The Development of Sayk's Cell Sedimentation Chamber: A Historical View on Clinical Cerebrospinal Fluid Diagnostics

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Keywords

Cytological techniques · Cerebrospinal fluid · Medical history, 20th century · Neurologic examination · Nervous system diseases

Abstract

Objective: The history of Sayk's cell sedimentation chamber is examined and reviewed in the context of clinically utilisable cerebrospinal fluid (CSF) cytology. **Methods:** A review of the literature was undertaken in PubMed and Google Scholar to search for primary and secondary sources on the history of CSF diagnosis. Moreover, documents in the archives of the Universities of Rostock and Jena, the Brain Research Institute of Cécile and Oskar Vogt in Düsseldorf and the Centre for Neurology at Rostock University were used. **Results:** The cell sedimentation chamber, developed by Johannes Sayk (1923–2005) at the beginning of the 1950s, enabled, for the first time, CSF cells to be presented with a quality comparable to the blood profile, with a low technological expenditure and using all staining methods suitable for haematological cells. The procedure could be performed quickly and cost efficiently, and therefore, it was easy to integrate it into clinical routine and it quickly became a widely used procedure. In Europe, it was considered the standard method for cytodiagnosis of CSF until the 1990s. **Conclusions:** The procedure revolutionised CSF diagnostics and paved

the way for modern qualitative CSF cytology. In course of time, the cell sedimentation chamber was superseded by the cytocentrifuge.

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Introduction

The development of the cell sedimentation chamber by the neurologist Johannes Sayk (Fig. 1) in the 1950s led to the initiation of a procedure that enabled the cells of the cerebrospinal fluid (CSF) to be presented and differentiated in everyday clinical practice [1]. By using various staining methods, it was possible, for the first time, to evaluate the CSF cells with a quality comparable to the blood profile. Although Sayk is repeatedly mentioned in historical overviews as the co-founder of a clinically feasible qualitative CSF cytology, so far, little is known about the history of the cell sedimentation chamber. To rectify this, for the first time, documents from the archives of the Universities of Rostock and Jena, the Brain Research Institute of Cécile and Oskar Vogt in Düsseldorf, and the Centre for Neurology of Rostock University were examined. Biographical details and Sayk's other accomplishments in the field of neurology will be mentioned only briefly here but can be found in the work of Kumbier and Zettl [2]. Johannes Sayk was born on September 28, 1923,

in Sgonn in East Prussia (today Zgon in Poland). Following his studies at the University Neurological Clinic in Jena and the Brain Research Institute of Oskar and Cécile Vogt in Neustadt/Black Forest, he became the Director of the Neurological Department of the University of Jena, where he set about establishing a brain research laboratory. In 1961, Sayk was appointed to the Chair for Neurology at the University of Rostock, which held until his retirement in 1989. Among many other accomplishments, Sayk co-founded the Research Group of Cerebrospinal Fluid of the World Federation of Neurology, received numerous awards, and was a Fellow of the Royal Society of Medicine in Great Britain. He died in Rostock on December 4, 2005.

The Precursors of the Cell Sedimentation Chamber

The introduction of transcutaneous lumbar puncture by Heinrich Irenaeus Quincke (1842–1922) in 1891 and its rapid dissemination as an examination method led, at the beginning of the 20th century, to the first cytological examinations of CSF [3, 4]. This required a practicable and reliable method of gaining cell profiles similar to those for blood [5]. Various methods for CSF diagnostics were developed, which primarily served to enrich the very sensitive CSF cells. As CSF has a very low cell concentration, cytodagnosis could only take place following cell enrichment. One of the early procedures was the so-called French method of Fernand Georges Isidore Widal (1862–1929), Paul Jean-François Ravaut (1872–1934) and Jean Marie Athanase Sicard (1872–1929), published in 1901 [6]. Its principle was based on centrifugation and subsequent staining of the CSF as well as counting or estimating the cells of one immersion field under the microscope. Although the centrifugation damaged the cells and impeded the cell differentiation, the procedure initially caught on, thanks to its quick implementation [7]. Another method suitable for quantitative cell analysis of CSF was the Fuchs-Rosenthal counting chamber developed in 1904, whose functioning principle, similar to that of a counting chamber for blood cells, was based on the staining and counting of the CSF cells with minimal dilution [8]. Yet another approach was offered by the procedure described by Alois Alzheimer (1864–1915) in 1907, according to which through precipitation, centrifugation, reworking of the sediment and embedding in celloidin, results comparable to those of a histological specimen could be achieved [9]. The application of this method

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Fig. 1. Johannes Sayk (1923–2005) in 1950 (Jena University Archives, promotions to Dr. med. 1949–1950, Inventory L, No. 359, p. 285).

in routine clinical practice was hindered by the preparation of the specimens, which required a great deal of time and effort.

Due to the unsatisfactory results of the techniques developed thus far, at the beginning of the 1930s, Otto Einstein (1876–1959) and Berthold Ostertag (1895–1975) developed a procedure based principally on salting out the CSF using an ammonium sulphate solution and subsequent centrifugation [10, 11]. Although this meant that the CSF no longer had to be examined directly after the lumbar puncture, as had previously been the case, the ammonium sulphate precipitation led to considerable damage to the CSF cells.

Thus, the procedures developed in the first half of the 20th century were either too laborious in clinical practice, or were unable to provide the optimal results required for cytological examination of CSF. In particular, the damage to the cells resulting from centrifugation was a considerable disadvantage. Moreover, the long preparation time required for the methods that did not involve centrifugation negatively influenced the cell profile. In clinical practice, however, a procedure was required which was simple, quick and as cost efficient as possible, and which provided results for cell differentiation that were easy to assess, similar to the results of the blood profile.

The Emergence of the Cell Sedimentation Chamber

These problems were also being encountered at the University Neurological Clinic in Jena. Therefore, at the beginning of the 1950s, the Clinic Director of the time, Rudolf Lemke (1906–1957), assigned the task of developing a new method to his employee Johannes Sayk; this method would overcome these difficulties and improve the analysis of CSF and in particular cell differentiation. It was no coincidence that Lemke's choice fell on Sayk. Even during his training, Sayk had concerned himself with CSF cytology on Lemke's suggestion [12]. Lemke himself had evidently been inspired by Georg Schaltenbrand (1897–1979) [13], who had already looked intensively at the possibilities of CSF examination [14].

Sayk indeed successfully managed to develop a procedure that enabled the CSF cell profile to be observed with a comparable quality to the blood profile. The sedimentation chamber, which he constructed in collaboration with the nationally owned company Carl Zeiss Jena, offered a cost-efficient and quick procedure that was therefore suitable for routine clinical practice. In 1954, Sayk published his CSF cytological examinations based on his new method for the first time, and in 1957, he presented them at the VI World Congress for Neurology in Brussels [15]. International interest in his sedimentation chamber also quickly followed, with scientists from home and abroad taking part in his courses on CSF cytology [16]. His research works brought him into contact with the Brain Research Institute in Neustadt/Black Forest, which was led by Oskar Vogt (1870–1959), where Sayk was able to conduct a research study in 1957 [17].

In 1960, with the monograph "Cytology of Cerebrospinal Fluid," Sayk presented a pioneering and internationally acclaimed work [18]. One year later, he was called to the newly founded Chair for Neurology at the University of Rostock [19]. Here, he continued to work on improving his cell sedimentation chamber and focused on CSF research [20]. In 1962, he established the CSF laboratory, which became the leading national establishment for CSF analyses, where reference values for examination standardization were established and scientists from home and abroad were trained [16].

The Design and Function of the Cell Sedimentation Chamber

The sedimentation chamber was based on the construction of the so-called *sedimentator* of Ernst Trömner (1868–1930) and draws on the principle of spontaneous sedimen-

Fig. 2. Model of the cell sedimentation chamber with knurling screws ca. 1955 for pressure regulation (University of Rostock Media Centre, File: "Sedimentation chamber according to Sayk 8552a," courtesy of Dr. rer. nat. Reinhard Lehmitz).

tation of cells, as had been previously employed by Hans Schöenberg (1915–1994) [21, 22]. Sayk accelerated the sedimentation through the suction effect of a strip of absorbent paper. Additionally, he paid precise attention to the proportions to enable him to achieve an optimal hydrostatic ratio [18]. The structure of the chamber is formed by a glass cylinder with a diameter of approximately 12 mm and a variable height of a maximum of 20 mm, which is set in a rubber tube. By means of a device for pressure regulation, the cylinder can be pressed onto the mounting plate below. A microscopic slide forms the bottom of the cylinder, upon which there is a strip of absorbent paper with a hole in the middle, which is identical to the diameter of the glass cylinder. The cells of the CSF sediment on the slide surface, and the strip of absorbent paper surrounding the cylinder continuously extract the fluid containing few or no cells. Through pressure regulation, it was possible to regulate the speed of fluid removal, thus reducing the danger of damage to the cells. The first models of the cell sedimentation chamber possessed knurled screws (Fig. 2), which meant that due to the sensitive adjustment of pressure and the consequent irregularities in the capillarity of the absorbent paper, the results were strongly dependent on the examiner's experience. Therefore, this mechanism

was later replaced by an easy-to-use weight lever (Fig. 3), with which the examiner could adjust the necessary pressure. Through the gentle sedimentation procedure, the cells remained exceptionally well preserved in comparison to all other hitherto known methods, and could be treated further with all staining methods, making the quality of the CSF specimen for cell differentiation comparable to that of a blood or bone marrow smear. The simple structure of the chamber and the equally simple handling were also advantageous, enabling the quick realization of replicas and use in many laboratories. However, there were disadvantages as well. Through the absorbent paper, a certain amount of cell loss could not be avoided; the loss ranged between 40 and 70%, which needed to be considered in the quantitative assessment (absolute cell count determination) particularly of CSF containing few cells [23, 24]. Moreover, the CSF drainage led to vortex formations, which could potentially lead to deformations of the cells. And the fluid absorbed by the paper was no longer available for further analysis. Nevertheless, Sayk's sedimentation chamber spread throughout large areas of Europe, and was to become the standard method for gaining CSF cells. It enabled, for the first time, a cytodiagnosis of CSF in clinical practice.

The Dissemination of the Cell Sedimentation Chamber

The cell sedimentation chamber was sold at home and abroad by the (East) Berlin company Ing. Wolfgang Dorenburg KG (limited partnership). Due to insufficient production capacities, production there ceased in 1973 and was taken over by the company, Dr. Günter Lange KG. The serial production as well as the export abroad led to a wide dissemination of this cell sedimentation chamber. However, as not all clinics were able to acquire this chamber, several replicas emerged, as shown by reports from the former East Germany or Poland. With the introduction of the method in Finland in 1974, the sedimentation chamber had established itself as a procedure for CSF cytological analysis throughout the whole of Europe, 20 years after it was first introduced [16]. A survey conducted at the end of the 1970s showed that Sayk's method was used in 90% of West German clinics in the neurology and neurosurgery departments [25]. According to Sayk, Schaltenbrand had also tested the sedimentation chamber in the Clinic of Neurology in Würzburg, contributing substantially to the dissemination of the new method [26]. As Schaltenbrand was also in touch with the American neurologist Percival Bailey (1892–1973), the sedimentation chamber method also reached the United

Fig. 3. Model of the cell sedimentation chamber with weight lever ca. 1960 for pressure regulation (University of Rostock Media Centre, File: "Sedimentation chamber according to Sayk 8542a," courtesy of Dr. rer. nat. Reinhard Lehmitz).

States. Subsequently, its areas of application were broadened, and the sedimentation chamber was used, among other things, for the cell analysis of urine [27], peritoneal fluid [28], and for amniotic fluid analysis [16].

The Sorption Chamber as a Further Development

Apart from minor modifications such as the replacement of the knurled screws with a weight lever and the insertion of additional micro-adjustment screws, no fundamental changes were made to the cell sedimentation chamber for a number of years. Rather, only extensions such as the preparation for electron microscopic examination were done. Nevertheless, Sayk worked constantly on improving his cell sedimentation chamber. The successor model, which aimed to eradicate the aforemen-

tioned problems, was developed by Sayk in collaboration with his colleague Reinhard Lehmitz (* 1948). Introduced in 1979 and patented in 1989, it represented a major advancement in this method [29]. The chamber, described as a sorption chamber, was similar in design to the sedimentation chamber. The chamber sleeve is made from porolith, a porous material, and broadens towards the base. It is coated from the inside with a liquid film, the presorber, leading to a temporally limited impermeability to fluid. After feeding CSF into the chamber, the cells first sink to the slide located on the base. Depending on its chemical composition, the presorber dissolves after a predetermined time and enables the porous chamber sleeve to absorb the now virtually cell-free CSF. The cone-like shape, which broadens to the base, ensures an even drainage of CSF despite strong suction capacity, without disruptive vortex formations. This chamber technology enabled cell loss to sink to 10%.

The development of modern cytodiagnostic procedures revealed, however, a considerable disadvantage of the sorption chamber procedure. Due to residues of the ceramic material of the chamber sleeve coating, many examinations were not possible, including certain cytochemical reactions and cell markings, but also electron microscopy. Only the further development of the chamber finally allowed an unrestricted use for scientific and clinical examinations, as the hard, porous chamber sleeve was replaced with a strip of absorbent paper.

Conclusion

In Europe, the sedimentation chamber procedure was seen as the standard clinical method for several decades. The simple principle and the possibility of cost-efficient replication ensured that the cell sedimentation chamber could also be used in countries with economy of scarcity

[3]. The cell sedimentation chamber developed by Sayk, and the subsequent cytochemical staining methods, enabled various inflammatory diseases to be better detected through differential diagnosis, such as viral and bacterial meningoencephalitis, Guillain-Barré syndrome, or Bannwarth's syndrome (neuroborreliosis). However, the particular strength of the method is shown in the detection of "atypical cells" in CSF, for instance, in the context of neoplasia, metastases or subarachnoid haemorrhage. Despite the international interest, the cell sedimentation procedure was ultimately unable to assert itself in the Anglo-American area because the filter procedures developed there were being preferred. In contrast to the cell sedimentation chamber, the filter procedure has the advantage that the remaining CSF is not lost. Moreover, at up to 90%, the cell yield is clearly higher, and comparable to that of the sorption chamber. Only at the beginning of the 1990s another procedure found its way into clinical CSF diagnostics, which completely replaced Sayk's sedimentation chamber. The cytocentrifuge constructed by the Shandon company (Great Britain) combined the centrifugation of the cells on a slide with the absorption of the remaining CSF by means of a strip of absorbent paper or drainage by a special device, meaning that it was available for further analyses. Of clear advantage here are the quick and simple production of CSF cytological specimens and the low cell loss. By contrast, a prevailing advantage of Sayk's cell sedimentation chamber is the very well-maintained cytomorphology, which, particularly in the diagnosis of neoplastic and inflammatory diseases, can still be highly relevant to this day [30].

Disclosure Statement

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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