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Online links

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APC | axin | β-catenin | CDK4 | CDK6 | *c*-jun | CK2 | *c*-myc | cyclin D1 | DVL | DYRK | eIF2B | FRAT | frizzled | GBP | glycogen synthase | GSK3 | IGF1 | insulin | insulin receptor | IRS1 | IRS2 | MAPKAP-K1 | mTOR | PDK1 | 6-phosphofructo-1-kinase | PI3K | PKA | PKB | retinoblastoma | S6K | Tau | WNTs
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TIMELINE

Giulio Bizzozero: a pioneer of cell biology

Paolo Mazzarello, Alessandro L. Calligaro and Alberto Calligaro

The Italian pathologist Giulio Bizzozero began his haematological investigations more than 130 years ago. Among his outstanding achievements was the discovery of the role of platelets in haemostasis and the identification of the bone marrow as the site of production of blood cells. One hundred years after his untimely death, the significance of these, and many more of his findings, is still recognized.

In the 4 January 2001 issue of *Nature*, John L. Heilbron and William F. Bynum rightly acknowledged Giulio Bizzozero (1846–1901) — "the Italian microscopist who described red blood cells forming in the bone marrow and platelets circulating in the bloodstream"[1] — among the men of science and scientific events that they recommended for anniversary recognition. Bizzozero has also been recognized as one of the leading Italian biologists of the last two centuries by Neidhard Paweletz in his recent article on the birth of life science in Italy[2]. These are among the few references in the recent international science press that give credit to an outstanding figure of eighteenth-century biology and medicine, who made important discoveries and exerted a central role in promoting medical and biological studies in Italy.

The making of a scientist

Giulio Bizzozero, the son of Felice Bizzozero, a small-time industrialist, and of Carolina Veratti, was born in Varese, not far from Milan, into a middle-class family that was deeply involved in the *Risorgimento* — the social and political activities directed towards the liberation of Italy from Austrian domination. In 1859, while his older brother Cesare fought against Austria, his mother volunteered as a nurse at the Hospital of Varese, which was full of war-wounded patients.

Bizzozero grew up in a very stimulating cultural environment, and in 1861, after his high-school classical studies, he enrolled at the University of Pavia as a medical student[3,4,5] (BOX 1). During his medical-student training, Bizzozero worked for a year in the Laboratory of Physiology under the supervision of Eusebio Oehl, a steadfast advocate of microscopic research. Enrico Sertoli — who identified the cells of the seminiferous tubules of the testis — was also trained in this laboratory. Soon afterwards, Bizzozero began to carry out histological and histopathological research under the direction of Paolo Mantegazza who, in 1861, had founded the Laboratory of Experimental Pathology — the first of its kind in Italy. Very gifted as a scientific illustrator, Bizzozero helped Mantegazza to prepare his publications by drawing illustrations of histological preparations.

In June 1866 at the age of 20 (FIG. 1), having published several papers on different topics — including the morphology of bone marrow, the structure of skin, bones and cerebral neoplasm and the development of connective tissue — Bizzozero graduated in medicine and received the Mateucci Prize, which was awarded to the student who had achieved the highest grade in all courses. On gaining his degree, Bizzozero participated as a military medical doctor in the Third Independence War against Austria. Soon afterwards, he travelled abroad to expand his scientific knowledge, visiting the laboratories of the histologist Heinrich Frey in Zürich, and the founder of cellular pathology, Rudolf Virchow, in Berlin. In 1867, back in Pavia, he began his academic career as a deputy professor of general pathology and lecturer of histology, becoming the Italian prophet of the new theories propounded by Virchow on the cell structure of living matter and on cellular pathology[4,5].

In an academic environment that was dominated at the time by old-fashioned doctrines and dogmatic teaching, which presented scientific matter as truth *ex cathedra* and scoffed at anyone who 'looked into the hole' (that is, used a microscope)[6], Bizzozero preached that science was 'knowledge in progress' that must remove its veil of mystery and authoritarianism: "…the

Box 1 | **The University of Pavia**

In the nineteenth century, the University of Pavia (pictured) was the main cultural centre in Lombardy. This dated back to 1361, following an act by Emperor Charles IV, who designated it a 'Studium Generale' and prescribed that students, rectors, doctors and functionaries should be allowed the same immunities and privileges enjoyed by the students of Paris, Bologna, Oxford, Orléans and Montpellier[3]. The Studium was renowned for the great scholars that had studied and taught there, including:

- Girolamo Cardano (1501–1576), a mathematician, who invented the cardan joint and provided the first printed explanation of a procedure for solving cubic equations
- Lazzaro Spallanzani (1729–1799), a biologist who, among others, disproved the spontaneous generation of microscopic living beings and carried out artificial fertilization in the dog
- Antonio Scarpa (1752–1832), the anatomist famous for the anatomical eponyms *Scarpa triangle* and *Scarpa ganglion* of the ear
- Gaspare Aselli (1581–1625), an anatomist who discovered the chyliferous vessels in the gut and peritoneum
- Alessandro Volta (1745–1827), the physicist who invented the voltaic pile
- Samuel August Tissot (1728–1797), a clinician famous for his works on nervous diseases
- Johann Peter Frank (1745–1821), the founder of modern public hygiene

In the nineteenth and early twentieth centuries, other famous scholars linked with the University of Pavia included:

- Camillo Golgi (1843–1926), one of the founders of modern neuroscience and after whom the 'Golgi complex' or 'Golgi apparatus' of the cell was named
- Agostino Bassi (1773–1856), the first person to succeed in the experimental transmission of a contagious disease
- Alfonso Corti (1822–1876), known for his discoveries on the anatomical structure of the ear
- Angelo Dubini (1813–1902), who identified *Ancylostoma duodenale*
- Enrico Sertoli (1842–1910), the discoverer of the cells of the seminiferous tubules of the testis that bear his name
- Adelchi Negri (1876–1912), who identified what later became known as 'Negri bodies' in the brains of animals and humans infected with the rabies virus
- Emilio Veratti (1872–1967), who described the sarcoplasmic reticulum
- Battista Grassi (1854–1925), who discovered that mosquitoes were responsible for transmitting malaria between humans, and received the Darwin Medal from the Royal Society of London
- Antonio Carini (1872–1950), who discovered *Pneumocystis carinii*, which is responsible for recurrent pneumonia in patients with AIDS

(Photograph of the seventeenth-century building of the University of Pavia kindly provided by the Museum for the History of the University of Pavia.)

teacher should not present science as a series of dogmas supported by the prestige of a name, … but instead expose it in its true condition, with its doubts and its questions"[7]. According to Bizzozero, laboratory training must allow the activity of many individuals to be put to the service of science, "…so that new discoveries, previously the privilege of an elite, are now not infrequently due to the perseverance and well-directed activity of a student"[7].

A good-humoured, dynamic young man, graceful and sociable — albeit with a pugnacious side to his character — Bizzozero became a hero to the many students and young medical doctors, often older than he was, who were beginning their scientific careers under his direction. His authority was never imposed, but was always accepted as a consequence of his magnetic personality, which gave him the hallmark of a natural leader. Among his followers was Camillo Golgi, who carried out investigations into the structure of the central nervous system that eventually led him, in 1873, to the discovery of the 'black reaction' (now known as 'Golgi impregnation' or 'Golgi staining'). This discovery revolutionized neuroanatomical research by allowing, for the first time, a full view of single nerve cells and their processes[6,8]. In 1877, Golgi strengthened his personal relationship with Bizzozero when he married Bizzozero's niece, Lina Aletti.

First discoveries

Haematopoiesis in the bone marrow. Before his graduation, Bizzozero began to investigate the histology of bone marrow. At that time, there were two main views on the functions of this tissue, which dated back to the ancient time of Hippocrates and Aristotle. According to these views, the bone marrow either constituted an 'excrement' of the bone (*excrementum ossium*), or, on the contrary, it represented its nutritional 'matrix'. Bizzozero discovered a particular kind of nucleated red cell in this tissue, and he considered these cells to be the precursors of the mature red cells of the circulating blood (FIG. 2). Moreover, he realized that bone marrow is the site of production of white blood cells and also exerts a role, like the spleen, in the destruction of old cellular elements. Bizzozero's conclusions were confirmed by experiments of bleeding under controlled conditions, carried out on chickens and pigeons, which amplified the blood-cell-forming phenomenon in bone marrow[9–11].

The finding that bone marrow was able to produce blood was also made at the same time by the Prussian haematologist Ernst Neumann, a professor at the University of Königsberg, who published part of his results in 1868, a month before the Italian researcher[12].

Nodes of Bizzozero in the skin. Another field of interest for Bizzozero concerned the histological structure of the epidermis and, in particular, the points of contact between adjacent cells of the *stratum spinosum*, erroneously considered at the time to be 'intercellular bridges' that established a cytoplasmic continuity. Bizzozero's attention was captured by some peculiar shapes that he observed in these cells, such that the processes projecting from the adjacent cellular elements met end-to-end in small, dense and dark nodules, later known as 'nodes of Bizzozero'. Bizzozero perceptively concluded that there was no cytoplasmic continuity between these cells, and that the dense nodules that we now call desmosomes or 'connecting bodies' (from the

Figure 1 | **Giulio Bizzozero around 1866, aged 20.**

Greek words *desmos* for 'bond' or 'ligament' and *soma* for 'body'), were, in fact, bipartite adhesive structures to which both cells contributed[13–15]. Bizzozero's conclusion was not accepted at the time, owing to the influence of Louis Ranvier, who propounded the opposite idea of cell-to-cell continuity. The definitive resolution of this controversy came with the advent of the electron microscope, which revealed a protoplasmic discontinuity at the level of the intercellular bridges, so confirming Bizzozero's theory[16–18].

Phagocytosis in the eye. Following the discovery of the haematopoietic function of the bone marrow and the identification of the nodes of Bizzozero, Bizzozero's third important scientific contribution came from an investigation into the mechanisms by which pus elements are produced in the inflammation process in the anterior chamber of the eye. Bizzozero gave a clear description of phagocytosis in two papers that were published in 1871 and 1872 in the Italian medical magazine, *Gazzetta Medica Italiana – Lombardia*[19,20], the same journal that published Camillo Golgi's first communication on the 'black reaction' in 1873 (REF. 21). Using *in vivo* experimental conditions in rabbits and clinical observations in humans, Bizzozero described great cellular elements (now known to be macrophages) "…that have the faculty to devour the surrounding elements"[20]. He stated that "…when an irritating process affects the wall of the anterior chamber (of the eye) … some great and lively contractile cellular elements develop, which can swallow and introduce into their protoplasm [by means of active amoeboid movements] the elements that are immersed in the surrounding liquid"[20]; that is, red blood cells, pigment granules and pus particles. In the concluding remarks of his 1872 paper, Bizzozero clearly explains this phenomenon as the method for eliminating pus particles or effused blood in the anterior chamber of the eye, giving an exact pathophysiological interpretation of phagocytosis. In 1873, Bizzozero even hinted at the role exerted against infection by connective reticular phagocytes of lymph nodes. In a study on the structure of lymphatic glands, he stated that reticular cells could ingest infective particles that were carried by the lymphatic liquid[22]. He added that: "…this fact is, perhaps, the cause of the stoppage of some infections to the lymphatic glands which are connected to the part covered by the infection through the lymphatic vessels"[22]. So, the mechanism of phagocytosis as a biological phenomenon was precisely described by Bizzozero in the period between 1871 and 1873, more than 10 years before Elie Metchnikoff described it[23]. However, if we consider a structured 'theory' or 'doctrine' of phagocytosis; that is, a theory or doctrine of immunity, this implies a mechanism of defence from external biological aggression that could be fully proposed only after the microbiological discoveries of Louis Pasteur and Robert Koch. And this concept was structured and articulated by Metchnikoff from 1882 onwards. However, the great Russian founder of immunology never sought credit for being the first to describe the phenomenon of phagocytosis, and in his masterpiece *Immunity in Infective Diseases*[24] gave full credit to Bizzozero for having "…first recognized … an amoeboid cell which had ingested pus corpuscles".

Establishing a research laboratory

In 1872, after this extremely productive period of research, Bizzozero, at the age of 26, was appointed full Professor of General Pathology at the University of Turin in Piedmont, a region in the north of Italy. His arrival in this town was soon followed by a period of struggle to impose experimental methods against the vitalistic philosophy that still dominated the old Piedmontese medicine, according to which the human body was under the influence of a vital force that was independent of physico–chemical law. The old academics ridiculed the microscope as the instrument "…which showed what one wanted to see and not what one must see"[25]. To overcome the difficulty in obtaining space from the university, Bizzozero set up a private laboratory in his house, which soon became an important experimental centre for morphological research. Things began to improve from 1876, when Bizzozero was granted a laboratory and funding for an assistant and a laboratory technician from the university.

Meanwhile, he founded the magazine *Archivio per le Scienze Mediche* (Archive for Medical Sciences) and, in 1879, published the first edition of his *Manuale di Microscopia Clinica* (Handbook of Clinical Microscopy), which was subsequently reprinted many times and translated into several languages including German and French.

At the end of the 1870s, Bizzozero's scientific activity increased enormously in the laboratory at the University of Turin, and outstanding scientific results soon followed. In 1879, he described a new instrument he had invented, named the 'chromo-cytometer', which allowed alterations in the content of haemoglobin in the blood to be quantified[26] (BOX 2). With this instrument, Bizzozero, in collaboration with Golgi, was able to study the variation of haemoglobin in various pathological conditions and during blood transfusions into the peritoneal cavity, a procedure that allowed considerable quantities of blood to be transfused without

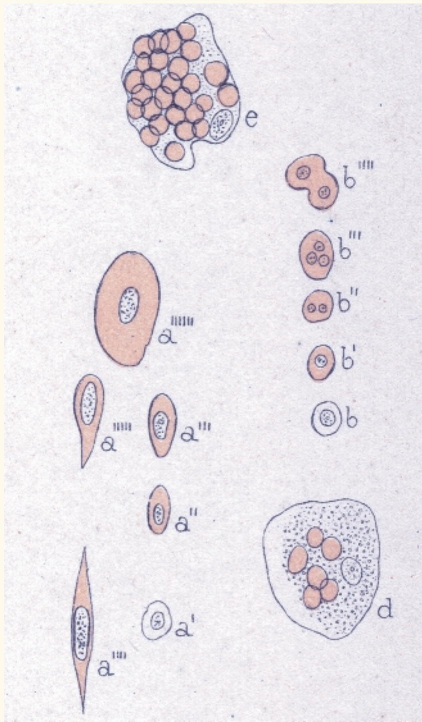
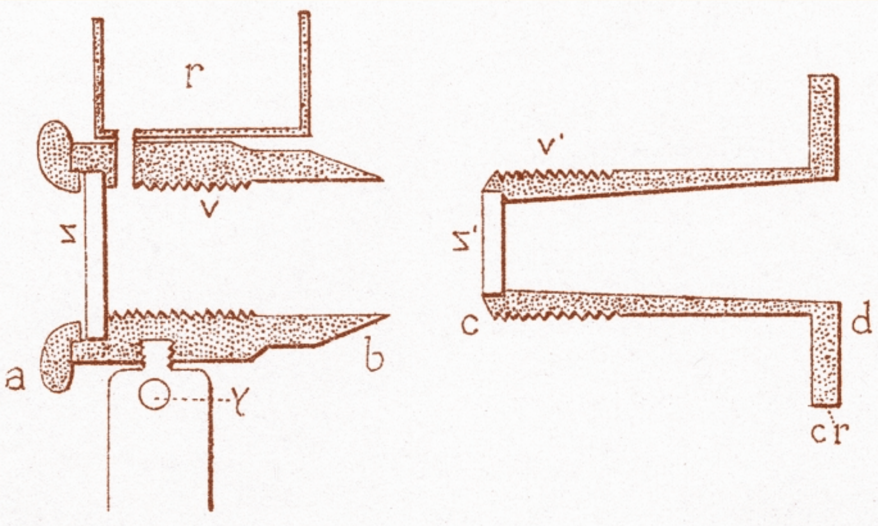


Figure 2 | **Bizzozero's drawings showing developmental stages of red blood cells.** Series **a** shows red blood cells in the bone marrow of the frog, series **b** shows red blood cells in the bone marrow of humans, and **d** and **e** show macrophages that have ingested erythrocytes. Reproduced with permission from REF. 14 © (1905) Hoepli, Milano.

Box 2 | **The chromo-cytometer**



Bizzozero's chromo-cytometer was composed of two tubes, one inserted inside the other (see Bizzozero's drawing of a vertical section of the chromo-cytometer). Both tubes were closed at one end with a glass disk, and the tube on the right was screwed onto the other until the two tubes were joined. To use the instrument as a cytometer (that is, to assess the amount of haemoglobin without disrupting red blood cells), measurements were made in a dark room at a fixed distance from a candle. The blood — diluted in a saline solution to avoid haemolysis — was put in the chamber (r), and the tube on the right was slightly unscrewed to allow the blood to pass into the space between the two disks. The thickness of the diluted blood (read on a scale in the larger tube) that allowed the contour line of the candle flame to be clearly observed through the glass disk of the tube on the left, was converted into the amount of haemoglobin by means of a standard curve or a transformation table.

To use the instrument as a chromometer (that is, to assess the amount of haemoglobin after the disruption of red blood cells), the blood was first diluted in distilled water, and the observations were made in natural light, using a coloured glass as a standard. The thickness obtained was again converted into the amount of haemoglobin using a standard curve or a transformation table. Reproduced with permission from REF. 14 © (1905) Hoepli, Milano.

the dangerous reactions that often occurred before the discovery of blood groups[27,28].

The platelets: a new blood corpuscle

Discovering 'discoid corpuscles'. In 1881, Bizzozero reached the pinnacle of his scientific productivity with the discovery of a "…constant blood particle, differing from red and white blood cells", the existence of which "…has been suspected by several authors for some time"[29–31]. Bizzozero named this third morphological element of the blood *piastrine* — the Italian for 'small plates'; they were called *Blutplättchen* in German and *petites plaques* in French (subsequently called *plaquettes*), and in English they were later named 'platelets'. He described them as discoid corpuscles without a nucleus, consisting of a membrane and a matrix in which there were a few dispersed granules — haemoglobin was never present. Before his investigations, several researchers had observed platelets in the blood, but they regarded these particles either as degenerated and disintegrated leukocytes, or as clots of fibrin or a particular kind of microbe. Between 1877 and 1879, just before Bizzozero made these observations, the renowned French haematologist George Hayem published a series of papers with a fairly clear description of platelets[32,33]. However, he considered them to be the precursors of red blood cells; that is, 'haematoblasts'. In 1880, Ernst Neumann still related platelets to erythrocytes, but instead of considering them as their precursors, he thought they were artefacts derived from faded and altered red blood cells as a result of the incorrect techniques that were used to study blood[34,35].

Bizzozero was the first person to clearly regard platelets as a third morphological element of the blood, unrelated to erythrocytes and leukocytes. He observed them *in vivo* under the microscope as colourless and transparent particles, around 2–3 μm in diameter, which circulated in the blood of the mesenteric vessels of anaesthetized rabbits and guinea pigs. Moreover, in many species of mammal, including humans, he observed platelets as isolated corpuscles in the first blood drops taken from a wound, but only after very rapid preparation, as they were unstable elements that soon underwent morphological changes to form granular aggregates (FIG. 3a,b).

An important role for platelets. Bizzozero's outstanding merit was the discovery of the role of platelets in blood thrombosis and haemostasis. In 1856, Rudolf Virchow had considered thrombosis as a clot that developed within blood vessels. Subsequently, Paolo Mantegazza — Bizzozero's mentor during his Pavian years — showed that a thread introduced into a vessel of a living experimental animal developed a coated white clot, which he considered to be an accumulation of leukocytes and fibrin. Also according to Mantegazza, following damage to the vessels, the white blood corpuscles stopped at the particular point where the endothelium was damaged, forming the white thrombus, which he considered to be composed mainly of leukocytes[36]. Bizzozero challenged this hypothesis, which was supported by other researchers, and showed that in mammals, the main elements responsible for the formation of a white thrombus, instead of being the white blood cells, were, in fact, the platelets (FIG. 4). He clearly understood the role of the platelets "…in stopping haemorrhages by closing discontinuity in the vessels", therefore clarifying their role in physiological conditions. He also suspected a role for these blood constituents in diseases characterized by increased thrombosis, but did not recognize their involvement in haemorrhagic conditions such as purpura.

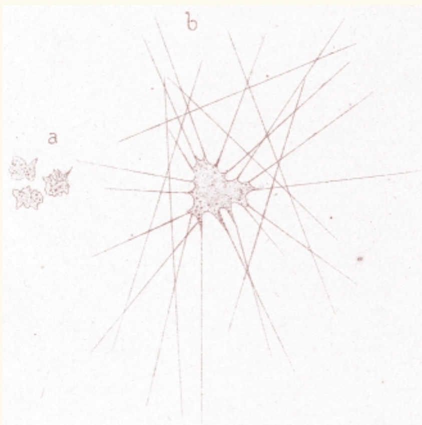


Figure 3 | **Bizzozero's drawings showing early deformation of platelets in the dog.**
a | Platelets observed immediately after blood extraction. **b** | Platelets after eight minutes, when they are found fused in a single mass with fibrin threads merging within the aggregates.
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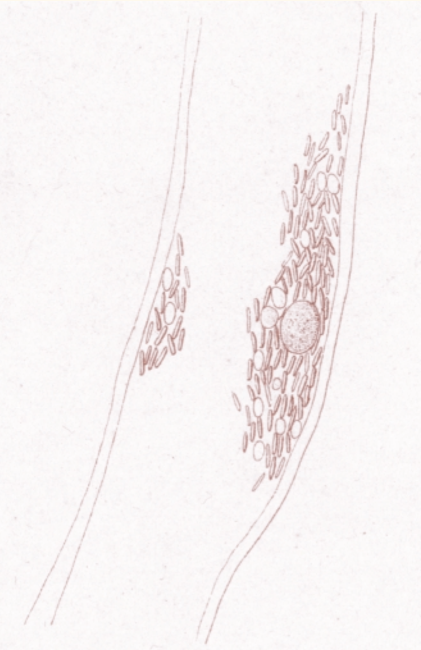

Figure 4 | **Bizzozero's drawing showing two parietal thrombi in a small mesenteric vessel of guinea pig.** Among the platelets is a small white blood cell. Reproduced with permission from REF. 14 © (1905) Hoepli, Milano.

Bizzozero was aware of the possible origin of platelets from bone marrow, but was unable to prove this hypothesis. Even though he observed megakaryocytes in 1869, only after his death were these elements identified as the precursors of platelets[34,37,38].

The discovery of platelets was immediately recognized internationally as a result of papers published in French and German by Bizzozero in 1882, and an article entitled 'A new blood corpuscle'[39,40], which appeared in the British medical journal *The Lancet* on 21 January 1882. However, a dispute developed between Bizzozero and Hayem as to who discovered the role of platelets in thrombosis and haemostasis, and the Italian gained full credit for his work only after 10 years of heated debate.

Labile, stable and everlasting tissue

The discoveries of Walther Flemming on cell division during 1874–1882 opened an extraordinary new field of investigation[41]. Bizzozero immediately recognized the importance of these observations and soon applied Flemming's methods and concepts to the study of cell proliferation in the bone marrow and the spleen (FIG. 5). The old problem of the origin of blood cells from the bone marrow was, therefore, completely reconsidered in the light of the new ideas on mitosis, tracing back the maturation of red cells to a process of cell fission and karyokinesis. But Bizzozero, with the aid of some of his pupils, was able to carry out an extensive investigation into the reproductive and regenerative capability of many tissues, especially glandular tissues, leading him to some general theoretical conclusions of great biological importance. One of Flemming's most important tenets was that regeneration in an organism occurs by cell division; accordingly, Bizzozero considered the proportion of mitotic cells observed in a tissue as an index of its regenerative capability. By scrutinizing the literature and his own experimental data, he reached the general conclusion that all the tissues of living organisms could be ascribed to one of the following three biological categories: 'labile', 'stable' or 'everlasting'[42,43]. Cells of labile tissues undergo a continuous reproduction by mitosis throughout life, with newcomers replacing the lost elements. To this category he assigned, among others, some glands (testicle, ovary, lymph, sebaceous, gastric and gut), the bone marrow and the spleen. Stable tissues are characterized by cells in which mitosis normally lasts until birth or some time later. However, they can also undergo regeneration through cell fission under particular pathological situations during post-natal life. Bizzozero considered liver, bones and smooth muscle among the stable tissues. As an example of regeneration in pathological conditions, Bizzozero quoted the experiments of Emil Clemens Ponfick[44], who had observed that removing a large proportion of the liver in experimental animals was followed by a subsequent regeneration that led to the restoration of its initial mass. Everlasting tissues include nervous and striated muscle; that is, tissues formed by post-mitotic cells. According to Bizzozero, their reservoir of germinal cells is exhausted during early embryonic development, so these tissues lack the potential to reproduce and regenerate.

Bizzozero presented these concepts to the Eleventh International Medical Congress that was held in Rome in Spring 1894, in front of many worldwide medical and biological celebrities, including his old master Rudolf Virchow[6,42]. Bizzozero's conclusions immediately became known everywhere and his classification of tissues as labile, stable and everlasting was held as a 'central dogma' of cell biology until recent times. But in science, dogmas are rarely everlasting, and recent studies on stem cells — one of the blossoming fields in biology — have undermined Bizzozero's tenets. Far from being completely lost after birth, cellular elements that retain their potential to divide are widely found, particularly in the nervous system[45,46]. However, having lasted almost 100 years, Bizzozero's 'dogma' is certainly one of the longer lasting in the history of biology.

An interrupted life

During his scientific life, Bizzozero made many other contributions to biology. He published papers on the structure of serous membranes (pleural and peritoneal) — perceived as a continuous layer; on the origin of meningiomas; on the structure of lymphatic glands; and on the development of granulation tissue. In 1893, while he was studying the structure of gastric epithelium, he discovered some spirilla in the stomach of dogs that could also be found inside epithelial cells. This was one of the initial findings that eventually led to the identification of *Helicobacter pylori* as one of the causative agents of gastric ulcers.

In the 1890s, Bizzozero interrupted his laboratory activities as a result of an eye condition that hindered his microscopic ability. Through his articles in the popular press, he subsequently became a pre-eminent proponent of social and political measures against the spread of infectious diseases, and an active promoter of public understanding of the benefits of science. Bizzozero wrote on the advantages of vaccination, and on the practical application of the principles of hygiene for the improvement of public health. He even realised that cancer and smoking are related and, in a

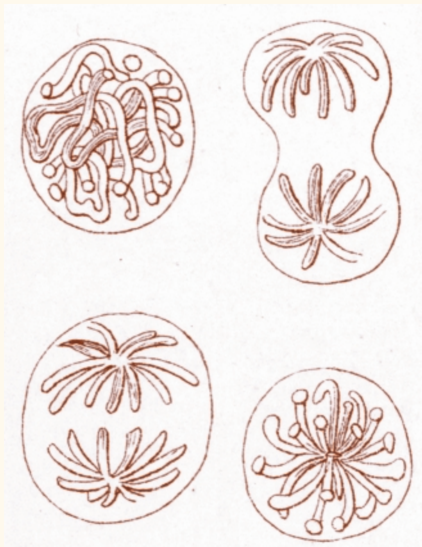

Figure 5 | **Early application of Flemming's concept.** Drawings by Bizzozero that show images of mitosis in the spleen of the triton (1883). Reproduced with permission from REF. 14 © (1905) Hoepli, Milano.

Figure 6 | **'The histological trio'.** From left to right: Giulio Bizzozero, Albert von Kölliker and Camillo Golgi in 1900. (Photograph kindly provided by Professor Vanio Vannini, Institute of General Pathology, University of Pavia.)

popular article, he suggested giving up smoking to reduce the spread of this disease[4]. For his competence, he was elected president of a number of medical societies and a member of several public health commissions. In 1890, he was appointed *senatore* — member of the Italian parliament. At the beginning of April 1901, while waiting for a visit from his friend Albert von Kölliker, the prophet of nineteenth-century histology (pictured together with Bizzozero and Camillo Golgi in FIG. 6), Bizzozero was stricken by a devastating bout of pneumonia. He died on 8 April. Among the many telegrams of condolence from Europe was that of Virchow, who remembered "…the most renowned man in our science" and "…one of the glories of this century"[47].

Bizzozero was a gifted scientist who opened new important fields to scientific exploration even if, as often occurred, he left the full development of his ideas and observations to other researchers. As a consequence, his name — apart from his contribution to the discovery of platelets and their function — was progressively forgotten in the history of cell biology.

His premature death deprived the scientific community of a man of strong will and great experimental skill, who worked hard for the development of cell biology and for the renewal of medical studies in Italy.

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