Colours and Shapes: an exploration of microscopy, tissues and cells

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Introduction

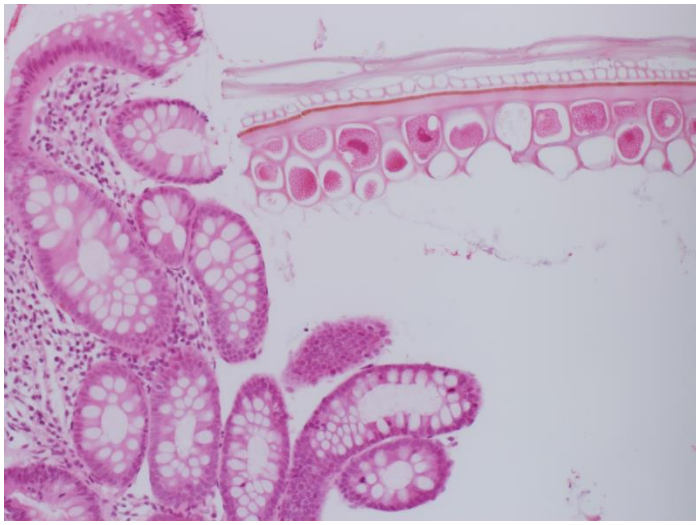
Cellular Pathology includes autopsy, histopathology and cytopathology. Dissection for elucidation of anatomy has been around, on and off, for a long time; the autopsy specifically to identify a cause of death is much more recent.
Cytohistopathology, relating disease to cells is a development of the past century and a half, and cytology and histology of about 200 years. Here I am going to examine the circumstances and sequences of events which led to recognition of the cell as the basis of all living organisms, a concept that underpins the specialty of cellular pathology. The idea of the basic cell unit has permeated scientific knowledge to an extent that today we almost cannot comprehend the thoughts of those in the past who only recognised disease at the whole body or organ level.

"Cell morphology can only be studied by microscopy, and the history of clinical cytology is almost synonymous with the history of the microscope applied to medicine" is quoted from *History of Clinical Cytology*, by Heinz Grunze and Arthur Spriggs. Similar statements appear in the introduction to most examinations of the development of the cell theory. This essay covers the evolution of the concept of the cell; the development of the microscope will be found in a separate paper.

For many centuries anatomy indicated that a body included organs but this was not particularly related to disease which was generally approached as a holistic or whole body matter. Cells really were not a concept that would fit with what the eye can appreciate as cell morphology can only be studied when tissue is magnified, thus the history of microscopes is intimately related to progress and errors in the development of the cell theory. Add to this techniques and equipment for cutting, staining and mounting and you begin to see how only the advent of relatively recent technology would allow this advance.

Seventeenth century – Botanists and Cells

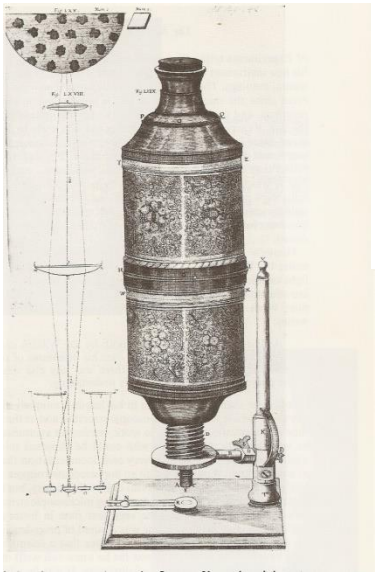
Botanists were well ahead of the medical men in their appreciation of microstructure, where the cell concept began its progress, though many medics were also botanists.

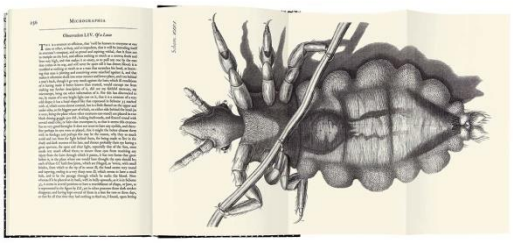


The advantage the botanists enjoyed was that plant cells are much larger than animal or human, and often could be obtained by peeling off a thin, sometimes single cell, layer whereas animal studies tended to be carried out on whole small items such as insects sometimes part dissected and squashed onto a slide. Plant cells also have a thicker, more obviously visible, membrane.
Left: this modern photomicrograph shows human colonic mucosa to the left and vegetable material to the right.

In the seventeenth century scientists, mainly botanists, considered microscopic structure in the development of a more logical and scientific taxonomy for plant life. One of the first to gain perception of such structures was Robert HOOKE (1635 - 1703) of Freshwater, Isle of Wight. He was a man of many talents – as a Baroque architect he worked with Wren on the renewal of London after the Great Fire and as a botanist he developed

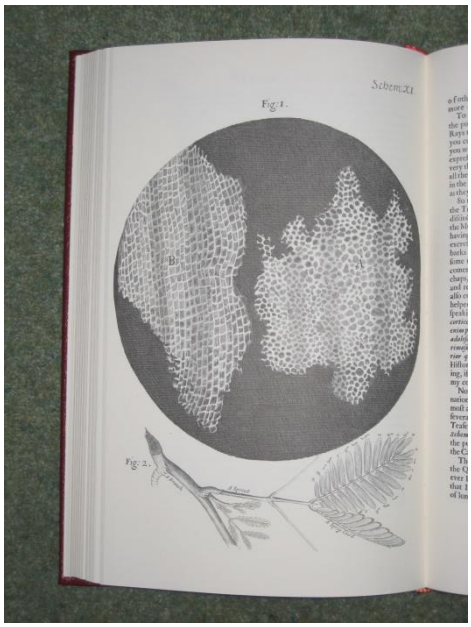
his own simple compound microscope circa 1665 for scientific observations, which boasted a screw focusing mechanism and used it to study cork. Like many of the scientists discussed in this essay he reported many of his findings by letter to the Royal Society of London, for which he was the first Curator of Experiments appointed in 1661.





Left: Hooke's simple compound microscope with screw focusing mechanism. Top right: modern photomicrograph of a head louse. Bottom right: pull out plate of a head louse in *Micrographia*.

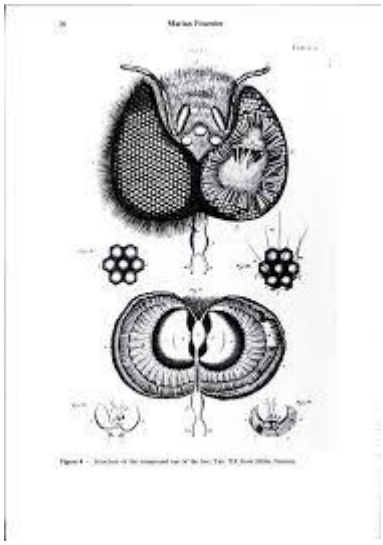
Hooke examined cork in 1667 in his *"Micrographia or some physiological description of minute bodies made by magnifying glasses*" reported that his sections "consisted of a great many little boxes". He used the term cell, after the room inhabited by a monk, but had applied it to the remnant supporting structures.



Hooke's drawing of cork in *Micrographia* (left) and a modern photomicrograph of a section of cork in an old Victorian slide from 1862 (right).

He expanded the scope of his observations writing "Nor is the kind of texture peculiar to cork only…." And finally "nature seems to perform several animal actions with the same schematism or organization that is common to all vegetables" a very early mention of plants and animals sharing the same common basic building block.

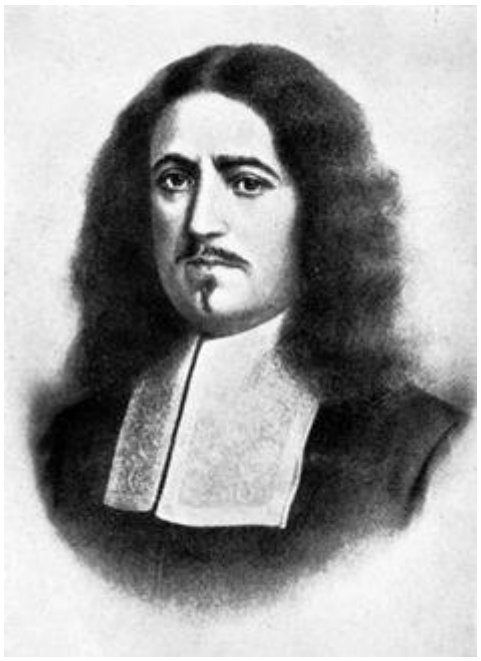
Hooke's rather skittish interest led to indiscriminate use of the microscope and the book randomly covers ground between gravel in urine and the stinging point of a nettle.



He was not the only one using a microscope – Dutch microscopist Jan SWAMMERDAM (1637 – 1680) drew the eye of a bee in his *Biblia naturae* 1737 and is credited with being the first to describe erythrocytes (red blood cells) in 1658 though at this time he thought there was a central body.

He was a contemporary of Anton van LEEUWENHOEK (1632 – 1728) who also described erythrocytes in 1687. For his involvement in the development of the microscope see separate essay.

Notable botanists included Marcello MALPIGHI (1628 - 1694) and his contemporary, Coventry born Nehemiah GREW (1641 - 1712) who are remembered for their systematic study of botanical subjects.



Marcello Malpighi

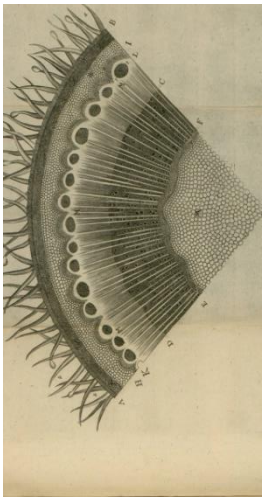
Nehemiah Grew

Grew was mainly a chemist who in 1695 established what gave Epsom's water its "medicinal" quality and made them the "bitter purging salts" as he called them in his book. He was granted a patent to manufacture the salt, which he did in Acton so there was no longer a need to visit Epsom to enjoy, if that's the word, the salts.

Malpighi named the boxy structures he saw down his microscope utricles or vesicles, distinguishing them from the tubes which are now called vessels and coined the term parenchyma. His work, *Anatome Plantarum*, 1671, appeared the year before Grew's *Anatomy of Vegetables begun*. In his book Malpighi announced that plants were "frequently constructed of exceedingly little bladders which viewed through a microscope are plainly visible". Their sometimes-acrimonious competition made good forward progress.

Left: frontispiece of *Anatome Plantarum,* 1671 Marcello Malpighi

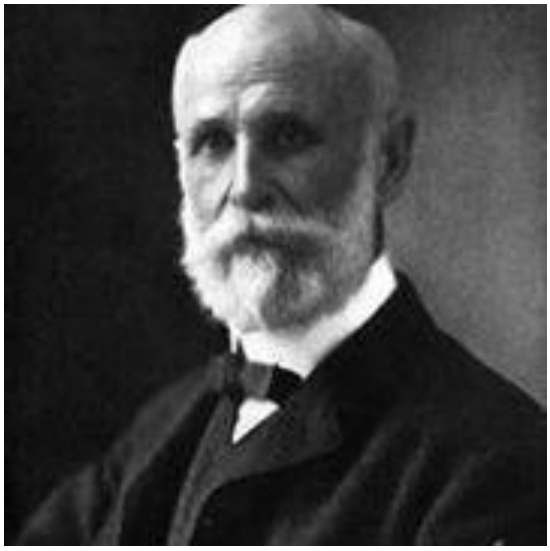
Right: plate from *Anatomy of Vegetables begun,* 1672 Nehemiah Grew. Later included in his collection of essays *The Anatomy of Plants1682.*



Botanists, and other scientists, seventeenth century did not generally work in isolation but frequently corresponded amongst themselves and communicated with the Royal Society. Then, and later, exchange of information and developments in this way meant that ideas were in general knowledge long before any formal publication appeared. All the aforementioned botanists and microscopists were contemporary and in communication with each other.

Eighteenth century – Zoologists and Cells

The histologists had now made a start as English physician Clopton HAVERS (1657 – 1702), who gave his name to the Haversian canals in bone, made animal studies in 1691 *(Osteologia nova, or some new observations of the bones, and the Parts belonging to them, with the manner of their accretion and nutrition*) and described bone marrow as a heap of pearls. In 1726 Alexander MONRO primus, Scottish surgeon and anatomist (1697 - 1767) recognised the clusters of pearls as being very similar to the tissue known as fat, reported in *The Anatomy of the Human Bones*.



Clopton Havers

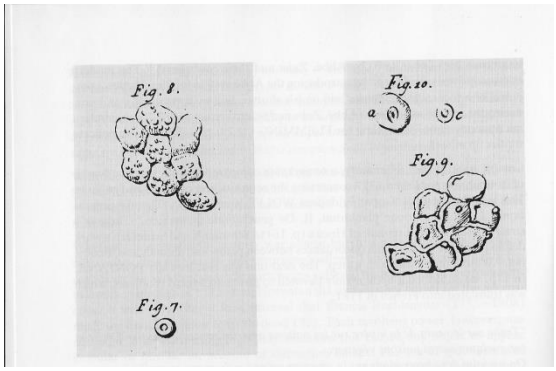
Photomicrograph of bone marrow

Albrecht von Haller

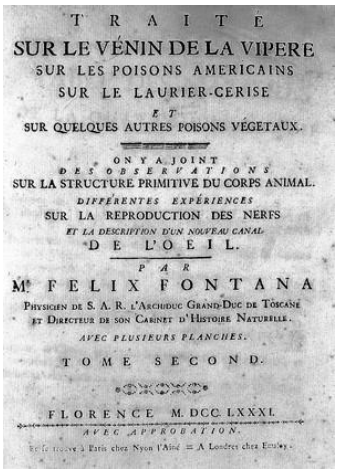
Progress then faltered as in 1757 Swiss physician Albrecht von HALLER (1708 – 1777) in his *Elementa physiologiae corporis humani* speculated that the solid parts of animals and plants were composed of fibre and an organised concrete. The elementary fibre was in turn made up of very fine fibrils which could not be seen.

Globulist Theory – an Aberrant Vision

The fibre theory lasted several decades and was then replaced by the globulists – a theory in which everything consisted of many small globules, probably based on a microscope artefact (see development of the microscope). Italian Felix/Felice FONTANA (1730 – 1805), generally termed a physicist and toxicologist, was a globulist but still managed to contribute decent pictures of cells, describe staining with syrup of violet and also the nucleus in 1767. In his Pharmacology text, *Traité sur le vénin de la vipère,* a French translation of the original Italian he included a study of eel's skin and by 1781 he regarded organs as masses of coiled cylinders!



Fontana's drawings of cells


TRAITE
SUR LE VÉNIN DE LA VIPERE
SUR LES POISONS AMERICAINS
SUR LE LAURIER-CERISE
ET
SUR QUELQUES AUTRES POISONS VÉGETAUX.
ON Y A JOINT
DES OBSERVATIONS
SUR LA STRUCTURE PRIMITIVE DU CORPS ANIMAL.
DIFFÉRENTES EXPÉRIENCES
SUR LA REPRODUCTION DES NERFS
ET LA DESCRIPTION D'UN NOUVEAU CANAL
DE L'OEIL.
PAR
Mr. FELIX FONTANA
PHYSICIEN DE S. A. R. L'ARCHIDUC GRAND-DUC DE TOSCANE
ET DIRECTEUR DE SON CABINET D'HISTOIRE NATURELLE.
AVEC PLUSIEURS PLANCHES.
TOME SECOND.
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AVEC APPROBATION.

Fontana's later book

Prochaska

In 1779 Czech anatomist and physiologist Georg PROCHASKA (1749 – 1820) supported the idea and described brain as composed of globules 1/8th the size of the blood particles, *De structura nervorum tractatus anatomicus*.

Many notable scientists confirmed the globalist approach, up to and including Henri MILNE-EDWARDS (1800 – 1885) in 1826 in *Recherches Microscopique sur la Structure intime des tissus organiques des Animaux* who records the globules as 1/300mm diameter. There are no figures in his work so it is not possible to deduce what structure or artefact he may have visualized.

Ignaz DÖLLINGER (1770 – 1841), 1828, suggested that the body was built up of blood corpuscles which move in wall-less channels in the tissues, *De vasis sanguiferis quae villis intestinorum tenduium hominis brutorumque insunt*.

Initially Rene Joachim Henri DUTRUCHET (1776 – 1847) also supported the globalists but later progressed noting, as had Hooke before him, the similarities between plant and animal structure. *Recherches anatomiques et physiologiques…* 1824.

Many of these observations, particularly "globules" were the result of poor optical instruments and the haloed spherical artefact appearances generated by the convex/concave lens combinations available.

Nineteenth Century – Better Microscopes; Clearer Visions

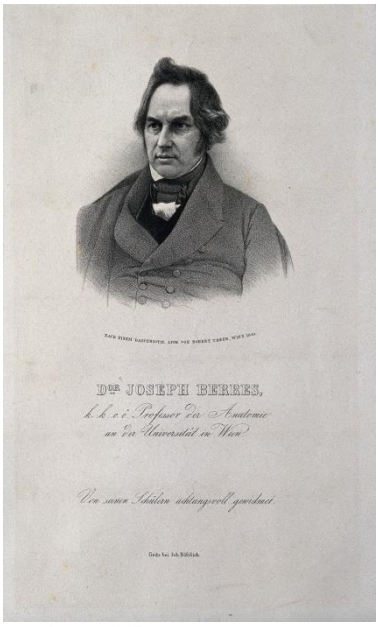
Joseph Jackson LISTER (1786 - 1869) was a successful London wine merchant who tinkered with optical instruments as a hobby. In the 1820s he turned his attention to the microscope and was then able to recombine the crown and flint glass lenses in such a way as to resolve the spherical aberrations. He published a paper on these principles in 1829 *On the Limit to Defining Power, in Vision with the Unassisted Eye, the Telescope, and the Microscope*, when his famous son was but two years old. These beautifully constructed, very ornamental devices were often regarded as of no practical use, but as a toy for the rich and curious.

Left: J J Lister with one of his microscopes.

Below: Thomas Hodgkin

Lister however, had already collaborated with Thomas HODGKIN (1798 - 1866) to publish a paper "Notice of some microscopic observations of the blood and animal tissues" in the Philosophical Magazine of 1827. Writing was very polite in those days and the paper opens: "the powerful compound achromatic microscope in the possession of JJ Lister, being, as I have reason to think, far superior to anything of the kind yet produced in this country, a short account of its application to animal structures will probably not be considered altogether uninteresting"
They outlined the appearances of various tissues evidently with sufficient resolution to describe the striations of skeletal muscle. They also described the red blood cell as biconcave with no nucleus but were considered incorrect as so many others had seen it. Had they also examined animal erythrocytes they might have realised how unusual the human cell is; sadly many disregarded their excellent research because of this supposed error!

The microscope was avidly taken up by the many technophiles in the German speaking world, one being Joseph BERRES (1796 - 1844) who was not only a surgeon, but occupied the Chair of Gross Anatomy in Vienna in 1830. In his studies he observed and described "small bubbles" but did not comprehend what he saw. This did not hinder his most beautiful published work, a copy of which is on display in the Collections of the Medical University, Vienna (known as the Josephineum), where it is labelled the first atlas of histology (*Der Mikroskopischen gebilde des Menschlichen Körpers*, Vien 1836) and for which he had invented a method of duplicating his minutely accurate pictures.



Joseph Berres Josephineum, Wahringer Strasse, 1090 Vienna

Defining the cell structure and spontaneous generation.

The botanists surged ahead with every new development in magnification. In his MD of 1759, *Theoria Generationis*, Berlin born Caspar Friedrich WOLFF (1733 – 1794) held that plant buds were composed of a gelatinous material, which collected into drops destined to be transformed into utricles (or cells). This appears to be the original suggestion of the free formation of cells. By 1768 he was formulating the germ layer theory of embryology, published in *De Formatione Intestinorum*.

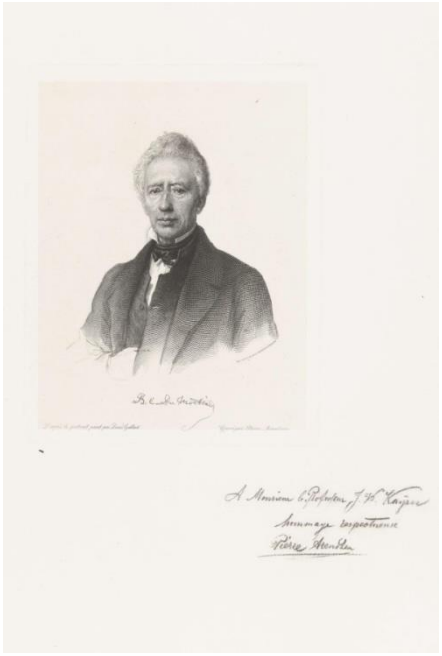
In the early 19th century botanists were beginning to elucidate the structure of cells. Charles Francois BRISSEAU DE MIRBEL (1776 – 1854) was the first to apply the term “cell” to vegetable elements in general, in his 1802 *Traité d’anatomie et de physiologie vegetale*. He was supported by many botanists who were by now developing a clear notion of cells as structural units, but still maintained that they were excavated from a homogenous fundamental substance.

In 1812 Prof Dr J J P MOLDENHAUER (1766 – 1827) macerated maize and succeeded in demonstrating that each, now separated, cell had its own wall, reported in *Beiträge zur Anatomie der Pflanzen*, Keil.

Even as the botanists progressed with practical observation thoughtful philosophy in the Galenic/Hippocratic tradition was far from dead, and indeed still maintained a respected position within scientific progress. Leader of the German Naturphilosophie movement Lorenz OKENFUSS, later shortened to OKEN (1779 – 1851) in 1805, *Die Zeugung* and French born Jean-Baptiste LAMARCK (1744 – 1829) in 1809 advanced hypotheses “thus every living body is essentially a mass of cellular tissue in which more or less complex fluids move more or less rapidly, so that if this body is very simple, that is without special organs, it appears homogenous and presents nothing but cellular tissue containing fluids which move within it slowly; but if its organisation is complex all its organs without exception, as well as their most minute parts, are enveloped in cellular tissue, and even are essentially formed of it”, *Philosophie Zoologique*. If that has a ring of some truth about it is worth pointing out that neither man is known to have observed tissue or to have used a microscope.

In *Recherches sur la Structure comparée et le Développement des Animaux et des Végétaux*, 1832 Barthélemy Charles Joseph DUMORTIER (1797 – 1878), a Belgian, described binary fission in plants with a mid-line partition within an existing cell leading him to reject both new cells arising within the old and the notion of spontaneous formation.

This discovery has often been attributed to German scientist Hugo von MOHL (1805 - 1872) in 1837, *Über die Vermehrung Pflanzen-Zellen durch Theilung,* however he did coin the term protoplasm for the material contained in the cell.



Dumortier

von Mohl

von Mohl's cells

In 1833 Robert BROWN (1773 – 1858) a noted Scotsman interpreted Fontana's 1767 observation "one saw a little body within the globule" and described, "this areola, or nucleus of the cell as perhaps it might be termed…" He also stated that in his opinion similarities could be observed in the fine structures of plants and animals.

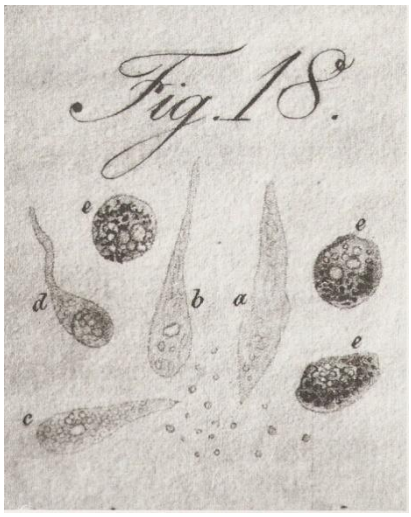
Left: epidermis from the orchid Cymbidium reproduced by Brian Ford using Brown's microscope and preparations.

Brown (of Brownian motion) described cell nuclei in *On the Modes of fecundation in Orchidaceae and Asclepiaceae*

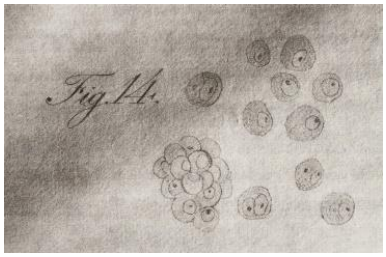
Clinical Application and the Cell Theory

In Germany political revolution had left the way open for scientists to fully embrace technology and relatively expensive equipment, such as the microscope, were acquired in their tens whereas more traditional (British) establishments could afford only one or two. At a time when many were announcing that the surgeons touch and experience of gross appearances would never be replaced by a fancy magnifying glass a School of Microscopic Anatomy and Biology was founded in Berlin by German comparative anatomist Johannes Peter MÜLLER (1801 - 1858).

Left: Müller



Middle and right: cells from carcinomata from *On the Nature and Structural Characteristics of Cancer and those Morbid Growths which may be Confounded with it*. 1838



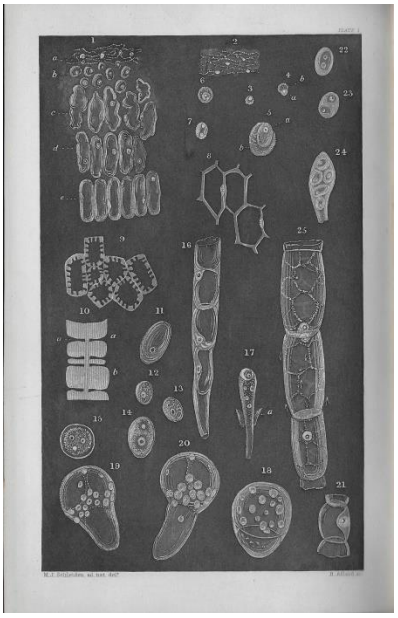
Müller himself published little however it is noteworthy to consider his 1838 publication including these drawings in which he illustrates cells of a melanoma (fig 18) with pigmented cells labelled e and cells of a carcinoma of breast (fig 14).

It is little wonder that he is often recognised as the initiator of clinical cytology. His true forte was to stimulate the researches of his students and academic staff. The work of the School was not confined to human, or even animal, research and several botanists were part of the staff. It was in any case not unusual for medically trained individuals to maintain an interest, or even second career in botany at this time. His pupils included Virchow, Henlé, Remak, Haekel, Waldeyer, Dubois-Reymond and Helmholtz.

Theodor SCHWANN (1810 - 1882) was a student of Johannes Müller, actively pursuing these microscopic considerations when meetings and conversation with Matthias Jakob SCHLEIDEN (1804 - 1881), a botanist also working with Müller, introduced him to the extensive research which had already established the cellular structure of plants.

First right:
Schleiden

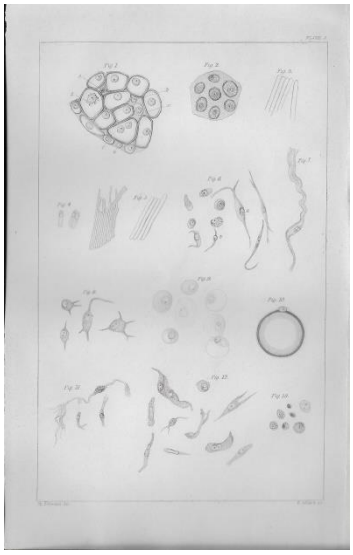
Second right: cells from *Contributions to phytogenesis*
Plate I
1838



Schwann conclusively established that **all living organisms are made up of cells**, which he termed the **"Cell Theory"** and published the details of his work in 1839 which was translated for the Sydenham Society in 1847 as *Microscopical Researches*, and is generally bound together with the closely related work of his co-worker Schleiden.

Considerable controversy then ensued as to the origin of the cells. It was Schwann's contention that cells developed from a formless blastema, and described the process as crystallisation of extravascular fluid around a granule with the later formation of a nucleus and finally the cell membrane, a version of the spontaneous generation theory.

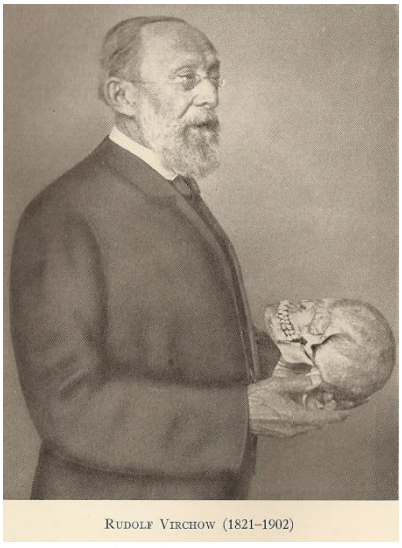
Schleiden had recognised in 1838 that cells may come from other cells but suggested they arose within the nucleus of old ones, or possibly from the cytoblastema (cytoplasm).



Far left: cells from *Microscopical Researches into the Accordance in the Structure and Growth of Animals and Plants*, plate III

Left: Schwann

In 1847 Rudolf VIRCHOW (1821 - 1902) published the first volume of his journal, *Archive for Pathological Anatomy and physiology and Clinical Medicine*, now generally referred to as "Virchow's Archiv" in which he reviewed the process and stated:



Virchow

1) All organization progresses through differentiation of a formless blastema.

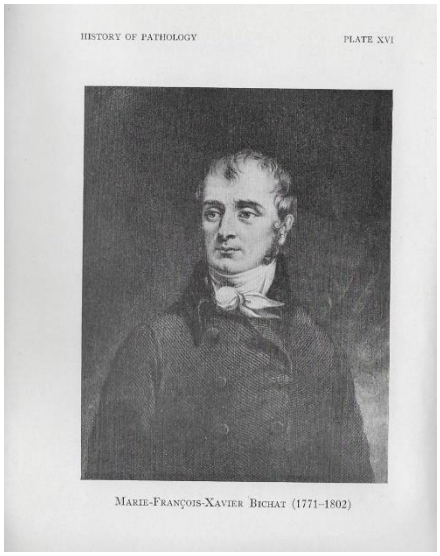
2) Blastema is primarily fluid and an exudate from vessels.

3) Differentiation within the blastema results in the formation of cells.

In these statements he is following the ideas of Schwann, and it may be noted that all of them are now deemed incorrect.

Relating Disease to the Cell – Histopathology

With normal histology making creditable considerable and accurate progress we may turn to the parallel concept of disease. Two hundred years ago the tragically short lived Marie Francois Xavier BICHAT (1771 - 1802) took the proposals of Philippe PINEL (1745 - 1826) his contemporary, that "inflammations" could affect different tissues within the individual organ.



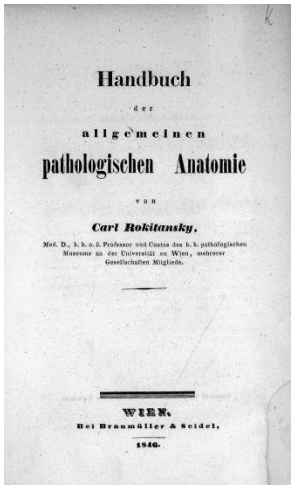
Bichat

Pinel

Bichat proposed that, and I quote - "we must be convinced of the necessity of considering local disease not from the standpoint of the compound organs, which are rarely affected as a whole, but from the standpoint of their different textures, which are almost always attacked separately" *Traité de l'anatomie descriptive*, 1802/1803. He defined 21 different textures, or tissues is the term we would use, including nervous, connective, vascular, muscular, osseous, absorbent, - and he achieved all this without a microscope - but by careful hand dissection!

Carl Freiherr ROKITANSKY (1804 - 1878) was born in Czechoslovakia two years after the death of Bichat from tuberculosis. He studied medicine in Prague and Vienna, and became an assistant prosector in the Algemeines Krankenhaus when he graduated. By 1832 he had become professor. He performed a huge number of post mortem examinations and insisted that they be done by specialist pathologists – perhaps the first suggestion of pathology as a medical specialty.

All this post mortem activity with careful annotation enabled Rokitansky to identify and correlate many pathologic entities at the localised organ level, and also to appreciate that many varied appearances were, in many cases, just the chronological development of a much smaller number of specific disease processes. He presented these findings in a magnificent 3 volume treatise the "*Handbuch der speciellen pathologischen Anatomie*" published in reverse order between 1842 and 1846. The format of the information will be familiar to the reader of the modern cellular pathology text: that of the first volume covering general pathology and the theories of disease, and volumes 2 and 3 dealing with organs by system with a methodical description of the relevant lesions. The book was adjudged so important that it was translated by the Sydenham Society almost immediately, the English version was available from 1845.



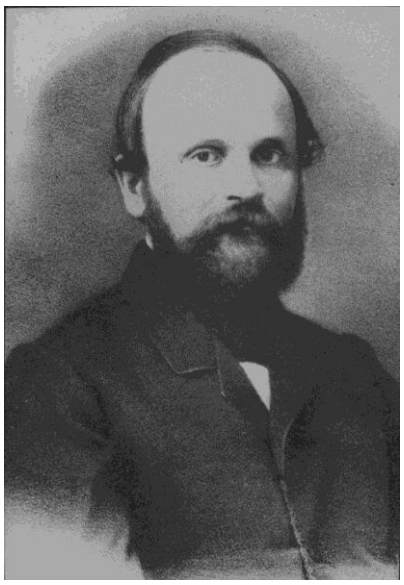
Far left: Rokitansky

Left: frontispiece of *Handbuch der allgemeinen pathologischen Anatomie*
1846

For possibly the only occasion in an otherwise ordered and methodical career, Rokitansky chose to communicate his ill-considered theory of crases within volume one published in 1846. The Sydenham Society translator considered it so frivolous that he simply omitted it. In the theory Rokitansky utilised Schwann's blastema (also supported by Virchow) and built an entire disease system around it. His reasoning was that if blastema is omnipresent then the only possible fluid candidate was blood, and more precisely the plasma protein, and that disease was caused by the imbalance of substances within the blood, in particular fibrin and albumin.

The theory was an entirely unsubstantiated final attempt to explain disease in the humoral tradition, heartily embraced by the clinicians of Vienna, but then seized upon by Virchow who deemed it a monstrous anachronism.

Loss of the spontaneous



Remak

Several individuals did risk the venom of Virchow and question the blastema origin of cells.

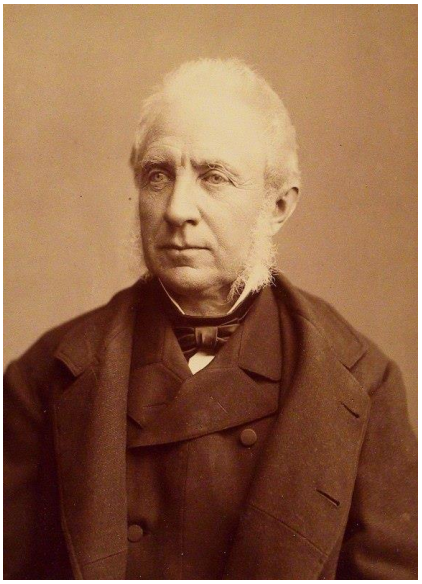
Jewish Polish/German Robert REMAK (1815 - 1865) another student of Müller had long been dubious, and in 1852 he stated "for myself the extracellular creation of animal cells has been, since this cell theory was made public, as incredible as the generation equivocal (spontaneous generation)", *Über die extracelluläre Entstehung tierischer Zellen und über die Vermehrung derselben durch Teilung*.
Shy retiring Remak was suggesting that cells arose from division but was mostly overlooked in favour of the far more flamboyant Virchow.

Rokitansky, who in many ways remained more open minded than Virchow, not only accepted Schwann blastema, but went on to consider other options including the possibility of the cells in some way developing further cells out of their own substance

One of Rokitansky's pupils was Carl WEDL (1815 – 1891) whom he encouraged to develop the microscope. Wedl, a native Austrian, collaborated with many of the clinicians in Vienna who by now had accepted histology and histopathology as a useful tool in medical practice.

In 1854 he published a textbook *Grundzüge der pathologischen Histologie* translated as the Rudiments of pathological histology, in which he felt able to assume the reader would be acquainted with normal histology from other sources. In his book he incorporated the Schwann's blastema but also described cell division as a mode of proliferation.

Whilst many of his observations regarding the cell content of tissues and some of their pathological appearances are just as valid today the incorporation of the blastema theory made his text out of date even as it was published, for Virchow suddenly executed an about turn worthy of any politician, which of course he was.



Wedl

In 1854, in his *Handbook on Special Pathology* Virchow credited Remak's theories of cell proliferation and announced "there is no life but through direct succession."

By 1855 Virchow had absorbed the available ideas of cell proliferation and condensed them into major statements which he proposed in his *Cellular Pathology*, and which he confidently hoped would become the pathology of the future. It is worth considering his statements here:

1) I formulate the doctrine of pathological generation and of neoplasia in the cellular pathological sense, in simple terms; omnis cellula e cellula (all cells arise from other cells)

2) If pathology is nothing but physiology with obstacles, and diseased life nothing but healthy life interfered with by all manner of external and internal influences, then pathology too must be referred back to the cell.

The first is the no-nonsense statement that cells only arise from other cells; ditching any thoughts of spontaneous generation, from blastema or other fluids and forming in the nucleus or cytoplasm. Descriptions of mitosis (cell division follow soon after).

The second establishes the idea that disease is related to the cell, and therefore that an examination of tissue utilising a microscope, recognising abnormal cells, can be the basis of diagnosis – which pretty much encapsulates my entire career.

As with all historical considerations of a significant event there are many known and unknown contributors to the final denouement. Historical figures are recognised mainly by their publications but the data is often widely acknowledged and utilised long before a book or paper is published. In this era printing might take years and a translation a decade.

Virchow was an influential and energetic advocate of his own theories and certainly played an important role but perhaps neglects Remak's prior recognition of cell division. Certainly all the players I have mentioned, and many I know not of, have contributed to this vital discovery.

Bibliography

In this essay I have tried to give the titles of the publication in which the relevant observation was reported to give you, the reader, an idea of where the information came from and so that you can look for yourself if you wish. I have not read them all – my technical German is acceptable, less so French and non-existent Latin and Italian. I have a small library of historical pathology texts, some reproductions, a collection of antique microscope slides and two antique microscopes which I have used in the images. Modern photomicrographs are also my own.

Books I have used include the following, most of which are also old and out of print.

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