FOREWORD

Francis BACON, in his *Novum Organum*, Robert BOYLE, in his *Skeptical Chemist* and René DESCARTES, in his *Discourse on Method*; all of these men were witnesses to the scientific revolution, which, in the 17^{th} century, began to awaken the western world from a long sleep. In each of these works, the author emphasizes the role of the experimental method in exploring the laws of Nature, that is to say, the way in which an experiment is designed, implemented according to tried and tested techniques, and used as a basis for drawing conclusions that are based only on results, with their margins of error, taking into account contemporary traditions and prejudices. Two centuries later, Claude BERNARD, in his *Introduction to the Study of Experimental Medicine*, made a passionate plea for the application of the experimental method when studying the functions of living beings. Twenty-first century Biology, which has been fertilized by highly sophisticated techniques inherited from Physics and Chemistry, blessed with a constantly increasing expertise in the manipulation of the genome, initiated into the mysteries of information technology, and enriched with the ever-growing fund of basic knowledge, at times appears to have forgotten its roots. In an epoch in which bioengineering is seen as an all-conquering "Open Sesame" that opens wide the doors to the temple of living beings, it may be salutary for the student or young researcher to look back upon the past and reflect, with respect, upon that which, in former times, inspired thinkers were able to achieve with the modest means at their disposal, means that were sometimes derisory when compared with contemporary technology. It may do them good to recognize that the harmonious edifice of current knowledge was built upon a solid foundation of fundamental discoveries made by famous thinkers of the past. They can only benefit from recalling the fact that audacious innovations based on experimental evidence, have, in their time, come up against the vindictiveness of an ill-prepared, ill-informed public that clings to its endless routine. One has only to remember that the introduction of asepsis and antiseptics into surgical practices in the 19^{th} century, which was based on acquired proof of the existence of infectious germs, did not always proceed smoothly.

This book relates the circumstances that have, over time, led thinkers and philosophers to ask questions about living Nature, to dare to explore using experimental devices and to lay out beacons that have prevented them from falling into any traps that would be prejudicial to scientific progress. By basing the discussion on concrete examples that refer to easily-understood experiments, my intention has been to provide the reader with food for thought, not only with respect to the experimental method itself, its history and its role in the objective analysis of the

In contrast to PARMENIDES, EMPEDOCLES of Agrigentum (490 - 438 BC) rehabilitated the role of the senses and observation. There are not one, but four, primordial elements, **water**, **air**, **fire** and **earth**, considered to be the reflection of certain states of matter, and the roots of things in Nature that are accessible to our senses. While incorporating solid bodies into the notion of earth, gaseous bodies into the notion of air and liquid bodies into the notion of water, EMPEDOCLES considered that beings and things are made up of a mixture or a combination of the four elements in proportions that determine their specificity. Thus wood contains some of the earth element, as it is heavy and solid, some of the water element, which it exudes when it is heated, and some of the air and fire elements, because it gives off smoke and produces flames when it burns. This theory of the four elements was accepted and taught until the Renaissance, and even beyond. It was popularized by geometric symbols represented on public buildings. These symbols are still visible on the remains of some monuments (Figure I.2).

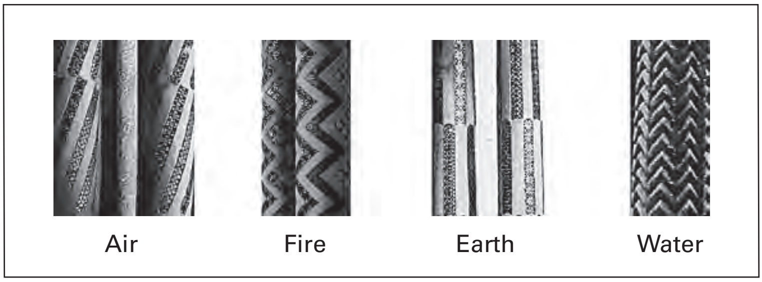


Figure I.2 - Symbols for the four elements, air, fire, earth and water, represented on columns of the Benedictine cloister at Monreale, in Sicily
(photograph by Henri PAYANT)

developed throughout the Middle Ages. These had only modest tools, which, even in the 13^{th}-14^{th} centuries, were limited to bellows furnaces, oil baths, retorts and stills (Figure I.5).

Figure I.5 - An alchemist's dispensary in the Middle Ages
(from a plate held in the French National Library - BnF)

In the **western alchemical tradition**, we find natural philosophers and physicians, such as Roger BACON and ALBERT the Great. In Montpellier, Arnauld DE VILLENEUVE (1235 - 1311), who was a doctor and regent of the school of medicine, perfected the procedures of distillation. He gave detailed descriptions of the operations required to obtain a certain number of products, including alcohol, which was already known, and was called *"eau-de-vie"*, or water of life. He provided a process for preparing turpentine. Another alchemist of the beginning of the 14^{th} century, Ramon LULL (1232 - 1316), who was of Catalonian origin, emphasized the idea of **quintessence**, a subtle principle that was present in many products, to which he gave a specific quality. Thus, brandy was considered as the quintessence in which resided the virtue of wine. Here we have the idea of **active principles** being present in natural substances, principles that modern chemistry would endeavor to purify, and which would prove highly useful in therapeutics. However,

The Italian thermometer represented in this figure was made up of a hollow glass tube that was closed and coiled around in a graduated spiral, with swellings at both ends. The lower swelling, which acted as a reservoir, and part of the spiral were filled with liquid (alcohol, known as spirits of wine). The rise or fall of the column of liquid in the spiraled portion made it possible to identify variations in temperature. This piece of scientific apparatus was bequeathed to posterity as an *objet d'art*. In addition to spiral thermometers, straight thermometers were manufactured for utilitarian purposes.

Figure II.9 - Spiral thermometer manufactured in Italy in the 17th century
(Photo Franca PRINCIPE, IMSS - Florence, with permission)

It took very little time for the advantages of using the **thermometer as an instrument to measure the temperature of living beings** to be appreciated. The first applications and discoveries soon appeared. The thermometer made it possible to demonstrate that the body temperature of Man and of mammals is maintained at a constant value that is independent of the outside temperature, which gave rise to the important idea of **thermal regulation** being a fundamental characteristic of life. The Italian doctor, mathematician, physiologist and astronomer, Giovanni Alfonso BORELLI (1608 - 1679) used vivisection experiments to prove that the heart is at the same temperature as the rest of the body of an animal, thus refuting the theory put forward by DESCARTES that the heart was a kind of kettle distributing its heat to the rest of the body. BORELLI had MALPIGHI as a pupil, and the latter was to become one of the pioneers of microscopic anatomy (Chapter II-3.2).

they were also able to discuss the experimental protocol and even refute the conclusions that were made. In short, BOYLE made experimentation a stimulus for progress in the sciences and **prohibited any pompous discussion or trivial speculation**, leading the Royal Society to choose as its motto *"Nullius in verba"* (on the words of no one). This way of doing science would continue in British scholarly circles, and would be marked by a concern for detail, the attention paid to performance and the reliability of instruments, and the precision shown in drafting experimental protocols.

6.3. RENÉ DESCARTES AND THE CARDINAL PRINCIPLES OF SCIENTIFIC RESEARCH

R. DESCARTES
(1596 - 1650)

René DESCARTES, a man of the laboratory, experienced in the practices of the physical sciences of the period, discussed experimental science from quite a different angle than Francis BACON. In his *Discourse on Method* (1637), which was a preface to three essays covering the topics of Dioptrics, Meteors and Geometry, DESCARTES stated and developed four principles for "rightly conducting one's reason and seeking truth in the sciences." These principles have not aged. They remain the plinth on which all experimental science stands. The first principle is **doubt**: "never accept anything as true which I cannot accept as obviously true." The second principle emphasizes the necessity of sticking to **limited questions**: "divide each of the problems I am examining into as many parts as I can, as many as shall be necessary to best resolve them." The third principle shows how to **move from the simple to the complex**: "begin with the simplest and easiest to understand matters, in order to reach, little by little, as if by degrees, the most complex knowledge." Finally, the fourth and last principle refers to the **ability to summarize**: "make my enumerations so complete and my reviews so general that I can be assured that I have not omitted anything." These were simple ideas that would be universally recognized and would have a considerable influence, both in the immediate and long after, due to their rational foundation.

The objective philosophy of DESCARTES was inspired by mathematical rigor as well as by his understanding of the laws of mechanics and of human anatomy. HARVEY's discovery of the circulation of the blood, the first mechanical representation of a physiological function, would lead DESCARTES, reasoning by analogy, to formulate the concept of the **animal-machine** that is found in the *Treatise on Man* (1664): "all living beings," he wrote, "are machines, automata, mechanisms. Man is different because he also has a soul." He also wrote: "I suppose that the body is nothing other than a statue or an earthly machine that God has formed deliberately to make it more similar to us than is possible." In his *Principles of Philosophy* (1644), he states: "I do not see any difference between the machines made by artisans and the

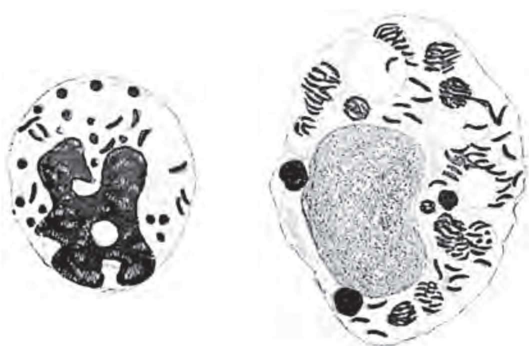
2.2.4. Advantages and traps involved in reasoning by analogy

"An analogy can deceive us as our instinct can lead us astray, and as we can stumble when walking: this is not a reason to renounce the use of our legs or to rebel systematically against our instinct and the senses."

Antoine Augustin COURNOT
Materialism, Vitalism, Rationalism - 1875

In the Middle Ages, analogical reasoning, which was honored in the Hippocratic tradition, was considered to be essential for building knowledge. The use of analogy in contemporary biological science has often had beneficial results and contributed to interesting discoveries. Sometimes, however, the analogy, an overly easy piece of mental gymnastics, can lead to the inappropriate interpretation of experimental results and give them an erroneous meaning. Constructive audacity in analogical reasoning and mistaken interpretation due to too hasty a comparison are illustrated here by memorable examples.

In the second half of the 19th century, while microbiology was establishing itself as a new discipline, certain bacteriologists convinced themselves that white blood cells, which were first called microphages and then neutrophils because of their tinctorial properties, were vectors of bacteria and spread infection (Figure III.3). In fact, **neutrophils** are in charge of the destruction of bacteria. They are **cellular** or **innate immunity** cells, in the same way as **macrophages**, cells that reside in the tissues.



A B

A - Guinea-pig neutrophil (microphage) filled with choleric vibrions, most of which are transformed into granules.

B - Guinea-pig macrophage filled with choleric vibrions not transformed into granules.

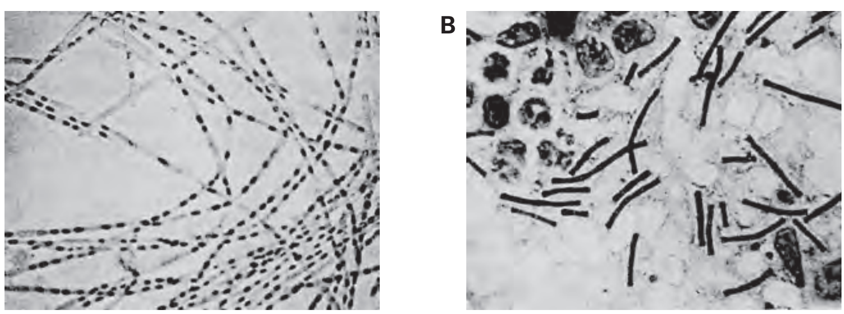
Figure III.3 - Internalization of choleric vibrions by the neutrophil or macrophage, two innate immunity cells
(from E. METCHNIKOFF - *Immunity in Infectious Diseases*, Masson, Paris, 1901)

This discovery was only accepted after a long controversy in which the naturalist of Russian origin, Elie METCHNIKOFF (1845 - 1916) was a pioneer. In this context, it

R. KOCH
(1843 - 1910)

which is an extract of a marine alga. In current practice, the agar is sterilized and fluidified beforehand by heating, and the nutrients necessary for bacterial growth are added to it. Cooled to a temperature below 40°C, the agar sets to form a solid gel. A droplet of a very dilute bacterial suspension is deposited on the gel and spread uniformly across it by means of a thin, curved spreader, which acts rather like a rake. The dish is covered with its lid and is placed in the oven and brought to 30° or 37°C. After 24 hours, it is found that, scattered across the gel, little mounds have formed. These correspond to bacterial colonies, each colony (or clone) arising from the iterative division of a single bacterial cell into several million daughter cells. Nowadays, the technique of producing bacterial isolates on PETRI dishes seems so obvious that it is difficult to imagine its beginnings. The first microphotographs of bacterial cells coming from pure cultures of the anthrax bacillus were taken by Robert KOCH from 1867 onwards (Figure III.13). Thus, making use of a technique that was astonishingly simple and very inexpensive, bacteriology established itself as an experimental discipline.

A B



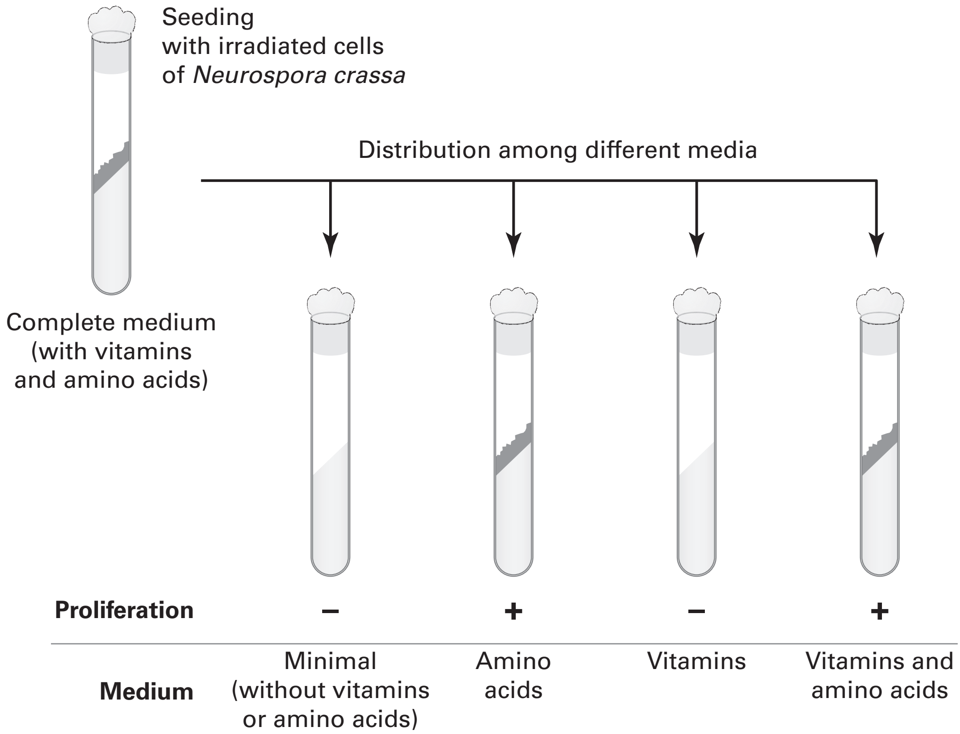
At the beginning of the microbiological era (end of the 19th century), the anthrax bacillus was a model microorganism for the study of infectious pathologies (easily identified illness in the animal, typical rod shape of bacilli).

A - Cells of anthrax bacillus (not-colored) showing the existence of spores that are refringent in light.

B - Smear of cells from the spleen of an animal infected with the anthrax bacillus, after coloration, showing the characteristic rods of the anthrax bacillus, in the middle of the spleen cells.

Figure III.13 - First photomicrographs of bacteria taken by Robert KOCH in 1877

A mastery of the production of pure cultures made it possible to make definite identifications of many previously-unknown bacterial species, to establish relationships between a particular bacterial species and the infection it causes in animals, and by extension, in Man, and also to protect against infection and contagion by applying the principles of asepsis. Experimental surgery in animals and human surgery benefited directly. It was during this period that the criteria regarded as

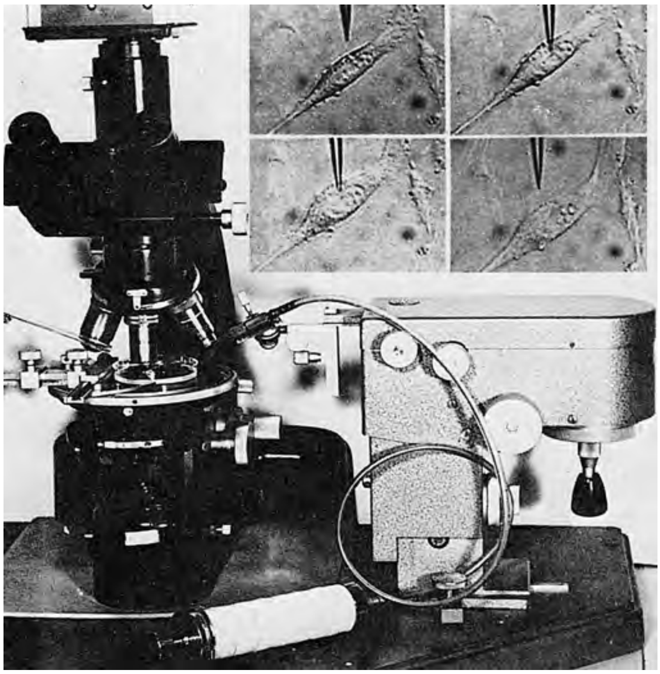


Cells of the mold *Neurospora crassa*, previously irradiated with X-rays or ultraviolet rays in order to increase the rate of mutations, are deposited on a complete culture medium (solid agar). After proliferation, the cells are distributed among different tubes, one of which contains a minimal culture medium (synthetic medium without vitamins or amino acids), and the others of which contain the minimal medium to which has been added a mixture of vitamins or of amino acids, or a mixture of vitamins and amino acids.

The absence of proliferation signifies a defect in the synthesis either of the vitamin(s), or of the amino acid(s). Here there is a defect in amino acid synthesis because the addition of the mixture of amino acids starts the proliferation up again. The operation is continued in order to identify precisely for which amino acid the synthesis has been blocked because of a mutation in one of the enzymes involved in the pathway of this synthesis. The synthesis of any amino acid involves a chain of precursors, which are generally well identified. The addition of these precursors, one by one, to the minimal culture medium makes it possible to locate the site of the mutation, by identification of the precursor for which the synthesis is blocked.

Figure III.15 - Principle of production and detection of mutants in the microscopic mold *Neurospora crassa*
(adapted from G.W. BEADLE (1946) *American Scientist*, vol. 34, p. 31)

In the 1940s, **microbial chemistry** was expanding rapidly. Bacteria became a preferred terrain to which the principles of molecular biology could readily be applied. A non-pathogenic strain of the enterobacterium ***Escherichia coli*** (***E. coli***) became the reference experimental model in numerous laboratories around the world. It is surprising to note that the **genetic code** (which was later recognized to be universal, or nearly so) was first deciphered based on experiments on *E. coli*,



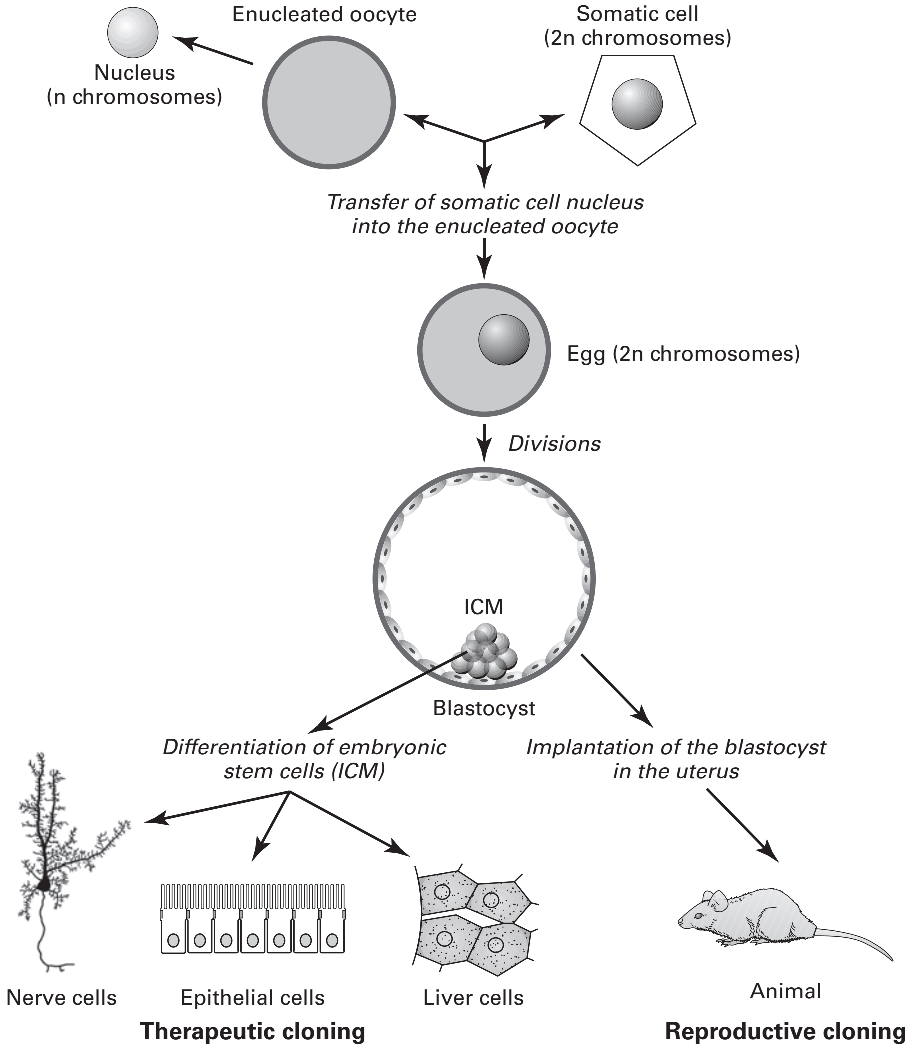
A very small volume of DNA (2.10^{-9} ml) is injected under the microscope into eukaryotic cells (HeLa cells in inset) using a micropipette with a very fine end that pierces the cell membrane. The swelling of the cells at the moment of injection can be seen (inset).

Figure IV.4 - Injection of DNA into individual cells by micromanipulation under the microscope (reproduced from J.E. DARNELL, N. LODISH and D. BALTIMORE - *Molecular Cell Biology*, Scientific American Books, W.H. Freeman and Company, New York, USA, 1986, p. 207, with permission of A. GRÄßMANN, Ph.D. thesis, 1968, Freie University, Berlin)

Supported by these successes, genetic engineering started to come to the fore as an application-oriented discipline. Levels of performance that would never have been imagined half a century before were achieved, such as the production of growth hormone, interferons, blood coagulation factors and vaccines. In the final decades of the 20th century, phenotype transformations using genetic modifications that had previously been carried out in bacteria and yeasts were successfully attempted in animals and plants. It was observed that a mutated DNA integrated into a plasmid and introduced into a fertilized mouse egg (by micromanipulation) modifies the mouse's genetic inheritance, which affects first the embryo and then the adult mouse with phenotype modifications. Such mice, which are said to be **transgenic** because of the stable integration of a foreign DNA into their genome, are now widely used as animal models in studies that aim to understand the mechanisms involved in high-incidence human pathologies such as cancer, diabetes, and rheumatoid conditions. In 1982, two American researchers [16], Ralph BRINSTER (b. 1932)

16 For review see: R.D. PALMITER and R.L. BRINSTER (1986) *"Germ line transformation in mice"*. *Annual review Genetics*, vol. 20, pp. 465-499.

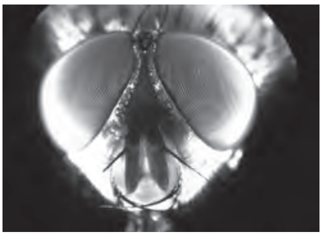
cows, goats, pigs, rabbits, cats, dogs, rats and horses. As far as ethical discussion about cloning is concerned (Chapter IV.5), it is essential to note that the demarcation line between reproductive cloning and therapeutic cloning is situated where decisions are made concerning the **destiny of the cloned blastocyst** (Figure IV.13).



The transfer of the nucleus of a somatic cell (liver, epidermis, muscle) containing 2n chromosomes into an enucleated oocyte gives rise to an egg (2n chromosomes) that is able to divide and to produce a blastocyst. The cells of the blastocyst inner cell mass (ICM) can be used as stem cells that can differentiate into different types of cell line (therapeutic cloning). On the other hand, if the whole blastocyst is implanted into a uterus, it will produce an embryo which, after birth, will grow into an adult animal (reproductive cloning). Reproductive cloning and therapeutic cloning therefore differ because of the fact that in reproductive cloning, the whole blastocyst is used, while in therapeutic cloning, only certain cells, corresponding to the inner cell mass (ICM) of the blastocyst, are used.

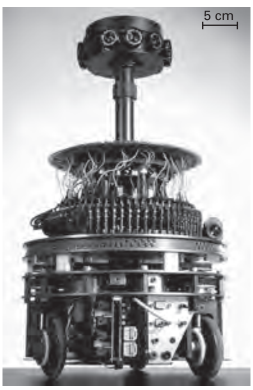
Figure IV.13 - Therapeutic cloning *versus* reproductive cloning

components of which correspond to around one hundred movement detectors in the fly, was incorporated into the head of a robot. The recorded light signals were transmitted to the moving components of the robot.

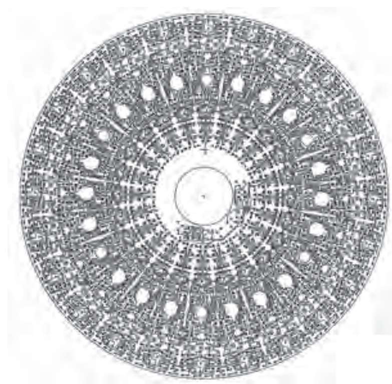


A *Photo N. Franceschini*

B *Photo F. Ruffier et N. Franceschini*



C *Photo N. Franceschini et C. Blanes*



D *Photo N. Franceschini et C. Blanes*

A - Head of the blowfly, *Calliphora*, seen from the front, showing the two compound eyes with their multifacetted array. Each eye hides 40,000 photoreceptors that drive various image processors based on a few hundred thousand neurons.

B - "Elementary Motion Detector" (EMD) neuron and its evolution over fifteen years: on the left, first generation (1989), using Surface Mounted Device (SMD) technology, compared to a one franc coin from that period; on the right, the 2003 version of the highly-miniaturized hybrid (analog + digital) EMD circuit (mass 0.8 grams), compared with a one euro coin.

C - Autonomous vehicle (12 kg) able to move around in a field of obstacles that it does not know about in advance. Its vision is based on a genuine compound eye, whose circuits are inspired by those of the fly. It includes a network of 114 "motion detecting neurons", transcribed electronically according to the principle analyzed in the fly's eye by means of microelectrodes and a specially-constructed microscope-telescope. This network is arranged around a ring that is about thirty centimeters in diameter. The recently-constructed roboflies, Oscar and Octave, only weigh around one hundred grams.

D - Routing of the electronic components (resistances, condensers, diodes and amplifiers that operate in their thousands) soldered onto the six-layer printed circuit-board that provides the connection between the sensors and the steering motor on board the autonomous mobile robot shown in (**C**).

Figure IV.20 - Neuromimetic robot based on the operation of fly's eye
(construction and photographs by N. Franceschini *et al.*, Biorobotics Laboratory, Institute of Movement Science [UMR 6233, CNRS & University of the Mediterranean, Marseille], reproduced with permission)