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Formalin use in anatomical and histological science in the 19th and 20th centuries

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Abstract: The introduction of formalin, a formaldehyde solution, as a disinfectant and fixative was an essential improvement in anatomical and histological science. This paper is an outline of the historical use of formalin based on primary source texts and historical studies. We describe how the discovery of acetaldehyde in the 18th century led to the development of formalin as the most common ingredient in embalming fluids in the 20th century and is still used today. Particularly important contributions to this process were made by Justus von Liebig, Alexander Butlerow and August Wilhelm Hofmann in the development of anatomical and histological preparation techniques, and by Ferdinand Blum, Ferdinand Julius Cohn, Frederick C. Kenyon and Victor Wehr in the practical uses of formaldehyde solutions in preservation and fixation of soft tissues. However, formalin is not without its drawbacks and as its toxicity became more understood, method to mitigate its effects were demanded. Eventually safer preparation techniques were developed, including Hagens' plastination and Thiel Embalming Method. These techniques may someday largely replace high-concentration formalin solutions but they both still require at least small quantities of formaldehyde to preserve tissues for study.

Key words: formaldehyde solution, formalin, scientific preparatory techniques, history of anatomy, history of histology.

Introduction

In this paper, we present the history of the development of formalin and its introduction into anatomical and histological preparation techniques on the basis of existing studies and primary source texts. Formalin is a saturated (35–40%) aqueous solution of formaldehyde, it is a colorless liquid with a pungent, irritating odor and taste, readily miscible with ethanol. It exhibit strong fixative and preservative properties. A 3–4% aqueous solution is used for fixing plant tissues and various concentrations for animal and human tissues for microscopic study [1]. A 1–2% aqueous solution can be used as a disinfectant, antiseptic or antiperspirant. Formalin is also used in the manufacturing of furniture, clothing, cosmetics, and paper. Prior to the early 20th century, formaldehyde was used mainly for surgical and hospital disinfection or for preserving anatomical preparations.

History

The history of formalin dates back to the Age of Enlightenment when medieval alchemy, steeped in magic and astrology, slowly gave birth to chemistry, its modern offspring. Ancient lore was evolved into scientific understanding by scientists like German-Swedish chemist Carl Wilhelm Scheele who discovered a number of elements and compound. In 1777, he discovered the substance that came to be known as acetaldehyde [2]. Its synthesis was described later in the 18th century by the French scientists Antoine François Fourcroy and Louis Nicolas Vauquelin, who isolated it in the course of their experimental research on sulfuric acid. While studying the oxidation of alcohols in 1835, Justus von Liebig categorized acetaldehyde as a "dehydrogenated alcohol" and calling it at the time "aldehyde" [3]. With this growing understanding of aldehyde chemistry, Alexander Butlerow became the first to synthesize formaldehyde in its gaseous form and as a polymer in solution in 1859 [4, 5]. Nine years later, in 1868, August Wilhelm Hofmann, proposed a simplified method of producing formaldehyde from methanol and determined its molecular structure [6].

As scientists discovered the properties of formaldehyde, industrial engineers of the time quickly developed commercial applications for it as well. This led to a demand for large amounts of the compound and by 1891 the first patent for a large scale production process was obtained in Germany [7]. Production of formaldehyde solutions quickly spread to other countries in Industrial Revolution-era Europe. These commercially-produced solutions varied significantly in concentration and other ingredients and bore a variety of names. In England it was usually called formic acid. In Germany we find formol and formalin, the term also widely used in the United States. In some countries, formaldehyde was sold under the trademark Formal [7].

With this increase in industrial production, these various solutions became less expensive and more available to scientist as well.

As more scientist gained access to formaldehyde, medical applications for the compound were gradually discovered. Between 1880 and 1890 its antiseptic properties were recognized [8], leading to promising tests of clinical applications [4]. In 1892, the French scholar Jean Auguste Trillat observed formaldehyde hardening soft tissues and triggering coagulation [9]. The following year Ferdinand Blum, working intensively on the use of formaldehyde solution in antiseptic procedures, reported that the skin of the fingertips became visibly thickened after prolonged exposure, having become impregnated [10, 11].

Applications for these preservative properties of formaldehyde were quickly recognized and by the mid-1890s some scientists were already experimenting with various solutions in anatomical and histological preparations [12]. An earlier pioneer was the German physician and biologist Ferdinand Blum who compared the effectiveness of formaldehyde to that of traditionally-used preserving agents such as alcohol [13]. By 1896, Blum had shown that formaldehyde was more effective at maintaining the color and shape of tissues without affecting the microscopic structure of the preparation [14, 15].

Fig. 1. Ferdinand Julius Cohn (1828–1898).

Blum's results were confirmed by Ferdinand Julius Cohn (Fig. 1), Frederick C. Kenyon, by his own father, Isaac Blum and by others [16]. In 1893, Cohn experimented with a 40% solution of formaldehyde (later to be called formalin) and confirmed its antibacterial and fixative effect on internal structure of animal soft tissues and proteins [17]. This was confirmed by Isaac Blum who at the end of 1893 used 10% and 20% solutions of formaldehyde to preserve different types of tissue in

many animal and plant species, including fish, amphibians, reptiles, small mammals and invertebrates, as well as flowers and fruit. The elder Blum also used his solutions to preserve human embryonic and fetal specimens. Similar work with formalin was done by Kenyon who published his results in a paper in 1895. In it, Kenyon describes how he was able to reproduce the results obtained by Blum and other researchers, supporting formaldehyde's effectiveness in the preparation of various types of animal tissues. Additionally, he found that even when formalin caused shrinkage of a tissue, it did not alter its microscopic structure. On a macroscopic scale, he noted that the shape and color of tissue was preserved with only blood-filled vessels becoming discolored, that the eyeballs maintained a nearly life-like appearance, and that plant material could be prepared successfully as well. In his paper, Kenyon also gave practical guidelines for the use of formalin, including the simultaneous use of alcohol for fixing histological sections.

With this proven effectiveness and practical guidelines, the use of formalin in anatomy and histology increased gradually during the mid-1890s. It also became an essential ingredient in the embalming human bodies. From 1841 to 1879, not one of the 13 formulas commonly used for embalming fluids included formalin. By 1899, this number grew to 25 of 159 registered recipes. From the turn of the century until 1954, formalin was present in 192 of 413 formulas [18]. This increase in formalin use was helped by further improvements in the manufacturing process such as a process of producing formaldehyde from methanol, developed by the German chemist Oscar Loew [19]. By 1895, the cost of formaldehyde dropped to below the cost of other commonly used fixatives, including alcohol [7]. Before the end of the 19th century, formaldehyde had mostly replaced alcohol as the main ingredient in anatomical and histological preparation techniques.

The use of formalin in Poland was introduced by the surgeon Victor Wehr. A disciple of another famed Polish surgeon, Louis Rydygier, he described the uses of the solution during a session of the Seventh Congress of Polish Physicians and Naturalists held in Lvov in 1894. He presented a number of reports in the scientific literature and described his own uses [20]. Wehr began using formalin in 1893 to preserve pathologically altered organs and tissues removed during surgical operations. He was especially interested in structure of tumors. He reported: "resected tumors or organs are inserted immediately after the operation into a 1–2% aqueous solution of formalin or formol. On the second or third day after this, the contaminated fluid is changed once. After a further 8–14 days preparations can be removed and stored in a glass jar with a glass stopper. It should be remembered that a little of the fluid must remain at the bottom of the jar, so the preparations are constantly under the influence of at least a minimal amount of formol vapor. In preparations stored in this way I did not see, even after more than half a year, any trace of decay, or destructive bacteria, or yeast or mold" [20].

Wehr also confirmed the durability of formalin preparations and that organs retained their normal color and shape. He found that organs or tissues saturated with blood (e.g. placenta) darkened and turned brown but that the natural color could be restored through an alcohol treatment. This confirmed the observations made by Blum and Kenyon. Wehr's lecture sparked interest in the Polish medical community. The following year, Wehr present sections of human brains preserved in formaldehyde [21]. These finding were supported in a report by Viktor Chęciński, the pathologist and prosector in Odessa Hospital, detailing his experiences with formalin for preserving whole brains and their parts [22]. The usefulness of formaldehyde in creating histological sections was also confirmed by Henry Frederick Hoyer Jr. [23]. During his research on the nervous system, this Polish scholar used formalin fixation at the initial stage of preparation of histological specimens, which he then stained using the technique developed by Camillo Golgi.

Fig. 2. Henryk Hoyer.

Solutions of formaldehyde were used in anatomical preparations throughout the 20th century. The methods of preparation their long-term effects were continually investigated. Numerous studies compared the results achieved using different concentrations of formaldehyde with those achieved with other preservative agents. During the 1950s, experiments comparing formalin, Carnoy's solution (fixative composed mainly of ethanol, chloroform and glacial acetic acid) and spirits were conducted by many scientific groups: Sandritter *et al.* (1955), Harbesa and Neumann (1955), and Hartleib *et al.* (1956) [24–26]. Most of these trials demonstrated negligible loss of proteins from formalin-fixed organs (below the detection limits of the time). This finding was of particular importance, since it meant that formalin-treated tested further using other methods and their protein content analyzed.

Health Concerns

As formalin use became commonplace, its adverse health effect became known. One of the first reports concerning its toxicity was published in 1905 by American physician Martin H. Fisher. The results of his investigation warned that inhalation of even small quantities of formaldehyde vapors could result in pneumonia, bronchitis and kidney in kidneys [27]. The allergenic effect of formaldehyde are well demonstrated [28]. It is a particularly strong contact allergen and for this reason, it is not used to produce drugs applied externally (de Groot *et al.*, 2009). There are also strong data suggesting that direct contact with formaldehyde and prolonged exposure to its fumes can lead to various chronic diseases including cancer [29]. By the end of the 20th century, some countries such as Sweden and Japan banned the use of formaldehyde in cosmetics, and many others limited the concentration of it permitted in industrial products. Despite these concerns, the use of formalin remains widespread.

Formaldehyde solutions were still common in the dissection room where their health risks can be mitigated. The toxic hazard can be significantly reduced by using masks and special ventilation systems [30]. Low-concentration formalin solutions can still be effective as embalming fluids when they are combined with certain salts or industrial methanol spirits [31, 32]. The toxicity of formaldehyde can also be reduced with the addition of substances such as ammonium carbonite [33], monoethanolamine [34] or Infuntrace™ [35]. While toxicity is the greatest drawback of formaldehyde, it is certainly not the only one. Because it causes rapid coagulation of blood, tissue with a high blood content can undergo color and structural changes when treated. Formalin preparations do degrade over time despite their durability and have a strong, unpleasant odor [1]. Nevertheless its low cost and high effectiveness outweigh these disadvantages and therefore continues to be used to preserve cadavers.

New Development

Viable alternatives to formalin began to surface in last decades of 20th century but these still made use of some quantities of formaldehyde [1]. Among the earliest was plastination, developed by German anatomist Gunter von Hagens in late 1970's. Its process included fixation, dehydration and forced impregnation of a liquid polymer into the specimen. The early stages of the plastination process require temporary preservation of tissues in with a solution of formalin and alcohol or acetone along with potassium nitrate and potassium acetate [36, 37]. The specimen would then be dehydrated and finally impregnated with the polymer. Depending on the desired outcome, Von Hagens used silicone rubber, polyester or epoxy resin [38]. The plastination process produces specimens of high quality and durability that are now extensively used in anatomical research and medical training [39].

Another notable technique was developed in the early 1990's by the German anatomist Walther Thiel from Graz Institute of Anatomy. His technique was the results of over 30 years of experimentation in the long-term preservation of human cadavers. His aim was to find a preservation method that would maintain the natural color, consistency and transparency of a tissue for as long as possible. He prepared a total of 977 complete cadavers and numerous post-autopsy corpses [40]. The best method involved a mixture of salt compounds with low amounts of volatile formaldehyde. The precise composition of embalming fluid varied and is still constantly being refined [41]. Thiel named his technique Soft Embalming Method though it is also sometimes called the Graz Embalming Protocol or Thiel Embalming Method. Cadavers preserved with Thiel's method are very well suited for anatomical research and medical training [42, 43]. They have no detectable odor, preserve the shape and color of muscles, viscera and vasculature. There is no stiffening of tissues and the cadaver maintain life-like pliability [44]. Despite these obvious advantages, the Thiel Embalming Method has not spread beyond Germany as fast as would be expected. This is partly due to higher cost, the need for additional equipment and, critically, that the papers describing the process were initially published only in German [45].

Summary

The formaldehyde solution formalin has a long history in anatomical and histological preparations. The discovery of formaldehyde in the middle 19th century was followed by significant scientific interest in its properties. Its preservative effects were observed in the 1890's and formulas of varying concentrations were used to prepare anatomical and histological specimens. When it began to be produced on an industrial scale, formalin became widely available to scientist. It became the leading fixing agent in the dissection room and in microscopic preparations. It was the subject of much scientific interest with some important observations concerning the use of formalin for preparations of central nervous system made by Polish scholars as early as the closing years of 19th century. In first decades of 20th century, the toxicity of formaldehyde became a growing concern. With no viable alternatives, its use was continued while attempting to mitigate the health effects [46–50]. The extensive efforts to reduce the toxic effect of formalin show that it continued to be the best solution for the preparation of cadavers, both fetal and adult even after more than one hundred of search for alternative [51, 52]. Even though strong formalin solutions are being slowly replaced by other processes in the 21st century, these still use small quantities of formaldehyde to preserve the living tissues scientists wish to study.

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