

Historical Note

Creatinine determination according to Jaffe—what does it stand for?

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Abstract

In 1886, Max Jaffe discovered a reaction of creatinine with picric acid in an alkaline environment. Although the manuscript describes the nature of a precipitate and does not deal with an analytical assay, Jaffe's landmark paper elucidated the basic principles of the creatinine determination method (originally developed by Otto Folin), which became immensely popular and has easily withstood the test of time. Despite the advent of the enzymatic creatinine analysis, the analytical method is still popular due to its simplicity and low cost. As there is no standard recipe for the 'Jaffe' method, much methodological variation has occurred over time. This lack of methodological standardization implies that even in the 21st century, improving the interchangeability of Jaffe results is still an issue.

Keywords: alkaline picrate reaction; creatinine; Otto Folin; standardization

Introduction

Apart from glucose, creatinine is the most common analyte in clinical chemistry. Even in the beginning of the 21st century, the majority of clinical laboratories refer to the 'Jaffe method' when assaying creatinine. The method is still in use 125 years following the discovery of the principle, which is a fact unparalleled in clinical chemistry. Despite the advent of the enzymatic creatinine analysis, the Jaffe method is still popular due to its simplicity and low cost. However, few people are aware that, in fact, Max Jaffe never published an analytical method, but rather he discovered the nature of urinary compounds, reactive with picric acid in an alkaline environment. The following paper highlights the discovery of Jaffe and its consequences.

Max Jaffe's life

Max Jaffe (often misspelled as Jaffé) was born in Grünberg in Silesia (nowadays Zielona Gora in Poland) on 25 July 1841. He was a German pharmacologist, biochemist and pathologist of Jewish descent. He received his medical education in Berlin, where he qualified in 1862. Ludwig Traube and Wilhelm Kühne were among his academic

teachers. While still a student, he took a keen interest in chemical investigations and worked in the pathological laboratory under the direction of W. Kühne. Travel during his studies took him to Prague, Vienna and Paris. Thereafter, he became an assistant in the medical clinic at Königsberg in East Prussia from 1865 to 1872 under Ernst von Leyden, with whom he published a work on putrid sputum. This led to the discovery of the spirilla and leptothrix characteristic of putrid processes in the lungs. In 1867, he obtained a habilitation in internal medicine. As a doctor, he participated in the Franco-German war (1870–1871) and was decorated with the Iron Cross Second Class. In 1872, he was awarded the title Extraordinary Professor of Medicinal Chemistry. From 1873 until his death, he was the first Ordinary Professor of Pharmacology at the University of Königsberg (present day Kaliningrad, Russia). After his promotion to director (1878) of the Laboratory for Medical Chemistry and Experimental Pharmacology (belonging to the pathology institute), he became a member of the Deutsche Akademie der Naturforscher Leopoldina in 1882. From 1910 onwards, this laboratory became an independent institution [1].

His principal work consisted of the discovery of urobilin and urobilinogen in urine and their origin in bile. Besides studying indican and creatinine, his investigations focused on urocanic acid in the urine of dogs, as well as on ornithine in the excrement of birds, the biotransformation of various exogenous compounds and analytical chemistry [1, 2].

Professor Max Jaffe (Figure 1) enjoyed a high reputation as a teacher and as a scientist. In 1901, he was mentioned among the list of Germany's greatest 19th century doctors [3]. Next to his employment at the University, he had a private consultant practice. He died on 26 October 1911 in Berlin. His tomb is situated at the Jewish cemetery Berlin-Weißensee.

Jaffe and the Jaffe principle

Among kidney researchers, Jaffe is well known for having given his name to an analytical principle for assaying creatinine in human body fluids. By 1875, it was acknowledged that adding saturated picric acid solutions to human urine produced crystals, which were then attributed to uric acid



Fig. 1. Photograph of Max Jaffe (1841–1911).

[4]. In 1886, Jaffe observed that a red colour formed when creatinine reacted with picric acid in an alkaline medium [5] and observed the needle-formed crystals under the microscope. Furthermore, he could demonstrate the nature of the precipitated compound as being a double salt of potassium and creatinine with picric acid by the typical precipitation of creatinine with zinc chloride (Neubauer reaction) [6] and by carrying out the Weyl's test [7] (adding a dilute solution of sodium nitroprusside and then putting in a few drops of sodium hydroxide that induces a ruby red colour, changing to blue on warming with acetic acid). In his landmark paper, Jaffe discussed that the alkaline picrate reaction could also be observed to a much lesser extent with a number of organic compounds (e.g. acetone, glucose) (Figure 2). These compounds were later designated as pseudochromogens and are a source of unspecificity in the Jaffe reaction.

One year before, creatinine had been synthesized for the first time by Jan Horbaczewski (1854–1942, professor of medicinal chemistry at the Czech medical faculty in Prague) [8]. At the time of Jaffe's discovery, creatinine (the degradation product of creatine) was considered to be an excretion product of nitrogen production. As the creatinine precursors are synthesized by the liver, the first interest for creatinine, from a clinical perspective, was liver insufficiency.

Folin and the Jaffe reaction

At the end of the 19th century and at the beginning of the 20th century, creatinine was generally assayed using the Neubauer reaction (adding an alcoholic solution of zinc chloride to a creatinine-containing solution, yielding a complex with two molecules of creatinine and one molecule of zinc chloride). After weighing the precipitate and multiplying the result by 0.642, the amount of precipitated creatinine was found [6].

Ueber den Niederschlag, welchen Pikrinsäure in normalem Harn erzeugt und über eine neue Reaction des Kreatinins.

Von
M. Jaffe.

(Aus dem Laboratorium für medicin. Chemie zu Königsberg i. Pr.)
(Der Redaktion zugegangen am 24. Juni 1886.)

In der physiolog.-chemischen Literatur finden sich nur sehr spärliche Angaben über das Verhalten normalen Harns auf Zusatz von Pikrinsäure.

In K. B. Hofmann's Lehrbuch der Zoochemie heisst es S. 402: «Fügt man gesättigte Pikrinsäurelösung zu Menschenharn, so bleibt derselbe anfänglich klar, dann setzt sich (nach 3–4 Stunden) ein mässiger, gelbgefärbter, aus Harnsäurekrystallen und feinen Nadeln bestehender Bodensatz ab». H. Beaunis (Nouveaux éléments de Physiologie humaine) sagt S. 776: «l'acide picrique en precipite des cristaux d'acide urique» und H. Huppert in seiner Bearbeitung von Neubauer-Vogel's Analyse des Harns giebt S. 124 an, dass normaler Harn beim Kochen mit Pikrinsäure einen starken flockigen Niederschlag giebt.

Die gelegentliche Verwendung der Pikrinsäure zum Nachweise von Eiweissstoffen war für mich die Veranlassung die Erscheinungen, welche diese Säure in normalem Urin hervorruft, genauer zu verfolgen.

Es ergab sich Folgendes:

Wenn man menschlichen Harn mit conc. wässriger Pikrinsäurelösung versetzt, so bleibt er anfangs klar, scheidet aber im Laufe der nächsten Stunden meistens ein spärliches krystallinisches Sediment ab.

Fig. 2. Front page of the manuscript of the landmark paper dealing with the alkaline picrate reaction of creatinine (*Z Physiol Chem* 1886; 10: 391–400).

In the spring of 1900, Otto Folin (1867–1934), one of the fathers of modern American clinical chemistry, was offered a position as research biochemist at the McLean Hospital in the suburbs of Boston. Folin, who had been trained in Sweden and Germany, decided to study the protein metabolism of normal versus mentally disturbed individuals by measuring as accurately and as completely as possible all the known nitrogenous and other products excreted in the urine, hoping thereby to learn the normal range of variation in the partition of the total nitrogen among the known products and residual fraction and then to consider possible abnormal variations. This first goal was essential to developing more and better quantitative methods before any worthwhile surveys could be started. These were the considerations that led to Folin's interest in devising suitable quantitative methods for urine and blood analysis, an interest that held his attention for the rest of his life. The methods he developed enabled Folin to explore normal and abnormal features of the metabolism with consequences not yet foreseen. Folin no longer used the cumbersome Neubauer reaction and started to work with alkaline picrate solutions and called it the Jaffe reaction [9].

Folin's first years at McLean were mainly devoted to devising and testing methods for determinations of urea, ammonia, uric acid, creatinine and creatine, sulphates and urine acidity. The first of Folin's colorimetric methods was that for creatinine, an application of a colour reaction of that

substance with picric acid noted by Jaffe many years earlier. Although other colour reactions had been used long before to estimate biological products, such as Nessler's reagent for ammonia in water analysis, Folin's method for creatinine, using a more delicate and precise instrument for colour comparison (the Duboscq colorimeter), is commonly regarded as the introduction of colorimetry into modern biochemical procedures. In the meantime, the methods were used in studies of metabolism of normal individuals and selected hospital patients, each on uniform diets of known composition. The amounts of the above metabolic products excreted in the urine were carefully collected. In 1904, Folin proposed creatinine as a marker to test the completeness of urine collection. Following an additional hydrolysis step, the method proved to be useful for the determination of the precursor molecule creatine as well [10].

Shortly after Folin's publication, Dr Georg Dorner, a pupil of Jaffe, started to use the colorimetric method developed by Folin based on the Jaffe principle in Königsberg [11]. The reaction time variation among Jaffe recipes was huge from the beginning. Folin initially gave a time limit of 5–10 min for the development of the Jaffe reaction. Later, he restricted the time limit to 5 min and this period was later adopted by most workers. However, from the very beginning, the pioneers of creatinine analysis never used a standard recipe: Benedict and Myers incubated only 3.5 min, Mellanby 5 min, Dorner 5–15 min and Mendel and Rose 10 min. Similarly, a huge reaction temperature variation among Jaffe recipes was observed [12].

Isidor Greenwald (1888–1976, Harriman Research Laboratory, Roosevelt Hospital, New York) was the first to make a systematic study of the chemistry of the Jaffe reaction [13]. He ascribed the red colour to a salt of creatinine, picric acid and sodium hydroxide and noted that there were at least two places in the creatinine molecule where a shift in a hydrogen atom could produce a tautomer: a lactam–lactim rearrangement between positions 3 and 4 or a keto–enol change between positions 4 and 5 [14].

By 1909, it was recognized that urinary excretion of creatinine was low in muscle disease, especially in muscular dystrophy [15]. The introduction of creatinine into the clinical chemistry was a slow process.

Creatinine and glomerular filtration rate

The significance of creatinine as a renal marker molecule only became clear due to the pioneer work of Poul Kristian Brandt Rehberg (1895–1989). In 1926, Rehberg (Zoophysiological laboratory, University of Copenhagen) suggested that creatinine was filtered through glomeruli and concentrated in the tubules, being neither reabsorbed nor secreted [16]. He first proposed the use of creatinine as an exogenous administration. Because there were many substances in the serum that were not really creatinine but gave Jaffe's reaction, one could not rely on endogenous creatinine. However, since tubular secretion of creatinine often counterbalanced the overestimation in the serum by non-creatinine chromogens, a marriage of convenience was born because the measurement of an endogenous creatinine clearance did not require the inconvenience of the admin-

istration of an exogenous substance. By the time micro-puncture studies confirmed the advantage of insulin over creatinine, one knew that creatinine clearance was indeed an inadequate measure of glomerular filtration rate (GFR) [17]. However, creatinine was still used due to its convenience. By the late 20th century, we had so much experience with the creatinine clearance that we really knew more about human diseases and symptoms at any given creatinine clearance than at the true GFR [18].

Analytical issues

In the century that followed Folin's analytical breakthrough, many adaptations and improvements of the Jaffe reaction were proposed. Improving the specificity of the reaction by reducing the interference caused by the pseudochromogens has been a continuous challenge to clinical chemists for over a century. Unfortunately, a standard Jaffe recipe could never be achieved. The introduction of automated chemistry analysis by Leonard Skeggs (1918–2002) [19] signified a major milestone. There was no longer a need for sample deproteinization—native serum could be used for analysis. This important practical advantage, however, introduced a protein error in the Jaffe method. On average, the pseudochromogen effect caused by plasma proteins artificially increased creatinine values by ± 0.3 mg/dL or ± 27 $\mu\text{mol/L}$ [20].

At the time being, the broad variety of Jaffe recipes is a major source for analytical variation [21]. Trueness verification of actual creatinine assays in the market demonstrates a disappointing variability that needs substantial improvement [22]. The recent introduction of the global creatinine standard material SRM 967 [23] has had a major impact on the magnitude of serum and plasma creatinine values. The unspecificity caused by the pseudochromogens has to be removed from the result. For Jaffe-based creatinine assays, the only solution is to 'compensate' for the analytical error using a mathematical correction [20]. Also the coefficients of estimated GFR formulae have been adapted to the new standards [24]. However, global standardization will not be able to uniformize creatinine assays since there is no standard adaptation explaining how *in vitro* diagnostics companies will have to adapt their creatinine assay calibration to the new isotope dilution mass spectrometry standard. Implementation of calibration traceable to higher order reference measurement procedures and reference materials does not correct for analytical interferences of field methods (non-specificity bias). To account for the sensitivity of alkaline picrate-based methods to non-creatinine chromogens, some manufacturers have adjusted the calibration to minimize the pseudo-creatinine contribution of plasma proteins, thereby producing results more closely aligned with the reference method (isotope dilution mass spectrometry). This strategy makes the assumption that the non-creatinine chromogen interference is constant among samples, which is an oversimplification. In consequence, there have been recommendations to abandon the Jaffe method in favour of the enzymatic method [23]. However, the favourable cost of Jaffe-based creatinine testing appears to be a major practical hurdle in this process.

Conclusions

The initial observations by Jaffe in 1886 have induced a breakthrough in biomedical research. The analytical principles described earlier by Neubauer and Weyl were not suitable for routine work. In contrast, Jaffe's findings allowed Folin to develop a practical analytical method, which has withstood the test of time, even in the absence of a standard recipe. Unfortunately, Jaffe never realized the diagnostic potential of creatinine assays for nephrology. Despite the introduction of more specific enzymatic assays, the remarkable simplicity of the Jaffe principle will warrant an extended life span of this unique test.

Conflict of interest statement. None declared.

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