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The history of cytogenetics

Portraits of some pioneers◇

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1. Preamble

In his book "*La Mémoire, l'Histoire, l'Oubli*" Paul Ricoeur reminds us of "*the difficult path that must be traversed between memory and its historic representation*". It is hazardous to try it. But it is worth attempting to sketch some faces—those that oblivion has not erased—and to find again the climate of the times, sometimes full of sound and fury, progressing from the polytene chromosomes of diptera to the human chromosomes of today, fluorescent and multicolored, thanks to technological advances that now permit us to explore all their secrets. It has not always been so and if, henceforth, human cytogenetics plays an authoritative role in the classification of animal and vegetable karyotypes, we would be wrong to forget that all began with flies and corn.

2. Nonhuman chromosomes

Thomas Hunt Morgan (1866–1945) was working on the sea urchin, when, at the age of 43, on the advice of W.E. Castle of the University of California, he decided to use the vinegar fly as an experimental tool. Son of a captain of the Confederate army (the war of secession ended in 1865), he was rigorous, modest, and methodical. He examined hundreds of generations of these highly prolific flies (one generation occurs every 12 d). He made a good choice. The *Drosophila* was an ideal animal for comparative studies of phenotype and chromosomal structure:

- It can be observed with a binocular magnifying glass.
- It possesses only four pairs of chromosomes.
- The salivary glands of its larvae possess giant chromosomes (called polytenes [1]), whose transverse striations are quite visible.
- Mutations, which modify characters compared to the "wild" type, occur frequently and are accompanied by modifications in the chromosomal striations.

A careful worker, who did not wish to make any hasty conclusions, T.H. Morgan preferred to create the term "crossing over" (which corresponds to recombinations estimated statistically) rather than to use the term already in existence of "chiasmatype" (crossings observed with the microscope), so as not to infer without proof a suspected phenomenon ... which would moreover be proven soon by his work and those of his students: Bridges found the first spontaneous mutation (*white eye*), Sturtevant established the relation between physical distance and genetic distance, and Muller demonstrated the highly mutagenic action of ionizing radiation. Thus, "The Chromosomal Theory of Heredity" was elaborated and published in 1920.

Just as *Escherischia coli* was "the beast of burden" for molecular geneticists, one could say that *Drosophila melanogaster* was the beast of burden for the chromosomal geneticists in the beginning.

Even today, it is amazing to think of all these genes, localized on the chromosomes of the fly in a first genomic map, then cloned, sequenced and placed in data banks, permitting researchers to know, in silico, their human homologues and to understand better their role! Although Morgan alone received the Nobel Prize in 1933, his research reflected the work of a brilliant and productive group with which he managed to surround himself [23].

◇ Translated

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[1] Polyteny is due to absence of the separation of chromatid strands which duplicate themselves hundreds of times without the cell dividing, and which accumulate as a thick fiber.

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2.1. The discovery of transposons

Barbara McClintock was born in Brooklyn (New York) in 1902 just as the work of Mendel was being re-discovered. She earned her doctorate in Science in 1927 at the University of Cornell. She rapidly became an expert in the cytogenetics of corn, and when Lewis Stadler of the University of Missouri sent her lines of irradiated corn, she discovered chromosomes in rings and became aware of the role of their extremities—which she called "telomeres"—in the non-adhesiveness of normal chromosomes. In 1936, she rejoined the University of Missouri where she conducted her work on chromosome breakage, observed recombinations, which resulted in aneuploidy during mitosis. Although she was not an ambitious person, the rivalry between her and Mary Guthrie could have led to her departure from Missouri in 1942 for Cold Spring Harbor. Initially on a temporary status, she ended up in a tenured position and conducted her research on corn lines. She made the unusual observation that certain genes passed from one location to another within the nucleus. In order to demonstrate the reality of these curious phenomena, she repeated the experiments. From 1944 onwards, she became certain of the existence of transposable elements, but waited until 1951 to publish her work at a Cold Spring Harbor Symposium. The reception was skeptical, even hostile, and a painful surprise to her. It so happened that she was going along with the theories of Richard Goldschmidt, who had strongly irritated the scientific community with his repeated refutations of the celebrated theory of Beadle and Tatum: one gene→one enzyme [2] using vague arguments concerning regulatory genes [6]. For Barbara, who had an almost mystic concern for the truth, this was a terrible disappointment. Later, she conducted studies in ethnobotany, spending many seasons in South America with Indians in order to study the evolution of their corn cultures.

She chose an independent and solitary life and distanced herself from society. When she won the Nobel Prize in 1983, more than 30 years after her discovery, when transposons and retrotransposons made their entry in force in Genetics, she remained the same, quite whimsical, eccentric, and without concessions. She continued to work until her death in 1992, at the age of 90, hiding her honors, which were for her an agony, and saw only those "who had something to say" and not those who would have wanted to say that they met Barbara McClintock.

Only her longevity brought about the scientific notoriety that she should have earned a long time before [3].

3. The culture of cells

It would not be possible to understand the history of human cytogenetics without mentioning cell cultures. Since even if many doctors—pediatricians, neurologists or ophthalmologists—and geneticists above all, were preoccupied with congenital malformations or hereditary illnesses, nothing could have been accomplished without the establishment of this technique whose history appears to us difficult and marked with errors—sometimes very beneficial—as we will see.

Since 1885, Roux demonstrated that cells of chick embryos could be maintained alive in a saline solution. But proliferation in vitro was first obtained by Rolf Harrison in 1907 in the USA: these were fragments of spinal marrow of the frog in a plasma clot. However, it is the name of Alexis Carrel, which remains indissolubly linked to cell cultures, whose chick embryo cells were cultivated without interruption at the Rockefeller Institute from 1913 to 1946! However, numerous hypotheses have been formulated concerning these extraordinary cells: fraud, malignant transformation, addition of fresh cells during the changes of the nutritional medium ... [29].

Although the remarkable history of this person extends greatly beyond that of cell multiplication in vitro, it appears necessary to bring it up here, since it prefigures in the events that follow.

3.1. Alexis Carrel (1873–1944)

Born at Sainte-Foy-Lès-Lyon, he was a brilliant student at the school of St. Joseph, then at the Faculty of Medicine in Lyon where he became prosector of Anatomy in 1898 under Professor Testut. At the same time he specialized in reparative vascular surgery. Four years previously, President Sadi Carnot died in Lyon from blows inflicted by an Italian anarchist. Surgeons had been unable to repair the injuries to his portal vein. During the course of several semesters, Carrel established techniques for the sutures of blood vessels while working with Marcel Soulier, professor of Therapy. But he failed the hospital-university competition for René Leriche and considered leaving Lyon where he felt constrained. At the request of Canadians who had come to learn his techniques, he left first for the Hotel-Dieu in Montreal, then, offered several posts in the USA, he opted for the Rockefeller Institute where he carried out work not only on cell cultures but also on innovative experimental surgery that were to be the origin of cardiovascular surgery and of organ grafts. He was to become, in 1912, the youngest scientist to receive the Nobel Prize in Medicine.

After the war of 1914–1918, during which he set up a military hospital and invented with Henri Dakin the antiseptic that bears Dakin's name. Then he returned to the USA. There he invented, among other things, an apparatus for conserving transplanted organs, with his friend the celebrated aviator, Charles Lindbergh. Covered with honors and medals: grand Commander of the Legion of Honor, the

[2] Since 1940, abandoning their work on insects to turn towards microorganisms, they provided irrefutable proof for this theory beginning with *Neurospora crassa*.

[3] Sadly, Rosalind Franklin did not have this opportunity. She could have won the Nobel Prize, but she died at the age of 37 of ovarian cancer. She had produced excellent X-ray diffraction images of DNA that Maurice Wilkins, her collaborator, took as his own, to entrust them to Watson and Crick before their publication on the double helix [28].

Swedish order of Polar Star, the order of Leopold of Belgium, member of pontifical societies, he achieved enormous success in 1933 with his book "*Man, this Unknown*".

Sadly, the end of his life was a disaster. In 1940, after the defeat of France, he was appointed by Marshal Petain to create an Institute of Man: "*La Fondation française pour l'Etude des Problèmes humains*" for which he received the equivalent of $8 million. Alas, his Institute espoused the worst racist theories and was involved in denouncing foreigners, making their deportation from France easier. In a report of the Cahiers de la Fondation, one can read: "*The presence of groups of undesirable foreigners is of certain danger from the biological point of view for the French population*". This is the reason why, after the Liberation, Valéry Radot, then Minister of Health, conducted an investigation into the Foundation [7]. Considered a collaborator, Alexis Carrel nevertheless avoided a trial and prison on the grounds that it would aggravate his cardiac condition. He had his first heart attack in 1943. His death, the following year, drew little attention in the media. Despite the unquestionable racism of his "Foundation", posterity would perhaps have remembered only the prestigious contributions of this man, had not the Extreme Right made Alexis Carrel a symbol of their struggle in the 1990s. There ensued a violent war of words between the revisionists and the antiracists which is far from over and whose echoes resound today.

3.2. Cells by the thousands

Since viruses are obligatory parasites of living cells, their study and research on vaccines necessitate large quantities of cell cultures for research laboratories and diagnostics in virology. The use of trypsin to dissociate cells before placing them in culture was the first step in their serial propagation (Rous and Jones, 1916). But above all, the "immortalization" of cell lines was a revolutionary technical achievement.

In 1952, George and Margaret Gey succeeded in obtaining the first continuous cell line. These were cells taken from a cancer of the uterine cervix of Henrietta Lacks, a 31-year-old American of African origin, who died 8 months later, in the hospital's segregated ward for blacks. Called Hela cells, they were distributed to laboratories all over the world, where their chromosomal material evolved on their own way in different ways. Even today, they are used in many studies in cell biology. The odd legacy of this young black woman has troubled many researchers [14] and a poet even dedicated an ode to her [4]. Numerous other cell lines were thus created in the laboratory and, from the 1950s onwards, the enormous industrial needs for the culture of vaccines against poliomyelitis created an important industry for the production of cell cultures and nutritive media (Eagle, 1955; Ham, 1965; Sato, 1960).

But a better knowledge of these "immortalized" lines, with their erratic chromosomal transformations, soon led researchers to prefer diploid cells—whose replication is limited—for their many studies in cell biology.

4. Human chromosomes

4.1. A long series of errors

The first optic microscopes having appeared in the 15th century, the observers gradually became familiar with the morphology of cells in the centuries that followed. Cell division was known in the 19th century, and in 1888, Waldeyer gave the name of chromosomes to the colored bodies that appeared in the cell.

But how many chromosomes are there in the human cell, and is the number of chromosomes stable in all individuals? This was not known at the beginning of the 20th century. To resolve the problem, cells fixed in histologic sections were examined throughout their thickness by means of a micrometer. As the result of his laborious work on sections of gonads, von Winiwarter published in 1912 a memoir that became a reference: he estimated that there were 47 chromosomes in men and 48 in women. Then in 1921, Painter discovered the Y chromosome in testicular cells from three males from the Texas State Insane Asylum who had been castrated because of "*excessive self abuse coupled with certain phases of insanity*". There were thus 48 chromosomes in the human with the sex chromosomes XX or XY.

Rather than work on histologic sections, other cytologists resorted to cell cultures (Kemp, 1929) or to leucocytes (Kroutchov and Berlin, 1934) in attempts to find the 48 chromosomes, the number of which was established by consensus ... so well established that a research couple, the Melanders, abandoned their research on human embryos because they were unable to find 48 chromosomes in their cultures but found only 46!

However, the 1950s were extremely productive years for the human cytogeneticists thanks to a succession of events, which permitted the observation of mitosis without the need for the tedious technique of Winiwarter:

- In 1952, T.C. Hsu, in Houston, somewhat by chance, noted that a hypotonic solution added to the culture before fixation, caused the cells to swell and permitted dispersion of the chromosomes.
- In 1953, Murray Barr, in London (Ontario, Canada), located the body that could be found only in female nuclei, at first in the epiphysis of the cat, then in all nuclei including human females. Thanks to this observation, the diagnosis of a disease linked to the X chromosome was made possible in Copenhagen from 1960 onwards.
- But above all, it was at Lund, in the laboratory of Albert Levan that on the 22nd of December 1955 at 02:00 h in the morning, after long hours of work, Joe Hin Tjio, a Chinese from Indonesia and in charge of a course in agronomy at Saragosse, obtained some excellent samples of mitosis from cells of human embryonic cul-

[4] Marcel Thiry. Prose des cellules Hela, 1969.

tures, using a technique established in plants. All the cells had 46 chromosomes. Certain of his finding, he published his work [24] and presented it at different meetings. The following year, in sections of testicular tissue, Ford and Hamerton [8] confirmed the constant presence of 46 chromosomes in the somatic cells of the human [5].

All was now ready for studies of abnormalities in chromosome numbers in human pathology.

5. Discovery of the first chromosomal abnormality

"To each his own truth" Anatole France.

5.1. The revelation

At the end of the International Congress of Genetics, in Montreal, in August 1958, Jérome Lejeune announced the presence of a supplementary chromosome in Down's syndrome to Clark Fraser and several other geneticists at McGill University. His announcement was received with interest but also with skepticism: for this congenital malformation that some attributed to a dominant mutation of an autosomal gene, was it not a simple polymorphism? Then a publication in the C.R. of the Academy of Sciences of Paris on the 26th of January 1959 [6] reported the observation of human chromosomes in tissue culture where the case of three children with Down's syndrome with an extra chromosome was mentioned. Finally, on the 16th of March 1959, the publication in this same journal on the presence of a small supernumerary chromosomes in nine children affected with Down's syndrome confirmed the existence of the first chromosomal aberration in a disease and inaugurated a new discipline: human cytogenetics [20].

5.2. The discoverers

Since the authors of the publications mentioned above have not formed a definite group, it is appropriate to mention them separately.

5.2.1. Raymond Turpin (1895–1998)

He was a part of the generation, which remained in the turmoil of World War I (WW I) (Fig. 1). He had just completed his medical studies when he was mobilized at the age of 20 and appointed to the fighting corps of the military fortresses of Verdun. Although it was not mentioned in his biographies, the memory of this hell must have affected him profoundly. After victory, he resumed his studies and succeeded in the competition for the Internat of the Hospitals of Paris in 1921. He chose Pediatrics at a time when tuberculosis was rampant but when prevention was possible: the BCG vaccine developed by Calmette and Guerin had just been shown to be effective in animals. One now had to make the serious decision of testing it in the human. R. Turpin participated in the first vaccination campaigns in infants born of tuberculous mothers. Then, around 1930, when infectious diseases were beginning to be controlled, he turned towards hereditary diseases because he had always been interested in problems of the innate and the acquired. In 1941, he inaugurated the teaching of Genetics in the Faculty of Medicine. He founded the Institute of Progenesis in 1958 and created the first chair in Fundamental Genetics. He may thus be considered along with Professor Maurice Lamy as the one who originated Medical Genetics in France.

Fig. 1. Raymond Turpin (1895–1998).

First at the *Hôpital Saint-Louis*, then at the *Hôpital Trousseau*. Turpin began studies on the etiology of Down's syndrome (then called "mongolism") and on its clinical manifestations. With his student, Alexandre Caratzali, he gathered more than a hundred families with an affected child and studied the cases where there were several in the same family. Very early on, he considered the hypothesis of an anomaly analogous to the Bar mutation in *Drosophila*, at the same time as Waardenburg did, for Down's, the possibility of a duplication or loss of chromosomal material [27].

Still it was necessary to demonstrate it, but this was impossible before 1956. On his return from the Congress of Genetics in Copenhagen where the chromosome number was definitively recognized as 46, R. Turpin wanted to establish rapidly the techniques that would permit one to observe and count them, all the more since Marthe Gautier had just returned from training in the USA where she had learned the techniques of cell culture.

Later, he replaced Jean Cathala in the Chair of Paediatrics at the *Hôpital Necker–Enfants Malades*, a post that he held until his retirement.

[5] But for several years more, 48 was the chromosome number as taught in schools and colleges. The media at that time did not pay much heed to scientific discoveries.

[6] Five days later, Patricia Jacobs and Strong [17] published a paper in *Nature* on anomaly in sex chromosomes in Klinefelter's syndrome: XXY.

5.2.2. Marthe Gautier

She was the fifth child of six siblings in a family of farmers in the Seine and Marne, a department southwest of Paris (Fig. 2). Thus, nothing would have destined her for a career in medicine if it were not for the support of her mother who wanted her daughters to attain higher education whatever the sacrifices. It was thus that her elder sister, Paulette, entered the Faculty of Medicine of Paris where Marthe joined her in 1942. Unfortunately, although she had prepared for the internat competition, Paulette was killed in 1944 by the Germans, who, in retreat, avenged themselves of their defeat by firing on innocent civilians. Martha continued her studies. She passed the externat, then the internat despite the difficulties that weighed heavily on women at that time in succeeding in these competitive examinations—where anonymity was restricted only to the written part of the examinations. In her promotion in 1950, out of 80 appointed, only two were women! Her internat was directed towards Pediatrics. During her last post with Professor Robert Debré, she was particularly interested in acute rheumatoid arthritis, which was to be the subject of her thesis in 1955. It was then that Professor Debré proposed that she go to Benedikt F. Massel at Harvard (Massachusetts) in the United States. Since the end of WW II, this country was a magnet for science, and promising students were sent there to study. After a sea voyage with Jean Aicardi and Jacques Couvreur (the airplane was too expensive for those supported by grants), she discovered that besides the clinical part she was also assigned to work in the cell culture laboratory. The techniques there were well established, and Marthe Gautier carried out cultures of aortic explants removed in the course of surgery. She even co-authored a publication, but she was interested above all in the teaching of Massel on acute rheumatoid arthritis and that of Alexander S. Nadas on congenital cardiopathies.

She returned in 1956 to a fait accompli: the post of head of clinics under Professor Lelong, which was planned for her before her departure. She thus found herself in the service of Professor Turpin. The latter, at that time, wanted a cell culture facility in his department to verify the number of chromosomes in infants with Down's syndrome. Marthe offered to take charge, but everything had first to be organized. She needed space, glassware, cock plasma, chick embryonic extracts, human serum, in brief, everything that was needed at this time when research laboratories did not exist and pharmaceutical firms only sent nutritive medium for roller cultures destined for Virology. Laboratory space was found in the Parrot pavilion of the Hospital Trousseau. Jacques Lafourcade, who at that time held the title of "*Médecin des Hôpitaux*", came sometimes to see Martha to ask questions about her work (expressing his concern, with gentle irony, about when the fibroblasts would end up invading the staircases!). Marthe Gautier procured the material (including a rooster, proposed plasma donor, which disturbed the surroundings with its morning crowing!), in waiting for the samples of skin biopsies of children with Down's syndrome that Monsieur Turpin obtained in 1957 after having obtained the authorization of the parents and the consent of the surgeons. To avoid any artifacts, she studied cells only as primary cultures and used neither trypsin nor colchicine, fearing that they might induce changes. Some excellent mitoses were finally obtained, but there was no photomicroscope in the laboratory. The photos had to be done elsewhere by Jérôme Lejeune.

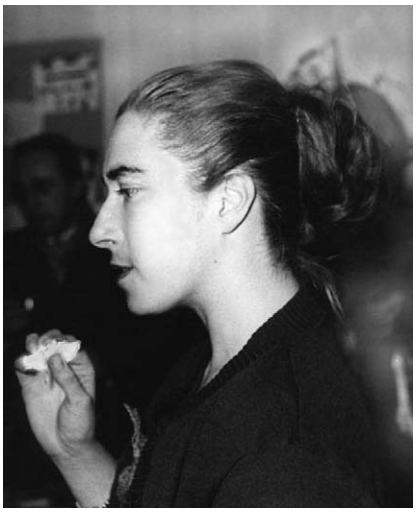

Fig. 2. Marthe Gautier.

After having co-authored the first publications [19,20], Marthe decided to leave the service of professor Turpin where from then on she no longer felt comfortable. She succeeded in a competition for the position of *Assistant des hôpitaux* and decided to devote herself entirely to Cardiopediatrics, preferring human contact with children and their families, in the service of Professor Nouaille who had just opened up his service in at the *Hospital Kremlin Bicêtre*. Subsequently, she became Master of Research at the INSERM.

Currently in retirement, she spends part of her time painting fruits and wildflowers on porcelain.

5.2.3. Jérôme Lejeune (1926–1994) [7]

Coming from a family of three boys, he was a "child of Montrouge" as it pleased his friend, Jean de Grouchy, to recall [15] (Fig. 3). In fact, his paternal grandfather was mayor of this town in the Ile de France. His father, Pierre Lejeune, became mayor of Etampes during the war of 1939–1944. Due to this, he was to be accused during the Liberation of collaborating with the Germans, arrested and freed for lack of evidence after an imprisonment of 5 months. He came out of it clearly broken, and one can easily imagine the impact of this event on his son, Jérôme, who was 18 years old at the time.

[7] This important figure in French cytogenetics is well-known to all. The details of his life were published by his daughter, Clara Gaymard Lejeune, in a book [21] from which this review owes much.

Fig. 3. Jérome Lejeune (1926–1994).

He studied medicine and in 1951 presented his thesis entitled "Contribution to the study of the regression of the masculine index in multiple pregnancies" [22].

Thus, even at that time, it seems that he was interested in Genetics problems, in particular the origin of mongolism. In the department of Professor Turpin, he was in charge of making dermatoglyphs of mongolian children and noted the analogy of a unique palm line with that of monkeys [25]. Then he undertook his military service at which time he made the acquaintance of Jean de Grouchy. The latter was at the *Hôpital Necker-Enfants–Malades* in the service of the other pioneer of Genetics in France, Maurice Lamy. From this chance encounter, a long friendship was born.

It was at this time that J. Lejeune met in Paris a young Danish woman, Birthe Bringsted, whom he married in Odense, Denmark, despite the reservations of his family: this young woman was a foreigner, she came from a modest family, and moreover, she was a Protestant. They had five children. Madame Lejeune espoused all the causes of her husband and continues after his death to be occupied, without respite, with the foundation that bears his name. Among the first publications of Jérôme Lejeune, one is surprised to find the name of his wife, who must have assisted in the fastidious work of dermatoglyphics [26], even before the birth of their first child. Then, shortly thereafter, he asked Marie-Odile Réthoré to collaborate with him. She accepted gladly and at the same time agreed to be the godmother of the newborn little girl. Moreover, M.-O. Réthoré was to be the godmother of all the Lejeune children, about which the academician that she became remains proud even today.

Lejeune became a trainee in research at the CNRS and, with M.-O. Rethoré, was interested then in the effects of ionizing radiation. Appointed an expert by the Commission of Radiation, it was during a congress that he brought up, as we have said, the presence of a supernumerary chromosome in mongolian infants (despite the recommendations of Professor Turpin to be very discreet on the subject). Then, there were the two first publications in the Academy of Sciences, already mentioned above. The open road proved to be extremely productive. Consequently, when Marthe Gautier left the service, he continued research on chromosomes and discovered, with Jacques Lafourcade and M.-O. Réthoré, a very large number of anomalies. At the end of his life, he had authored more than 500 publications. First at Trousseau, then at the *Hôpital Necker-Enfants–Malades*, his laboratory became the center where all researchers and doctors interested in cytogenetics would flock. The large majority of those responsible for the other French cytogenetics laboratories were formed there, and that does not account for all the scientists who came from numerous other countries. In 1963, he was awarded the Kennedy Prize, which was handed to him by the President himself. In 1964, he was appointed to the first chair in Fundamental Genetics of the University of Paris. Every Saturday in the company of Madame Lejeune, devoted mistress of the house, he invited to his home Jacques Lafourcade—who became professor of Pediatrics—and Jean de Grouchy, where they continued their scientific discussions and no doubt, philosophical-religious ones as well. Since Jérôme Lejeune was also a fervent and uncompromising believer, he became more and more shocked by changes in morals. In 1967, the authorization for women to use contraceptives seemed to him at odds with morality. He lived sadly through the events of May 1968. In 1970, he was outraged when he learned that the Peyret law proposed to render legal the interruption of pregnancy in the case of fetal abnormalities. He then undertook a fight without mercy, seeking to rally doctors and jurists. He succeeded, for in 1973, the Declaration of the Doctors of France won the support of 18,000. The seriousness of this act, which is totally against the spirit of the Hippocratic oath, raised questions in everyone's conscience. Many gynecologists will refer to the "clause of conscience" when, in 1975, the "law of Veil" was adopted, authorizing voluntary interruptions of pregnancy.

But in view of the hold taken by the intransigent positions of Jérôme Lejeune, the French geneticists were divided. Some disapproved of its intransigence and wanted instead to provide genetic counseling to families at high risk of having a child suffering from a malady of "extreme gravity".

Since then, having become a member of the *Académie Pontificale des Sciences* and later President of the *Académie Pontificale pour la Vie*, he rarely attended international scientific meetings in Genetics where foreign geneticists (Anglo-Saxons in particular) did not understand his attitude. He sought desperately for a cure for children affected with trisomy 21 and turned towards other battles, like that of restoring to favor the authenticity of *Saint Suaire* which was considered to be false after carbon^{14} dating [8]. It is clear that the attitude of Jérôme Lejeune cost him the Nobel Prize for his work in cytogenetics and that the French cytogeneticists would be undermined for a long time, while other branches of Genetics, clinical or molecular, would develop with greater vigor.

[8] J.-M. Le Méné. Le Professeur Lejeune. Fondateur de la Génétique moderne. MamE Edit 1997.

6. Prenatal cytogenetics

We must now go back in time to consider the status of medical knowledge about birth defects. Before it was possible to visualize the fetus by ultrasound, fetal abnormalities remained undetected until birth, except for the most serious cases leading to arrested development, that is, a miscarriage or expulsion of a dead fetus, often decomposed.

6.1. Birth of Prenatal Medicine

As André Boué wrote in his book on Fetal Medicine [1]: "*On the epidemiological level, it was impossible to evaluate the frequency of spontaneous abortions: on the one hand, there is the tendency to present an induced abortion as a spontaneous one; on the other hand, women who had spontaneous abortions do not report it in retrospective studies*".

There was a very repressive legislation against abortions at the time. Under the Vichy regime, a woman who had performed abortions was even condemned to death and executed. Secret abortions were often practiced under conditions of deplorable hygiene, placing the lives of women in danger.

Nevertheless, two exemplary studies carried out during the 1940s opened the way for research on fetal pathology:

- one in Australia, which demonstrated the pathogenic effect of the rubella virus on the fetus. An ophthalmologist, Norman Gregg, established the relation between the occurrence of a rubella (German measles) epidemic and the presence of a congenital cataract in 78 newborns several months after birth;
- the other was in Boston (USA) where Arthur Hertig carried out between 1936 and 1941 a pathological study of 1000 products of spontaneous abortions. He noted that three-quarters of them were blighted ova and concluded that this was a case not of an external cause but of a defect in the egg itself, all the more as the expulsion occurred later, several months after the developmental arrest.

Spontaneous abortions and fetal malformations could thus be due to genetic or environmental causes.

Everything had yet to be done and from the 1950s onwards, we witnessed the birth of Prenatal Medicine.

Prevention of the exogenous causes was undertaken with success:

- prevention of Rh iso-immunization in Rh-negative women,
- vaccination against rubella, avoiding a primary infection during pregnancy,
- a multivitamin regime with folates to lower the frequency of neural tube anomalies.

As to the prevention of endogenous causes, the discovery of the high frequency of chromosomal abnormalities in spontaneous abortions opened up this field of inquiry.

The story of André and Joëlle Boué is appropriate to insert here.

6.2. Joëlle and André Boué

Although Joëlle Poirier was born in Paris, it was only in Prague before the war that her eyes were first opened to the realities of the world. At the German school that she attended, the songs that she learned were in Yiddish—but it was not until much later that she realized it. From 1935, new students kept arriving and swelled the ranks of the classes. They were young girls of German-Jewish families fleeing Nazism and the abuses of the black shirts.

In 1936, her family, who was not Jewish, moved to Paris. But as an only child, she was alone with her mother. Her father, who was in civil aviation, had committed himself, like André Malraux and so many others, to fighting fascism in Spain.

In Medicine, Joëlle Poirier wished to become a surgeon before she met André Boué. He was trained in a new discipline: anesthesiology. Without doubt the couple dreamed of other places, since they decided to go to Teheran. An Iranian surgeon, a friend of the family who wanted to do cardiovascular surgery of high quality, was looking for an anesthetist in order to work under better conditions.

When the Boués arrived in Iran in 1950 to begin their work in anesthesiology and intensive care, there was much to do. There were neither sterile solutions nor blood for transfusions. Luckily, the director of the Pasteur Institute welcomed them with open arms. Marcel Balthazard offered them the possibility of setting up a laboratory with a Blood Bank. Thus began the first studies of the frequency distribution of blood groups in the Iranian population: Moslems, Kurds, Turkomans, Afghans, as well as the Gabars, later disciples of Zarathrustra, who were very close to the Parsis. Variations in blood groups were a reflection of the history of migrations in this corner of the world [2,3].

The standard procedure at the time was to work with cell cultures for which calf serum and amniotic fluid were necessary (or so it was believed then). For André Boué, who had helped his veterinarian father in the Norman countryside, keeping a heifer was child's play... which sometimes turned into a fight when the beast ran off before Joëlle could remove any blood! Studies on the enterovirus were expanding. But to provide evidence for the cytopathogenic effect of poliomyelitis, cell cultures of monkey kidneys were needed. But monkeys were not to be found in Iran. This did not deter Joëlle. Pilots of Iranian Airways used to bring some back illegally from Bombay... until the day that one of the primates escaped and grabbed the veil of an Iranian woman in the airplane's toilet!

They thus had to abandon their project or to find another solution. Perinatal mortality was quite high in Teheran. Stillborns could be found in the mornings in the maternity wards, abandoned without a burial place. Their tissues provided ample samples for cultures. With the passing of years, the *Centre de Transfusion* and *Laboratoire* increased in size and was a full-time occupation for André and Joëlle Boué. A child, François, was born to them, and then another, Nicolas.

Fig. 4. Joelle Boué surrounded by Margaratha Mikkelsen (Denmark) and Ted Galjaard (The Netherlands) at left, and at right Marie Ferguson-Smith (UK) and Eva Sachs (The Netherlands).

But when the latter died at 9 months of age of a very serious infection, Iran lost its charm and color for them.

They decided to leave. Several posts were offered to them. France, Canada? They had enough pioneer spirit to try the New World as another adventure. But the bucolic charm of the Château of Longchamp, where Robert Debré was looking for a virologist, held André back, while Joëlle gave birth in 1959 to a baby girl. Very anxious, she devoted 2 years to her child. Then she rejoined André at Longchamp and like him, joined the CNRS. She went in Turpin's department to initiate cytogenetic techniques, from then on an important supplement for cell cultures.

In order to round off their training, they left together for the *Wistar Institute* (Philadelphia, PA, USA) during the academic year 1963–1964. They arrived at just the right time! A combination of events was going to determine the rest of their work:

- An epidemic of rubella was rampant in the USA, which demonstrated the pathogenic effect of the virus on embryos;
- It was observed that an effect of this virus was to arrest mitosis in the cultures depending upon the tissue of origin;
- They learnt that chromosomal abnormalities were observed in spontaneous abortions by David Carr (London, Ontario) [5].

The problem was thus the following: in the course of development, particularly in spontaneous abortions, there must exist:

- exogenous disorders (viral infections, for example);
- endogenous disorders (abnormalities in programming).

On their return to Longchamp, they embarked then on this study, complete with comparative pathologic analysis of embryos and/or placentas [4]. For this work, they received the silver medal of the CNRS in 1968, given for the first time to two persons jointly.

Prenatal diagnostics is only the logical follow-up of this research.

In June 1970, they carried out the first prenatal diagnosis for recombination aneuploidy. The mother, a carrier of the balanced translocation, left for London, accompanied by gynecologists from the *Maternité de Port Royal* and the *Maternité Baudelocque*, to undergo amniocentesis: a part of the amniotic fluid was given to Kurt Hirschhorn (then at the *Galton Institute*), and the other part was placed in culture the same evening at Longchamp by Joëlle Boué. Unfortunately, the same aneuploidy (which had previously caused the death of her elder, malformed brother) was found again, and the first abortion for a chromosomal abnormality was carried out in France.

From then on, and for many years, in the absence of legislation, numerous couples at risk, from all the provinces in France and from foreign countries, were allowed to attempt another pregnancy.

Afterwards, they would become leaders of very important European studies devoted to spontaneous abortions and to prenatal diagnostics and would foster friendly relationships with numerous foreign cytogeneticists (Fig. 4).

7. A glance at the United Kingdom

Although the French discovered the first chromosome abnormality, Sweden is indisputably the cradle of human cytogenetics: the quality of the metaphases obtained by Joe Hin Tjio are amazing, even today. Besides the Scandinavian countries, other countries of Europe also possessed a good infrastructure of technique during the 1950s. Unfortunately, in this already long resumé, it would be impossible to review all the pioneers who have contributed to the birth of our discipline. We will thus limit ourselves to two great figures of the United Kingdom.

7.1. Charles Edmund Ford (1912–1999)

He was the eldest of six siblings (Fig. 5). He was a brilliant student. At the age of 16, he entered *King's College* of London and earned a Ph.D. at the age of 23. As one of the most celebrated human geneticists, he had an astonishing career, full of risks, trips, but also hard work. He began his studies in botany, first with the chromosomes of the *Oenothera* (a flowering plant selected for this type of study because the spontaneous alterations were described by Hugo de Vries at the end of the last century), then on the *Malva* and

Fig. 5. Charles Ford (1912–1999).

Lavatera. From 1938 to 1946—with an interruption during WW II when he was a lieutenant in the Royal Artillery—he traveled to Ceylon (Sri Lanka) to do research as part of a program of study on the *Heveas*, and on the production of rubber.

In 1946, he was in Canada where the Ministry of Supplies had transferred a part of its services for greater security. There he studied the effects of radiation on the roots of *Vicia faba*. But when the Ministry returned to the United Kingdom, studies on radiation were entirely taken over by the Medical Research Council (MRC). He, therefore, joined the Radiobiology Unit of the MRC at Harwell in 1949. There he became chief of the section of cytogenetics where he spent the most productive years of his career. He established, with John Hamerton, a technique for studying chromosomes that he applied to numerous wild animals in the hope of discovering one whose chromosomes would be particularly easy to study. In 1953, he observed that in shrews, the chromosomes varied in number: from 22 to 27 in males and from 22 to 25 in females, the variation being the result of acrocentric fusions [9]. It was the first time that a chromosomal polymorphism was demonstrated in animals. Then he undertook studies of radiation-induced translocations in the mouse, since he was interested in chromosomal alterations associated with leukemias and tumors [10].

It was at this moment that a surgeon, who was visiting his laboratory and impressed by the quality of his pictures of chromosomes, asked him to give him testicular biopsies to study human chromosomes. At first, Charles Ford did not see the usefulness of this, since it was known that the chromosome number in humans was 48. But as soon as he learned, in 1956, that Tjio and Levan found 46 chromosomes, he immediately got in contact with the surgeon, and soon confirmed the presence of 23 bivalents in the spermatogonia [8]. 1959 was an opportune time for the discovery of numeric anomalies. In Turner's and Klinefelter's syndromes, because of the discordance between the sex and Barr body, anomalies were suspected by Paul Polani who provided Ford with some biopsies. Ford then demonstrated the loss of an X chromosome in Turner's syndrome [11] and discovered the first mosaic in a Klinefelter XX/XXY [12]. Then, in the course of his career, he continued his work in cytogenetics on man and mouse, and his brilliant and passionate speeches, in refined English, still resound in the ears of those who have heard him. In 1979–1980, he spent his last year of activity as an "Invited Professor" at the University of Leiden (The Netherlands), where he left a message for everyone: "*Treasure your exceptions*" [13].

7.2. Patricia Jacobs

It was an unimaginable event of our time that when Patricia Jacobs discovered 47 chromosomes in Klinefelter's syndrome, she was 23 years of age with a technician who was only 16 (Fig. 6)!

Michael Court Brown, then director of research on the effects of radiation in the group at MRC newly created at Edinburgh, recruited her as a scientist to study chromosomes in spontaneous and radiation-induced leukemias.

He sent her first to train at Harwell and Oxford, where for 4 months, she was initiated into the cytogenetics of mammals with Charles Ford and cultures of bone marrow with Lazlo Lajta. On her return, since leukemias were not very numerous, she practiced the techniques on diverse samples. An endocrinologist, Dr. John Strong, provided her with a sample from a subject affected with Klinefelter's syndrome, whose smears showed the presence of a Barr body. We are the beginning of the summer of 1958. Preparations of mitoses were not excellent, but it seemed that there were 47 chromosomes in this subject. P. Jacobs left on vacation to let things settle, by asking Muriel Brunton, her young technician, to prepare slides of the subject and of controls without labeling them so that she could look at them on her return. When she examined them, she found not only one but two lots of slides with 47 chromosomes. She thought that she was mistaken and that it must be an artifact. But, smiling and very conscious of her strategy, Muriel explained to her that she had prepared two trays of slides from the same subject. This time, Patricia took part in the game and after several hours became convinced that this subject, and he alone, had 47 chromosomes. She reported this to Court Brown who saw immedi-

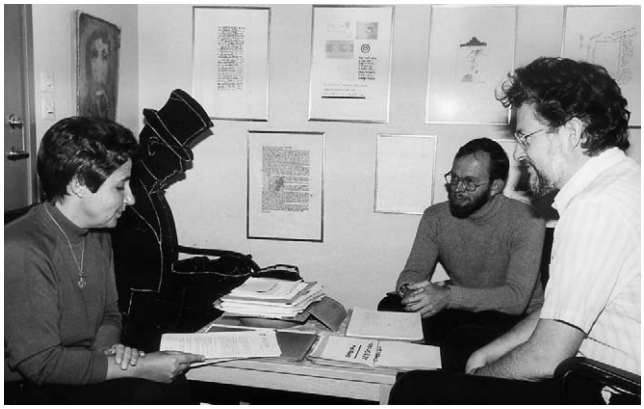

Fig. 6. Patricia A. Jacob, N.E. Morton (who became her husband) and J. Linsten (Sweden).

ately the importance of this discovery and had her write a note to be published in *Nature* several weeks later [17].

As P. Jacobs herself emphasized [18], her history merits consideration in many respects:

- the use of blind methods is vital for biological analysis,
- the current prolongation of studies will no longer permit one so young such an opportunity as she had,
- recognition for work accomplished and encouragement to carry it out on one's own terms in writing and signing a publication give to young researchers the confidence that they need to continue and to blossom.

The long career of Patricia Jacobs continued on numerous studies of the cytogenetics of populations, first in Scotland, then in Hawaï (USA) at the University of Honolulu where she later established with her group the area of prenatal diagnosis. Still active, she is at present in Great Britain at the University of Salisbury.

8. Conclusions

Descriptions of cytogenetic techniques can be found in books. Constitutional abnormalities were the subject of an atlas [16] and some catalogs, some of which are now computerized. The implications of these chromosomal studies are numerous in Cell Biology and extend widely into the field of Human Genetics. Rather than review the history of normal and pathologic chromosomes, it seemed preferable to retrace the lives of several pioneers. The history of Science is also the history of the men and women who made it.

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References

[1] A. Boué, La médecine du fœtus, Odile Jacob, Paris, 1995.
[2] A. Boué, J. Boué, Etude sur la répartition des groupes sanguins en Iran, Sang 26 (1955) 705–713.
[3] A. Boué, J. Boué, II Étude sur la répartition des groupes sanguins en Iran, Ann. Inst. Pasteur 91 (1956) 898–911.
[4] J. Boué, A. Boué, P. Lazar, Les aberrations chromosomiques dans les avortements, Ann. Genet. 10 (1967) 179–187.
[5] D.H. Carr, Chromosomes anomalies as a cause of spontaneous abortion, Am. J. Obstet. Gynecol. 97 (1967) 283–293.
[6] N.C. Comfort, Two genes, no enzyme: a second look at Barbara McClintock and the 1951 Cold Spring Harbor Symposium, Genetics 140 (1995) 1161–1166.
[7] A. Drouard, Une inconnue des sciences sociales: la fondation Alexis Carrel, Maison des Sciences de l'Homme/INED, Paris, 1992.
[8] C. Ford, J.L. Hamerton, The chromosomes of man, Nature 178 (1956) 1020–1023.
[9] C.E. Ford, J.L. Hamerton, G.B. Sharman, Chromosome polymorphism in the common shrew, Nature 180 (1953) 392–393.
[10] C.E. Ford, J.L. Hamerton, D.W.H. Barnes, J.F. Loutit, Studies of radiation chimaeras by the use of chromosomes markers, in: G.C. de Hevesy, A.G. Forssberg, J.D. Abbatt (Eds.), Advances in Radiobiology, Edinburgh, 1957.
[11] C.E. Ford, K.W. Jones, P.E. Polani, J.C. de Almeida, J.H. Brigg, A sex chromosome anomaly in a case of gonadal dysgenesis, Lancet 1 (1959) 711–713.
[12] C.E. Ford, P.E. Polani, J.H. Briggs, P.M.F. Bishop, A presumptive human XXY/XX mosaic, Nature 183 (1959) 1030–1032.
[13] C.E. Ford, Treasure your exceptions Univ. Leiden ISBN 90 6021 376 9. 1981.
[14] M. Gold, A Conspiracy of Cells: One Woman's Immortal Legacy and the Medical Scandal it Caused, State Univ. New York Press, 1968.
[15] J. de Grouchy, Jérôme Lejeune 1927–1994, Méd./Sci. 10 (1994) 624–625.
[16] J. de Grouchy, C. Turleau, Atlas des Maladies Chromosomiques, 2ème édit, Expansion Scientifique, Paris, 1982.
[17] P.A. Jacobs, J.A. Strong, A case of human intersexuality having a possible XXY sex-determining mechanism, Nature 183 (1959) 302–303.
[18] P.A. Jacobs, The William Allan memorial Award Address: human population cytogenetics: the first 25 years, Am. J. Hum. Genet. 34 (1982) 689–698.
[19] J. Lejeune, M. Gautier, R. Turpin, Les chromosomes humains en culture de tissus, C.R. Acad. Sci. Paris 248 (1959) 602–603.
[20] J. Lejeune, M. Gautier, R. Turpin, Étude des chromosomes somatiques de neuf enfants mongoliens, C.R. Acad. Sci. Paris 248 (1959) 1721–1722.
[21] C. Lejeune, La vie est un bonheur, Criterion, 1997.
[22] J. Lejeune, Contribution à l'étude de la régression de l'indicie de masculinité des grossesses multiples, Thèse Médecine, Paris, 1951.
[23] A.H. Sturtevant, A History of Genetics, Harper et Rowe, New York, 1965.
[24] H.J. Tjio, A. Levan, The chromosome numbers of man, Hereditas 42 (1956) 1–6.
[25] R. Turpin, J. Lejeune, Analogie entre le type dermatoglyphique palmaire des singes inférieurs et celui des enfants atteints de mongolisme, C.R. Acad. Sci. Paris 238 (1954) 395–397.
[26] R. Turpin, J. Lejeune, M. Génin, M.B. Lejeune, Recherche d'une éventuelle association entre l'hémophilie et certaines particularités des dermatoglyphes, Semaine des Hôpitaux de Paris (Annales de la Recherche Médicale; n°3). 1955.
[27] P.J. Waardenburg, Das Menschliche Auge und seine Erbanlagen, Bibliogr. Genet. 7 (1932).
[28] J.D. Watson, The Double Helix, Robert Lafont, 1968.
[29] J.A. Witkowski, Dr Carrel's immortal cells, Med. History 24 (1980) 129–142.