin abnormalities were not confined to any single group of people.

In 1927, Hahn and Gillespie showed that sickling of the red cells was related to low oxygen tension. In 1948 Janet Watson, a paediatric haematologist from New York suggested that the paucity of sickle cells in the peripheral blood of neonates resulted from the presence of fetal haemoglobin in the red cells, which consequently did not have the abnormal sickle haemoglobin seen in adults. Using the new technique of protein electrophoresis, Pauling and Itano showed in 1948 that the haemoglobin from patients with sickle cell disease is different than that of normals. This made sickle cell disease the first disorder in which an abnormality in a protein was known to be at fault.

In 1956 Ingram and Hunt sequenced sickle haemoglobin and showed that a glutamic acid at position 6 was replaced by a valine in sickle cell disease. Using the known information about amino acids and the codons that coded for them, they were able to predict the mutation in sickle cell disease. This made sickle cell disease the first genetic disorder whose molecular basis was known. In 1984 bone marrow transplantation performed in a child with sickle cell disease produced the first reported cure of the disease. The transplantation was done to treat acute leukaemia. The child's sickle cell disease was cured as a side-event. The procedure nonetheless set the precedence for later transplantion efforts directed specifically at sickle cell disease. Hydroxyurea became the first and only drug with proven ability to prevent complications of sickle cell disease in a multicentre study completed in 1995. The discovery that sickle-cell disease protects against

malaria showed that this apparently inferior trait was a result of a beneficial adaptation against a dangerous disease, and explained its different geographical distribution.

Sickle cell disease provides an excellent model representing the scientific, diagnostic and therapeutic advances which are occurring in medicine. It is important that we scientists, biologists and physicians continue to pursue technological understanding of disease without allowing such technologies to structure our thoughts and our feelings?

Additional Reading

Ferrata A. (1918)
Le emopatie. Vol. 1 & 2. Società Editrice Libraria, Milano

Starr D. (2000)
Blood: an epic history of medicine and commerce. Quill

Tapper M. (1999)
In the blood: sickle cell anemia and the politics of race.
University of Pennsylvania Press

Wailoo K. (1999)
Drawing blood: Technology and disease identity in 20th Century America. The Johns Hopkins University Press

Wintrobe M.M. (1980)
Blood pure eloquent. McGraw Hill

Wintrobe M.M. (1985)
Haematology, the blossoming of a science: A story of inspiration and effort. Lea & Febiger