Da descoberta da circulação sanguínea aos primeiros factos hemorreológicos (2ª Parte)* [109]

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SUMÁRIO

Nesta segunda e última parte é recordada, em suas secções (A e B) a contribuição de mais duas personalidades geniais, respectivamente Marcello Malpighi e Antoine Van Leeuwenhoek, os quais, pela originalidade dos seus trabalhos, confirmaram as observações pioneiras de Harvey, completando-as ainda com factos relevantes. Malpighi aplicou o microscópio óptico, até então quase só utilizado como o curiosidade mundana, ao estudo in *vitro* de componentes dos tecidos corporais de várias espécies. Deste conjunto de observações pioneiras destaca-se a visualização, pela primeira vez, das estruturas capilares e dos glóbulos sanguíneos, de que resultaram a definição da composição do sangue e a conceptualização, inédita, sobre a respectiva fluidez. Adicionalmente, Malpighi confirmou a continuidade (estrutural e funcional) da circulação sistémica, que Harvey não tivera possibilidade de demonstrar. Por seu lado, Van Leeuwenhoek, apesar de desprovido de preparação académica soube ver, através de lentes por ele construídas, as particularidades microscópicas da natureza orgânica e inorgânica que o rodeavam. Entre inúmeras e pormenorizadas observações em todo o tipo de matéria merecem destaque as con-

ABSTRACT

From the discovery of the circulation of the blood to the first steps in hemorheology: Part 2

In the second and last part of this article, the contributions of two more brilliant figures are described in two sections, A and B, respectively Marcello Malpighi and Antonie van Leeuwenhoek. Through the originality of their work, both confirmed the pioneer William Harvey's observations and went on to add important details. Malpighi applied the optical microscope, until then used almost exclusively as a mere curiosity, to the in vitro study of the body tissues of various species. Outstanding among these pioneering observations was the visualization, for the first time, of capillary structures and blood corpuscles, on the basis of which he defined the composition of blood and, also for the first time, the concept of its fluidity. Furthermore, Malpighi confirmed the structural and functional continuity of the systemic circulation, which Harvey had been unable to demonstrate. Although van Leeuwenhoek lacked an academic education, he was able to see, through lenses he built himself, the microscopic details of the organic and inorganic world around him. Among numerous detailed

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clusões que obteve sobre a natureza do sangue e dos glóbulos vermelhos, a que atribuiu a coloração própria do sangue arterial e venoso. Calculou com rigor apreciável a dimensão dos eritrócitos. Soube distinguir o sangue arterial do venoso, confirmou a função cardíaca como origem do impulso propulsor do sangue pelas artérias e da ritmicidade do pulso e compreendeu o retorno do sangue até a coração por vasos diferentes, as veias. Visualizou a bifurcação das artérias em vasos com dimensões cada vez mais estreitas até formarem redes capilares que originavam, por seu lado, canais venosos sucessivamente mais alargados. Demonstrou a existência de anastomoses arterio-venosas. Verificou o fenómeno da sedimentação do sangue e a deformabilidade dos eritrocitos (que variava entre a forma original, discoide ou oval, observada nos troncos vasculares mais volumosos até às formas achatadas, adaptáveis ao diâmetro dos vasos mais estreitas). Neste aspecto distinguiu as características da circulação dos eritrócitos e as alterações subsequentes à coagulação sanguínea, sendo um introdutor perspicaz de conceitos hemorreológicos aplicados à medicina.

observations of all types of substances, particularly important were the insights that he obtained into the nature of blood and red blood cells, to which he attributed the color of arterial and venous blood. He calculated the size of erythrocytes with striking accuracy. He was able to distinguish arterial from venous blood, confirmed the heart's function as the force behind the propulsion of the blood through the arteries and of the rhythmic nature of the pulse, and elucidated the return of the blood to the heart by different vessels, the veins. He visualized the bifurcation of the arteries into increasingly narrow vessels until they gave way to capillary networks, which in turn became successively wider venous channels. Van Leeuwenhoek demonstrated the existence of arterial-venous anastomoses. He discovered erythrocyte sedimentation and deformability (their shape varying from the original discoid or oval form, observed in larger vascular segments, to flat forms, adapted to the diameter of narrower vessels). In the process he defined the characteristics of red cell circulation and the alterations that follow blood coagulation, and was thus a perceptive pioneer of the concepts of hemorheology applied to medicine.

A- O início da observação microscópica dos tecidos corporais por Malpighi

Marcello Malpighi (*Figura 1*) nasceu em 1628 perto de Bolonha, em cuja universidade foi admitido com 17 anos para estudar filosofia aristotélica. Após algum tempo de interrupção devido à morte dos pais, Malpighi regressou à universidade em 1649 para estudar medicina, tendo concluído o curso quatro anos mais tarde; três anos depois foi admitido como docente de prática médica. No ano seguinte, a convite do rei Ferdinando II da Toscânia, foi nomeado professor de medicina

A. Malpighi's microscopic observations of body tissues

Marcello Malpighi (*Figure 1*) was born near Bologna, where he entered the university at the age of 17 to study Aristotelian philosophy. His studies were interrupted due to the death of his parents, but he returned in 1649 to study medicine, finishing the course four years later. Three years later he was appointed a teacher in medical practice, and in the following year was invited by Ferdinand II of Tuscany to take up the professorship of theoretical medicine in Pisa, where he remained for three years before teórica em Pisa, onde permaneceu cerca de três anos, até regressar a Bolonha, em 1659. Dois anos depois foi convidado para professor de *primarius*, na cidade siciliana de Messina, donde voltou novamente para Bolonha ao fim de mais quatro anos, desta vez para ficar na sua *Alma Mater* nos vinte e cinco anos seguintes. No fim da vida aceitou o convite para médico particular do Papa Inocêncio, em Roma, onde viria a falecer três anos depois [(1)].

Parece que Malpighi se interessou pela microscopia ainda estudante. Esse interesse terá sido provavelmente incentivado, como se infere de um dos seus trabalhos, intitulado *Opera omnia* [(2)], pela repercussão popular das descobertas de Galileu com o telescópio e, depois, com os primeiros resultados que estavam a ser obtidos com um novo instrumento, o microscópio. Na senda daqueles desenvolvimentos começara a verificar-se, no início do século XVII, grande entusiasmo pelo microscópio entre a população das camadas sociais superiores da Europa, pelo qual qualquer pessoa poderia distinguir, com ampliações de pelo menos dez vezes, a dimensão real, pormenores materiais indetectáveis com a visão normal [(3)].

Naquele ambiente social, movido por motivações puramente académicas, Malpighi desenvolveu notável e plurifacetado trabalho de investigação, sendo autor de muitas descobertas, algumas das quais permanecem associadas ao seu nome. Pelo conjunto de observações inéditas realizadas, Malpighi é, há muito, considerado um precursor da Anatomia Microscópica, Histologia e, ainda, da Embriologia e da Botânica. Grande parte desses resultados foi baseada na vivissecção animal e na observação microscópica (*Fig 2*) dos respectivos tecidos, designadamente a pele, pulmões, fígado e rins de diversas espécies animais, e ainda também vegetais. No fundo, Malpighi pretendia interpretar os desígnios e a uniformidade de actuação biológica da Natureza através da identificação e comparação da estrutura e organização dos diversos

MARCELLO MALPIGHI.
From an engraving of the oil-painting by A. M. Tobar, presented to the Royal Society by Malpighi.

Figura 1- Marcello Malpighi (1628-1694), médico e anatomista italiano, foi o primeiro a utilizar sistematicamente o microscópio para completar os estudos anatómico e identificar a organização estrutural dos diversos tecidos, animais e vegetais.
Cortesia/ Courtesy: Wikimedia Commons

Figure 1. Marcello Malpighi (1628-1694), Italian physician and anatomist, was the first to use the microscope systematically for anatomical studies to determine the structure of animal and plant tissues.
Courtesy of Wikimedia Commons.

returning to Bologna in 1659. Two years later he accepted the position of *Professor Primarius* at Messina, Sicily, where he stayed for four years, and then returned to Bologna and his *alma mater* for the next twenty-five years. Towards the end of his life he was invited to be personal physician to Pope Innocent XII in Rome, where he died three years later [(1)].

Malpighi's interest in microscopy, which appears to have begun while he was still a student, was probably stimulated (as suggested in one of his works, titled *Opera omnia* [(2)]) by the widespread fame of Galileo's discoveries with the telescope, and later by the first findings from a new instrument, the microscope. In the

[a] Segunda de duas partes.
§ Professor Catedrático (aposentado) da Faculdade de Medicina da Universidade de Lisboa.
NB - A transcrição de parte dos textos originais citados foi adaptada à grafia actual.

Figura 2 - Microscópico Divini, semelhante a um dos instrumentos ópticos em que Malpighi realizou parte dos seus trabalhos. A perda de todo o equipamento no incêndio da residência de Malpighi impossibilita a reprodução de um microscópio próprio.
Cortesia/Courtesy: Dra Ariane Dröscher e G.Minelli: All'origine della biologia moderna. La vita di un testimone e protagonista: Marcello Malpighi nell'Università di Bologna. Milano: Editoriale Jaca Book, 1987, pp. 60 – 63).

Figure 2. A microscope made by Divini, similar to one used by Malpighi. All Malpighi's equipment was lost in a fire in his house, and so no images of his actual instruments exist.
Courtesy of Dr. Ariane Dröscher and Minelli, G: All'origine della biologia moderna. La vita di un testimone e protagonista: Marcello Malpighi nell'Università di Bologna. Milano: Editoriale Jaca Book, 1987, pp. 60-63.

tecidos e órgãos de diferentes seres vivos, inspirando-se na perspectiva mecanicista iniciada pela atomística de Demócrito, depois expandida pelos conceitos mecanicistas de Galileu. Malpighi também advogava que o esclarecimento dos mecanismos que constituem todas as coisas deveria abranger toda a Natureza e o estudo das estruturas mais pequenas (*minima ou atoma*). Estas seriam as suas "máquinas orgânicas", incluindo as que eram observadas somente ao microscópio ou ainda mais pequenas e ainda impossíveis de detectar, numa óbvia antecipação ao progresso registado nos séculos seguintes [2, 4].

As primeiras observações de capilares

Em Pisa, Malpighi lançou as bases do seu trabalho, que punha em causa o saber e os ensi-

wake of these developments, there was great enthusiasm in the early 17th century among the upper classes in Europe for the microscope, with which anyone could see details invisible to the naked eye, using magnifications of ten times or more [3].

Against this social background, but driven solely by the desire for knowledge, Malpighi carried out a remarkable program of diversified research that yielded many discoveries, some of which still bear his name. Due to the range of his ground-breaking observations, Malpighi has long been considered a pioneer of microscopic anatomy and histology, as well as embryology and botany. Many of his findings were based on vivisection of animals and microscopic observation (*Figure 2*) of their tissues, including the skin, lungs, liver and kidneys of various animal and plant species. Malpighi set out to interpret the designs of nature and to discern the similarities in biological activity by identifying and comparing the structure and organization of various tissues and organs from different organisms. He was inspired by the Greek philosopher Democritus' theory of atomism and the mechanistic concepts of Galileo. Malpighi also considered that the quest to understand the mechanisms behind things should be widened to cover all of nature, including the smallest structures (*minima or atoma*). These "organic machines", as he called them, included those that could only be seen with the microscope, or were even smaller – invisible in his day – clearly anticipating the advances of future centuries [2, 4].

The first observations of capillaries

In Pisa, Malpighi laid the foundations for his life's work, which called into question the then prevailing medical knowledge and teaching; during this period he first took an interest in the color of blood. However, it was after he returned to Bologna and then left for Messina that his first discoveries began to take shape. [5]. Observing the lungs of frogs through the microscope, Malpighi was able to determine the shape and arrangement of the alveoli at the end of the smallest branches of the bronnamentos médicos então prevalecentes. Data deste período o seu primeiro interesse pela coloração do sangue. Porém, foi no período entre o termo do seu primeiro regresso a Bolonha e a sua instalação em Messina, que os primeiros resultados de Malpighi começaram a tomar corpo (5). Utilizando como material de observação microscópica os pulmões de rã, Malpighi pôs em evidência a forma e disposição dos alvéolos na extremidade das ramificações brônquicas mais finas e demonstrou que o parênquima[a] pulmonar não era uma estrutura homogénea de sangue acumulado (6):

"*The substance of the lungs is commonly supposed to be fleshy because it owes its origin to the blood, and it is believed to be not unlike the liver or the spleen, and all agree that the lung in the foetus is red when nourished by the blood alone, and consequently that it operates as a fleshy viscus of warm and humid temper. With greater care the senses and the reason seem to show an opposite nature of the substance. By diligent investigation I have found the whole mass of the lungs, with the vessels going out of it attached, to be an aggregate of very light and very thin membranes, which, tense and sinuous, form an almost infinite number of orbicular vesicles and cavities, such as we see in the honey-comb alveoli of bees, formed of wax spread out into partitions. These (vesicles and cavities) have situation and connection as if there is an entrance into them from the trachea, directly from the one into the other; and at last they end in the containing membrane.* "

Quatro anos após a morte de Harvey, verificou (e descreveu no seu primeiro texto, *De Pulmonibus*, em 1661[b]) que o sangue não se misturava com o parênquima pulmonar (*Fig 3*), antes fluía em correntes muito finas e tortuosas que seguiam diversas direcções até se reunirem num trajecto final único, ao mesmo tempo que antecipava as trocas gasosas alvéolo-capilares (7):

chioles, and demonstrated that the pulmonary parenchyma[1] was not a homogeneous structure of accumulated blood (6):

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Four years after William Harvey's death, he confirmed in his first book, *De Pulmonibus*, in 1661[2], that the blood did not mix with the pulmonary parenchyma (*Figure 3*), but flowed in extremely small and winding streams that followed different routes before eventually uniting in a single vessel, and anticipated the discovery of alveolar-capillary gas exchange (7):

Between these membranes there run out very many very small vessels from the lobules, which enter into those (lobules) opposite. By these membranes air is received or ejected as in the larger sinuses which have mutual communication so that air can be passed from one to the other ....The structure of the lungs is such that it may be enabled to mix the blood more thoroughly. The branches of the vessels, even to the smallest, creep through the lung mass, so much so that when the included substances are thence sent on they are broken in the divisions and mingled at the divarications of the vessels as if by dashing together; at the same time they merge

[a] O termo parênquima derivou do Grego parenkhuma, por erradamente ser aceite desde a Antiga Grécia que a polpa de todos os órgãos sólidos, como o fígado e o baço, e também os pulmões, seria constituída por sangue escoado para o seu interior.
[b] O texto foi inicialmente redigido em duas cartas (Epistolae De Pulmonibus) que Malpighi endereçou ao seu antigo mentor filosófico e amigo de Pisa, o matemático e naturalista Giovanni Borelli, sendo posteriormente publicado como livro em Bolonha (1661), depois republicado em Leiden e em outros locais.

[1] The word "parenchyma" derives from the Greek *parenkhuma*, a pouring in, from the mistaken belief since classical times that the soft tissue of solid organs, such as the liver and spleen, as well as the lungs, was formed from blood draining into their interior.
[2] The text was originally written as two letters (*Epistolae De Pulmonibus*) addressed to Malpighi's former mentor in philosophy and friend at Pisa, the mathematician and naturalist Giovanni Borelli, and subsequently published as a book in Bologna in 1661, later being republished in Leiden and elsewhere.

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Na realidade, Malpighi estava essencialmente interessado em esclarecer a estrutura dos pulmões e não a dos capilares, acabando porém por os identificar [(8)]:

"Observation by means of the microscope will reveal more wonderful things than those viewed in regard to mere structure and connection: for while the heart is still beating the contrary (i.e., in opposite directions in the different vessels) movement of the blood is observed in the vessels,-though with difficulty,-so that the circulation of the blood is clearly exposed. This is more clearly recognized in the mesentery and in the other greater veins contained in the abdomen. Thus by this impulse the blood is driven in very small streams) through the arteries like a flood into the several cells, one or other branch clearly passing through or ending there…. Thus by this impulse the blood is driven in very small (streams) through the arteries like a flood into the several cells, one or other branch clearly passing through or ending there. Thus the blood, much divided, puts off its red color, and, carried round in a winding way, is poured out on all sides till at length it may reach the walls, the angles, and the absorbing branches of the veins."

Malpighi confirmou, em pulmões e ainda

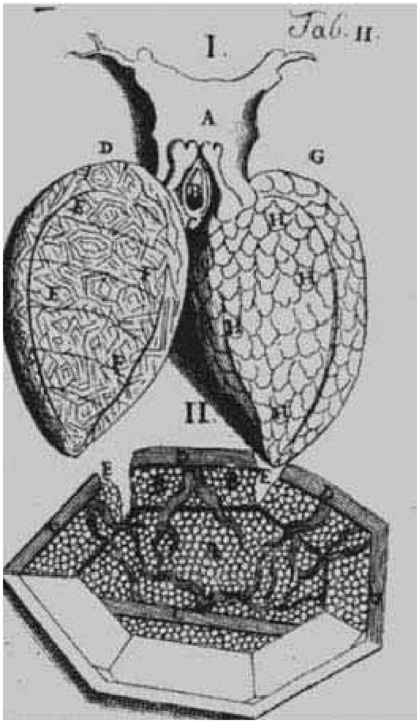

Figura 3 - Estrutura de pulmão. Reprodução em "De pulmonibus observationes anatomicae" (1661). A parte superior (I) põe em evidência a laringe (A) e o o pulmão de rã visto do exterior (à esquerda) e do interior em corte longitudinal (desenho da direita). Em F é assinalado o prolongamento da artéria pulmonar e em H o da veia pulmonar para os vértices. Em II observa-se a reprodução dos alvéolos e dos vasos sanguíneos. Posteriormente, Malpighi identificou a rede capilar que envolvia os alvéolos e a continuidade destes com as ramificações terminais traqueo -brônquicas. A demonstração de uma rede capilar entre as veias e as artérias confirmou o modelo da circulação que havia sido proposto por Harvey.
Cortesia/Courtesy: Wikimedia Commons.

Figure 3.Structure of the lung, as shown in Malpighi's De pulmonibus observationes anatomicae (1661). The upper part (I) shows the larynx (A) and lung of a frog, seen from the exterior (left) and in longitudinal section (right). The course of the pulmonary artery is indicated by F and that of the pulmonary vein towards the lung apex by H. The lower part (II) shows a representation of alveoli and blood vessels. Subsequently, Malpighi identified the capillary network surrounding the alveoli and the latter's continuity with the terminal branches of the tracheobronchial tree. His demonstration of the existence of a capillary network between veins and arteries confirmed the model of the circulation proposed by Harvey.
Courtesy of Wikimedia Commons.

[a] O termo parênquima derivou do Grego parenkhuma, por erradamente ser aceite desde a Antiga Grécia que a polpa de todos os órgãos sólidos, como o fígado e o baço, e também os pulmões, seria constituída por sangue escoado para o seu interior.
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em preparações de bexiga de rã, que aquelas correntes avermelhadas tinham formato semelhante ao de vasos sanguíneos, pelo que o sangue nunca extravasava [9]:

"…Also that, although in the lungs of perfect animals the vessels seem sometimes to gape and end in the midst of the network of rings, nevertheless, it is likely that, as in the cells of frogs and tortoise, that vessel prolonged further into small vessels in the form of a network and these escape the senses on account of their exquisite smallness. Also from these things can be solved with the greatest probability the question, of the mutual union and anastomosis of the vessels. For if Nature turns the blood about in vessels, and combines the ends of the vessels in a network, it is likely that in other cases an anastomosis joins them ; this is clearly recognized in the bladder of frogs swollen with urine, in which the above described motion of the blood is observed through the transparent vessels joined together by anastomosis…"

Implicitamente, Malpighi concluiu que o sangue das artérias passava para as veias através de uma rede de canalículos [10], consolidando assim o modelo da circulação definido por Harvey e, também as indicações anteriormente sugeridas por Leonardo da Vinci quanto à sua existência [11].

Natureza do sangue

Ainda em *De Pulmonibus*, Malpighi define claramente o que entende por sangue, classificando-o como uma substância que flui continuamente através das artérias e veias, que contém um "número infinito de partículas", e que é constituída por duas partes diferentes, uma esbranquiçada (a que chama soro) e outra vermelha [12]:

"By blood I do not understand the aggregate of the four common humours-both biles, blood and pituita, but all that which flows continuously through the veins and arteries, and which consists of an almost infinite number of particles. All these seem to be comprehended in two parts, alike in some degree to our unaided sense-that is to say-the whitish part, commonly called the serum, and the red".

De seguida, antecipando os conceitos de fluidez e viscosidade sanguíneas, que seriam definidos matematicamente quase três séculos depois, teoriza sob a fluidez da matéria em geral e a do sangue em particular, admitindo

better into one nature, the air being wedged in the vesicles, agitating in a measure those substances. These (vesicles) pressing the vessels on all sides, while they are emptied and filled successively, are able to mix the whole material by pressure continued through their alternations.

Malpighi was mainly interested in elucidating the structure of the lungs rather than identifying the capillaries, but that is in fact what he did [8]:

Observations by means of the microscope will reveal more wonderful things than those viewed in regard to mere structure and connection: for while the heart is still beating the contrary (i.e., in opposite directions in the different vessels) movement of the blood is observed in the vessels–though with difficulty–so that the circulation of the blood is clearly exposed. This is more clearly recognized in the mesentery and in the other great veins contained in the abdomen. Thus by this impulse the blood is driven in very small [streams] through the arteries like a flood into the several cells, one or other branch clearly passing through or ending there... sent down through the walls, at length runs into the area... Thus blood, much divided, puts off its red colour, and, carried round in a winding way is poured out on all sides till at length it may reach the walls, the angles, and the absorbing branches of the veins.

Studying the lungs of frogs and preparations of frog's bladders, Malpighi discovered that these red streams had a similar form to that of blood vessels, which was why blood never escaped from them [9]:

Also that, although in the lungs of perfect animals the vessels seem sometimes to gape and end in the midst of the network of rings, nevertheless, it is likely that, as in the cells of frogs and tortoise, that vessel is prolonged further into small vessels in the form of a network, and these escape the senses on account of their exquisite smallness. Also from these things can be solved with the greatest probability the question of the mutual union and anastomosis of the vessels. For if Nature turns the blood about in vessels, and combines the ends of the vessels in a network, it is likely that in other cases an anastomosis joins them; this is clearly recognized in the bladder of frogs swollen with urine, in which the above described motion of the blood is observed through the transparent vessels joined together by anastomosis...

Malpighi deduced that arterial blood passed into the veins through a network of que todos os corpos e matérias, mesmo os mais secos e aparentemente mais consistentes, são constituídos por partículas que podem agregar-se ou desagregar-se consoante for o meio (agente físico) utilizado [12]:

"…in Nature, there are bodies which were not originally endowed with fluidity but have their smallest parts ready for connection and union, so that, only with greatest force can they be separated, and, when separated, they endeavor to bring about mutual union. Again, these bodies, by admixture with another interposed body, become fluid."

Nessa perspectiva, o sangue poderia estar fluido ou seco, atingindo neste caso a dureza de uma pedra. Refere então a consistência dos coágulos, apresenta razões para o sangue coagular e explica como evitar a coagulação, mantendo-o em permanente agitação. Neste aspecto refere-se à circulação do sangue com um processo natural em que, na presença do componente esbranquiçado, a "parte vermelha" flui [7]:

"Nor is there a doubt that there are parts in the blood mass which are inclined to easy union, and may attain so much solidity that they rival a stone in hardness.

The evidence of this is in the red part of the blood, which, separated from the serum and dried so that it bears the nature of a stone, can be rubbed down into small pieces of definite form… These particles therefore, duly mixed, form a certain fluid, for the serum is first fluid by admixture of watery substance… From the serous or white part, the fluidity of the red part arises. We see this in the blood drawn from a divided vein. For, separated, the smallest red particles now mixed together, perhaps excited to movement by the warm particles issuing outside, are united to those like themselves, and the serous substance, pressed out all round on every side, is separated… All this is confirmed by common experience, for to prevent the blood issuing from the still living animal, from being divided into its parts by clotting, women are accustomed to crush it with the fingers or a rod and shake it up, i.e., in order that the thorough mixtures of the white and red be maintained. Therefore, in order that this mixing may succeed best, and that the smallest part of the white may fall between and touch the smallest part of the red, and the mass of the blood be renewed and made by steady mixing,…"

As invejas e controvérsias que aquelas descobertas suscitaram entre os restantes académicos terão motivado Malpighi a trocar

small channels [10], thereby confirming Harvey's model of the circulation, as well as Leonardo da Vinci's previous suggestions that such connections existed [11].

The nature of blood

In *De Pulmonibus* Malpighi also clearly defines what he understands blood to be, describing it as a substance flowing continually through the arteries and veins that contains an "almost infinite number of particles" and that is composed of two parts, one whitish (which he called "serum"), and the other red [12]:

By blood I do not understand the aggregate of the four common humours–both biles, blood and pituita, but all that which flows continuously through the veins and arteries, and which consists of an almost infinite number of particles. All these seem to be comprehended in two parts, alike in some degree to our unaided sense–that is to say–the whitish part, commonly called the serum, and the red.

He went on to anticipate the concepts of blood fluidity and viscosity, which were only expressed mathematically nearly three centuries later. He put forward theories concerning the fluidity of matter in general and of the blood in particular, asserting that all bodies and materials, even the driest and apparently firmest, are composed of particles that can aggregate or separate depending on the physical medium involved [12]:

...in Nature, there are bodies which were not originally endowed with fluidity but have their smallest parts ready for connection and union, so that, only with greatest force can they be separated, and, when separated, they endeavour to bring about mutual union. Again, these bodies, by admixture with another interposed body, become fluid.

In his view, blood could be fluid or dry, in the latter case becoming as hard as a rock. He notes the consistency of blood clots, presenting reasons for the blood to coagulate, and describes how to prevent blood from coagulating by constant mixing. In this context he refers to the circulation of the blood as a natural process in which the "red part" of the blood flows in the presence of the "white part" [7]:

Bolonha por Messina, onde prosseguiu as suas investigações e o ensino da medicina. Data deste período um conjunto de resultados importantes, tais como a identificação das papilas gustativas da língua humana, e a estrutura dos diferentes segmentos do sistema nervoso de insectos.

Evidência microscópica de eritrócitos e estruturas vasculares

Em *De polypo cordis* (publicado em 1666)[c], Malpighi referiu que o líquido hemático resultante da lavagem de coágulos sanguíneos retirados de um coração humano continha grande número de corpúsculos avermelhados (*atoma rubra*)[d], aos quais atribuiu a cor do sangue; além das observações microscópicas dos glóbulos, Malpighi descreveu a composição dos coágulos sanguíneos e a diferença entre os coágulos do coração direito ou esquerdo. Na secção de *De viscerum structura exercitatio anatomica* (*Fig 4*) que se refere ao rim (Renibus), Malpighi descreveu os capilares (que viriam a ser conhecidos por corpúsculos de Malpighi e, finalmente, por glomérulos renais) que identificou após injectar água corada numa artéria renal, os quais, pelo seu aspecto esférico, lhe pareciam maçãs pendentes das artérias interlobulares (*veluti poma*). O prestígio granjeado por aqueles trabalhos suscitou que fosse nomeado em 1669 para membro honorário da Royal Society de Londres, em cujo jornal publicou a maior parte da sua obra desde então. [13]

De regresso a Bolonha, prosseguiu o estudo microscópico de diversos órgãos, donde concluiu que a maior parte da matéria viva tinha organização glandular. À semelhança de Harvey, interessou-se pelo desenvolvimento embrionário do pinto, onde identificou, em 1673, o sector vascular que inclui o seio ter-

Nor is there a doubt that there are parts in the blood mass which are inclined to easy union, and may attain so much solidity that they rival a stone in hardness.

The evidence of this is in the red part of the blood, which, separated from the serum and dried so that it bears the nature of a stone, can be rubbed down into small pieces of definite form...These particles therefore, duly mixed, form a certain fluid, for the serum is first fluid by admixture of watery substance... From the serous or white part, the fluidity of the red part arises. We see this in the blood drawn from a divided vein. For, separated, the smallest red particles now mixed together, perhaps excited to movement by the warm particles issuing outside, are united to those like themselves, and the serous substance, pressed out all round on every side, is separated... All this is confirmed by common experience, for to prevent the blood issuing from the still living animal, from being divided into its parts by clotting, women are accustomed to crush it with the fingers or a rod and shake it up, i.e., in order that the thorough mixtures of the white and red be maintained. Therefore, in order that this mixing may succeed best, and that the smallest part of the white may fall between and touch the smallest part of the red, and the mass of the blood be renewed and made by steady mixing...

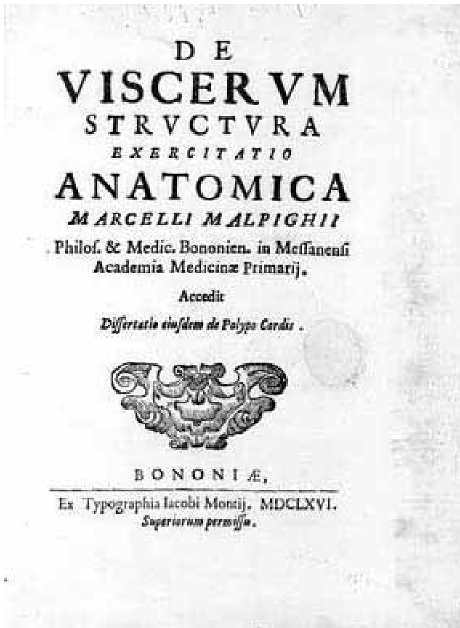
The controversy and envy aroused by these discoveries among his colleagues prompted Malpighi to leave Bologna for Messina, where he continued his research and medical teaching. During this period he made a number of important discoveries, including the identification of taste buds on the human tongue, and the structure of the different segments of the nervous system of insects.

Microscopic evidence of erythrocytes and vascular structures

In *De polypo cordis*, published in 1666[3], Malpighi reported that the liquid resulting from washing blood clots taken from a human heart contained a large number of red corpuscles (*atoma rubra*)[4], to which he attributed the color of blood. He also studied the composition of blood clots and the difference between clots from the right and the left heart. In the

[c] *De polypo cordis* é um cinco textos que constituem a obra mais importante de Malpighi, *De viscerum structura exercitário anatomica* (*Fig. 6*), cuja 1ª edição foi publicada em Bolonha, 1666 (ano do grande incêndio de Londres).
[d] A existência de eritrócitos no sangue de rá teria sido observada já em 1658, pelo biólogo e microscopista holandês Jan Swammerdam.

[3] *De polypo cordis* is one of the five texts that make up the most important of Malpighi's works, *De viscerum structura exercitatio anatomica (Figure 6)*, first published in Bologna in 1666, the year of the Great Fire of London.
[4] The existence of erythrocytes in frog's blood had been observed in 1658 by the Dutch biologist and microscopist Jan Swammerdam.


DE
VISCERVM
STRVCTVRA
EXERCITATIO
ANATOMICA
MARCELLI MALPIGHII
Philos. & Medic. Bononien. in Messanensi
Academia Medicinæ Primarij.
Accedit
Dissertatio eiusdem de Polypo Cordis.

BONONIÆ,
Ex Typographia Iacobi Montij. MDCLXVI.
Superiorum permissu.

Figura 4 - Capa da 1ª edição do mais importante tratado de Malpighi (De viscerum structura exercitatio anatomica).
Cortesia/Courtesy: Carl W. Gottschalk Collection, Rare Book Collection, University of North Carolina at Chapel Hill.

Figure 4. Cover of the first edition of Malpighi's most important work, De viscerum structura exercitatio anatomica.
Courtesy of Carl W. Gottschalk Collection, Rare Book Collection, University of North Carolina at Chapel Hill.

minal, o tubo cardíaco e o arco aórtico, além das pregas neurais e somitos.

Detractores e a *Risposta* póstuma de Malpighi

Na última década de vida somaram-se-lhe acontecimentos infaustos, pessoais e familiares. Além de uma saúde débil, Malpighi continuou a ter a oposição de um meio académico invejoso e reaccionário à ciência moderna; para cúmulo das desgraças a sua residência ardeu, causando-lhe a perda irreparável de aparelhos e microscópios, livros e demais documentação. Estes acontecimentos, no fim de quase quarenta anos de estudos microscópicos pioneiros do maior alcance e projecção científica e médica - mas que, para os seus contemporâneos não tinham valor por não contribuírem para melhorar a prática clínica da época - decerto apressaram a sua frustração e retirada, em 1691, para Roma. Em

section of *De viscerum structura exercitatio anatomica* (*Figure 4*) dealing with the kidneys (*Renibus*), Malpighi described capillaries, identifying them by injecting colored water into a renal artery, which because of their spherical appearance looked like apples (*veluti poma*) hanging from the interlobular arteries. These were initially called Malpighian corpuscles and are now known as renal glomeruli. As a result of the prestige that followed these discoveries he was made an honorary member of the Royal Society in London, which went on to publish most of his subsequent works [13].

On his return to Bologna, he continued to study various organs with the microscope, concluding that most living matter was organized in the form of glands. Like Harvey, he was interested in embryonic development in chicks, in which in 1673 he identified the vascular sector, including the terminal sinus, the cardiac tube and the aortic arch, as well as the neural folds and somites.

Malpighi's detractors and his posthumous *Risposta*

In the last decade of his life Malpighi suffered a series of personal misfortunes. He was in poor health; he faced the continuing hostility of envious and reactionary colleagues unwilling to accept modern science; and worst of all, his house burned down, destroying his microscopes and other equipment, books and other documents. These events, coming after almost forty years of pioneering microscopic studies of enormous range and immense scientific and medical importance that nevertheless were little appreciated by his contemporaries on the grounds that they did not lead to improvements in clinical practice, undoubtedly contributed to his frustration and to his decision in 1691 to move to Rome. In response to his detractors, Malpighi sent the Royal Society a series of comments to be published after his death. They appeared in 1697 under the title of *Opera posthuma*[5], and contained the following note, part of the *Risposta* (response) to Giovanni Sbaraglia, which faithfully reflects the dispute between different resposta aos seus detractores, Malpighi endereçou à Royal Society um conjunto de comentários a serem publicados após a sua morte. Foram-no em 1697, sob o título de *Opera posthuma*[e], em que a nota que a seguir se transcreve (incluída na Risposta a Giovanni Sbaragli) espelha fielmente a disputa entre diferentes concepções de ciência, de um lado a moderna e do outro, a dos cultivadores da medicina antiga[14]:

"I have not and will never lend faith to that vulgar concept whereby some men, because of a concatenation of causes unknown to us, are subjected and condemned to a perpetual molestation and vexation, not only in domestic affairs, but also in literary ones, which are the most important. Yet, I find that concept evidently proved and realized in me, since as soon as I became doctor, I started seeing sarcastic writings against the doctrine that I was privately professing with the due respect toward everyone. I then read, during the course of time, books printed against me with ignominious titles and full of jokes. I heard sarcastic public lectures, especially on anatomy. In the academies discourses have been presented against my works that were pure satires. We have seen ignominious [lunari] and almanacs, and publicly defended conclusions that were pure libels. Lately we have seen a circular letter against my studies titled De recentiorum medicorum studio dissertatio epistolaris ad amicum, in which the author disparages and attacks rational medicine, and tries to prove the uselessness of anatomy, embracing empirical medicine."

B- Leeuwenhoek e as primeiras observações microscópicas *in vivo*

As observações preliminares dos glóbulos vermelhos por Malpighi e "Jan Swammerdam" viriam a ser confirmadas e completadas pelo naturalista autodidacta holandês Antoni van Leeuwenhoek (*Fig 5*), também comerciante de tecidos, camareiro e agrimensor municipal, reconhecidamente um dos fundadores da microscopia e precursor da microbiologia, da parasitologia e da experimentação biológica [15,16].

No ramo de negócio de tecidos em que Leeuwenhoek trabalhava, era costume obser-

conceptions of science, the modern approach versus that of the followers of ancient medicine [14]:

I have not and will never lend faith to that vulgar concept whereby some men, because of a concatenation of causes unknown to us, are subjected and condemned to a perpetual molestation and vexation, not only in domestic affairs, but also in literary ones, which are the most important. Yet, I find that concept evidently proved and realized in me, since as soon as I became doctor, I started seeing sarcastic writings against the doctrine that I was privately professing with the due respect toward everyone. I then read, during the course of time, books printed against me with ignominious titles and full of jokes. I heard sarcastic public lectures, especially on anatomy. In the academies discourses have been presented against my works that were pure satires. We have seen ignominious *lunari* and almanacs, and publicly defended conclusions that were pure libels. Lately we have seen a circular letter against my studies titled *De recentiorum medicorum studio dissertatio epistolaris ad amicum*, in which the author disparages and attacks rational medicine, and tries to prove the uselessness of anatomy, embracing empirical medicine.

B. van Leeuwenhoek and the first *in vivo* microscopic observations

The first observations of red corpuscles by Malpighi and Swammerdam were subsequently corroborated and extended by the self-taught Dutch naturalist Antonie (or Antoine) van Leeuwenhoek (*Figure 5*). By profession a cloth merchant, chamberlain and municipal surveyor, van Leeuwenhoek is recognized as one of the founders of microscopy and a pioneer of microbiology, parasitology and experimental biology [15, 16].

In the commercial world of textiles, it was common practice to inspect textiles closely with small lenses similar to glass beads. Van Leeuwenhoek probably took one of these primitive lenses on his only trip to London, in 1668, in order to examine the cloth he was interested in purchasing. It was probably on

[e] Além de dois textos de *Risposta* (a dois dos seus mais acérrimos opositores, Sbaraglia e Lipari, que defendiam a medicina empírica e questionavam a utilidade clínica das investigações anatómicas e microscópicas), o volume *Opera posthuma* incluía uma extensa autobiorafia (*Vita a seipso scripta*) em que Malpighi reafirmava conceitos e defendia os resultados que obtivera durante uma vida dedicada à investigação.

[5] Besides the two sections entitled *Risposta* (addressed to two of his fiercest opponents, Sbaraglia and Lipari, who advocated empirical medicine and questioned the clinical value of anatomical and microscopic studies), *Opera posthuma* included an extensive autobiography (*Vita a seipso scripta*), in which Malpighi reaffirmed the theories and defended the findings that were the fruit of a lifetime of research.

Figura 5 - Antoni van Leeuwenhoek (1632-1723), pintura em tela de Jan Verkolje. Natural de Delft, Holanda, Leeuwenhoek foi um dos pioneiros da microscopia e precursor da Microbiologia. Embora não tivesse qualquer formação académica, foi homenageado pela Royal Society de Londres pelo mérito dos seus trabalhos, sendo admitido em 1680 na categoria de fellow, mediante proposta de Robert Hooke. Leeuwenhoek ficou muito sensibilizado e nunca esqueceu aquela distinção. Em reconhecimento, preparou um armário vistoso com prateleiras, onde dispôs 16 dos seus microscópios construídos em prata, encarregando a sua filha Maria de os enviar após a sua morte à Royal Society.
Cortesia/ Courtesy: "Museum Boerhaave, Leiden, The Netherlands"

Figure 5. Antonie van Leeuwenhoek (1632-1723), oil on canvas by Jan Verkolje. Born in Delft, The Netherlands, van Leeuwenhoek was a pioneer of microscopy and of microbiology. Although he had no academic training, he was honored for the excellence of his work by the Royal Society of London, who made him a Fellow in 1680 following nomination by Robert Hooke. Van Leeuwenhoek was deeply touched and never forgot this accolade, in acknowledgement of which he had a fine cabinet made on whose shelves he set out sixteen of his microscopes, made in silver, to be presented to the Society by his daughter Maria after his death.
Courtesy of Boerhaave Museum, Leiden, The Netherlands.

var em pormenor os têxteis através de pequenas "contas" de vidro. Na única vez em que foi a Londres, em1668, Leeuwenhoek terá levado uma dessas lentes primitivas para examinar melhor os tecidos que pretendia adquirir. Terá sido também nessa ocasião que Leeuwenhoek veio a conhecer um livro (*Micrographia*) de Robert Hooke (17,18), onde este reproduzira desenhos de diversos objectos ampliados (incluindo tecidos) que observara através de

the same journey that he came across a book by Robert Hooke[6], *Micrographia* (17, 18), which contains drawings of various materials (including textiles) enlarged through a microscope. Inspired by what he saw, van Leeuwenhoek set out to improve the quality of his lenses, using a grinding method that he kept secret, as well as reducing their diameter to as little as one or two millimeters, while keeping them sharply curved. Such small sizes made them extremely difficult to handle and focus, so van Leeuwenhoek fixed his lenses between two brass or silver plates held together by iron screws (*Figure 6*) (18). While Hooke and other microscopists were more concerned with magnifying objects and organisms that were large enough to see, van Leeuwenhoek, now in his forties, concentrated on observations of things invisible to the naked eye. He was thus the first to detect the existence of minute organisms (which he called *diercken* or "animalcules") moving in his water or biological samples. Besides revealing the microscopic details of all kinds of natural materials, including minerals, cells, hair, small insects, plants, drops of water and other liquids, he also attempted to study the explosion of gunpowder (19). He is estimated to have studied around 200 different species; to observe this "new world" he made over 400 microscopes and lenses over the course of his life.

On the urging of his compatriot Regnier de Graaf[7], an admirer of his studies who had introduced him to Henry Oldenburg, then Secretary of the Royal Society, van Leeuwenhoek sent Oldenburg his first manuscript, together with an explanatory letter in which he set forth his previous misgivings concerning his work's acceptance (20):

> 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 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 to stand blame or refutation from others. Pressed by Dr Reg. de Graaf I have thought better of my intention and given him a memorial of what I observed... which he (Mr de Graaf) has for-

um microscópio. Inspirado pelo que vira, Leeuwenhoek dedicou-se a melhorar a qualidade das suas lentes, através de um processo de polimento que manteve secreto, além de lhes reduzir o diâmetro até 1 a 2 milímetros, ainda que mantendo uma curvatura acentuada. Tão reduzidas dimensões criavam grandes dificuldades de manuseamento e focagem, pelo Leeuwenhoek decidiu fixar as lentes entre duas placas idênticas de latão ou prata (*Fig 6*), completando o instrumento com parafusos em ferro [18.]. Enquanto Hooke e outros microscopistas se mantinham interessados em revelar ampliações de objectos e pequenos seres já visíveis, Leeuwenhoek, já com cerca dos 40 anos de idade, começou a interessar-se pelas observações microscópicas do que não se via; foi, por isso, o primeiro a constatar e a divulgar a existência de pequenos seres vivos minúsculos (a que chamou, no seu idioma, "dierccken", animaizinhos) que se movimentavam em amostras líquidas e biológicas, além de ter revelado os pormenores microscópicos de toda a espécie de matéria constituinte da Natureza (tais como minerais, células, cabelos pequenos insectos, plantas e gotas de água e outros líquidos) e, inclusivamente, também tentou observar a explosão de pólvora [19]. Estima-se que as suas observações abrangeram amostras de cerca de 200 espécies biológicas. Esse "novo mundo"

warded to you and informed me of your reply. I see from this that my observations were not unwelcome to the Royal Society.

Following this favorable reception, van Leeuwenhoek began a lifelong correspondence with the Royal Society that continued for nearly 50 years, from 1673 until his death in 1723. The results of his observations, and later his experiments (written in Dutch and then translated into Latin), were described in detail in letters, written in a colloquial and at times repetitive style. Most (around 200) were addressed to the Secretary of the Royal Society, which published them – some in full, some as extracts, translated into English – in its journal, the *Philosophical Transactions* [16, 21]. In another letter to the Society, written in 1685 but only delivered, by his daughter, in 1710, owing to difficulties in communication, he paid homage to his compatriot and friend Johannes (or Jan) Vermeer, the painter. In this letter Van Leeuwenhoek recalls his first observations with magnifying lenses and his intention to fix them in a frame to make it easier to examine specimens; he also remembers the shared enthusiasm and encouragement he received from Vermeer to communicate the "secret universe" he had discovered. One day, when he was bemoaning his inability to describe in words what he could see through his microscope, because it sounded too

[f] Robert Hooke (1635-1703), natural de uma aldeia próxima de Isle de Wright, Inglaterra, foi experimentador de ciência, inventor de novos instrumentos, auto-proclamado fundador da meteorologia. Trabalhou como assistente de Robert Boyle, tendo construído a primeira bomba de ar feita em Inglaterra, que permitiria aquele descobrir a lei física conhecida pelo seu nome. Em 1662 foi nomeado Curador da Royal Society e, de 1677 a 1682, seu Secretário. Seria popularizado como microscopista após publicar em 1665 o tratado "Micrographia", onde introduziu o termo "célula" com o sentido biológico actual, e cujas imagens (que permaneceram famosas nos séculos seguintes) haviam sido obtidas através de um microscópio composto por si construído. O tratado incluía ainda um conjunto de outras preciosidades, tais como a descrição do barómetro de roda, um higrómetro, a definição do ponto de congelação como o nível zero na escala dos termóemetros, discussão sobre estrutura dos cristais (com modelos esféricos), a definição de calor, expansão térmica e combustão, e ainda considerações sobre Astronomia. Demonstrou que o stress é proporcional à tensão (lei de Hooke), afirmou que fósseis eram um registo de épocas passadas (inédito para uma época, em que aqueles vestígios geológicos eram interpretados de modo fantasista) Recebeu o título de médico honorário.

[6] Robert (1635-1703), was born on the Isle of Wight, England. He was an experimental scientist, an inventor of new instruments, and the self-proclaimed founder of meteorology. Working as assistant to Robert Boyle, he built the first air pump made in England, with which Boyle, discovered the physical law that bears his name. In 1662 he was appointed Curator of the Royal Society from 1677 to 1682. He became famous as a microscopist after the publication in 1665 of his treatise titled *Micrographia*, in which he was the first to use the term "cell" in its modern biological sense; the images in the book, which remained famous for centuries, were obtained using a compound microscope he built himself. *Micrographia* also contained many other remarkable features including a description of a wheel barometer and a hygrometer, the definition of the zero on the thermometer scale as freezing point, discussions on the structure of crystals using spherical models, definitions of heat, thermal expansion and combustion, and thoughts on astronomy. He showed that stress is proportional to strain (Hooke's law), and argued that fossils are a record of past ages, an unprecedented claim in an age when fossils were interpreted in a whimsical rather than scientific manner. He was awarded the title of honrary physician.

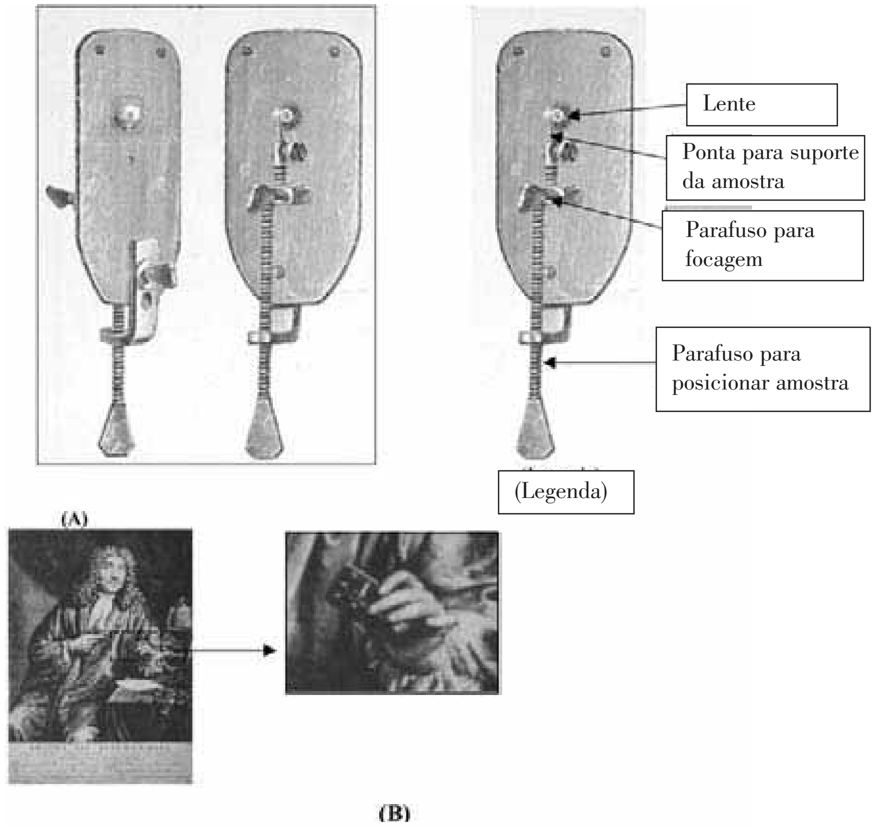


Figura 6 (A) - Reprodução de um dos microscópios feitos por Leeuwenhoek, observado de frente e reverso. O instrumento mede em comprimento cerca de 7 a 9 centímetros (as dimensões relativas são bem visíveis em B). É constituído por duas placas metálicas perfuradas na mesma altura, entre as quais está fixada uma lente com diâmetro muito reduzido (alguns milímetros) na zona do orifício. Três parafusos interligados permitem fixar a amostra, focar e, o mais comprido, para segurar o instrumento e para subir, descer ou rodar a amostra. Sendo a distância focal de cerca de 1 mm, a lente tinha de se posicionar junto ao olho do observador, além de requerer boas condições especiais de iluminação. Este microscópio (que, na realidade, é uma lente biconvexa de aumentar) possibilitava ampliar objectos até cerca de um terço dos microscópios ópticos modernos. Numa das suas cartas, Leeuwenhoek indicou a melhor forma de observar a amostra: colocava-se num aposento em relativa penumbra onde entrava um feixe de luz do dia, para o qual orientava a amostra a examinar. Além dos 26 microscópios oferecidos à Royal Society após a sua morte, foram inventariados no seu espólio mais 247 instrumentos completos (a maior parte dos quais ainda conservava amostras dispostas para observação) e 172 lentes montadas entre placas, o que perfaz um total de 445 instrumentos.
Cortesia/Courtesy: 'John Innes Archives courtesy of the John Innes Foundation'.

(B) Um dos textos publicados por Leeuwenhoek em holandês (HYPERLINK "http://www.gutenberg.org/etext/18929" Den Waaragtigen Omloop des Bloeds) incluiu um retrato semelhante ao do quadro da Fig 6, que o representa a segurar um dos seus microscópios e cuja legenda anota a vinculação à Royal Society.

Figure 6. (A) A microscope made by van Leeuwenhoek, seen from front and back. The instrument is around 7-9 cm in length (its overall size is clearly visible in B). It consists of two metal plates, perforated at the same point, between which a very small lens (a few millimeters in diameter) is fixed in the orifice. Three interconnected screws are used to fix the specimen and to focus; the longest screw is used to hold the instrument and to raise, lower or rotate the specimen. As the focal length was around one millimeter, the lens had to be positioned very near the observer's eye, as well as requiring special lighting. This microscope, which is based on a biconvex magnifying lens, was able to enlarge objects to around a third of the amplification of modern optical microscopes. In one of his letters, van Leeuwenhoek explained that the best way to observe the specimen was in a relatively dark room in which a single ray of sunlight entered, and to point the microscope at it.
Besides the 26 microscopes he left to the Royal Society, another 247 complete instruments were found after his death, most of them still containing specimens prepared for observation, and 172 lenses mounted between plates, making a total of 445 instruments.
John Innes Archives, courtesy of the John Innes Foundation.

(B) A book published by van Leeuwenhoek in Dutch (Den Waaragtigen Omloop des Bloeds) contained a portrait similar to that in Figure 5, showing him holding one of his microscopes (detail). The caption refers to his links with the Royal Society.
Courtesy of Project Gutenberg (http://www.gutenberg.org/etext/18929)

foi persistentemente observado por Leeuwenhoek através de mais de quatro centenas de microscópios e lentes que construiu ao longo da vida.

Persuadido por Regnier de Graaf [g], conterrâneo e admirador dos seus estudos, que previamente o havia apresentado a Henry Oldenburg (então secretário da Royal Society), Leeuwenhoek enviou a este, em 1673, o seu primeiro manuscrito, junto com uma carta de reconhecimento em que justificava anteriores dúvidas quanto à aceitação do trabalho [20]:

> *"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 to express my thoughts properly, secondly because I have not been brought up in languages or arts, but in trade, and thirdly because I have not feel to stand blame or refutation from others. Pressed by Dr Reg. de Graaf I have thought better my intention and given him a memorial of what I observed …. He (Mr de Graaf) has forward to you and informed me of your reply. I see from this that my observations were not unwelcome to the Royal Society."*

No seguimento daquele favorável acolhimento, Leeuwenhoek prosseguiu uma assídua correspondência com a Royal Society, que se manteve durante quase 50 anos de actividade (de 1673 a 1723, ano em que morreu). Os resultados das observações, no início, e, depois, também experiências (redigidos em holandês, depois traduzido para latim) eram pormenorizadamente descritos em cartas (de estilo coloquial, por vezes repetitivo) a maior parte das quais (cerca de duzentas) foi endereçada ao secretário da Royal Society de Londres, que as publicava (completas ou extractos, traduzidos em inglês) nas *Philosophical Transactions* daquela sociedade [16,21]. Leeuwenhoek redigiu em 1685 uma outra carta (remetida pela filha à Royal Society somente em 1710, devido a dificuldades de comunicação)[h] onde homenageava postumamente o seu conterrâneo e amigo Johannes Vermeer, afamado pintor. Leeuwenhoek recorda, a propósito das suas primeiras observações com lentes de aumentar (e a "phantasmagorical" for the members of the Royal Society, the artist suggested that he should draw what he saw, teaching him and helping him with his first drawings. Some years later, van Leeuwenhoek decided to employ a draftsman to ensure that his observations were faithfully reproduced and could be identified by others [22].

As well as his observations, van Leeuwenhoek felt free to add his own interpretations and theories on a great variety of subjects, albeit at times over-imaginative [16]. This less favorable aspect of his contribution (although hardly surprising considering the reports presented to the Royal Society at this time by other members who did have an academic training) does not diminish the extraordinary quality and originality of his work. Analysis of the meticulously researched descriptions in his publications, and of the painstaking effort needed to spend hours on end observing details magnified hundreds of times through a tiny lens, leaves one in awe of the enormous enthusiasm and dedication with which van Leeuwenhoek carried out his studies.

Van Leeuwenhoek probably built his first microscope in 1673 and continued thereafter to produce successively better models, many specifically designed for what he wished to observe [18]. He set out in particular to identify unknown characteristics of living things, which he showed consisted of 80% water plus a variety of minuscule components; he also discovered, described and drew many single-celled organisms and, indeed, cells, particularly red blood cells, spermatozoa, muscle fibers (showing their striated patterns in detail), tendons, bone, bacteria and protozoans.

On September 7 1674 he sent the Secretary of the Royal Society a report of his first observations of microorganisms, probably protozoans, in water samples from a canal in Delft. In this letter, which has been hailed as the birth of microbiology [19], van Leeuwenhoek expresses his amazement and delight at these tiny animals and algae, whose existence he had never suspected [23]:

intenção de as fixar numa moldura para examinar melhor as espécimes), assim como o entusiasmo partilhado e o incentivo que teve por parte de Vermeer para divulgar "o universo desconhecido que descobrira". Num dia em que se lamentou a Vermeer da incapacidade que sentia em descrever por palavras o que observava ao microscópio, "porque pareceria demasiado fantasmagórico aos membros da Royal Society", aquele sugeriu-lhe que desenhasse o que via, ensinando-o e ajudando-o nos seus primeiros desenhos. Alguns anos mais tarde, Leeuwenhoek decidiu-se a contratar um ilustrador para que as suas observações fossem fielmente reproduzidas e identificadas por outros. [22]

Leeuwenhoek não hesitava em incluir, juntamente com os factos observados, também as suas interpretações ou extrapolações, por vezes demasiado imaginosas, sobre os mais variados assuntos [16]. Esta faceta menos favorável (mas que não surpreende, no contexto dos relatos apresentados na época à Royal Society por outros *fellows* com formação académica), não faz esquecer, porém, o grande mérito e originalidade das suas pesquisas e resultados. Cotejando a minúcia das descrições publicadas e tendo em conta o grande esforço que lhe terá sido necessário para durante horas seguidas visualizar pormenores, ampliados centenas de vezes, através de uma pequeníssima lente, fica-se com a certeza do grande entusiasmo e dedicação com que Leeuwenhoek desenvolveu os seus estudos.

Leeuwenhoek terá construído o seu

About two leagues from this town [Delft] there lies an Inland-Sea, called Berkelse-Lake ...This water is in Winter very clear, but about beginning or midst of Summer it grows whitish and there are then small green clouds permeating it... Passing lately over this Sea at a time, when it blew a fresh gale of wind, and observing the water as above described, I took some of it in a glass-vessel...I found moving in it several earthy particles, and some green streaks, spirally ranged, after the manner of the copper or tin-worms, used by distillers to cool their distilled waters; and the whole compass of each of these streaks was about the thickness of a man's hair on his head. Other particles had but the beginning of the said streak, all consisting of small green globules interspersed; among all which there crawled abundance of little animals, some of which were roundish; those that were somewhat bigger than others were of oval figure. Of these latter I saw two legs 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 in number. These animals had diverse colours, some being whitish, others pellucid; others again were green in the middle, and before and behind white, others greyish. 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 cheese, wheat flour, mould, and the like.

In 1693 he described five different types of bacteria from his own mouth [24], and continued to identify new microorganisms until 1716. Between 1680 and 1701 he spent much time observing the microscopic structure of insects by means of microdissection [25].

Van Leeuwenhoek's observation of red corpuscles in various animal species

In another of his early observations, to his surprise he found in a drop of his own blood

[g] Reginer de Graaf (1641-1673), anatomista e médico holandês, contribuiu com descobertas relevantes sobre o aparelho genital, em especial o feminino, e a biologia da reprodução. O seu nome ficou associado aos folículos do ovário (de Graaf), cujo desenvolvimento descreveu.

[h] Entre 1685 e 1693 e, num segundo período, de 1712-1720, não houve troca de correspondência ou as cartas de Leeuwenhoek não foram publicadas no jornal da Royal Society. Essa interrupção coincide com e mandato de Edmond Halley nos cargos de secretário e, depois, editor de *Philosophical Transactions*. Perante esta inaceitável prepotência Leeuwenhoek decidiu dar a conhecer os seus resultados a outras individualidades públicas e científicas, publicando-os em holandês ou latim.

[7] Regnier (or Reinier) de Graaf (1641-1673), a Dutch anatomist and physician, made important discoveries about the genital apparatus, particulary in females, and reproductive biology. He described the development of the ovarian (or Graafian) follicles that are named after him.

[8] Between 1685 and 1693 and again from 1712 to 1720, the correspondence between van Leeuwenhoek and the Royal Society ceased or his letters were not published in the *Philosophical Transactions*. This hiatus coincided with the period during which Edmond Halley was the Society's Secretary and then editor of the *Transactions*. In the face of this opposition, van Leeuwenhoek decided to publish his findings elsewhere, in Dutch or Latin.

primeiro microscópio em 1673, ao que se seguiram sucessivos e aperfeiçoados modelos, aparentemente adaptados ao que pretendia observar[18]. Leeuwenhoek procurava, em particular, identificar as características desconhecidas da matéria viva, que demonstrou ser constituída em cerca de 80% por água e o restante por elementos diversificados e minúsculos; também descobriu, descreveu e reproduziu em desenho alguns seres ou elementos unicelulares, com destaque para os eritrócitos humanos, espermatozóides, fibras musculares (pormenorizando-lhes o padrão estriado), tendões, osso, bactérias e protozoários

As primeiras observações de microrganismos, provavelmente protozoários existentes em amostras de água de um canal de Delft, foram enviadas em 7 de Setembro de1674 ao secretário da Royal Society. Nessa carta, referida como o da "fundação" da microbiologia[19], Leeuwenhoek não esconde a surpresa e encanto pelos minúsculos seres e algas que não supusera existirem[23]:

"About to leagues from this town (Delft) there lyes an Inland-Sea, called Berkelse-Sea …This water is in Winter very clear, but about beginning or midst of Summer it grows whitish and there are then small green clouds permeating it… Passing lately over this Sea at a time, when it blew a fresh gale of wind, and observing the water as above. described, I took some of it in a glass-vessel, which …I found moving in it several earthy particles, and some green streaks, spirally ranged, after the manner of the cooper or tin-worms, used by distillers to cool their distilled waters; and the whole compass of each of these streaks was about the thickness of a man's- hair of his head. Other particles had but the beginning of the said streak, all consisting of small green globules interspersed; among all which there crawled abundance of little animals, some of which were roundish; those that were somewhat bigger than others were of oval figure. Of these latter I saw two legs 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 in number. These animals had diverse colours, some being whitish, others pellucid; 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 wonder at it. I judge, that some of these little creatures were above a thousand times smaller than the smallest ones, which I

small round globules (*Figure 7*), circulating in suspension[26]:

I have divers times endeavoured to see and to know, what parts the Blood consists of, and at length I have observed taking some blood of my own hand, that it consists of small round globules driven through a crystalline humidity or water.

Professor Brian Ford has demonstrated that, using one of van Leeuwenhoek's original lenses, it is possible to see clearly and in detail the morphology of erythrocytes and leukocytes, thereby confirming these observations[18].

In the same year (1674), he sent a letter presenting further observations of these "globules" and the plasma, which he called the "crystalline liquor", and noting two interesting phenomena: erythrocyte sedimentation and aggregation, which occur slowly after the blood is extracted, and the darkening of extracted blood resulting from deoxygenation and coagulation[27]:

The small red globules in the blood...are heavier than the crystalline liquor in which they are carried, because soon after that the blood is let out of the veins, those globules by little and little subside towards the bottom; and being made of soft fluid corpuscles, and many lying upon one another, they do unite themselves close together, and by close conjunction the blood that is under the surface alters its colour, and becomes darkred or blackish....

In the same document, van Leeuwenhoek refers to his discoveries concerning the size, changes in color, and movement of red blood cells in suspension, which he observed by passing blood through extremely narrow glass tubes that he made for the purpose, and the enhanced effects when a length of the tubing was heated. The following year he presented what must be the first hemorheological observation applicable to clinical practice, when he reported that his own blood cells appeared more rigid when he was sick, and more flexible when he recovered his health. Demonstrating remarkable insight, not only for his time but also for someone who had no medical or other academic training, he went *have hitherto seen in cheese, wheaten flower. mould, and the like."*

Em 1693 descreveu cinco tipos diferentes de bactérias colhidas da própria boca [24], tendo continuado a identificar novos microrganismos até cerca de 1716. Entre 1680 e 1701 aprofundou a observação da constituição microscópica de insectos, recorrendo à microdissecção [25].

Visualização de glóbulos vermelhos em diversas espécies animais

Em outra das suas observações iniciais, viu, com surpresa, que numa gota do seu próprio sangue também existiam pequenos corpúsculos circulares de cor vermelha que rodopiavam na suspensão [26]:

"I have divers times endeavoured to see and to know, what parts of the Blood consists of, and at length I have observed taking some blood of my own hand, that it consists of small round globules driven through a crystalline humidity or water."

O Professor Brian Ford demonstrou recentemente que, utilizando uma das lentes originais construídas por Leeuwenhoek, conseguia observar bastante bem os pormenores morfológicos de eritrócitos e leucócitos (*Fig 7*), confirmando as observações citadas [18].

Numa carta enviada no mesmo ano (1674), apresenta observações mais completas sobre os glóbulos e o plasma (que designou por "crystaline liquor"), anotando ainda dois factos interessantes, o da sedimentação e agregação globulares (que decorriam lentamente após a extracção do sangue, a par com o escurecimento do sangue (decerto resultante da desoxigenação e eventual coagulação) [27]:

"The small red globules in the blood… are heavier than the crystalline liquor in which they are carried, because soon after that the blood is let out of the veins, those globules by little and little subside towards the bottom; and being made of soft fluid corpuscles, and many lying upon one another, they do not unite themselves close together, and by close conjunction of the blood that is under the surface alters its colour, and becomes dark-red or blackish…."

on to hypothesize that the deformability of these cells was essential for the blood to circulate in the capillaries under normal conditions [28]:

And on this occasion I very well remember, that, about two years ago, I divers times observed my own blood, and noted, that those sanguineous globules that make blood red, seemed then to be firmer and harder than they are in my blood now; at which time my body was very indisposed, so that I fell into a sickness, which held near three weeks: but now I find those globules of my blood softer, and more sticking to one another, and my body in a good state of health. I know not, whether sickness, and even death itself, may not sometimes proceed from the hardness of those globules. I am apt to imagine, that those sanguineous globules in a healthy body must be very flexible and pliant, if they shall pass through the small capillary veins and arteries, and that in their passage they change into an oval figure, reassuming their roundness when they come into a larger room.

Similarly, some years later van Leeuwenhoek noted the relationship between the shape of red blood cells in tadpoles, and the velocity of blood flow, and the animal's functional status [29]:

...these particles [of blood], though in this creature they were of a flat and oval shape, yet sometimes by reason of the smallness of the artery, assumed a kind of oblong round figure, and when the animal, by being taken out of the water, grew languid, the blood in these very minute arteries began to stagnate, and when it again acquired motion, many of the globules appeared twice as long as broad, and also pointed at their extremities.

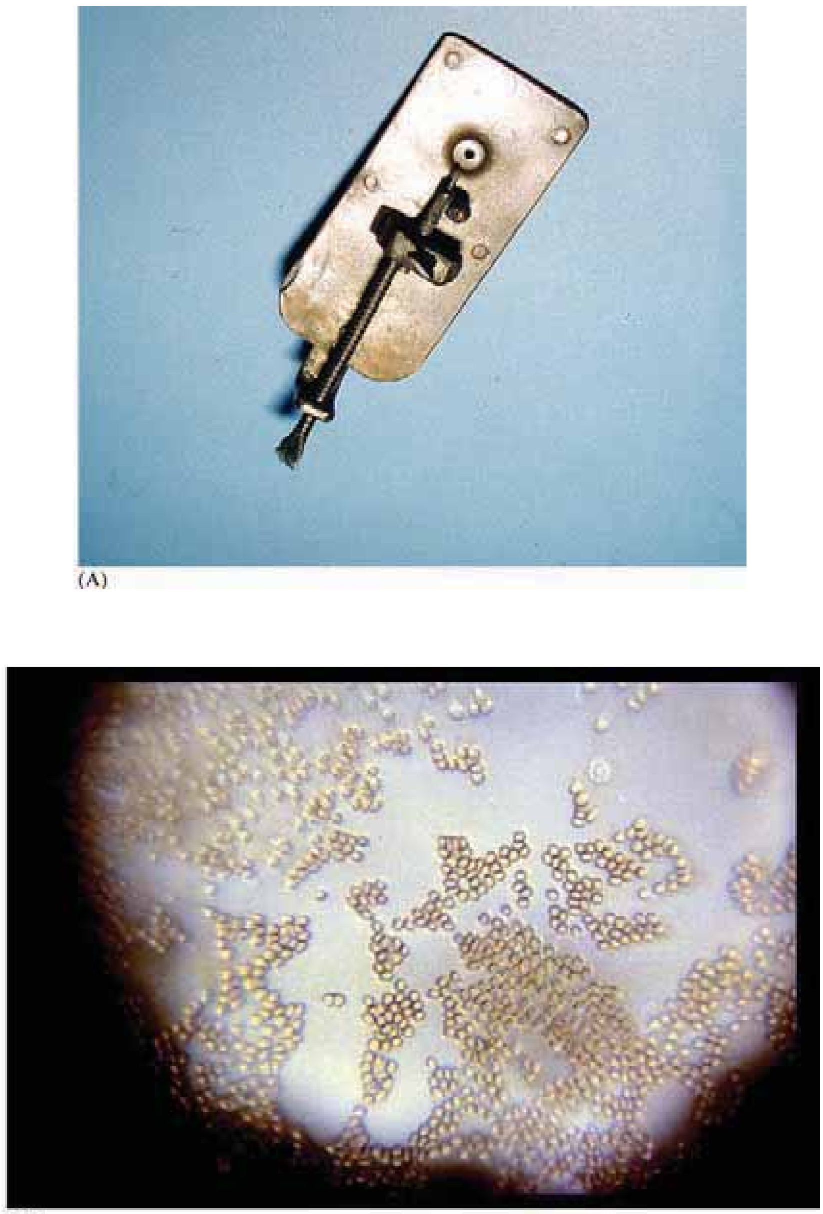
His descriptions of erythrocytes are more precise than those of Malpighi and Swammerdam; he arrived at a figure remarkably close to 8.5 μ for the size of these "globules", using (as in all his observations) a grain of sand seen through the microscope for comparison [30]:

The blood is composed of exceeding small particles, named, globules, which, in most animals, are of a red colour, swimming in a liquor, called, by physicians, the serum; and by means of these globules the motion of the blood becomes visible, which otherwise would not be discoverable by the sight. These particles, or globules, are so minute, that one hundred of them, placed side by side, would not equal the diameter of a common grain of

No mesmo documento, Leeuwenhoek refere-se aos resultados que obteve, designadamente, as dimensões, alterações de cor e o movimento dos glóbulos em suspensão (que se

sand; consequently, a grain of sand is above a million times the size of one such globule.

In a 1683 communication to the Royal Society, van Leeuwenhoek noted that each



(A) (B)

Figura 7 - (A) Exemplar de microscópio de Leeuwenhoek, construído em latão e com uma lente esférica que amplia cerca de 300 vezes. Em (B) é apresentada uma fotografia de eritrócitos obtida pelo Professor Brian J Ford com aquele instrumento. É de notar, no quadrante superior direito, o pormenor em que se observa um granulócito neutrófilo.

Figure 7. (A) One of van Leeuwenhoek's microscopes, made of brass, with a spherical lens with 300x magnification. (B) Photograph of erythrocytes taken by Professor Brian J. Ford with this microscope. Note the detail of the neutrophil granulocyte at upper right.
Courtesy of Professor Brian J Ford (http://www.brianjford.com/wavrbcs.htm).

acentuavam quando aplicava calor a um segmento do tubo) ao fazer passar o sangue através de tubos de vidro muito estreitos que construíra para o efeito. No ano seguinte apresentou a que terá sido a primeira observação hemorreológica aplicada à clínica, ao revelar como os seus eritrócitos pareciam mais rígidos quando estivera doente, e mais flexíveis após recuperar a saúde. Indo mais longe (demonstrando notável perspicácia, não só para os conhecimentos da época mas, sobretudo, por parte de quem não era médico e não possuía outra formação académica) acrescentou que a deformabilidade globular era essencial para que circulação do sangue pudesse ocorrer nos capilares em condições normais [28]:

" And on this occasion I very well remember, that, about two years ago, I divers times observed my own blood, and noted, hat those sanguineous globules that make blood red, seemed then to be firmer and harder that they are in my blood now; at which time my body was very indisposed, so that I fell into a sickness, which held near three weeks: but now I find those globules of my blood softer, and more sticking to one another, and my body in a good state of health. I know not, whether sickness, and even death itself, may not sometimes proceed from the hardness of those globules. I am apt to image, that those sanguineous globules in a healthy body must be very flexible and pliant, if they shall pass through the small capillary veins and arteries, and that in their passage they change into an oval figure, reassuming their roundness when they come into a larger room".

Alguns anos depois Leeuwenhoek viria, de modo semelhante, a relacionar a forma dos glóbulos de girino e a rapidez de movimentação do sangue com o estado funcional do animal [29]:

"…these particles (of blood), though in this creature they were of a flat and oval shape, yet sometimes by reason of the smallness of the artery, assumed a kind of oblong round figure, and when the animal, by being taken out of the water, grew languid, the blood in these very minute arteries began to stagnate, and when it again acquired motion, many of the globules appeared twice as long as broad, and also pointed at their extremities."

A descrição dos eritrócitos é mais precisa do que as registadas por Malpighi e de Swammerdam, obtendo com notável aproximação ao

frog erythrocyte appeared to contain small bubbles surrounded by a halo; this was subsequently identified as the cell's nucleus [31]. He found that the erythrocytes of fish, frogs, birds and some other animals were oval in shape, and that they gave the blood its red color. It is also noteworthy that he distinguished between the erythrocytes of terrestrial mammals (spherical) and of birds and fish (oval) [32]:

...the matter causing the redness of our blood was constituted of globules; I examined the blood of oxen, sheep and rabbits; and observed no difference in magnitude, between the globules of those animals, and those of men: so that, I conceived that the matter which in general made all blood red, was globules. But, after I had tried the blood of a salmon, a cod, of frogs [etc] and found that the matter, which caused the redness therein was made of parts oval, and flattish (as I had before said) I examined the blood of several birds; and have also observed, that the matter causing the redness of their blood, was also composed of like oval flattish parts, with those of fishes: so that I now concluded, that all animals, whether birds, fish, or other creatures that live in the water, have the parts causing the redness of their blood, consisting of the said oval flattish parts, and if hereafter I chance to find the contrary, I will advise you thereof.

He stated repeatedly that erythrocytes could be discoid, oval or flattened in shape, but occasionally returned to the idea that they were discoid (*Figure 8*). Some appeared to him to be thicker and darker around their edges [33], which fits the biconcave discoid model that is currently accepted. However, van Leeuwenhoek was unable to explain why he saw oval forms when they should have been spherical. He was also convinced that erythrocytes were composed of clusters of four to six small globules; this could have been due to confusion with the phenomenon of red cell crenation, in which the spicules appear as small formations surrounding a smaller central area, the cytoplasm; or he may have been looking at clusters of erythrocytes. He made a series of observations of the early stages of tadpole development and noted [34]:

To satisfy myself further, I cut off a piece of the tail from several little butts ... in order to view the blood out of the vessels: for I was not satisfied that the particles in valor de 8,5μ para a dimensão de cada glóbulo, utilizando para comparação, como em todas as suas outras observações, a de um grão de areia observado ao microscópio [30]:

"The blood is composed of exceeding small particles, named, globules, which, in most animals, are of a red colour, swimming in a liquor, called, by physicians, the serum ; and by means of these globules the motion of the blood becomes visible, which otherwise would not be discoverable by the sight. These particles, or globules, are so minute, that one hundred of them, placed side by side, would not equal the diameter of a common grain of sand; consequently, a grain of sand is above a million times the size of one such globule."

Numa comunicação que apresentou em 1683 à Royal Society, Leeuwenhoek anotou a existência, em cada eritrócito de rã, do que lhe pareciam pequenas "bolhas" envolvidas num halo, o qual seria posteriormente identificado como o núcleo celular [31]. Verificou que os eritrócitos de peixe, rã, aves e outros animais tinham forma oval, e que era a sua presença que conferia a cor vermelha ao sangue Merece referência a distinção apresentada entre os eritrócitos de mamíferos terrestres, esferóides, e os de aves e peixes, ovais [32]:

"*…the matter causing the redness of our blood was constituted of globules; I examined the blood of oxen, sheep and rabbits; and observed no difference in magnitude, between the globules of those animals, and those of men: so that, I conceived that the matter which in general made all blood red, was globules. But, after I had tried the blood of a salmon, a cod, of frogs [etc] and found that the matter, which caused the redness therein was made of parts oval, and flattish (as I had before said) I examined the blood of several birds; and have also observed, that the matter causing the redness of their blood, was also composed of like oval flattish parts, with those of fishes: so that I now concluded, that all animals, whether birds, fish, or other creatures that live in the water, have the parts causing the redness of their blood, consisting of the said oval flattish parts, and if hereafter I chance to find the contrary, I will advice you thereof."*

Concluía repetidamente que a forma dos eritrócitos poderia ser discoide, oval e também achatada, embora retornando de quando em vez ao conceito discoide (*Fig 8*); parte dos glóbulos parecia-lhe ser mais espessa e the blood of fishes were naturally oval; nay, I rather fancied that in their perfect state they'd come nearer to a round than an oval form; going upon this position, that the blood of fishes consists of six little globular bodies making up the particles, as well as that of men and other animals; for I could observe several particles broken in 4, 5 and some few in six pieces; and what I looked upon as very remarkable, I saw oval, and other figures become roundish, and at last perfectly round.

Pursuing the same line of thinking, he made a wax model to illustrate his theory that human erythrocytes were made up of six spheres, each of which contained six smaller spheres, and so on down to unimaginably small dimensions. Although this is incorrect, what is important is van Leeuwenhoek's atomistic approach, assuming that what he saw was only what could be visualized with the available equipment, and that there were equivalent smaller and smaller forms that might be detected with more powerful lenses [35]:

I have asserted formerly, that every one of the little globes, six of which make up one globe, does consist of 6 other globes; and the more we divide such a globe of blood by our imagination into smaller and smaller parts, the little parts that enter into the composition will still be inconceivably lesser...supposing I could discover the figure and shape of parts lesser than a globe of blood by a thousand million, I should still be far from reaching the first constituent parts.

The vascular network and intracapillary circulation

In a letter to the Royal Society in 1699, van Leeuwenhoek demonstrated that he understood the circulation of the blood and the difference between arteries and veins. He reported that he had shown several gentlemen who had visited him for that purpose the difference between arteries and veins in the tadpole, adding that they were more easily seen in tadpoles than in eels because the latter moved around more while being observed. He also noted that blood corpuscles separate when flowing in the smallest veins [36]:

....and when some learned men of this country sent me a word...they had in mind to come and give me a visit; I did agree...to show these Gentlemen the circulation of the blood (which all learned men dive into) and corada nos bordos [33], o que está de acordo com a morfologia em disco bicôncavo, actualmente mais aceite. Todavia, Leeuwenhoek nunca conseguiu justificar por que observava formas ovais quando deveriam ser esféricas. Estava também convicto de os eritrócitos serem constituídos por agregados de quatro a seis pequenos glóbulos (talvez confundido pelo fenómeno da crenação globular, em que as espículas simulam pequenas formações envolvendo uma área central mais reduzida, representada pelo citoplasma ou, em alternativa, agregados globulares). A propósito, numa série de observações em fase precoce do desenvolvimento de girinos, anotou o seguinte[34]:

"To satisfy myself further, I cut off a piece of the tail from several little butts … in order to view the blood out of the vessels: for I was not satisfied that the particles in the blood of fishes were naturally oval; nay, I rather fancied that in their perfect state they'd come nearer to a round than an oval form; going upon this position, that the blood of fishes consists of six little globular bodies making up the particles, as well as that of men and other animals; for I could observe several particles broken in 4, 5 and some few in six pieces; and what I looked upon as very remarkable, I saw oval, and other figures become roundish, and at last perfectly round."

Na mesma ordem de pensamento, construiu um modelo em cera pelo qual propôs que os eritrócitos humanos seriam constituídos por seis esferas, cada uma das quais incluíria outras seis esferas mais pequenas, ou seja, cada glóbulo conteria outros seis glóbulos mais pequenos que, no conjunto, configurariam uma esfera, e assim sucessivamente, até dimensões mínimas inimagináveis (*Fig 8*). Ainda que a suposição esteja incorrecta, é de realçar a perspectiva atomística de Leeuwenhoek, ao admitir que a imagem observada reflectia somente o que era possível visualizar com os meios disponíveis, pois que, como admitiu, haveria formas equivalentes mais e mais pequenas (admissivelmente, a serem evidenciadas com "lentes" mais poderosas)[35]:

"I have asserted formerly, that every one of the little globes, six of which make up one globe, does consist of 6 other globes; and the more we divide such a globe of blood by our imagination into smaller and smaller parts,

that the more, because these tadpoles are apt to lie quieter than eels, and secondly because one may more exactly discern the arteries from the veins in them than in any other animal, and then because even in the smallest or thinnest veins one may see the red globules of the blood run further asunder.

Further on, on the basis of observations of a vascular sector which he had divided into arterial and venous segments (to which he allocated different letters), he differentiates them according to the direction in which they carried blood[37]:

...we must call these blood vessels ABCDFG, and ABEFI arteries, because they carry the blood from the heart first in G and I: and the blood vessels GHK and IHK we must call veins, because they carry the blood to the heart again. In another place I saw the blood run in an artery that was so big, that about twenty of these red globules could run together at one time through it.

He extends this observation in another study of the arteries and veins and the capillary network between them, noting that blood flowed in the arteries in the opposite direction to the veins[38]:

...I let my eye run upon the great artery and vein, which was so close to another, that there was not above the distance of the fourth part of the breath of the hair of a man's head between them...the blood did run upwards in the artery, and downwards in the vein, and that with an equal velocity; yet what was most remarkable was, to see the manifold small arteries, that came forth from the great one, and which were spread into several branches, and turning came into one again, and were re-united, that at last they did pour out the blood again into the great vein...

In another study, he demonstrated the distribution and direction of the circulation in arteriovenous anastomoses and the resulting mixing of arterial and venous blood[39], the first time this had been observed *in vivo* (*Figure 8*).

Following closer observation of the vasculature of various animal species, he concluded that the different layers making up the walls of even the smallest arteries were not permeable to the "juices of the blood" (nutrients). He argued that they would have to be absorbed in the smallest peripheral vessels, which is in fact how the exchange of gases and nutrients takes place in the capillaries[40]:

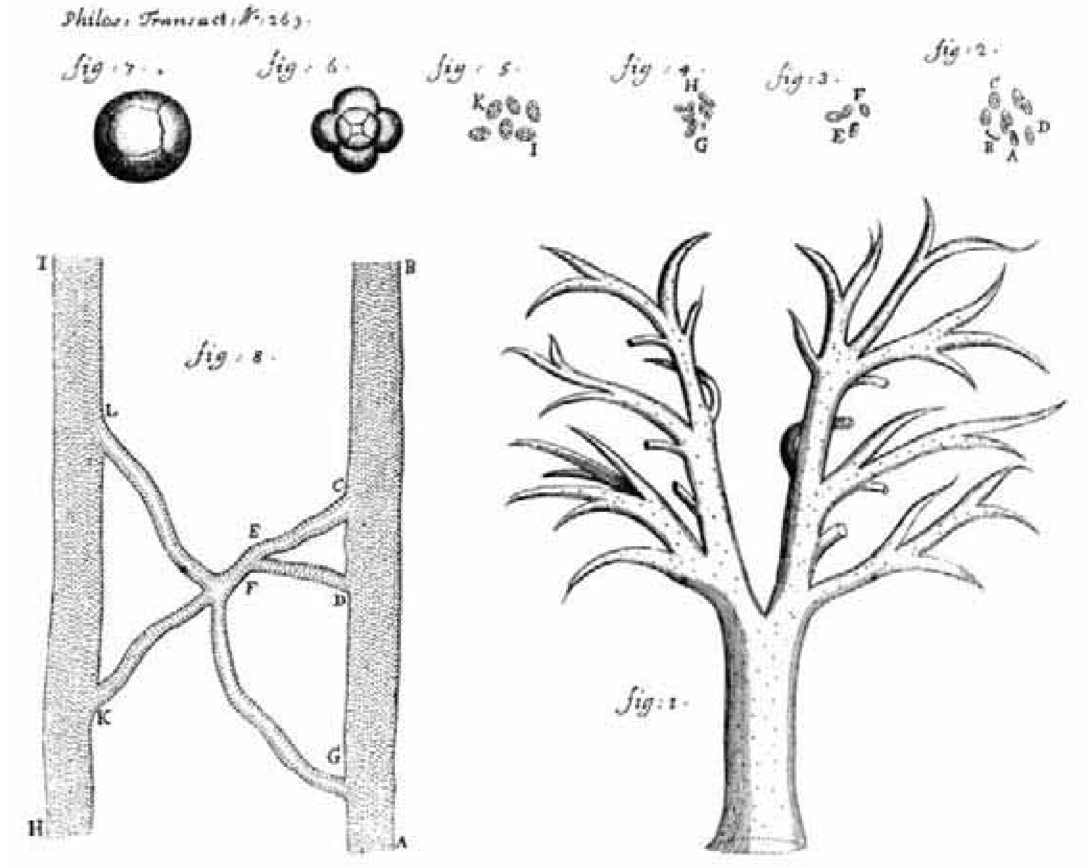


Figura 8 – Conjunto de desenhos de um estudo de Leeuwenhoek em girinos, sobre a circulação do sangue e eritrócitos. Em 1 é representada a ramificação de uma artéria em vasos cada vez mais finos. As imagens 3 a 5 evidenciam eritrócitos de girino observados com lentes de ampliação crescente, mostrando a pretensa constituição de cada eritrócito em seis glóbulos mais pequenos em formado reduzido (em 3) e em imagem ampliada (em 4). Em 6 e 7 é proposta a organização esférica dos seis glóbulos mais pequenos em um só eritrócito. Como termo de comparação, para evidenciar que todos os peixes e batráquios têm glóbulos ovais, com dimensões idênticas em todas as espécies, são apresentados eritrócitos de salmão, em que no interior de cada um qual são visualizadas partículas representativas dos seis glóbulos constituintes. Em 8 é apresentado o esquema de anastomoses artério - venosas (LKFECDG): parte do sangue que de I para H flui nas artérias também passa para as anastomoses, misturando-se com o sangue venoso proveniente do fluxo principal (de A para B), de modo a que o sangue das artérias e veias seja constituído parcialmente por aquela mistura.

Figure 8. Drawings from a study by van Leeuwenhoek of blood circulation and erythrocytes in tadpoles. Drawing 1 shows an artery branching into successively smaller vessels. Drawings 3 to 5 show erythrocytes seen at progressively greater magnifications, with the supposed composition of each cell as six smaller globules, at smaller (3) and larger (4) sizes. Drawings 6 and 7 represent van Leeuwenhoek's proposed organization of a single erythrocyte as a sphere made up of six smaller globules. For the purposes of comparison, and to demonstrate that all fish and amphibians have oval red blood cells and that these are the same size in all species, salmon erythrocytes are presented, in the interior of which can be seen particles representing the six constituent globules. Drawing 8 is a diagram of arteriovenous anastomoses (LKFECDG): some of the blood flowing in an artery from I to H passes through the anastomoses, mixing with venous blood from the main venous flow from A to B, meaning that the blood in arteries and veins consists partly of this mixture.

the little parts that enter into the composition will still be inconceivably lesser…supposing I could discover the figure and shape of parts lesser than a globe of blood by a thousand million, I should still be far from reaching the first constituent parts."

Particularidades da rede vascular e da circulação intracapilar

Numa carta de 1699 que enviou à Royal Society, Leeuwenhoek demonstra conhecer a

I am assured that the coats of the arteries are so formed, as not to permit the least particle of the blood to pass through them, until those arteries become divided into very small branches...and here, through the extreme thinness of the coats of the vessels, the finer juices of the blood may find a passage on all sides for the nourishment in parts adjoining.

He went on to hypothesize that nutrients are transported by arterial blood, which accounts for its brighter color, and that their absorption circulação do sangue e a diferença entre artérias e veias. Nessa carta relatou que, na presença de diversas testemunhas que o visitaram para o efeito, lhes mostrou a diferença entre artérias das veias, de girinos (acrescentando que seriam mais facilmente observáveis do que em enguias, por estes animais se agitarem muito enquanto durava a observação), notando ainda que os glóbulos sanguíneos fluíam separados entre si nas veias mais finas [36]:

"….and when some learned men of this country sent me a word…they had in mind to come and give me a visit; I did agree to it… to show these Gentlemen the circulation of the blood (which all learned men dive into) and that the more, because these tadpoles are apt to lye quieter than eels, and secondly because one may more exactly discern the arteries from the veins in them than in any other animal, and then because even in the smallest or thinner veins on may see the red globules of the blood run further asunder."

Mais adiante, tomando como base de observação um sector vascular pré-delimitado em segmentos arteriais e venosos, diferencia estes consoante o sentido em que transportam o sangue [37]:

"…we must call these blood vessels ABCDFG, and ABEFI arteries, because they carry the blood from the heart first in G and I: and the blood vessels GHK and IHK we must call veins, because they carry the blood to the heart again. In another place I saw the blood run on an artery that was so big, that about twenty of these red globules could run together at one time through it."

Aquela observação é completada noutro estudo, em que relaciona as artérias e veias com uma rede capilar interposta, observando ainda que o sangue fluía nas artérias em sentido oposto ao das veias [38]:

"I let my eye run upon the Great artery and vein, which was so close to another, that there was not above the distance of the fourth part of the breath of the hair of a man's head between them…the blood did run upwards in the artery, and downwards in the vein, and that with an equal velocity; yet what was most remarkable was, to see the manifold small arteries, that came forth from the great one, and which were spread into several branches, and turning came in one again, and were re-united, that at last they did pour out the blood again into the great vein…"

by the tissues explains the darker color of venous blood as it returns to the heart [41]:

...these things, I say, being duly weighed and considered, it seems clear that the arterial blood, coming from the heart, must contain more subtle and fluid parts than when in its passage to the heart. For the blood will not be deprived of its subtle fluids, when in the larger arteries; to prevent which, I imagine that they are provided with thick and solid coats. And here the particles of blood from which its redness proceeds, swimming in a thin juice, are of a bright red colour; but in the smallest arteries, some of its parts are drawn off for the support and nourishment of the body, whereby the blood, when returning in the veins, being deprived of those thin juices, assumes a darker red, and as more of the thin juices are taken away, it will appear blackish.

In 1683, he observed extremely small vessels, possibly capillaries, in the intestines, together with others containing a whitish liquid similar to milk, which would have been lymphatic capillaries. However, he was uncertain as to what their function might be in the circulatory system [42]:

Briefly put, they seem in my opinion, to have been created for the greater part to carry back to the heart the blood in the outermost parts...

In the same year, he used the term "capillary" for the first time while pondering the function of a tiny vein he was observing in a frog [43]:

Now if there be capillaries in the body a 1000 times less than this I examined, how thin must the threads necessarily be of which the coat is made?

However, in the following year, while dissecting the cornea of an ox, he noted a large number of transparent interlinked stripes, which he also considered to be capillaries [44]:

...which I judge to be, many of them, blood vessels, but so small that they contain none of the globules which cause the redness in the blood. By rubbing of our eyes, with our hands, we may so press these blood vessels, until they become so stretched out, that some of these bloody globules may get in, and remain there for some time, which may cause that redness in our eyes...

He found minuscule vessels in tadpoles, through which blood corpuscles passed one at a time, and extrapolated this finding to humans and other animals [45]:

Noutro estudo evidenciou a distribuição e sentido da circulação em anastomoses arterio-venosas e a consequente mistura de sangue arterial e venoso [39], na que terá sido a primeira demonstração realizada *in vivo* (*Fig 8*).

Após uma melhor observação da rede vascular de diversas espécies animais, considerou que as diversas camadas que constituem a parede das artérias, mesmo as mais finas, não seriam permeáveis aos nutrientes (*juices*). Em alternativa, admitiu que essas trocas teriam de ocorrer nos canalículos periféricos mais finos, o que está de acordo com o mecanismo das trocas gasosas e de nutrientes a nível dos capilares [40]:

"I am assured that the coats of the arteries are so formed, as not to permit the least particle of the blood to pass through them, until those arteries become divided into very small branches…and here, through the extreme thinness of the coats of the vessels, the finer juices of the blood may find a passage on all sides for the nourishment in parts adjoining."

Aquela observação era completada pela hipótese de que seria o sangue arterial a transportar os referidos nutrientes, associando a cor mais viva do sangue ao conteúdo de nutrientes transportados por este, pelo que, após serem cedidos aos tecidos, se explicaria a coloração vermelha - escuro do sangue venoso, de volta ao coração [41]:

"…these things, I say, being dully weighed and considered, it seems clear that the arterial blood, coming from the heart, must contain more subtle and fluid parts that when in its passage to the heart. For the blood will not be deprived of its subtle fluids, when in the larger arteries; to prevent which, I imagine that they are provided with thick and solid coats And here the particles of blood from which its redness proceeds, swimming in a thin juice, are of a bright red colour; but in the smallest arteries, some of its parts are drawn of for the support and nourishment of the body, whereby the blood, when returning in the veins, being deprived of those thin juices, assumes a darker red, and as more of the thin juices are taken away, it will appear blackish".

Em 1683, observou vasos sanguíneos muito finos (capilares?) nos intestinos, juntamente com outros que continham um líquido esbranquiçado, semelhante a leite, que seriam

For, if we now plainly perceive, that the passage of the blood from the arteries into the veins of the tadpole, is not performed in any other than those vessels, which are so minute as only to admit the passage of a single globule at a time, we may conclude that the same is performed in like manner in our own bodies, and in those of other animals. And if so, it must be impossible for us ever to discover the passage from the arteries into the veins in the human body, or of any terrestrial animal; first, because a single globule, when lying in one of those minute arteries, has not any visible colour, and secondly, because, in those vessels which are so large as to come within our sight, the blood has not any perceptible motion...

He was filled with astonishment and delight by the sight of a network of connections in a tadpole's tail made up of tiny vessels, supposedly arteries and veins, but actually the same thing – and now understood to be capillaries [46]:

Upon examining the tail of this creature a sight presented itself, more delightful than any that my eyes ever beheld... For I saw, not only that the blood in many places was conveyed through exceedingly minute vessels, from the middle of the tail towards the edges, but that each of these vessels had a curve or turning, and carried the blood back towards the middle of the tail in order to be again conveyed to the heart. Hereby it plainly appeared to me, that the blood-vessels I now saw in this animal and which bear the names of arteries and veins, are, in fact, one and the same; that is to say, that they are properly termed arteries so long as they convey blood to the farthest extremities of its vessels, and veins when they bring it back towards the heart.

He was struck by the "inconceivable" organization of the microvasculature in the tail of a small fish [47]:

...at the part where the fin begins, I there saw, to my great admiration, a large artery dividing itself into the before-mentioned smaller ones; and close to it, numbers of the minute veins returning from the fin, and uniting in one large vein. In short, here was such an agitation, or motion of the blood driven out of the large artery to the farthest end of the tail and into the fin, and running back in the small veins, into the large one, as is inconceivable.

As he extended his observations, he discerned the dense network of vessels in the tails of small eels, showing keen insight into capilares linfáticos. Porém ainda não estava bem certo da função que esses vasos teriam na circulação sanguínea [42]:

"Briefly put, they seem in my opinion, to have been created for the greater part to carry back to the heart the blood in the outermost parts…"

Ainda no mesmo ano, ao observar numa rã as camadas constituintes de uma pequena veia que teria a espessura de cabelo, utilizou pela primeira vez a designação capilar ao interrogar-se quanto à sua estrutura [43]:

" Now if there be capillaries in the body a 1000 times less then this I examined, how thin must the threads necessarily be of which the coat is made?

Contudo, no ano seguinte, ao dissecar a córnea de um olho de bovino, notou grande abundância de listras entrelaçadas, transparentes, que também considerou serem capilares [44]:

"…Which I judge to be, many of them, blood vessels, but so small that I they contain none of the globules which cause the redness in the blood. By rubbing of our eyes, with our hands, we may so press these blood vessels, until they become so stretched out, that some of these bloody globules may get in, and remain there for some time, which may cause that redness in our eyes…"

Verificou a presença em girinos de vias minúsculas por onde passava um glóbulo de cada vez, extrapolando o mesmo para o homem e outros animais [45]:

For, if we now plainly perceive, that the passage of the blood from the arteries into the veins of the tadpole, is not performed in any other than those vessels, which are so minute as only to admit the passage of a single globule at a time, we may conclude that the same is performed in like manner in our own bodies, and in those of other animals. And if so, it must be impossible for us ever to discover the passage from the arteries into the veins in the human body, or of any terrestrial animal; first, because a single globule, when lying in one of those minute arteries, has not any visible colour, and secondly, because, in those vessels which are to large as to come within our sight, the blood has not any perceptible motion…"

Espantou-se, deliciado, ao observar na cauda de girino uma rede de conexões consti-

the possible constitution of the vascular wall and the function of the capillaries [48]:

...we may conclude how exquisitely must be the vessels in which the circulation is performed; and if it were not so, how could all the parts of our bodies be continually supplied with nourishment?... And if we reflect, that each of these very small vessels must be formed with the same kind of coat as the larger ones though of a thinness proportioned to its size; and further, if we consider of what wonderfully fine and invisible membranes the coats of the smallest vessels must be formed, and how easily the finest part of the arterial blood may find a passage through those coats, to the end that every part of the body may, from thence, be continually supplied with necessary and suitable nourishment...

However, his observations of the brains of various animals led him to the conclusion that erythrocytes could only cross the capillary network after breaking up into particles that were small enough to do so, but did not take into account the variations in shape that he had seen in different flow conditions and in vessels of different diameters [49]:

Many of these blood-vessels were so minute that (to judge by the eye) if one of the red globules in the blood of the turkey, or other bird, were to be divided into five hundred parts, not one of these parts could be contained in the cavity of those vessels... These very minute blood-vessels appeared of a deeper colour where three or four of them lay one on another, without any interjacent [interfacing] substance. From these appearances, I was more firmly of the opinion than before, that the globules of blood, whence its redness proceeds, are divided into smaller parts, when they come to such minute vessels as they cannot enter without being divided...

On the basis of these observations he concluded that, after fragmenting into six (or more) smaller "globules" according to the size of the vessel and passing through the capillaries, each erythrocyte then regained its original shape [50]:

...and I moreover took notice of a great number of globules, each about a sixth part of the size of a globule of human blood, and which, I judged, issued from the vessels which were broken; and 1 concluded, that six of those globules would join to make up one globule of blood when they passed into larger vessels, for they were very inferior in transparency to the other globules adjoining. I also imagined that these globules, of which six would make up an ordinary sized globule, when they tuída por "vasos minúsculos a que se chamam artérias e veias, mas que são o mesmo", os quais, de acordo com a concepção actual, não eram senão capilares (46):

"Upon examining the tail of this creature a sight presented itself, more delightful than any that my eyes ever beheld; . . . For I saw, not only that the blood in many places was conveyed through exceedingly minute vessels, from the middle of the tail towards the edges, but that each of these vessels had a curve or turning, and carried the blood back towards the middle of the tail in order to be again conveyed to the heart. Hereby it plainly appeared to me, that the blood-vessels I now saw in this animal and which bear the names of arteries and veins, are, in fact, one and the same, that is to say, that they are properly termed arteries so long as they convey blood to the farthest extremities of its vessels, and veins when they bring it back towards the heart."

Completando aquela observação, destacou, admirado, a "inconcebível" organização vascular da microcirculação numa cauda de pequenos peixes (47):

"…at the part where the fin begins, I there saw, to my great admiration, a large artery dividing itself into the before-mentioned smaller ones ; and close to it, numbers of the minute veins returning from the fin, and uniting in one large vein. In short, here was such an agitation, or motion of the blood driven out of the large artery to the farthest end of the tail and into the fin, and running back in the small veins, into the large one, as is inconceivable."

Ao aprofundar aquelas observações, comprovou a grande densidade da rede microcirculatória existente na cauda de pequenas enguias, ao mesmo tempo que revelava grande percepção a possível constituição da parede vascular e tipo de funcionalidade reservada aos capilares(48):

"…we may conclude how exquisitely must be the vessels in which the circulation is performed; and if it were not so, how could all the parts of our bodies be continually supplied with nourishment?" … And if we reflect, that each of these very small vessels must be formed with the same kind of coat as the larger ones though of a thinness proportioned to its size ; and further, if we confider of what wonderfully fine and invisible membranes the coats of the smallest vessels must be formed, and how easily the finest part of the arterial blood may find a passage through those coats, to the end that every part of the body may, from thence, be continually supplied with necessary and suitable nourishment …"

came to vessels so small as not to admit them, must be again divided into still smaller parts, and then those vessels would become colourless.

At all events, van Leeuwenhoek undoubtedly mistook schistocytes resulting from the manipulation of samples under observation, or other types of cells or fragments of nerve tissue, for erythrocytes.

Cardiac function and pulsation

Van Leeuwenhoek showed remarkable insight in his observations of the beginning of cardiac function in tadpoles and the internal movement of the fluid as it began to take on a reddish color (46):

When these tadpoles were about eight or ten days old, I could perceive a small particle moving within their bodies, which I concluded to be the heart; and the fluid which was protruded from it began to assume a red colour.

The excitement he displayed in a letter in 1688, confirming the existence of blood circulation in a variety of animal tissues (a rooster's comb, tails of tadpoles and eels, frog's feet, rabbit's ear and the skin of a bat's wing), is understandable, as these findings resolved his doubts, as well as providing additional evidence for the model of the circulation that Harvey had put forward sixty years before. At the same time, he did not omit to arrange for credible witnesses to his observations (51):

Nay, I saw this movement as clearly as I, or anyone else, could ever imagine the whole propulsion of the blood from the heart, and the transition of the arteries (at the place where they join up together) into the veins. Although I contemplated this sight many times to my exceedingly great pleasure, I did not want to keep it to myself, but I showed this circulation of the blood to five prominent gentlemen, who declared to me that they had never yet seen anything of mine that was so worthy of being beheld.

On repeating his observations of the blood circulation in the tails of tadpoles, he saw that the "wave of propulsion" of the blood, clearly visible in the vessels near the heart, almost disappeared in the smallest vessels at the end of the tail, although the flow appeared to be uninterrupted and faster with every pulsation (52):

Porém, atendendo à disposição observada em cérebro de diversos animais, admitiu que os eritrócitos atravessavam a rede capilar depois de se fragmentarem em partículas com dimensões adequada aqueles canalículos, não valorizando assim a variação de conformação globular que referira em diversas condições de fluxo ou de diâmetro vascular [49]:

"Many of these blood-vessels were so minute than (to judge by the eye) if one of the red globules in the blood of the turkey, or other bird, were to be divided into five hundred parts, not one of these parts could be contained in the cavity of those vessels… These very minute blood-vessels appeared of a deeper colour where three or four of them lay one on another, without any interjacent[interfacing] substance. From these appearances, I was more firmly of opinion than before, that the globules of blood, whence its redness proceeds, are divided into smaller parts, when they come to such minute vessels as they cannot enter without being divided…"

No seguimento daquelas observações concluiu que, nos vasos pós-capilares, cada eritrócito era reconstituído na sua forma original a partir de seis glóbulos mais pequenos (ou múltiplos) resultantes de fragmentação anterior à travessia da rede capilar e com dimensões ajustadas ao respectivo calibre[50]:

"…and I moreover took notice of a great number of globules, each about a sixth part of the size of a globule of human blood, and which, I judged, issued from the vessels which were broken ; and I concluded, that six of those globules would join to make up one globule of blood when they passed into larger vessels, for they were very inferior in transparency to the other globules adjoining. I also imagined that these globules, of which six would make up an ordinary sized globule, when they came to vessels so small as not to admit them, must be again divided into still smaller parts, and then those vessels would become colourless."

Em qualquer dos casos, Leeuwenhoek decerto confundiu os eritrócitos com esquisócitos resultantes das manipulações associadas à observação, ou com outros tipos de células ou fragmentos do tecido neuronal.

Função cardíaca e pulsação

É notável a percepção com que acompanhou, em girinos, o início da respectiva função cardíaca e a movimentação interna de um

And one thing is here worthy of note, that in the very small vessels at the greatest distance from the heart, as in the extremity of the tail, there did not appear such a forcible and vehement protrusion as in the vessels near the heart; but though the blood in those small vessels appeared to move in an uninterrupted course, yet it could plainly be seen, that at every pulsation of the heart the course was a little accelerated...

In observations of the same animals he clearly states that, to his surprise, the rhythmic pulsation results from the distension of the arteries caused by the passage of blood impelled by the heart, after which the vessel returns to its initial diameter and some blood flows back, a process that is repeated with each pulsation [53]:

Now the blood...was by every pulsation of the heart impelled upwards...and in every moment of time that it was pushed upwards, it came also back again, in such a manner as if we saw before our naked eyes, a very quick motion of a saw that went backwards and forwards... This being so, we must conclude, that the tunic of the blood vessel between N and P, and also somewhat below N, is distended in wideness by every pulsation of the heart; and as quickly as this uncommon distention is performed, so quickly doth also the tunic of the vessel shrink again, whereby the blood that was pushed forth, is driven and forced to run back again.

Van Leeuwenhoek's observations of a fish's heart, which he managed to keep beating for four hours, enabled him to confirm that the blood expelled regularly with each contraction passed through a valve in the adjoining vessel that prevented it from returning (the aortic valve), which caused the "great artery" to dilate. On finding the same phenomenon in eels and other fish, he assumed that it should be the same for all animals. However, in this letter he attributed the pulsation of the blood to the movement of blood through the venous valves [54], thus contradicting previous studies in which he attributed it to the movement of blood in the arteries after cardiac contraction. In one of his works, he focuses on the pulsation of the blood and affirms his certainty that in all species the blood circulates in a closed circuit through vessels of different sizes, and he calculates their diameter by the number of red corpuscles passing together [55]:

líquido que começava a distinguir-se por coloração avermelhada [46]:

" When these tadpoles were about eight or ten days old, I could perceive a small particle moving within their bodies, Which I concluded to be the Heart; and the fluid which was protruded from it began to assume a red colour."

Compreende-se assim que, na carta de 1688 em que confirmou a existência de circulação sanguínea em diversos tecidos animais (nomeadamente na crista de frango, cauda de girino e extremidade de enguia, pata de rã, orelha de coelho e pele de asa de morcego), tenha revelado emoção (não se esquecendo também de apresentar testemunhas credíveis do que observara) por ver as suas dúvidas esclarecidas, e por conferir um suporte adicional ao modelo da circulação que Harvey propusera sessenta anos antes [51]:

"Nay, I saw this movement as clearly as I, or anyone else, could ever imagine the whole propulsion of the blood from the heart, and the transition of the arteries (at the place where join up together) into the veins. Although I contemplated this sight many times to my exceedingly great pleasure, I did not want to keep it to myself, but I showed this circulation of the blood to five prominent gentlemen, who declared to me that they had never yet seen anything of mine that was so worthy of being beheld."

Ao repetir a observação da circulação do sangue na cauda de girinos, verificou que a "onda propulsora" do sangue, bastante visível nos vasos junto do coração, como que desaparecia nos vasos mais finos da extremidade da cauda, embora o fluxo parecesse ininterrupto a cada pulsação e mais acelerado [52]:

"And one thing is here worthy of not^, that in the very small vessels at the greatest distance from the heart, as in the extremity of the tail, there did not appear such a forcible and vehement protrusion as in the vessels near the heart ; but though the blood in those small vessels appeared to move in an uninterrupted course, yet it could plainly be seen, that at every pulsation of the heart the course was a little accelerated…"

Ainda naqueles animais refere claramente, admirado com o fenómeno, que a pulsação ritmada resulta da distensão das artérias pela

I perceived, however, in many places, an artery and a vein, placed close beside each other, and of a size large enough to admit the passage of ten or twelve globules of blood at the same time; and in this artery the blood was protruded or driven forward with great swiftness, and flowed back through the vein, which was a most pleasing spectacle to behold. I could also most plainly perceive in the arteries, the rising, or pulsation, caused by the motion which the blood receives from the heart; these pulsations were so rapid that I judged seven strokes were performed in a second of time. The worm, or small animal which is produced from the spawn of frogs and is called a tadpole, afforded me a still more distinct view of this subject; for, upon placing one of them, which was newly hatched, before the microscope, I could definitely perceive the whole circuit of the blood.

Extrapolating this to humans, he calculated that the heart induced 108,000 pulsations every 24 hours [56]:

...it being...taken for granted, that the heart of a man doth push out the blood 75 times in a minute (some say 60 times, but I judge the first number to be the nearest) and that is 4500 in an hour's time, and 108000 in the space of a day and night.

Blood flow and hemorheological changes

Van Leeuwenhoek found that the appearance of blood cells and the fluid in which they were suspended tended to change according to the speed at which they circulated and the diameter of the vessels [57]:

I applied myself to view the circulation of blood with glasses more magnifying than I have yet used...Now the greater the magnifying glass is, the swifter does the circulation of the blood appear in the vessels. Having retarded this motion, I employed two or three seconds of time in observing the little veins, and found that in several small vessels the oval particles were undone, that I could neither see them nor those of which six had made up a particle of blood; but only a simple fluid matter, with faint colour running along the vessels; but in a large artery at the tail, the blood moved so slowly, that I could easily discern that the particles in that vessel were oval... the particles of blood are by a pressure so disjoined, and united with the fluid matter in which they move, that the whole appears as a simple moisture...perhaps the undone particles, when freed from the above mentioned straining, may return to their former figure...

Considering the composition and functions passagem do sangue, impulsionado pelo coração, a que se segue o retorno do diâmetro vascular ao estado inicial e algum refluxo, e assim sucessivamente a cada pulsação [53]:

" Now the blood…it was by every pulsation of the heart impelled upwards…and in every moment of time that it was pushed upwards, it came also back again, in such a manner as if we saw before our naked eyes, a very quick motion of a saw that went backwards and forwards…. This being so, we may conclude, that the tunic of the blood vessel between N and P, and also somewhat below N, is distended in wideness by every pulsation of the heart; and as quickly as this uncommon distention is performed, so quickly doth also the tunic of the vessel shrink again, whereby the blood that was push forth, is drove and forced to run back again"

Após observar um coração de peixe, que se manteve em funcionamento durante cerca de quarto horas, confirmou que o sangue expelido regularmente em cada contracção atravessava uma válvula do vaso a que estava unido, a qual lhe impedia o seu refluxo (válvula aórtica), originando a dilatação da " grande artéria". Verificando que o mesmo fenómeno ocorria noutros em enguias e peixes, considerou que o mesmo deveria suceder em todos os restantes animais. Porém, nesta carta de 1708 atribui a pulsação à passagem do sangue pelas válvulas venosas [54], contrariando os estudos anteriores em que a referira ao movimento do sangue nas artérias, na sequência da contracção cardíaca. Num desses trabalhos é destacada a pulsação sanguínea e a certeza de que a circulação decorria em circuito fechado em todas as espécies, por vasos de dimensões diferentes, cujo diâmetro calculava pelo número de glóbulos emparelhados [55]:

"I perceived, however, in many places, an artery and a vein, placed close beside each other, and of a size large enough to admit the passage of ten or twelve globules of blood at the same time; and in this artery the blood was protruded or driven forward with great swiftness, and flowed back through the vein, which was a most pleasing spectacle to behold. I could also most plainly perceive in the arteries, the rising, or pulsation, caused by the motion which the blood receives from the heart; these pulsations were so rapid that I judged seven strokes were performed in a second of time. The worm, or small animal which is produced from the spawn of' frogs and is called a tadpole, afforded me a still more distinct view of this subject; for,

of muscles, after relating the speed of blood circulation to the pulse and cardiac contraction, van Leeuwenhoek launched into the field of medical pathology, coming to the conclusion (which depends on principles of hemorheology that were only formulated two centuries later) that "thick blood", which he considered to be more common in people suffering from fever, made circulation more difficult and was responsible for what is now known as congestive heart failure[58]:

The blood in many feverish persons is very thick, and therefore passes slowly, and with difficulty, through the smaller arteries, and requires a very strong beating in the heart to force its way. When the blood is thick and makes this resistance, the heart upon contracting itself, cannot force it all out, but a great part remains behind in the ventricles.

In 1706 he described the development of a blood clot *in vitro* [59]. He had already seen *in vivo* that blood clotting in the tail of tadpoles could occur in larger vessels in which the blood flowed slowly or not at all. However, he claimed that in any animal, even after days of stagnation, cardiac contraction could restore circulatory flow and the original shape of the blood's "component particles" (red corpuscles), eliminating the clot. He thus believed that the propulsion of blood by the heart could prevent or reverse intravascular coagulation *in vivo* – which is in agreement with basic principles of blood flow and modern techniques of thromboembolic prophylaxis [60]:

But, since we now clearly see, that coagulated blood can, by the pulsation of the heart, in course of time, not only be put in motion, but also so far dissolved, that its component particles or globules may re-assume their primitive figure, we may fairly conclude, that blood, in any animal, which by a blow or bruise, is made to coagulate and stagnate in the vessels, may, in the space of some days, be restored to motion...we may easily conceive, that when a coagulation does happen, it may, by such frequent propulsions or pulsations as I have mentioned, be at length dissolved, and in all, or most of the vessels, restored to the same current or course as before.

He also noted that the formation of a blood clot blocked blood flow downstream, and this normalized when the stagnation disappeared [56]:

upon placing one of them, which was newly hatched, before the microscope, I could definitely perceive the whole circuit of the blood."

Extrapolando para o homem, calculou o coração induzia cerca de 108.000 pulsações por cada 24 h [56]:

"…taken for granted, that the heart of a man doth push out the blood 75 times in a minute (some say 60 times, but I judge the first number to be the nearest) and that is 4.500 in an hour's time, and 108.000 in the space of a day and night."

Fluxo sanguíneo e modificações hemorreológicas

Verificou que o aspecto dos glóbulos e suspensão sanguínea tendiam a modificar-se com a velocidade com que circulavam nos vasos e com o calibre destes [57]:

"…I applied myself to view the circulation of blood with glasses more magnifying than I have yet used…Now the greater the magnifying glass is, the swifter does the circulation of the blood in the vessels. Having retarded this motion, I employed two or three seconds of time in observing the little veins, and found than the in several small vessels the oval particles were undone, that I could neither see them nor those of which six had made up a particle of blood; but only a simple fluid matter, with faint colour running along the vessels; but in a large artery at the tail, the blood moved so slowly, that I could easily discern that the particles in that vessel were oval… that the particles of blood are by a pressure so disjoined, and united with the fluid matter in which they move, that the whole appears as a simple moisture…perhaps the undone particles, when freed from the above mentioned straining, may return to their former figure..."

A propósito da constituição e funções dos músculos, Leeuwenhoek, e após relacionar a velocidade da circulação sanguínea com o pulso e a contracção cardíaca, não hesitou em avançar pelo campo da patologia médica e para uma conclusão de base hemorreológica (que seria fundamentada cerca de duzentos anos mais tarde), ao admitir que o sangue espesso (que considerava ser mais frequente nas pessoas febris) dificultava a circulação e era motivo para um quadro de insuficiência congestiva [58]:

"The blood in many Feavorish [Febris] Persons is

So that now it doth plainly appear before our eyes, that the stagnated blood, cannot only be made to move again by the motion of the heart, which we call the beating of the pulse, nay, even in such a manner, that the coagulated red globules of the blood are uncongealed again, and assume their first figure.

He argued that the force of the heart's propulsion, in cases of circulatory stagnation, could open new paths for perfusion in areas of tissue with less resistance; the importance of this mechanism, by which collateral circulation develops in ischemic tissue, was only recognized in the 20th century [61]:

Moreover, it was my opinion that many of the large circulations of the blood, which I saw, when it began to stagnate, were not performed within the coats of the blood-vessels, but that, when the blood in the arteries was impeded in its course, the continued and strong propulsion from the heart caused it to form new canals, where the fish's skin made the least resistance...

He appears, once again, to have been the first to observe angiogenesis, in an example of local vascular self-regeneration [62]:

At another time, I observed an appearance of a different nature in the blood-vessels, which was occasioned by my having put a tadpole into a piece of clean paper, whereby a small spot in the very thinnest part of its tail stuck to the paper, and thereby received a small injury, so that some blood flowed from the wound, out of an artery which was of a size to admit about four globules of blood to past through it at a time. The blood thus flowing out, remained collected about the wounded part; but here another sight presented itself, which engaged all my attention; for, in this same artery, at about the half of an hair's breadth distance from the wounded part, another small branch appeared, wherein the blood pursued its course in the same uniform and distinct manner, as if the artery had remained uninjured.

Final comment

Given the importance and range of van Leeuwenhoek's pioneering studies, one would expect them to have had a corresponding impact on the scientific milieu of his time. But this was not the case. Apart from the generally warm reception and recognition from the Royal Society (with the exception of the periods mentioned above), as well as in his own country and from some foreigners, many of *very thick, and therefore passes slowly, and with difficulty, thro the smaller arteries, and requires a very strong beating in the heart to forces its way. When the blood is thick and makes this resistance, the heart upon contracting itself, cannot force it all out, but a great part remains behind in the ventricles."*

Em 1706 reportou o desenvolvimento da coagulação sanguínea *in vitro* [59]. Antes já verificara in vivo, na cauda de girinos, que coagulação sanguínea poderia sobrevir nos vasos de maiores dimensões em que o sangue fluía pouco ou estagnara. Porém, admitira que, mesmo após alguns dias de estagnação, a contractilidade cardíaca poderia restaurar, em qualquer animal, o fluxo circulatório (e eliminar a coagulação) e a forma original das partículas do sangue (glóbulos vermelhos). Por outras palavras, de acordo com um princípio básico da fluidez sanguínea e das actuais medidas de profilaxia da trombogénese, admitia, implicitamente, que a propulsão do sangue pelo coração evitava a coagulação intravascular ou a sua manutenção in vivo[60]:

"But, since we now clearly see, that coagulated blood can, by the pulsation of the heart, in course of time, not only be put in motion, but also so far dissolved, that its component particles or globules may re-assume their primitive figure, we may fairly conclude, that blood, in any animal, which by a blow or bruise, is made to coagulate and stagnate in the vessels, may, in the space of some days, be restored to motion…we may easily conceive, that when a coagulation does happen, it may, by such frequent propulsions or pulsations as I have mentioned, be at length dissolved, and in all, or most of the vessels, restored to the same current or course as before."

Por outro lado, notou que a formação de coágulos bloqueava o fluxo sanguíneo a jusante, o qual normalizava assim que aquela estagnação desaparecia [56]:

"So that now it doth plainly appear before our eyes, that the stagnated blood, cannot only be made to move again by the motion of the heart, which we call the beating of the pulse, nay, even in such a manner, that the coagulated red globules of the blood are uncongealed again, and assume their first figure"

Nas situações de estagnação do fluxo circulatório, admitiu que a força de propulsão sanguínea poderia abrir novas vias de per-

whom visited him on various occasions to see his discoveries for themselves, his findings were greeted at the time with skepticism. Thereafter, his work was ignored until the mid-19th century, when Delft city council organized a commemorative event in his honor. Renewed interest in his research is, however, mainly due to a monograph published by a British admirer, Clifford Dobell[63].

As well as the great number and variety of observations of which he left both written records and illustrations, van Leeuwenhoek confirmed the existence of the minute vessels of the capillary network that had been observed by Malpighi, and described the different forms taken by erythrocytes and the changes in the fluidity of blood in disease states. In doing so, he made vital contributions to refining the model of the circulation proposed by William Harvey, and to the emerging concept of the hemorheology of blood flow.

fusão em sectores teciduais com menor resistência, cuja importância, sob a forma de circulação colateral dos tecidos isquemiados, seria entendida somente no século XX [61]:

"Moreover, it was my opinion that many of the large circulations of the blood, which I saw, when it began to stagnate, were not performed within the coats of the blood- vessels, but that, when the blood in the arteries was impeded in its course, the continued and strong propulsion from the heart caused it to form new canals, where the fish's skin made the least resistance…"

Parece ter sido, mais uma vez, o primeiro a observar o que seria a activação da angiogénese numa situação de auto-regeneração vascular localizada [62]:

"At another time, I observed an appearance of a different nature in the blood-vessels, which was occasioned by my having put a tadpole into a piece of clean paper, whereby a small spot in the very thinnest part of its tail stuck to the paper, and thereby received a small injury, so that some blood flowed from the wound, out of an artery which was of a size to admit about four globules of blood to past through it at a time. The blood thus slowing out, remained collected about the wounded part; but here another sight presented itself, which engaged all my attention; for, in this same artery, at about the half of an hair's breadth distance from the wounded part, another small branch appeared, wherein the blood pursued its course in the same uniform and distinct manner, as if the artery had remained uninjured."

Observação final

Seria de crer que a importância e diversidade dos estudos pioneiros de Leeuwenhoek tivessem justificada repercussão no meio académico contemporâneo. Assim não sucedeu. Exceptuando o bom acolhimento geral e reconhecimento recebido da Royal Society (ainda que com alguns hiatos referidos) e, ainda, no seu país e entre individualidades estrangeiras (muitas das quais o visitaram por diversas vezes para observar directamente as suas descobertas), os resultados de Leeuwenhoek foram recebidos com alguma controvérsia na época. Por conseguinte, o conjunto da sua obra permaneceu ignorado até meados do século XIX, quando o município da sua cidade natal promoveu uma homenagem comemorativa . Porém, o renascido interesse pelas investigações de Leeuwenhoek ficou a dever-se à monografia publicada por um seu cultor inglês [63].

Aparte os muitos e diversificados resultados que deixou em registo escrito e desenhado, Leeuwenhoek, ao confirmar a existência os canalículos da rede capilar (já antes observados por Malpighi), e ao descrever as diversas representações assumidas pelos eritrócitos, e as possíveis variações da fluidez do sangue em estados de doença, contribuiu decisivamente para a definição do modelo da circulação proposto por Harvey e para o início da conceptualização hemorreológica do fluxo sanguíneo.

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ENCONTRO RENAL 2010

CURSO

"Risco Cardiovascular 2010 - O que importa saber?"

27 de Fevereiro

CENTRO DE CONGRESSOS DE VILAMOURA, PORTUGAL
HOTEL TIVOLI - PORTUGAL

O Curso decorrerá no sábado, 27 de Fevereiro, durante o Congresso Português de Nefrologia de 2010, que se realizará em Vilamoura de 24 a 27 de Fevereiro de 2010.

1º MÓDULO: 09.00h - 11.00h
Abordagem do risco cardiovascular no doente hipertenso com múltiplos factores de risco.
Moderador: Carlos Perdigão

1. Apresentação de caso clínico – Vera Martins
2. Estratificação do risco e controlo da pressão arterial – Carlos Perdigão
3. Padrões alimentares e controlo do peso – Maria Paes de Vasconcelos
4. Controlo glicémico – João Sequeira Duarte
5. Controlo lipídico – Alberto Melo e Silva

2º MÓDULO: 11.30h - 13.30h
Abordagem do doente com síndrome metabólica
Moderador: Alberto Melo e Silva

1. Apresentação de caso clínico - Helena Febra, Catarina Empis
2. Controle do peso e alimentação - Maria Paes de Vasconcelos
3. Actividade física – Joana Carvalho
4. Intensidade da intervenção farmacológica – Manuela Fiúza
5. Como actuar na aterosclerose subclínica – Cassiano Abreu Lima

3º MÓDULO: 14.30h - 16.30h
Prevenção cardiovascular após enfarte do miocárdio
Moderador: Cassiano Abreu Lima

1. Apresentação de caso clínico – Vera Martins
2. Alimentação e estilos de vida – Maria Paes de Vasconcelos
3. Papel da actividade física – Joana Carvalho
4. Controlo dos factores de risco – Carlos Perdigão
5. Cardioprotecção – Carlos Aguiar

4º MÓDULO: 17.00h - 19.00h
Risco cardiovascular e comorbilidades
Moderador: José Vinhas

1. Controlo dos factores de risco no doente com insuficiência cardíaca – José Carlos Silva Cardoso
2. Controlo dos factores de risco no doente com doença arterial periférica – Luís Mendes Pedro
3. Controlo dos factores de risco na criança obesa – Helena Fonseca
4. Controlo dos factores de risco no idoso diabético – João Sequeira Duarte