ACTA PHLEBOL 2015;16:41-51

Frogs' lungs and Malpighi's discovery of pulmonary capillaries

L. TONETTI

Department of Philosophy, Sapienza University Rome, Italy

In 1661, Marcello Malpighi (1628-1694), one of the most important 17th-century anatomists and physicians, published two epistles entitled *De pulmonibus* (On the lungs), both addressed to Giovanni Alfonso Borelli (1608-1679). In the first letter, thanks to the help of the microscope, Malpighi proved that the lungs were not fleshy, but that they had a vesicular structure. Moreover, with these new anatomical findings, he also revised the traditional view on pulmonary function: lungs do not cool the heart, as was previously supposed, but mix the blood. In the second letter, Malpighi decided to perform dissections of frogs. Assisted by his colleague and friend Carlo Fracassati (1630?-1672), he observed the arteriovenous anastomoses in the pulmonary circulation. Magnifying tools and new anatomical procedures allowed him to prove not only this mutual union of arteries and veins, but also that blood moves in opposed directions: thanks to these observations, Malpighi provided a strong evidence supporting Harvey's theory of blood circulation. However, Borelli, whose collaboration was pivotal, challenged Malpighi's view on pulmonary function. According to him, lungs do not mix blood, but divide blood particles into their smallest parts. Therefore, their function is not that of mixing, but that of generating blood, due to the triggering action exerted by the air particles. This paper aims at analysing all these issues within Malpighi's research program and showing the progress achieved by the Italian microscopic anatomy in the second half of 17th century.

Key words: History, modern 1601 - Anatomy - Lung - Arteriovenous anastomosis.

The 17th-century Italian research on anatomy was developed and improved by the Italian anatomist and physician Marcello Malpighi (1628-1694), who introduced the use of the microscope and focused his works on the key role played by microstructures.

The two letters *De pulmonibus* (On the lungs),[1-3] both published in 1661 and addressed to Giovanni Alfonso Borelli (1608-1679), represent not only Malpighi's first contribution to anatomy, but also his first attempt to implement the principles of his medical program. Thanks to Borelli's suggestions, Malpighi examined the structure and function of the lungs and, by the use of the microscope, provided an important evidence to support Harvey's theory, showing the anastomoses between arteries and veins in the pulmonary circulation.

The aim of this paper was to briefly reconstruct the genesis of Malpighi's discovery, focusing on three points: 1) lungs' vesicular structure; 2) the enigma of *rete mirabile*; and 3) the new interpretation of lung's function. In addition, I will analyse Borelli's objections to Malpighi's explanation of lungs' function.[4, 5]

Updating the traditional representation of the lungs

Marcello Malpighi carried out his anatomical observations on lungs in 1660 in Bologna, after three years (1656-1659) of apprenticeship in Pisa. This stay represented a key period for his career as anatomist: in fact, in Pisa he developed his philosophical orientation and improved his anatomical skills thanks to the collaboration with Giovanni Alfonso Borelli,[6, 7] an Italian physicist, mathematician, and physiologist. As Malpighi wrote in his autobiographical sketch *Vita a seipso scripta*,[8] Borelli educated him to the *libera philosophia*, by discussing the new philosophical questions from atomism and mechanistic program, and allowed him to approach the "new anatomy", thanks to his

Corresponding author: L. Tonetti, Department of Philosophy, Sapienza University, Rome, Italy. E-mail: tonetti.luca@gmail.com

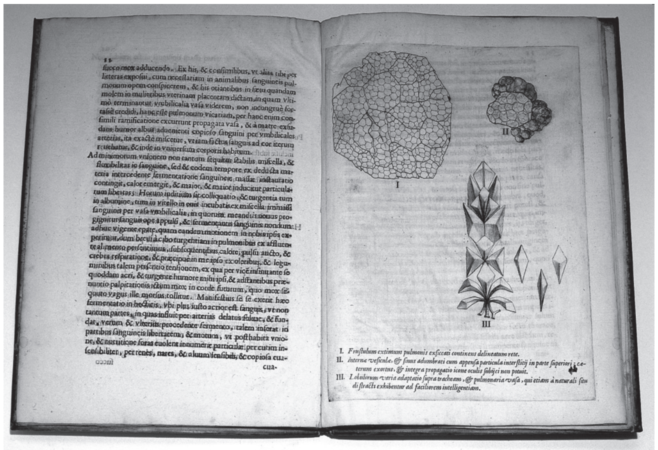

Figure 1.—Malpighi's anatomical table on the structure of the lungs.[11] (I) shows the "rete mirabilis" and (II) shows the vesicles and "interstitia". The last picture, (III), shows the lobules and their insertion.

circle. Probably, in the last years of 1650s, Malpighi started using also the microscope, although he did not talk about it explicitly in this section of his autobiography. After his return to Bologna, he worked jointly on experiments on live animals with Carlo Fracassati (1630?-1672),[9] an anatomist and lecturer at Bologna University (studium) too, and, finally, in 1660 he began his research on fishes' lungs, in order to study the motion of blood and the praecordium. These observations enabled him to completely modify the traditional representation of the lungs, with respect to their structure and function.[10]

At this stage, Malpighi realised that the substance of the lungs is very different from what was traditionally conceived: it is not "fleshy" and, therefore, it does not originate from the blood, as previously supposed. On the contrary, the lungs have a vesicular structure, similar to the honeycombs of bees, made of cavities and membranes. In the first letter to Borelli, he described lungs as follows:

"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".[3]

Three evidences supporting this view come from the use of both magnifying tools and new analytical procedures, such as fluid injection, insufflation and drying:

— thanks to the help of the microscope, he could demonstrate that when lungs are removed from animals and inflated with air, the vesicles are full of air;

— after inflation and drying, the external surface of the lungs shows vesicular prominences;

— when lungs are extracted from living animals, and cleaned up from the blood with water injections, they show an almost transparent substance. After the excess of water is removed with a small compression, and the lungs are inflated with air and then sun-dried, a white aggregate of vesicles is clearly visible.

Moreover, Malpighi supposed that this mass of vesicles, similar to a sponge, was the "continuation of the internal membrane of the trachea", so to ensure a continuous path to the air entering the lungs.

Overall, in his description of the lungs, Malpighi followed a morphofunctional criterion of "form and position". The lobes, which divide the pulmonary mass, can be further divided into a sort of unit of anatomical organisation named "lobule". Each lobule has its own internal structure: a membrane, a subsystem of vessels and a connection with the prolongations of the arteria aspera, *i.e.*, the trachea. However, Malpighi did not explain it fully: obviously, the lobules can be separated and easily identified by insufflation, incision or boiling. Nevertheless, they are "almost innumerable" and it is impossible for him to show their insertion into the trachea "for they vary under diverse circumstances". Finally, the "interstitial cavities" complete lungs' structure: although Luigi Belloni, an Italian historian of medicine, considered them an artefact produced by endotracheal insufflation technique,[2] they are not useless "bare vacuities and empty spaces". As we will see, in Malpighi's opinion, interstitia are actively involved not only in breathing mechanics, but also in the mixing of blood mass, due to their force of compression (Figure 1).

"Sacrificing an entire race of frogs…": the second letter to Borelli

There are two main problems that are not fully addressed within the first letter: 1) the enigma of the *rete mirabile*; 2) the existence of anastomoses between arteries and veins in pulmonary circulation.

As regards the first problem, Malpighi observed that the external and internal parts of a portion of the lung (in Latin, a *frustulum*), once exposed to the light, show a "mirabile quoddam rete", a wonderful network that seems to wrap all the vesicles. However, since he was not able to provide an explanation for its structure and function, he formulated three hypotheses:

— the network consists of vessels;
— the network consists of something nervous;
— the network is nothing but the membranous walls of the vesicles.

He provisionally chose the second hypothesis, as visible in the following passage:

> "For, in the interior cut part, certain nervous prolongations of this network seem to remain, either from particles turning out in drying, or lightly abraded by the knife, and in the outer part a certain shining, of the kind proper to the substance of nerves, seems to be observed. Hence I cannot deem it unlikely to be a nervous ligament of the vesicles united and mingled with the walls, as we see the semicircular cartilaginous prolongations in the trachea aspera, especially as it is probable, as I have said already, that those vesicles are continuations of the internal membrane of the trachea".[3]

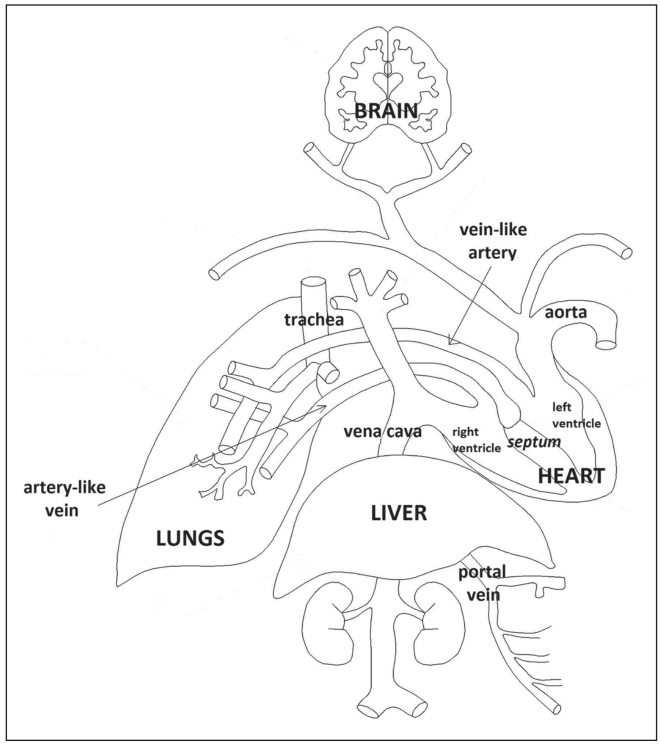

Figure 2.—Galen's blood system.

Malpighi introduced the second problem when he briefly mentioned the function of the pulmonary vascular system (trachea, pulmonary artery, pulmonary vein). We should explore this topic in some detail, because the pulmonary vascular system, in particular the relationship between the heart and the lungs, has played a pivotal role in Galen's physiology. Throughout the 17th century, in fact, Galen (AD 129-200/216) was the symbol of orthodoxy in medicine and the main source of medical learning. However, since the interpretations about this topic are not uniform and the secondary literature is vast, my historical reconstruction is necessarily partial.[11-13]

Galen defended a "dualistic conception" of the blood: there are two main distinct systems, the hepatic-venous system and the cardio-arterial one. This statement implies that: 1) there are two types of vessels, arteries and veins, divided according to the side of the heart which they are connected with; 2) the veins are the vessels connected with the right side, the arteries are those connected with the left side; 3) the arteries are full of blood, contrary to what Erasistratus claimed; 4) veins carry nutritive blood from the liver; 5) arteries carry vivified blood from the heart (left ventricle). Moreover, Galen claimed that:

— the blood does not circulate, but it is distributed along the parts of the body in order to feed them, just as the water irrigated fields;
— what makes possible blood's motion is not a pumping heart, but the attraction exerted by the peripheral tissues as well as the thoracic pressure.

These are the main principles on which Galen founded his theory.

Figure 2 represents Galen's blood system schematically. The stomach processes the food and converts it into a humour, the chyle, which needs further elaboration. The portal vein drains it to the liver that, since it is the organ responsible for the production of blood, turns the chyle into blood. The newly produced blood is ready to feed the upper and lower parts of the body through the vena cava, the vessel that originates from the hepatic veins, in the convex side of the liver. By the vena cava inferior, a part of the blood reaches also the right ventricle of the heart. The lungs totally depend on the right ventricle that provides them with the necessary nourishment, because they cannot receive the blood directly from the vena cava. For their own sake, Nature created a special vessel, the "artery-like vein", with a semilunar valve preventing the blood from going back. Galen adopted an anatomical vocabulary that may be ambiguous today: the pulmonary artery was named "artery-like vein" because, like a vein, it conveys nourishments, but it has the structure and the properties of an artery, *i.e.*, a thick coat able to protect the vessel from thoracic movements, and to filter the purest parts of the blood. Conversely, the pulmonary vein was named "vein-like artery" because, like an artery, it carries the pneuma to the heart, but it has the structure and the properties of a vein, *i.e.*, precisely one thin coat, able to withstand the thoracic pressure.[14]

At this point, it would be legitimate to ask Galen two questions: 1) how does the blood pass from the right to the left ventricle, in order to be further processed? 2) What role does the vein-like artery (pulmonary vein) play? Galen answered the first question by supposing the porosities of the interventricular septum: the blood would pass from the right to the left ventricle thanks to the pores of the septum that allow only the purest and thinner parts of the blood to pass through. However, does the blood pass through the pulmonary vein, too? Has Galen just guessed a form of pulmonary transit? We cannot answer these questions here and retrace the whole debate on pulmonary circulation's discovery, since we should assess how Arabic (Ibn Al-Nafis) [15] and Renaissance anatomists (Vesalius, Servetus, Colombo) [16, 17] have contributed to this problem. Nonetheless, it is worth to remember Galen's conclusions: first, the vein-like arteries (pulmonary veins) are strictly connected with the artery-like veins (pulmonary arteries) and the tracheo-bronchial system. Therefore, respiration (the lungs) and blood system (the heart) are strictly interrelated. Secondly, Galen supposed that there are the anastomoses between the vein-like arteries and the artery-like veins at the level of their capillaries in order to exchange blood and pneuma. We may conclude that, probably, being the pulmonary vein similar to an artery, it also includes a small portion of blood, although the septum still continues to be the left ventricle's main source of blood.

All these issues are involved in Malpighi's first letter, albeit implicitly. Several procedures for lung dissection would enable anatomists to study how the pulmonary vessels, even the smallest ones, branch out in the lung mass. Nevertheless, it is impossible for Malpighi to identify the anastomoses and, therefore, to prove their existence. He wrote:

> "Whether these vessels have mutual anastomosis in the sinuses or elsewhere, that thus the blood may be taken in by the vein by a continuous path, or whether they all gape into the substance of the lung, is doubtful and troubles my mind".[3]

Injection methods by mercury or coloured fluids, suggested by Borelli, do not reveal anything but the fact that "the introduced fluid makes more ways for itself which are not usual in the state of health". Those ways marked by the introduced liquids would seem artificial rather than natural.

Therefore, in summary, Malpighi answered the two problems just described above in this way:

— the rete mirabile consists of something nervous;

— it is impossible to show anastomoses among the pulmonary vessels.

The second letter to Borelli is a turning point: Malpighi realised that the two problems are strictly related. Assisted by his colleague Fracassati, he planned his new observations accurately, choosing the frog as animal model, and run so many dissections, as to be compared to a real "sacrifice" of the race of frogs, greater than the one described by Homer in *Batrachomyomachia* (Battle of frogs and mice).

Malpighi organised his observations in three steps. First, he analysed the lungs of the frog from a macroscopic point of view (first step), pointing out their "structure and connection". The mere observation of the abdomen dissected corroborated his view on lungs' substance (Figure 3):

> "They (the lungs) are nothing more than a membranous bladder, which at first sight seems to be spattered with very small spots, arranged in order after the fashion of the skin of the dogfish - commonly called Sagrino. In form and surface protuberances, it resembles the cone of a pine: but internally and externally, a certain texture of vessels diversely prolonged is connected together, which, by the pulse, by contrary movement, and the insertion of the vein, are pulmonary arteries. In the concave and interior part of this (bladder) it almost fades into an empty space devoted to the reception of air, but it is not everywhere smooth but is interrupted by the occurrence of alveoli. These are produced by membranous walls raised to a little height".[3]

Then, he proceeded with the microscopic investigation (second step). Contrary to Young's translation, at this point of his observation, Malpighi does not seem to use the microscope, yet, as we can see by referring to the Latin text and the English translation:

"His visis ad meram structuram, & compagem attinentibus mirabiliora, microscopica deteget observatio" [1] -(Observation by means of the microscope will reveal more wonderful things than those viewed in regard to mere structure and connection).[3]

Malpighi is talking about "observatio microscopica", rather than the microscope itself. This shade of meaning is not useless. In fact, here, I believe we can grasp a key aspect of his method: this passage can just be interpreted as a clear statement of his "microstructuralism".[19] In other words, according to Malpighi, anatomists should not consider the body and its parts as a whole, but in their constituents. Organic processes depend on "microstructures": identifying them and understanding their proprieties and functionality are necessary to infer how the body actually works. Malpighi wants to suggest a different and analytical point of view, also known as "resolutio ad minitum". "Resolutio" does not imply necessarily the use of magnifying tools. Rather, it involves a new philosophical perspective, that of "reduction" of the bodily parts to their components. Moreover, it is worth to highlight that "observatio microscopica" focuses on action rather than structure: this explains why Malpighi does not start to observe the structure of vessels, their conformation or connection, but the physiological activities, in which they are involved, *i.e.*, the blood motion in contrary directions. These observations could prove blood circulation. He noticed that the blood passing through the arteries and their smallest branches diffuses everywhere until the veins absorb it. Nevertheless, because of the limitation of sight, it is not possible to identify precisely anastomoses between arteries and veins:

"The power of the eye could not be extended further in the opened living animal, hence I had believed that this body of the blood breaks into the empty space, and is collected again by a gaping vessel and by the structure of the walls".[3]

This view is rapidly questioned by the use of the microscope ("perfectiori vitro oculis"). By magnifying a frog's dried lung (third step), Malpighi observed that arteries and veins branched so much that they form a sort of network: blood does not pour out in an empty space to be then absorbed by the veins, rather, it always passes through the branches of this network. Thus, Malpighi discovered the anastomoses:

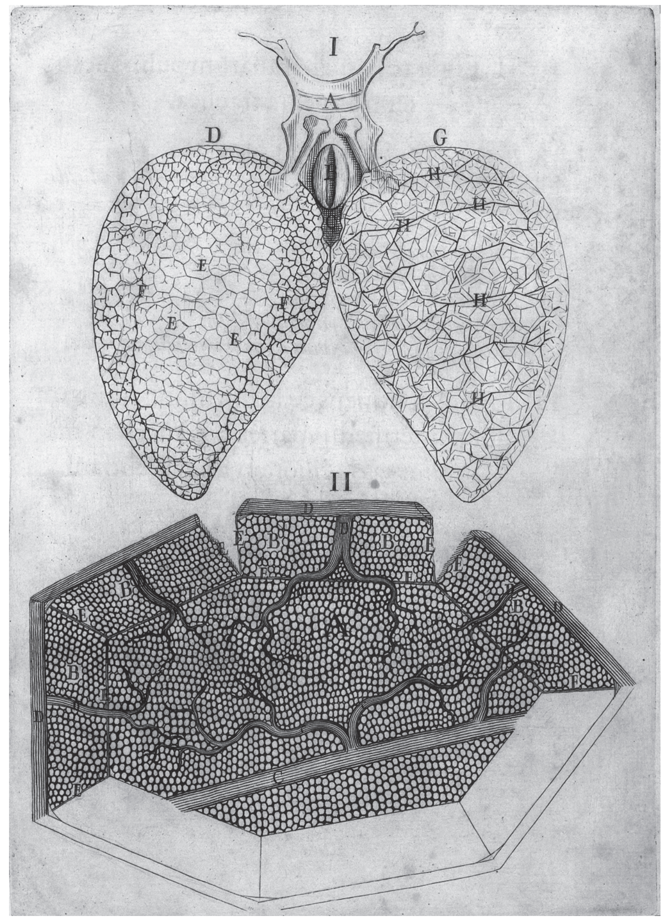

Figure 3.—Malpighi's anatomical table on the frog's lungs.[18] The picture (II) shows the anastomoses between the pulmonary artery (C) and the pulmonary vein (D).

"Here it was clear to sense that the blood flows away through the tortuous vessels, that it is not poured into spaces but always works through tubules, and is dispersed by the multiplex winding of the vessels. Nor is it a new practice of Nature to join together the extremities of vessels, since the same holds in the intestines and other parts; nay, what seems more wonderful, she joins the upper and the lower ends of veins to one another by visible anastomosis, as the most learned Fallopius has very well observed".[3]

Now, Malpighi can answer the two questions:
— the network previously believed to be nervous consists, instead, of vessels and allows the blood to pour out from the arteries to the veins;
— this network proves also the existence of a mutual union of arteries and veins.

These conclusions can support Harvey's theory only generalising these observations on frogs to all living beings, because of Nature's law of uniformity. As the Italian historian of medicine Domenico Bertoloni Meli says in his outstanding book on Malpighi:

> "By relying on analogy and on the widely held assumption of nature's uniformity and simplicity, Malpighi generalized his results on the lungs of some animals to the anastomosis of arteries and veins in the other parts of the body and all animals: he relied extensively on this crucial assumption in the rest of his work."[10]

I will try to briefly analyse some of these philosophical assumptions in the last section of this paper.

From structure to function: mixing blood

These new anatomical findings enabled Malpighi to challenge the traditional view on pulmonary function. Ancient scholars argued that the lungs were responsible for the cooling of the burning heat of the heart. This is, for example, what we read in one of the Plato's dialogues, *Timaeus*:

> "[70c] […] And as a means of relief for the leaping of the heart, in times when dangers are expected and passion is excited - since they knew that all such swelling of the passionate parts would arise from the action of fire -, they contrived and implanted the form of the lungs. This is, in the first place, soft and bloodless; and, moreover, it contains within it perforated cavities like those of a sponge, so that, when it receives the breath and the drink, it might have a cooling effect and furnish relief and comfort [70d] in the burning heat".[20]

Aristotle analysed this problem in depth in some of his minor works, such as *De juventute et senectute, De respiratione, De vita et morte* (On youth and old age, on breathing, on life and death),[21] deducing the cooling function and, consequently, the final cause of breathing from the importance of natural heat for life: "Life and the presence of soul", he said, "involve a certain heat" (474^{a} 25). All the organic processes depend on the "fire" that, however, might be managed responsibly, because it could cease to exist by "extinction" or by "exhaustion" (469^{b} 21-470^{a} 18). Anyway, "if the bodily heat must be conserved (as is necessary if life is to continue), there must be some way of cooling the heat resident in the source of warmth" (470^{a} 5-7). Galen confirmed this view: he supposed that the heart was hotter than the other organs of the body and, therefore, it was the seat of the innate heat. It should be remembered that the connection between tracheobronchial system and pulmonary vessels (vein-like artery and artery-like vein) entailed a strict relationship between respiration and blood system: on one hand, the lungs depend on the right ventricle for nourishments; on the other hand, the heart depends on the lungs for two tasks, cooling the innate heat as well as exhaling smoky waste. As we can notice, Galen assumed a double movement occurring in vein-like arteries: the pulmonary vein inhales the pneuma to the left ventricle and exhales warm and smoky vapors to the lungs, in order to expel them through the trachea.

Malpighi refuted this thesis: the lungs' function is not that of cooling the natural heat in the heart, but that of mixing blood. How did he come to this conclusion? The answer to this question is to be found in the development of anatomical knowledge in 17th century. The discovery of Gaspare Aselli (1581-1625) of the milky veins in 1622, although it was still framed in the Galenic tradition, questioned one of the most important "tenet" of Galen's anatomo-physiology: the "hematosis" in the liver. In other words, as already explained, Galen ascribed to the liver the function of transforming the chyle received by the portal veins into venous blood. Nevertheless, 17-century experiments on milky veins demonstrated the flow of chyle was very different from the traditional conception. In particular, after Aselli's observations, the French anatomist Jean Pecquet (1622-1674), in his *Experimenta nova anatomica* (1647), showed irrefutably that the chyle carried by the milky veins does not pour out in the liver, but in a "receptaculum" also known as "reservoir or cistern of Pecquet". This cistern is the base of the thoracic duct, which carries the chyle in the left subclavian artery, not in the liver. The implications are obvious: the liver does not produce the blood, as previously claimed by Galen. Consequently, the dualistic blood view falls too. Further studies on lymphatic system run by the Danish physician Thomas Bartholin (1616-1680) and the Swedish scientist Olaus Rudbeck (1630-1702) enriched this framework of knowledge.

In addition, it should be noted that even the physiologists' view regarding the blood changed: blood is not one of the four Hippocratic humours, but a fluid which flows uninterruptedly in the body and which can be analysed in its smallest constituents. Malpighi himself highlights this point in his letter: "By blood", he wrote, "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".[3]

These two heterogeneous parts, which the blood consists of, are originally solid. Like metals that become fluid thanks to "aqua regia, acids and the like disuniting things interposed", they get fluidity if correctly and duly mixed. Therefore, the blood fluidity is obtained through the right mixture of the particles that compose it. Otherwise, it solidifies. Lungs do exactly what women do to prevent blood clotting: they "crush it [the blood] 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".[3] Malpighi came to this conclusion thanks to the new anatomical data available at that time. The lungs receive the blood returning from the whole circuit by the pulmonary artery, and both the chyle and the lymph by the thoracic ducts. These two different substances cannot be mixed in the right ventricle of the heart: only the lungs can do it perfectly. Why? The reason is that: 1) the vessels branching through lung mass first force the division of the particles, then, due to their particular structure, combine them to form a new substance; 2) the air pressure exerted on the vessels by the alveoli mixes further these substances; 3) the fermentation occurring in the blood produces heat that increases the freedom of the particles.

In the light of what has been said, it is clear that Malpighi's explanation of lungs' function rests on a firm corpuscularistic perspective. However, as we will see in the next paragraph, Malpighi received several objections by Borelli.

Borelli's objections

In a letter dated February 18, 1661, Borelli challenged Malpighi's view on pulmonary function.[22] He claimed that the way the lungs work could not be simply compared to a sort of "pestle" aimed at crashing the white and the red, and mixing them. He did two examples to clarify his position.

Imagine two different fluids, water and black ink. The water flows in a large tube; the ink in a small tube. Now, add the small tube inside the large one in such a way that the ink can pour into the water: how do the two fluids mix themselves? Probably, there are only two ways: shaking them with irregular movements by a spoon or something else, or pressing the tube.

Now, imagine only a large tube branching in several small and thin channels: it is impossible to get the same mixture. In fact, in such a large tube, not all the water will be mixed with ink and vice versa. Therefore, some unmixed water will enter some of the small channels, and the same will happen to the ink. In order to mix them appropriately, the channels should be intertwined, so that one fluid can meet the other. Furthermore, the ink and the water should be poured together in a common vessel that, once compressed, finally may facilitate their mixture. This solution violates Nature's principle of simplicity.

These two examples were formalised by Borelli in the second part of his masterpiece *De motu animalium*, more precisely in chapter eight, which is devoted to the primary function of respiration. Among the erroneous opinions claimed by ancient and modern scholars, he challenged also the one availed by Malpighi, although he did not quote him directly. Not only respiration (1) does not cool the fire and the heat in the heart (proposition 96) and (2) does not expel the smoky vapours produced by the fire through expiration (prop. 97), but also neither (3) it mixes the heterogeneous particles of the blood (prop. 107-108), nor its main purpose is (4) transfusing the blood from the right to the left ventricle (prop. 111).[5]

Three propositions are to be assumed to prove Malpighi's mistake. First, in prop. 100, Borelli stated that "If part of a bag is occupied by white seeds and the bag is not completely filled by additional black seeds, both seeds can be mixed by repeated compression and squeezing of the bag".[5] If we look at figure 4A, we can see that the right part ALFCMB is completely filled with white

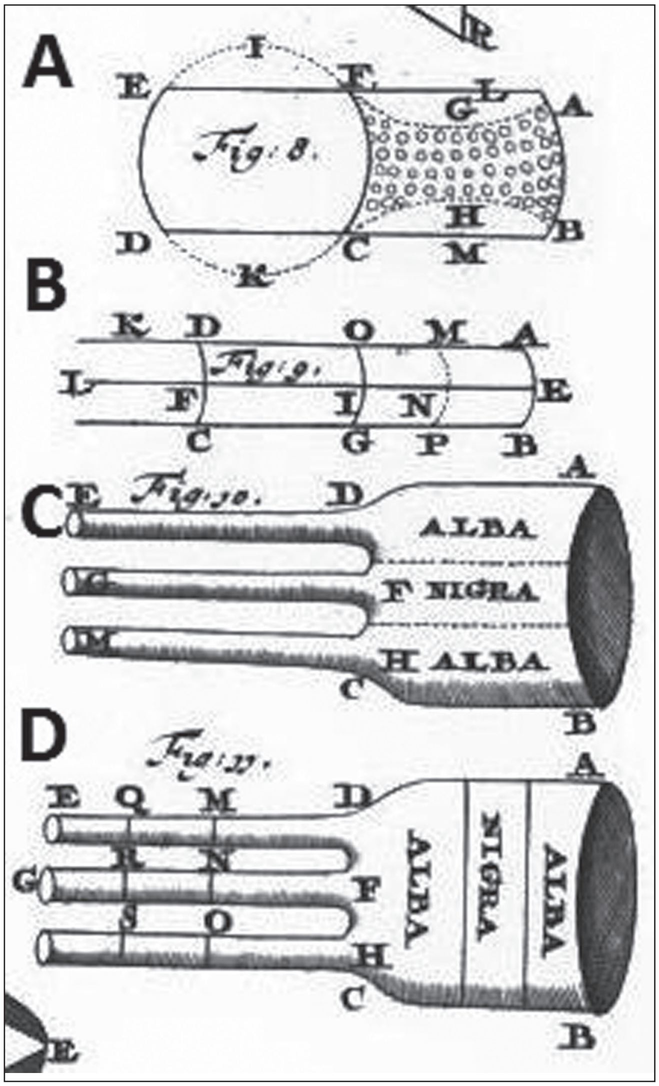

Figure 4.—Diagrams from "*De motu animalium*" (table XVIII).[23]

seeds, while the left part FEDC is incompletely filled with black ones. If we compress the right part in such a way that the area ALFCMB is reduced to AGFCHB, we can observe that the white seeds in ALFG and BHCM pour out in the left side, expanding it at the points I and K. Probably, at first, black seeds will move to I and K, but by repeated compressions, they will mingle with the white ones. The bag must not be rigid; otherwise, the mixture cannot occur (prop. 101).

Since liquids behave like particles, we try to replace the two seeds with two different fluids (prop. 103): "If there are two liquids, one white AG and the other black GD, in the narrow and soft canal AC and if one extremity DC is open and the other AB is closed, I claim that the liquids will not mix as a result of squeezing and compressing the canal".[5] In Figure 4B we can see a thin tube AC filled with two different fluids, AG (white) and GD (black), and the two sides, AB and DC, assumed to be respectively closed and opened. The mixture does not occur because, if we try to squeeze the tube reducing the area from ABCD to AEFD, the white fluid AG will move towards DF, the other end AB being closed, but the same will happen also to the black fluid GD. In other words, both can meet together if and only if the black liquid GD moves towards O, which is impossible from a physical point of view. Therefore, the two fluids cannot touch each other and, consequently, be mixed.

Now, Borelli proposed the second example of his previous letter of 1661 (prop. 105):

> "The conical and soft funnels ABCD is subdivided into several other funnels DE, FG, HM and into other thinner branches. Its base AB is closed. Fluids or white and black seeds are sent separately into the large funnel and they are compressed and shaken. I claim that they will not mix".[5]

Since seeds or liquids are separately arranged into the pipe (Figure 4C), only the white ones will enter DE and HM, and the black ones will enter FG, no matter how many times we try to compress the pipe or the small channels. No mixing occurs, as it happens to the water and the black ink of the example previously described. Even changing the arrangements of the layers, as in Figure 4D, white seeds/liquids will not mingle with black ones.

With prop. 100, 103, and 105, Borelli concluded that, contrary to what Malpighi claimed, "It is impossible that the heterogeneous components of the blood mix completely in the lungs however squeezed they are" (prop. 108).[5] Suppose two different kinds of particles, A and B. In order to have a successful mixture, opposite movements should push A-particles among B-particles. This is possible only in a bag-shaped place, as in Figure 4A. However, the lungs are very different because they consist of branches and small channels, with an extremity opened and the other closed, as in Figure 4B. For the reasons just explained in prop. 103, no mixing can occur. Similarly, by prop. 105, no mixing can occur also because lungs' vessels behave like the small channels in Figure 4C and 4D. Borelli wrote,

> "Consequently, although the movement of breathing squeezes and beats the blood vessels of the lungs, this does not imply that the heterogeneous elements of the blood must be mixed. Neither is the structure of the lungs such that intimate mixing of the components of the blood can be carried out in them".[5]

According to Borelli, in fact, lungs do not mix properly the blood, but divide the particles into their smallest parts (prop. 109). In fact, the blood entering the pulmonary artery is nothing but an altered mixture of different liquids, which requires the rearrangements of all their particles. Otherwise, a part of them could coagulate or would not be able to be distributed along the body. Furthermore, Borelli pays attention to the function of air. Empirical observations teach that inhaled air does not enter the veins nor, in any case, does it pass through the pores of the vessels. The mixing of blood with air seems to be untenable. However, the experiment of Torricelli shows that air can be mixed with a liquid and, in particular, with water. Foam is a good example. Therefore, Borelli believes that air mixes with the serous juice in the lungs' alveoli. The serous juice, consequently, becomes foamy. Now, this liquid, which inevitably includes small particles of air, can enter the pores of the veins and mingle with the blood (prop. 113). These particles act as spiral machines "which can be compressed by an external force and then spontaneously resile like springs" (prop. 125).[5] Once mixed with the blood, they produce oscillatory movements that continuously shake the blood. According to Borelli, the main function of the lungs is to generate blood, due to the triggering action of the particles of air. Thus, the altered blood entering the pulmonary artery, once divided in its minute parts, filtered, and mixed with aerial particles, gets the right mixture and composition. "From there", he said, "the portions of blood rejuvenated, vivified by spirits transmitted by the nerves and activated by the aerial machines are carried to the main trunk of the pulmonary vein which discharges them in the left ventricle. The blood then is ejaculated with a powerful force and distributed through the whole body of the animal" (prop. 129).[5]

Conclusions: *On the lungs* within the Malpighi's research program

Malpighi provided a clear definition of his medical program in his reply to Giovanni Girolamo Sbaraglia (1641-1710), a Bolognese conservative physician devoted to a radical form of "empiricism", who challenged him in a brief and controversial dissertation entitled *De recentiorum medicorum studio*. Sbaraglia claimed the uselessness of anatomy for the medical practice and, in a broader sense, the effectiveness of the empirical medicine over the rational. The Latin words "inutiles amputans", engraved on his commemorative medal, summarize his methodological concern: everything that is not strictly useful for therapy should be excluded from the medical practice. This is exactly the case of the anatomical investigations that occupied most of the rationalist physicians in the mid-seventeenth century. This clash between empiricism and rationalism, a *fil rouge* in the history of Western medicine from the Ancients to the Moderns, hits Malpighi at the end of his career as anatomist as well as clinician.[24-26]

In 1689, in his answer to Sbaraglia published posthumously in 1697, Malpighi defined the main features of his program:

> "Therefore, in the things of nature, which operates always uniformly by necessity, the sagacity of man is not of such a small character that cannot get to reveal many of his artifices. Thus, we see with admiration the discoveries in astronomy […]. We can say the same thing of the machines of our body, which are the basis of medicine […]. Man, examining these parts with the anatomy, learned by philosophy and mechanics, grasped their structure and their use, and proceeding in the same way *a priori*, came to build models of them, through which he reveals the causality of that effect and gives it a reason *a priori*".[27]

In this passage, as Bertoloni Meli says, Malpighi suggests a sort of "layered analysis" [26] that would allow physicians to understand the way Nature operates and to found both physiology and pathology. Strictly speaking, the art of medicine should be reformed according to a new method, which collects and processes the contributions from different fields of knowledge: the new anatomical findings, physics, and in particular mechanistic natural philosophy. Therefore, the "new anatomy", enhanced by magnifying tools and new techniques of investigation, compels conservative physicians to revise the traditional interpretation of the structure of most parts of the human body. The "new philosophy" invites them to explain their function in mechanistic terms. The "new physics", instead, allows them to build machines as "physical and experimental devices", in order to reproduce physiological and pathological phenomena.[28] Finally, the "new therapy", thanks to the different disciplines involved, forces them to overcome ancient therapeutics. Moreover, from a philosophical point of view, Malpighi's program rests on a key principle: the "uniformity of nature", which entails: 1) a comparative perspective; 2) the human-machine analogy; 3) necessity.

The epistles *On the lungs* are a good example. Thanks to "observatio microscopica" and the new techniques of anatomical investigation (insufflation, fluid injections, boiling), Malpighi updated the common representation of lungs' structure and discovered the anastomoses between arteries and veins in pulmonary circulation. Frog dissections were crucial because they showed bodily parts and physiological processes that, otherwise, would not be visible in "perfect" animals, like dogs or men themselves. Therefore, these new findings enabled him to reinterpret lungs' function, by questioning the traditional view claimed by the ancients (Plato, Aristotle, and Galen) as well as the moderns. Both the discovery of anastomoses and the observation of blood motion in opposed directions provide a strong evidence supporting Harvey's theory. This outstanding turning point rests on corpuscularism, suggested by Borelli: everything is explained in terms of particles and their motions. Physical proprieties depend on the particular arrangement the particles have: therefore, the lungs work as a machine that "rearrange" the main parts of the blood (the white and the red) in order to mix them and, consequently, maintain them fluid.

Malpighi's reply to Sbaraglia suggests several physical devices aimed at studying bodily processes, such as blood circulation or breathing mechanics. For example, a tube similar to an artery, filled with a fluid and then squeezed, as the heart would do, may enable physicians to study the pathology of blood circulation, cardiac arrhythmias or the variation of pulse. Anatomy and mechanics can also reproduce breathing thanks to an artificial thorax.

Finally, it should be noted the implications of this medical program on therapeutics. The epistles *On the lungs* discuss some morbid states. Obviously, contrary to Sbaraglia's claim, the new anatomical findings have an impact on pathology and therapeutics. For example, Malpighi describes blood fermentation's effects on consumptives: the excessive freedom of the blood particles, due to the fermentation, causes epistaxis, haematuria, and evacuation of the blood from the skin. This explains not only why the lungs are so damaged by tuberculosis, but also how fermentation can be reduced: milk, infusions and baths can inhibit the motions of the particles and have a positive effect on the health.

In conclusion, the focus on microstructures and their implications in medicine, which is pivotal in the two epistles *De pulmonibus*, will become a key aspect of Malpighi's work and, in particular, the interest in blood and morbid states related to it will be further developed in another important work, *De polypo cordis*.

Riassunto

I polmoni delle rane e la scoperta di Malpighi dei capillari polmonari

Nel 1661, Marcello Malpighi (1628-1694), uno dei più importanti anatomisti e medici del XVII secolo, pubblicò due lettere *De pulmonibus*, entrambe indirizzate a Giovanni Alfonso Borelli (1608-1679). Nella prima lettera, grazie all'aiuto del microscopio, Malpighi dimostrò che i polmoni non erano "visceri carnosi", ma avevano una struttura vescicolare. Inoltre, mediante i nuovi reperti anatomici osservati, corresse anche la concezione tradizionale della funzione polmonare: i polmoni non raffreddano il cuore, come precedentemente supposto, ma servono a mescolare il sangue. Nella seconda lettera, Malpighi decise di eseguire dissezioni di rane. Assistito dal suo collega e amico Carlo Fracassati (1630?-1672), poté osservare le anastomosi tra arterie e vene nella circolazione polmonare. Gli strumenti di ingrandimento e le nuove procedure anatomiche gli consentirono di dimostrare non solo questa connessione tra arterie e vene, ma anche il movimento del sangue in direzioni opposte: grazie a queste osservazioni, Malpighi esibì una forte evidenza a sostegno della teoria di Harvey. Tuttavia, Borelli, la cui collaborazione si rivelò fondamentale, mise in discussione la posizione di Malpighi sulla funzione polmonare: i polmoni non mescolano il sangue, ma si limitano a dividere le sue particelle nelle componenti più piccole. Dunque, la funzione dei polmoni non è mescolare il sangue ma generarlo, in forza del ruolo di attivazione esercitato dalle particelle dell'aria. Questo lavoro si propone di analizzare tutti questi aspetti all'interno del programma di ricerca di Malpighi e di mostrare il progresso raggiunto dall'anatomia microscopica italiana nella seconda metà del XVII secolo.

PAROLE CHIAVE: Storia della medicina moderna (1601-) - Anatomia - Polmoni - Anastomosi arterovenose.

References

1. Malpighi M. De pulmonibus observationes anatomicae. Bologna: Typis Jo. Baptistae Ferronii; 1661.
2. Malpighi M. Opere scelte. Turin: UTET; 1967. p. 71-99. (Italian translation by L. Belloni).
3. Young J. Malpighi's "De Pulmonibus". Proc R Soc Med 1929;23:1-11. (English translation by J. Young).
4. Borelli GA. De motu animalium. Rome: Angeli Bernabò; 1680-1681.
5. Borelli GA. On the movements of animals. Berlin: Springer; 1989. (English translation by P. Maquet).
6. Baldini U. Giovanni Alfonso Borelli. In: Dizionario Biografico degli Italiani. Vol. 12. Rome: Istituto della Enciclopedia Italiana; 1971. p. 543-51.
7. Montacutelli S. Giovanni Alfonso Borelli. In: Il Contributo italiano alla storia del pensiero. Scienze. Rome: Istituto della Enciclopedia Italiana; 2013. p. 259-262.
8. Malpighi M. Opera posthuma, Figuris Aeneis Illustrata. Quibus praefixa est ejusdem Vita à seipso scripta. London: A. & J. Churchill; 1697.
9. Belloni Speciale G. Carlo Fracassati. In: Dizionario Biografico degli Italiani. Vol. 49. Rome: Istituto della Enciclopedia Italiana; 1997. p. 533-535.
10. Bertoloni Meli D. Mechanism, Experiment, Disease. Marcello Malpighi and Seventeenth-Century Anatomy. Baltimore, MD: The Johns Hopkins University Press; 2011. Chapter 1, The New Anatomy, the Lungs, and Respiration; p. 31-55.
11. Malpighi M. De pulmonibus observationes anatomicae. Bologna: Typis Jo. Baptistae Ferronii; 1661. [Figure], Page 12 and facing plate; [first letter]. From: Wellcome Library, London. [Accessed 2015 March 14]. Available at: http://wellcomeimages.org/indexplus/image/L0021258.html
12. Harris CRS. The heart and the vascular system in ancient Greek medicine: from Alcmaeon to Galen. Oxford: The Clarendon Press; 1973. Chapter 6, Galen; p. 267-396.
13. For a review of the dispute on the "rete mirabile" and the discovery of pulmonary circulation in Galen, see Prioreschi P. Galenicae Quaestiones Disputatae Duae: rete mirabile and pulmonary circulation. Vesalius 1996;2:67-78.
14. Galen. L'utilità delle parti (*De usu partium*). In: Galen Opere scelte. Torino: UTET; 1978. Libro 6; p. 434-87. (Italian translation by I. Garofalo and M. Vegetti).
15. West JB. Ibn al-Nafis, the pulmonary circulation, and the Islamic Golden Age. J Appl Physiol 2008;105:1877-80.
16. Pagel W. Le idee biologiche di Harvey: aspetti scelti e sfondo storico: in appendice: William Harvey rivisitato. Milano: Feltrinelli; 1979. Capitolo 5, I predecessori di Harvey; p. 140-275. (Italian translation).
17. Stefanadis C, Karamanou M, Androutsos G. Michael Servetus (1511-1553) and the discovery of pulmonary circulation. Hellenic J Cardiol 2009;50:373-8.
18. Malpighi M. De pulmonibus observationes anatomicae. Bologna: Typis Jo. Baptistae Ferronii; 1661. [Figure], Engraving: figures I and II, showing lungs and capillaries; [second letter]. From: Wellcome Library, London. [Accessed 2015 March 14]. Available at: http://wellcomeimages.org/indexplus/image/L0031660.html
19. Duchesneau F. Leibniz le vivant et l'organisme. Paris: Vrin; 2010. Chapter 1, Un modèle de référence: Malpighi et les «petites machines» organiques; p. 25-46.
20. Plato. Plato in Twelve Volumes. Vol. 9. Cambridge, MA: Harvard University Press; 1925. (English translation by W.R.M. Lamb).
21. Aristotle. The works of Aristotle (*Meteorologica*, *De mundo*, *De anima*, *Parva Naturalisa*, *De spiritu*). Vol. 3. Oxford: Clarendon Press; 1931. (English translation by W.D. Ross).
22. Borelli GA. (Pisa, Italy). Letter to: M. Malpighi (Bologna, Italy). 1661 Feb. 18. Printed also in: Adelmann HB. The correspondence of Marcello Malpighi. Vol. 1 (1658-1669). Ithaca (NY) & London: Cornell University Press; 1975. p. 65-9.
23. Borelli GA. De motu animalium, pars secunda. The Hague: apud Petrum Gosse; 1743. [Figure], Diagrams of table XVIII; [at the end of the book]. [Accessed 2015 March 14]. Available at: http://books.google.it/books?id=E5o_AAAAcAAJ&hl=it&pg=PA385#v=onepage&q&f=false
24. Cavazza M. The uselessness of anatomy: Mini and Sbaraglia versus Malpighi. In: Bertoloni Meli D, editor. Marcello Malpighi, anatomist and physician. Florence: Olschki; 1997. p. 129-45.
25. Bertoloni Meli D. Mechanistic pathology and therapy in the medical Assayer of Marcello Malpighi. Med Hist 2007;51:165-80.
26. Bertoloni Meli D. Mechanism, experiment, disease. Marcello Malpighi and Seventeenth-Century anatomy. Baltimore, MD: The Johns Hopkins University Press; 2011. Chapter 11, From the New Anatomy to Pathology and Therapy; p. 303-30.
27. Malpighi M. Opere scelte. Torino: UTET; 1967. p. 512-3.
28. Bertoloni Meli D. Machines of the body between anatomy and pathology. In: Gaillard A, Goffi J-Y, Roukhomovsky B, Roux S, editors. Modèle Métaphore Machine Merveille. Bordeaux: Presses Universitaires de Bordeaux; 2012. p. 53-68.

Conflicts of interest.—The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Received on March 16, 2015.
Accepted for publication on April 8, 2015.