

The discovery of meiosis by E. Van Beneden, a breakthrough in the morphological phase of heredity

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*«The cytological discoveries of this period (1870-1900) reached their climax in the splendid researches of Edouard Van Beneden (1883-1884, 1887) on the history of the nuclei during the fertilization of the egg of the nematode *Ascaris megalocephala* which demonstrated that the chromosomes of the offspring are derived in equal numbers from the nuclei of the two conjugating germ-cells and hence equally from the two parents».*

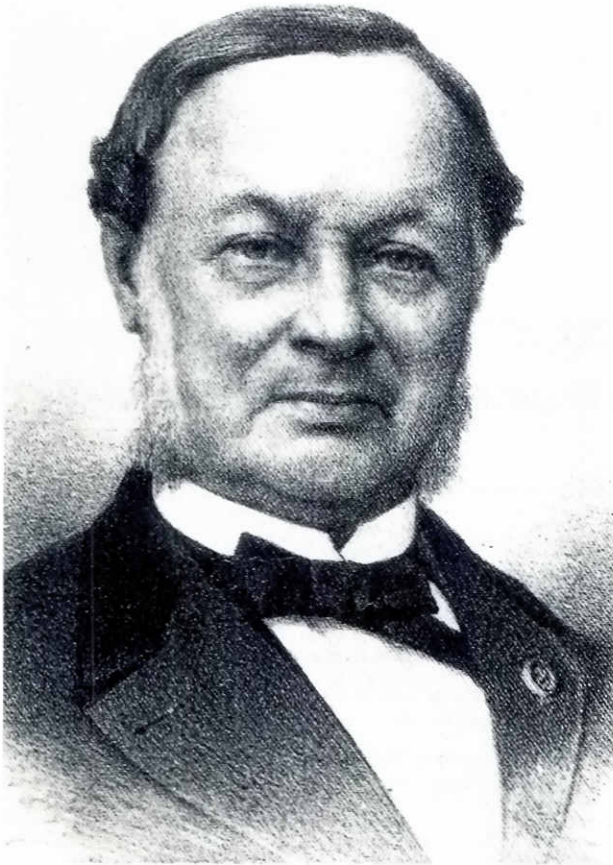
E.B. Wilson (1924)

The discovery of meiosis by Edouard Van Beneden in 1883-1884 is described in its historical perspective. At the dawn of cytology, this discovery is by far the most important advance in the morphological phase of heredity at the end of the 19th century and the beginning of the present one. The long way covered by Van Beneden (1846-1910) towards his discovery is outlined. The finding of the fusion of the two germ-cells in sea urchin eggs by Hertwig in 1876 led Van Beneden to seek some other biological material affording better insight into the maturation of the egg and fecundation. His choice of *Ascaris megalocephala* was a shrewd one, but his extensive study of maturation and fecundation ended in a blind alley. A final look at preparations made on female worms thrown live into dilute alcohol and left there for several months permitted the discovery of meiosis. Thanks to the unexpectedly low number of chromosomes, the demonstration of the halving of this number in germ-cells was unquestionable. His interpretation of maturation by pseudokaryokinesis was challenged by Boveri in 1887. The formation of the polar bodies follows the mitotic process. The first observations suggesting a side-by-side association of chromosomes were made in 1900 by a collaborator of Van Beneden's, Winiwarter, in a study of the formation of oocytes in the mammalian ovary. It finally became clear that the first maturation division is usually reductional and the second one, equational.

At the beginning of the 19th century, improvements in the microscope and the cellular theory of Schleiden and Schwann (1838-1839) gave a great impetus to the life sciences. The exploration of a new level of organization of the living world gave rise to new sciences. Histology developed quickly together with cellular pathology. New chapters of anatomy, zoology, botany and, above all, embryology were opened. Biologists and physicians, faced first with new fields of research, were soon involved in more fundamental problems. «The cell appears now to be a complex body whose mechanisms are still unknown; we must first disentangle the morphological changes which accompany the functions of life» (Van Beneden, 1883, page 286). Flemming (1882, page 2) expressed the same point of view somewhat more abruptly. «One has largely overlooked the interest of starting with the beginning; to build beings from unknown elements, to elaborate vital functions on bases which we ignore, is illogical.... It is surprising that research on the anatomy and the physiology of the cell should not have reached their full development earlier». It was the dawn of cytology.

The opportunity provided by a specific staining of chromatin created favorable conditions for elucidating mitosis and meiosis. Within the last twenty-five years of the 19th century, it became clear that this cellular component was the support of heredity. The great challenge of its inflexibility (Cuvier) or flexibility (Lamarck, Darwin)

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Professor Theodor Schwann (1810-1882), founder of the cell theory and Van Beneden's mentor at the University of Liège, where he taught from 1848 to 1879.

could be tackled on a new basis. The end of the century opened the way to the simultaneous rediscovery of Mendel's laws in Amsterdam (de Vries), Tübingen (Correns) and Vienna (Tschermak).

Edouard Van Beneden (1846-1910) was the son of Pierre-Joseph Van Beneden (1809-1894), who taught zoology at the University of Louvain and acquired a worldwide reputation on parasitism thanks to his discovery of the life cycle of tapeworms. Edouard grew up amidst the zoological collections accumulated by his father in the «Collegium Regium» where his family was living. He had however no propensity for natural history; contrary to his father, he was not keen on collecting shells or butterflies. At the time he entered University, he enrolled for both engineering and natural sciences. But one day, as he was making preparations just as a diversion in his father's lab, he made up his mind for zoology.

His first extensive investigation already reveals his inclination towards fundamental biological work. He made a large comparative study of the composition and formation of the egg (1870). This earned him the recognition of the Belgian Academy of Sciences and opened up a rapid university career for him. At the age of 24, he succeeded the entomologist Lacordaire to the Chair of Zoology at Liège.

He focused his research mainly on the ontogeny of mammals. He investigated the development of the egg in rabbits and bats and

summarized his results in a paper published in 1875. The study of fecundation allowed him to see spermatozoa around the egg in the perivitelline liquid but never inside the vitellus. This confirmed the theory of contact. Fertilization was due to the diffusion of a spermatogenic component through the cellular membrane of the egg which induced the formation of a peripheral nucleus. Another similar spherical formation appeared at the same time in the center of the egg. Then the superficial and the central pronuclei, as he called them, fused together to form the nucleus of the first embryonic cell.

Oskar Hertwig (1846-1922) conducted simultaneous investigations on sea urchin eggs on the Mediterranean coast. This was an outstanding material in view of its abundance, the fact that fertilization takes place in sea water, the transparency of the egg and its small size of about 0.1 mm allowing observation at high magnification. This brilliant study made it clear that fecundation consists of the union of the two germ-cells (1876). But the origin of the peripheral nucleus was misinterpreted: the nucleolus of the egg nucleus seemed to persist, contrary to the nucleus itself, so that the fusion seemed to be due to its association with the nucleus of the spermatozoon. Van Beneden (1876) immediately questioned this point. He had carried out some research on a variety of starfish on the Belgian coast, which clearly showed the disappearance of the nucleolus before that of the egg nucleus in the course of maturation. Hertwig's interpretation was not generally valid and should therefore be rejected. The female pronucleus must have another origin.

Hertwig's work was a fatal blow to the old contact theory of fecundation, but several steps remained obscure. The maturation of the egg with the formation of the polar bodies, the evanescence of the nucleus followed by the formation of the female pronucleus required further investigations. According to his research on rabbit and bat eggs (Van Beneden, 1880; Van Beneden and Julin, 1880), maturation rejuvenated the cell produced by the ovary; the expelled polar bodies and perivitelline components were assumed to correspond to male constituents. Fecundation compensated for these losses through the contribution of one or several spermatozoa.

After ten years of research on mammals from 1870 to 1880 and five on echinoderms (Hertwig, 1876; Fol, 1879), Van Beneden cast doubts on the adequacy of these materials: «Should not the main cause of the present disagreements be the small size of the spermatozoon and of its morphological constituents as well as the small size of the male pronucleus» (1883, page 277). His quest led him to the parasitic roundworm of the horse *Ascaris megalocephala*. This choice was neither original nor obvious. Earlier work on nematodes had been published without clear-cut results. But as Szent-Gyorgyi once said: «Genius consists in seeing what everybody sees and thinking what nobody thought». He immediately grasped the significance of the opportunity. «I have found, with the *Ascaris* of the horse, a wonderful material. I am convinced that the egg of this nematode will soon become a classical object of study to investigate and illustrate the phenomena connected with fecundation» (1883, page 277).

The advantages were numerous. This spermatozoon is as much as ten times larger than that of the sea urchin. It hoists a particularly large birefringent body which is like an identification flag. The egg can be made transparent when suitably treated. The various steps of its evolution take place simultaneously at the different levels of the genital tract: by cutting half a cm of the oviduct or the uterus, thousands of eggs showing the same stage of development can be obtained.



E. Van Beneden (1846-1910) with his second daughter Nelly in 1891 in his country-home near Liège.

The penetration of a spermatozoon can be seen very nicely. It moves to the center while the egg nucleus keeps a peripheral position. In this species, this triggers the two maturation divisions. Van Beneden obviously worked very hard to clarify this. He knew of course all about the recent progress made in the study of mitosis, to which he had contributed. A crucial advance had just been made by Flemming (1882), who made it clear that the chromosomes split *longitudinally* and seemed to distribute themselves equally between the two daughter-cells. Van Beneden wondered whether the formation of the polar bodies took place in the same way. His conclusion was negative. He suggested that the chromatic elements separated in a direction *perpendicular* to the normal one. In his opinion, the apparent similarity with the current karyokinesis was misleading. The isolation of the polar bodies followed a process of *pseudokaryokinesis*. After the expulsion of the second polar body, the spermatozoon, which so far remained unchanged, turned into the male pronucleus while what was left of the egg nucleus became the superficial female pronucleus. More than 230 pages were devoted to this meticulous and provocative description of maturation. But no further stage of evolution was present in the vagina. It

ended in a deceiving blind alley. How could this situation be overcome? «I did not have enough time to start egg cultures, an attempt which was likely to raise difficulties» (1883, page 497). As he was coeditor of the *Archives de Biologie*, the first part of the paper was sent to the printer in October 1883. This did not, however, exclude some rearguard investigations. Live female worms had been thrown into dilute alcohol and kept in this medium for several months. Why not stain these old preparations?

This supererogatory control reversed the situation completely. The eggs pursued their development; further steps, including segmentation, became observable. The alcohol made its way so slowly through the perivitelline layers that its penetration required several weeks. During this period of time, the embryo developed slowly; new stages occurred along the genital tract. These new data were hastily included. The drawings of the last two plates, XVIII and XIX, were suddenly somewhat unsatisfactory. They were completed by three new ones XVIII bis and XIX bis and ter. Volume 4 of the *Archives* was delayed. Printing was finished in March 1884, as Van Beneden took the opportunity of a visit to Liège by du Bois-Raymond to give him a copy on the 5th of April.

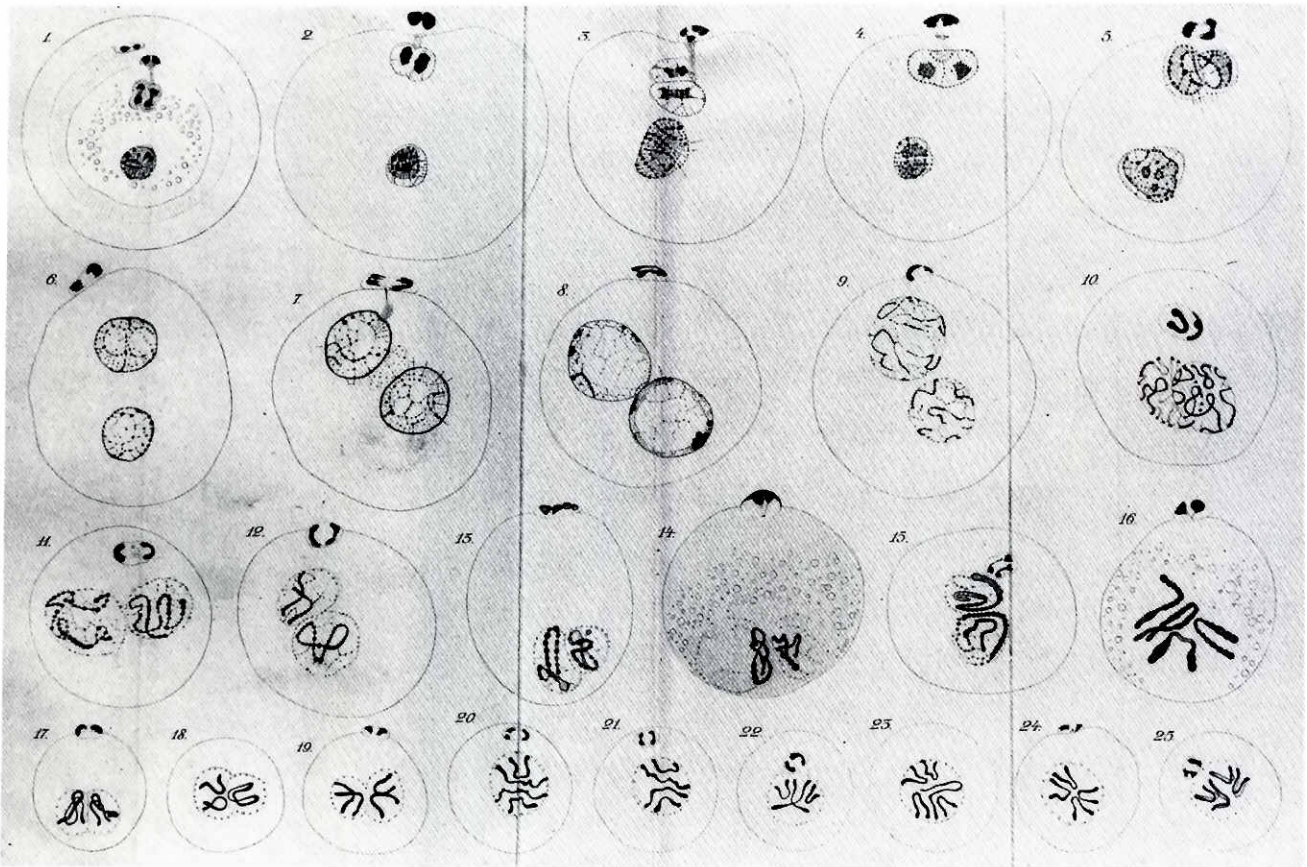


Plate I. Reproduction of plate XIX bis of Van Beneden, 1883. (1 to 8) Maturation of the pronuclei. (9 to 13) Spireme formation in both pronuclei. (14 and 15) Formation of the four primary chromatic loops («anses chromatiques»). (16 to 25) Various orientations of the primary chromatic loops in the equatorial plane. (23) A long supplementary chromatic loop is seen in the disc. (25) Duplication of the primary chromatic loops.

Nobody has told the story of Van Beneden discovering meiosis in the small poor lab of the Chair of Zoology. We have to rely on our imagination to bring back this climactic moment. His usual feverish activity must have been particularly great during those three months as he discovered these fundamental features, represented them and wrote the final 125 pages of the paper.

What a thrill to observe the extraordinary simplicity of the figures! The two pronuclei remained distinct and each chromatin condensed into only two elongated curved fragments, the *anses chromatiques*. What marvellous clarity contrasting with the confusing intricacy of other materials! Nietzsche says that «Winners never believe in chance». Historians of science should take care not to overestimate this factor but cannot deny its occurrence. Van Beneden was ready to grasp his chance thanks to his long quest and his exceptional intelligence. The last plates tell us the story of the discovery. The formation of the second polar body is illustrated by the two plates numbered XVIII. As the last observations allowed a slightly better description, a full bis-plate was added. The two chromatic elements remaining in the egg appeared heterogeneous: darkly stained regions alternated with clearer ones (Van Beneden, 1883, page 500). Many years will be needed to understand the occurrence of chromomeres. Plate XIX was also duplicated by the first 8 figures of plate XIX bis (Plate I). The maturation of the two pronuclei

consisting in the reticulation of their chromatin and their migration towards each other to the center of the cell was shown again in a more explicit and clearer way. Meiosis was finally described in the following figures of this plate and of plate XIX ter (plate II).

The drawings show the condensation of chromatin into a filament, the spireme as assumed at that time, in each of the pronuclei (I, Figs. 9, 10, 11). The alleged transversal splitting (I, Figs. 12, 15) gives rise to two loops in each pronucleus (I, Figs. 16, 17, 18, 19). These results were completely at variance with the fusion of the two germ-nuclei observed by Hertwig in sea urchin eggs. Each pronucleus evolved separately and their size made it possible to see very clearly that both contained two very similar if not identical chromatic elements. The occurrence of a supplementary component (I, Fig. 23) is exceptional and may be due to the accidental penetration of a second spermatozoon (1883, page 539). The equatorial plate which forms in the center of the cell corresponds to the juxtaposition of the two male and the two female chromosomes, as Waldeyer called these chromatic elements in 1888. Their central curved parts are turned towards the center of the plate and their ends towards the periphery (I, Figs. 19-24). Furthermore as they are ribbon-shaped (I, Fig. 15 and II, Fig. 1), their great axis is perpendicular to the equatorial plane so that the longitudinal splitting permits the separation of the two marginal colored parts interspaced by the

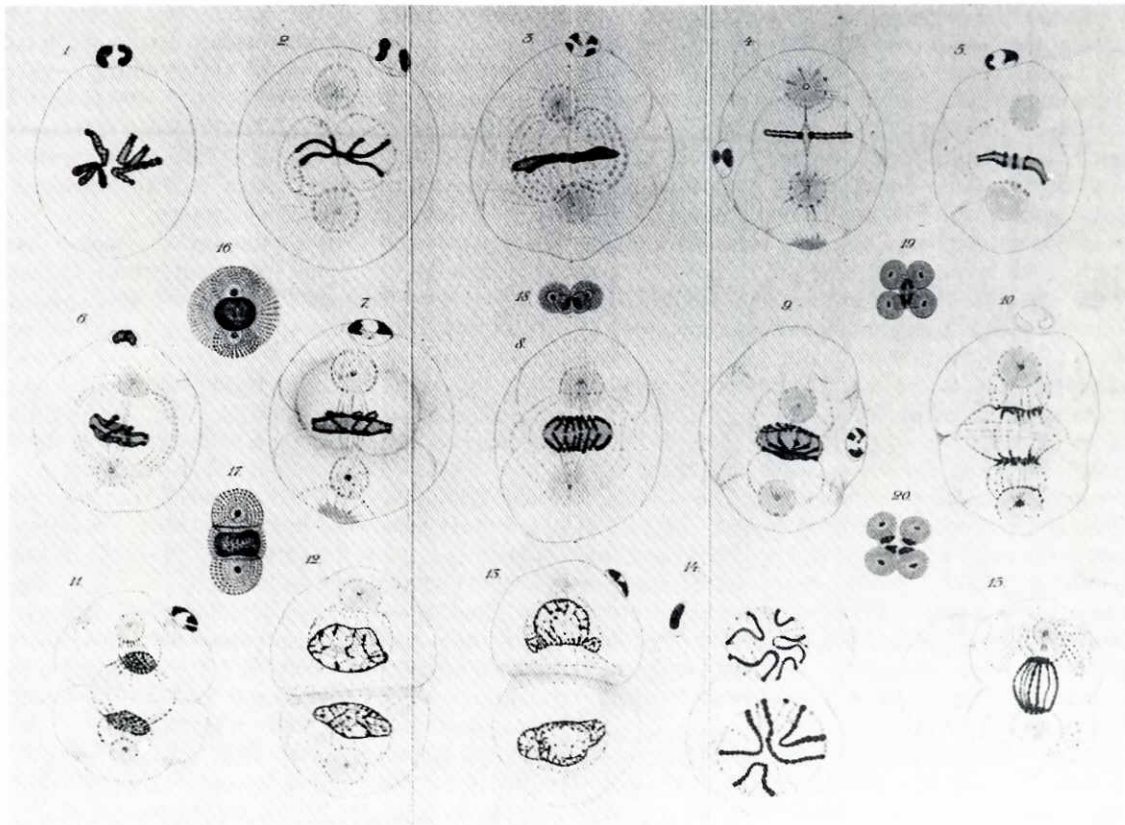


Plate II. Reproduction of plate XIX ter of Van Beneden, 1883. (1) The four primary chromatic loops are nearly completely duplicated. **(2)** Oblique view of the equatorial plane. The attractive spheres are visible. **(3 to 13)** Different steps of division. **(14)** Two-blastomere stage. In one cell, the four secondary chromatic loops are visible; in the other, the chromatic material is still in the spireme stage. **(15)** The splitting has progressed. The karyokinetic pattern has been represented in one of the blastomeres; the division of the other was complete. **(18 to 20)** Transformation of a spermatogonia into four spermatids. The elimination of darkly stained female «globules cytophoraux» was erroneously assumed by Van Beneden.

median, achromatic one (II, Figs. 1, 4, 5). The segregation of each of the twin chromosomes towards each of the two daughter-cells could be demonstrated. «The uniqueness of this material was due not only to the small number of the *anses chromatiques*, but also to their size and their accurate ordering in the equatorial plate» (1883, page 598). The migration of the two daughter chromosomes begins first in their central region (II, Figs. 6-9). At the end of the process, each of the first two embryonic cells is thus endowed with two female and two male chromosomes. It has a hermaphroditic nature and as the further divisions into four and eight cells show the reappearance of the four chromosomes, each cell of the adult should be hermaphroditic.

The cycle of nuclear events in germ- and embryonic cells was closed. The bold statement that the germ-cells were formed by pseudokaryokinesis was confirmed. While karyokinesis or mitosis according to Flemming (1882) was organized in such a way that all chromatic elements were minutely duplicated, maturation took place with the reduction of their numbers by half. «Each pronucleus is equivalent to a half-nucleus endowed, owing to its origin, with a

unisexual character» (1883, page 617). Fecundation allowed replacement of the ejected nuclear components with the male pronucleus.

Meiosis brought the final keystone to the construction which Van Beneden had been building with some kind of premonition for many years. His heretic view of pseudokaryokinesis appeared remarkably strengthened. The paper impressed the scientific world. The importance of the advance was emphatically recognized by Flemming (1885). *Ascaris megalocephala* became the subject of many studies. Boveri (1888) drew up a reference list of no less than 27 papers published between 1883 and 1888. The even share of chromatin in fecundation suggested strongly that it was the support of heredity. If Van Beneden remains in the history of biology as the discoverer of meiosis, his main tribute is due to his having focused the study of heredity on the chromosomes. He made a seminal discovery.

The long road to Van Beneden's discovery and his apparently sudden breakthrough in December 1883 are two inseparable steps. The 15-year period of pre-meiosis had prepared this turning

point. But the post-meiotic period is also of interest to appreciate Van Beneden's contribution. His next paper on this subject appeared only three years later (Van Beneden and Neyt, 1887a,b). He wanted to fill in some gaps in the earlier work, to examine some objections, to increase the objectivity of the experimental data through photography (Neyt was an expert in photography) and, last but not least, to examine more closely the cytoplasmic changes occurring in mitosis and meiosis. His earlier comments on this point had been limited to a few pages (1883, pages 547-553) and to the corresponding pictures of plate II. In fact, as Boveri said later (1888, page 817), «the nucleus does not divide; it is divided». The achromatic spindle which commands the division originates in the duplication of a unique cell organelle. The two asters, each with a small central body, arise from a single one which splits into two before the nuclear changes. This observation was published by Boveri on the 3rd of May (1887a) and in a much more extensive way by Van Beneden and Neyt on the 20th of August (1887a). This gave rise to an unyielding fight for priority which was reported by Rabl (1915) and is outside the scope of this paper.

After earning his doctoral degree *summa cum laude* in 1885, Boveri (1862-1915) read Van Beneden's paper and found that *Ascaris* was an excellent subject for further studies. He wanted to examine Van Beneden's interpretation of maturation. While the latter maintained in his paper with Neyt that it occurred according to pseudokaryokinesis (1887b), Boveri's reappraisal concluded that the general process of mitosis was valid. His description of the elimination of the polar bodies (1887b, 1888) has become classical. But the halving of the chromosomal number remained unshaken; it was even confirmed by a further comparative study carried out at the zoological station of Naples (Boveri, 1890).

The contradiction inherent in a normal mitosis on the one hand and the halving of the chromosome number on the other puzzled biologists for many years at the end of the last century. Although Van Beneden did not publish further work on meiosis, he contributed indirectly to the elucidation of this problem. His exclusive dedication to science was not that of a recluse. He valued his teaching duties and enjoyed discussing and collaborating with assistants and researchers. His excellence as a leader led to the foundation of a brilliant and enthusiastic school.

The most prominent investigations of his collaborators are probably those of Winiwarter (1901) and Winiwarter and Saintmont (1909) on the development of the ovary in mammals (rabbit, human, cat). It made it possible to follow early changes occurring in the nuclei of the oocytes and to determine their sequence. Its intricacy could be resolved into the following steps: resting stage → leptotene (or thin threads) → synapsis (or lateral association of two thin threads; the term was replaced later on by zygotene) → pachytene (shortening and thickening of the threads) → diplotene (decondensation into double thin filaments) → new resting stage (before maturation). There was a lively discussion at that time on the kind of association of spireme fragments inducing the reduction of the number of chromosomes. Telosynapsis (end-to-end association) was opposed to parasynapsis (side-by-side association). According to Wilson (1924), «the first definite suggestion of a side-by-side conjugation of leptotene-threads came from Winiwarter in 1901 as a result of a study of mammalian oogenesis (rabbit, human) though he did not fully commit himself to this conclusion until several years later (Winiwarter and Saintmont, 1909). In the meantime, the theory of parasynapsis was placed on a firm basis in both animals and plants by the work of many

observers, among them Janssens and A. and K. Schreiner».

It finally became clear then that the two chromatin tetrads of *Ascaris megalcephala bivalens* are due to the association side-by-side of two homologous duplicated chromosomes. The dissociation occurs generally in the first meiotic division, which therefore reduces the number of different chromosomes by half. The second meiotic division separates the two identical chromatids. In contrast, Weismann suggested in 1887 that the first division was equational and the second reductional. Van Beneden's work, which pointed out that the female pronucleus was identical to the second polar body, may have influenced his choice, as did the absence of the second polar body in diploid parthenogenesis.

We owe the story of the human facet of parasynapsis to Winiwarter (1946): "After a few short papers, Van Beneden advised me to start a long and exacting work which he regarded as very important. It required several years and much hard labor... As I had nearly finished my observations, I was alone in the lab one winter evening. I was about to go when I thought I should glance through my drawings again to make sure that there were no gaps in the sequences. I displayed them on my table and was looking through them when the door opened and Van Beneden came in. When he saw my drawings, in which he seemed warmly interested, he sat down and asked me many questions. I was the more happily surprised as I had the feeling my observations and my interpretations did not convince him.

"After some time, William, the lodgekeeper, came to let him know that supper was ready and that he was expected. He did not answer and remained quite still. The same scene happened two or three times. Van Beneden went forth with his scrutiny which lasted nearly two hours! I had the great satisfaction to convince him and to come to an agreement with him concerning my conclusions. When he left, he shook hands lengthily and said that he congratulated me for bringing this difficult subject to a successful end, that my work was fundamental, which I would understand later, and that he was happy that it had been done in his lab.

"The approval of Van Beneden, usually chary of praise, was the finest reward I could get. Suddenly, disappointments, discouragements, weariness and so on were swept away; the boundless joy of success overwhelmed me. How I managed to walk down the steps of the zoological institute, I can't remember! ... It was thanks to the 'Patron' that I had won the first round; it is thanks to his memory and his method that I won some more."

What better present for the discoverer of meiosis and what more adequate tribute for one of his young collaborators! And for us, how precious is the record of an exceptional moment between a brilliant assistant and a great scientific torchbearer of the last century!

Acknowledgments

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