Good practice report

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How to visualize the different lactose content of dairy products by Fearon's test and Woehlk test in classroom experiments and a new approach to the mechanisms and formulae of the mysterious red dyes

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Abstract:

The article connects historical developments in analytics with contexts of today and school experiments: Woehlk test and Fearon's test are historically known as the reactions of lactose and maltose with ammonia and methyamine, respectively. Both lead to characteristic red dyes whose formation and structural formulae had not been of interest or had even been reported incorrectly until 2015. Even though these tests, developed in the first half of the twentieth century, are obsolete in medicinal analysis today, they pose interesting experiments for school chemistry, when investigating the topic of macromolecules or sugars. In an inquiry or context-based teaching approach, tests visualizing different lactose concentrations in different dairy products would fit into the contexts milk and chemical detection of diseases. The experiments can also be used in a historically based problem-oriented approach in which the scientists, their work and lives can be the central perspective. As both methods of analysis result in brightly colored solutions, they are easily interpretable as well as aesthetically appealing to students. As the test developed by Fearon is quicker and makes use of less dangerous chemicals, it is the one to be preferably used in school.

Keywords: dairy products, detection reaction, Fearon, history of medicine and chemistry, lactose, life sciences, mechanism, school experiment, Woehlk

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Introduction

The history of chemistry is often applied in chemistry teaching with regard to conceptual developments, such as the development of different models of atoms, bonding or types of reaction (e.g. Mamlok-Naaman, Ben-Zvi, Hofstein, Menis, & Erduran, 2005; Scheffel, Brockmeier, & Parchmann, 2009). However historical elements also present fascinating insights into the Nature of Science (e.g. McComas, Clough, & Alamzroa, 1998) for other areas, such as the development of analytical processes. Often, the search for analytical reactions was the focus of research. In some of these cases, an application as a detection reaction was found later.

The following project lays out the parallel and iterative developments of detection reactions for carbohydrates, originally important in medical contexts. While the story itself is already fascinating, the application of these detection reactions gain new relevance due to today's observations of growing problems with lactose intolerance. The underlying chemistry can therefore be embedded in different conceptual approaches, such as history-based, inquiry-based or context-based learning, as discussed in the later part of this paper.

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The story of the historical development of detection tests for lactose

Solutions of methylamine or ammonia can be used to differentiate lactose from glucose: In 1904 in Copenhagen, Denmark, Alfred Woehlk found a reaction of ammonia solution with lactose producing a characteristic salmon red dye (cf. Woehlk, 1904). Only 1 year later, Hans Malfatti in Innsbruck, Austria, improved the “Woehlk reaction” with 3–4 drops of potassium hydroxide solution to detect a reducing sugar in the urine of women who had just given birth: neither Fehling reaction nor Benedict’s test, available at that time, were able to differentiate lactose from glucose, but the Woehlk test did. This was important for the medical treatment as lactose in urine of a woman in childbed is not dangerous, but glucose indicates a gestational diabetes.

Then, in 1942 in Dublin, Ireland, William Robert Fearon proposed to use methylamine instead of ammonia for this test. In the original manuscript, Fearon advised: “To 4 mL of the neutral solution add 3 or 4 drops of a 5 per cent aqueous solution of methylamine hydrochloride. Boil the mixture for about 30 s. Remove the tube from the flame, and at once add 3–5 drops of 20 per cent sodium hydroxide solution.” (Fearon, 1942, p. 130). Fearon’s test also resulted in a reddish color (“cherry”) if the solution contained lactose, and in a yellow color in the presence of glucose (Figure 1), but it was faster and more reliable than the Woehlk test: “I have found these ammonium hydroxide tests erratic and untrustworthy. When positive, they are probably due to the formation of an amine (by interaction between an aldehyde broken off from the sugar, and the ammonia), and subsequent reaction between the amine and the carbohydrate.” (Fearon, 1942, p. 132).

Figure 1: Resulting colors of both tests with different sugars: Fearon’s test with lactose (“cherry”), Woehlk test with lactose (“salmon”), Fearon’s or Woehlk test with glucose (“yellow”) (from left to right). (Photo: Klaus Ruppersberg).

The chemistry of lactose detection reactions: submicroscopic considerations

Despite numerous efforts to uncover the exact mechanisms of both tests, they have remained unknown or were reported incorrectly (“pyrrole red” in Grob, 2000) for a long time. The structures of the red dyes are also still a mystery because they are stable only in a strongly alkaline environment (pH 12–13) (cf. Nickerson, Vujicic, & Lin, 1976) and after addition of 30 mg sodium sulfite per test tube with 4 mL solution. They cannot be extracted with known solvents (cf. Ruppersberg & Blankenburg, 2018).

Figure 2: Molecules giving a red dye with ammonia or methylamine.

Nevertheless, comparative studies have now revealed some insights into the process (Kussler & Ruppersberg, 2019): initially only lactose and maltose were specified as detectable substances for these popular tests in the areas of urology and food-chemistry; on closer inspection cellobiose (homodimer of β -1,4-linked glucose molecules) can also be detected. All these disaccharides contain a reducing sugar molecule part (glucose) while

the rest of the molecule (Figure 2) obviously does not matter in the two test reactions of Woehlk and Fearon's test.

Hypothesis for the mechanisms of the Woehlk- and Fearon-reaction

If roughly half of the molecule doesn't seem to matter, will a protection group in position 4 then help to produce the red dye? The hypothesis was that a glucose molecule protected in position 4 should result in the same color reaction in both tests. This hypothesis could now be verified at the university of Kiel, Germany, with an application of 4,6-O-ethylidene-D-glucopyranose and 4,6-O-benzylidene-D-glucopyranose (Figure 3) (Kussler & Ruppersberg, 2019).

Figure 3: Protected glucose molecules: 4,6-O-ethylidene-D-glucopyranose (left) and 4,6-O-benzylidene-D-glucopyranose (right).

Also, lactulose and maltulose (Figure 4), which are produced in the alkaline state by a Lobry-de-Bruyn-van-Ekenstein rearrangement, react in the same way. In the case of Woehlk and Fearon's test, the color formation in the rearrangement products lactulose and maltulose develops much faster than in lactose and maltose; in the protected glucose the color develops even faster than in the rearrangement products (Figure 5).

Figure 4: Rearrangement products lactulose (left) and maltulose (right): keto-furanose instead of aldo-pyranose.

Also the resulting yellow color, typical of monosaccharides, occurs faster in fructose, being a ketose sugar, than in glucose, being an aldose sugar. (Fructose is not actually a reducing sugar, but forms – as known from Fehling's test and Benedict's test – rearrangement products in alkaline solutions according to Lobry-de-Bruyn-van-Ekenstein, which have a reducing effect.)

Furthermore, it was found that Woehlk and Fearon's test are comparable with the Maillard reaction (box).

Figure 5: Results of Fearn's test; top row: results after 5 min in a 70 °C water bath; bottom row: results after 12 min in a 70 °C water bath, each test tube with 4 mL methylamine solution and in addition, from left to right, with 8 mg, 4 mg, and 2 mg of protected glucose (EtGlc=4,6-O-ethylidene-D-glucopyranose), and 8 mg each of lactose(=Lac), lactulose(=Lul), maltose(=Mal), maltulose(=Mul), cellobiose(=Cel) and 4 mg each of the monosaccharides glucose(=Glc) and fructose(=Fru) (Photo: Klaus Ruppersberg).

Non-enzymatic browning:

Three stages of Maillard reaction (i.e. non-enzymatic browning) according to Hodge (1953):

1. Initial stage (colorless, no absorption in near-ultraviolet)

- A Sugar-amine-condensation
- B Amadori rearrangement

2. Intermediate stage (colorless or yellow, with strong absorption in near ultra-violet)

- C Sugar dehydration
- D Sugar fragmentation
- E Amino acid degradation

3. Final stage (highly colored)

- F Aldol condensation
- G Aldehyde-amine-polymerization, formation of heterocyclic nitrogen compounds

Further investigation through UV-Vis-spectrometry

Meanwhile, UV-Vis-spectrometric studies have been carried out to reveal the structure of the red dye. In these tests, instead of ammonia solution, methylammonium chloride is used, releasing methylamine in the alkaline pH range (Fearn's test), because Fearn's test is faster, more reliable and needs a less dangerous substance. The resulting dye absorbs in the visible range at 541 nm and is easier to study than the more unstable Woehlke dye (absorbing at 527 nm). Both dyes can be stabilized by an addition of sodium sulfite for approximately 6 h.

The following information is presently available: the difference between the absorption maxima at 527 nm (with ammonia) and 541 nm (with methylamine) indicates that it could be a dye molecule, its absorption red-shifted by 14 nm due to an exchange of hydrogen for the methyl group in the molecule (Kussler & Ruppersberg, 2019).

Hypothesis for the structure of the dye molecule

Since the reactions according to Woehlk and Fearon are comparable with the early and middle Maillard reactions, there is not only the red-colored end product but there are many (colorless and/or UV-absorbing) intermediate and end products, with only one in each case giving a maximum at 527 nm (with ammonia) and 541 nm (with methylamine). Key to these intermediates is the consideration and evaluation of the much higher maxima in the UV range. Since Fearon's test can be applied more reliably than Woehlk test, this method was preferred in subsequent structural investigations. Various sugar solutions (lactose, maltose, lactulose, glucose, fructose) were investigated with Fearon's test and determined spectrometrically in the range between 250 nm and 650 nm (Figure 6).

Figure 6: Eight milligram Lactulose in 4 mL solution of Fearon's test, measured in a heatable cuvette in the UV-Vis spectrometer Specord 50 plus, from 250 nm to 650 nm, 65 °C. Enlarged area: 450–650 nm, with additional measurements after 15, 17, and 19 min (maturation) accounting for the brown, grey and red curves, respectively.

The maturation of the dye (up to 40 minutes) is the reason why Fearon's test should not be used for absolute quantitative measurements. It can only be used for a semiquantitative overview in simultaneous experiments.

In Fearon's test, a conspicuous UV band at 312 nm and at 541 nm in terms of cherry-red color impression could be found (Kussler & Ruppersberg, 2019). Such color bands usually result from a charge transfer and occur in betaine compounds with charge separation. Spectra comparable in habitus and in band intensity are also shown by the mold poison moniliformin (sodium salt of semisquaric acid) (Scharf & Frauenrath, 1980) and azulene (Matenova et al., 2014).

For further interest

Postulation of a chromophore

Until recently there were no or only insufficient ideas about the constitution of the Woehlk and Fearon dyes and their formation. Based on the reactions known in the chemistry of carbohydrates, a structure was created which is consistent with previous experimental observations and has the corresponding features of a chromophore. It was assumed that in the Woehlk and Fearon test for lactose and maltose, the reaction of ammonia and methylamine occurs via the formyl group in C₁ and the hydroxyl group in C₅ forming a partially saturated

heterocyclic pyridine ring. An exposure of reducing sugars to alkali hydroxide initially results in endiolate anions, which can fragment further via aldol cleavage into triosereductone and other small fragments when exposed to alkaline conditions (Euler von & Hasselquist, 1950; Euler von & Eistert, 1957). Therefore, it was assumed that the second sugar molecule in the C_4 position functions as a protective group and thus prevents this fragmentation. Furthermore, it could then not function as a leaving group, something that is essential for the dye formation. It was further assumed that the dehydrogenation reactions of Schiff's base 2, which were initially not further discussed, also oxidized the 6-hydroxymethyl group to the formyl group and the alkali metal salt of 6-formylpyridin-1-ium-3,4-diolate 3 with a protected 5-hydroxy group (R^* =glucose in maltose and R^* =galactose in lactose) and $R=H$ (Woehlk test) resp. $R=Me$ (Fearon test) is formed, the formyl group being essential for the chromophore (Figure 7) (Kussler & Ruppersberg, 2019).

Figure 7: Postulated chromophore 3: $R=H$ for the Woehlk test, $R=Me$ for Fearon's test; R^* =galactose, if lactose/lactulose is used, or R^* =glucose, if maltose/maltulose is used.

However, the results of further investigations soon cast doubts on the assumed constitution of the dye, since these were not compatible with the proposed structure and the mechanism of formation. It was not comprehensible why ketoses reacted faster than the corresponding aldoses, if the ring closure actually occurred via the 1-formyl group. Likewise, it was surprising that the 4,6-O-ethylidene-protected glucopyranose reacted faster in both test reactions than the ketoses and also produced the red dye, even though a protected 6-hydroxymethyl group cannot readily be oxidized to a formyl group. After detecting the smell of benzaldehyde in the reaction with 4,6-O-benzylidene glucopyranose, it was clear that the protective group had to have been split off and that the assumed constitution could not be true.

In order to find a structure that would fit the new results, it was now assumed that lactose and maltose and the 4,6-O-protected glucopyranoses in a Lobry-De Bruyn-Van Ekenstein rearrangement (in the alkaline milieu) easily converted into the corresponding ketoses 4 and that the hydroxy methyl group could be oxidized in the 1-position of the saccharide to the carboxyl group, forming 2-keto carboxylic acids 5 in the process (cf. Spengler & Pfannenstiel, 1935). These acids can be converted into the corresponding 2,3-dioxido-1-carboxylate 6 in another Lobry-De Bruyn-Van Ekenstein rearrangement (cf. Eistert & Haupter, 1958) (cf. Hesse, Raemisch, & Renner, 1956). The reaction of 6 with ammonia and methylamine, respectively, leads to a new chromophore 8 (alkali salt of 6-hydroxymethyl-pyridin-1-ium-2,3,4-trisolate) via the hypothetical intermediate 7 with elimination of water and the second sugar molecule and cleavage of the protecting group, respectively (Figure 8) (cf. Arndt, Eistert, Scholz, & Aron, 1936). The ring closure is also completed via C_1 and C_5 . With this structure, the observations made so far in the Woehlk and Fearon tests can be described quite well.

Figure 8: Postulated chromophore 8: $R=H$ for the Woehlk test, $R=Me$ for Fearon's test; R^* =galactose, if lactose/lactulose is used, or R^* =glucose, if maltose/maltulose is used.

However, a short time later, structure 8 was also called into question, as newer results were no longer compatible with this constitution. The lack of cherry-red color in Fearon's test with isopropyl-, sec-butyl- and tert-butylamine argues against this structure and especially against its formation mechanism, while the adequate reaction with n-propyl, n-butyl and iso-butylamine, on the other hand, is successful. Also with aniline and

phenylhydrazine the test reactions are different. Thus, aniline gives an orange-yellow and phenylhydrazine a red dye, but their absorption spectra are fundamentally different from the spectrum typical of the Woehlk and Fearon dyes. For a positive color reaction, it therefore seems essential that a CH_2 -group („spacer“) is in the immediate vicinity of the amino group. The structure **8** was finally refuted when it succeeded, using HPLC-DAD-MS, to determine the molecular mass in a series of neutralized samples of the Fearon dye obtained from sugars and various amines.

The evaluation of the mass spectra showed that the different Fearon dyes have the empirical formula $\text{C}_6\text{H}_6\text{O}_2$ (Nalk) and the molecular mass is thus smaller than it would be according to the structures **3** and **8**. The dyes are thus formed formally by cleavage of water and the second sugar molecule (or the protective group) without oxidation by atmospheric oxygen. The fact that all color reactions are positive even in the absence of oxygen was confirmed experimentally. It was also shown that the mass spectra of Fearon dyes from 4,6-O-ethylidene-glucopyranose (and lactulose) and amines are identical. The observation of dye formation in Fearon's test by H-NMR shows that in the (hetero) aromatic proton region only singlets are present, which means that all protons have no ortho hydrogen atoms. Since, however, the MS and NMR results did not allow the development of a suitable structural formula with ring closure via C_1 and C_5 , it seemed obvious that Woehlk and Fearon dyes might be formed through ring closure via C_2 and C_6 . Through this finding, a suitable constitution could be found with the alkali metal salt of 2-methylpyridinium-3,5-diolate **16** ($\text{R}=\text{H}$ for the Woehlk dye, $\text{R}=\text{Me}$ for the Fearon dye), which satisfies all previous experimental results (Figure 9).

Figure 9: Postulated chromophore **16**: $\text{R}=\text{H}$ for the Woehlk dye, $\text{R}=\text{Me}$ for Fearon dye; $\text{R}^*=\text{galactose}$, if lactose/lactulose is used, or $\text{R}^*=\text{glucose}$, if maltose/maltulose is used.

Since the color formation with ketoses is faster than with the corresponding aldoses, the saccharides react with ammonia or the amine via the ketose form **4** to the Schiff base **9** (Figure 9), which must be capable of enamine-imine tautomerism. In the case of amines without a CH_2 spacer group, the base-catalyzed rearrangement to the enamine **10** may be absent due to steric hindrance and the reaction may stall or take a different course. Under base-catalyzed vinyllogous β -elimination of water (cf. Hollnagel & Kroh, 2000) vinylamine **11** is formed. Vinylamine **11** rearranges into the corresponding Schiff base with elimination of the second sugar molecule (or the protective group) resulting in the 2-imino-1-deoxyoson **12**. This in return rearranges into the tautomeric 2-imino-1-deoxyosone **13**. The OH-protecting group in the 4-position is necessary for two reasons: (a) to prevent fragmentation into smaller fragments (cf. Smuda, Voigt, & Glomb, 2010), (b) to avoid other reaction paths such as formation of formoines, 4-pyranones and furanones (cf. Voigt & Glomb, 2009; Voigt, Smuda, Pfahler, & Glomb, 2010). Via the tautomeric form **14**, the anhydrobase **15** forms in a pericyclic ring closure reaction, which then dissociates into the pyridinium-3,5-diolate **16** and a hydroxyl ion.

What can these tests be used for in school?

Since their discovery, both Woehlk test and Fearon's test have mainly been used to detect the reducing disaccharide lactose in urine in medical laboratories. Although there had already been a number of wet-chemical detection methods to identify sugars before, such as Fehling's test or Benedict's test, those analyses could only differentiate reducing from non-reducing sugars. Since both glucose and lactose molecules have an aldehyde group in the open-chain form necessary for a reaction, the result is positive in both cases. Only Woehlk or Fearon's test allow a safe differentiation between glucose and lactose. Thus, a relatively harmless milk congestion during pregnancy leading to more lactose in the urine (lactosuria) could be differentiated from a dangerous gestational diabetes.

Under modern considerations, methylammonium chloride is less dangerous than a 10 % ammonia solution (Table 1). Therefore, Fearon's test should be preferred and the Woehlk test with ammonia should only be used if a chemical school collection does not include methylammonium chloride (Ruppersberg, 2016).

Table 1: Consideration of dangers of ammonium vs. methylamine-hydrochloride.

Name	CAS	Physical	Inhalation	Contact	Swallow	Environment
Ammonia, w=10 %, c=5,6 mol/L	1336-21-6	Very high risk: H221: Flammable gas, H290: May be corrosive to metals	High risk: H331: Toxic if inhaled, EUH071: Corrosive to the respiratory tract	High risk: H314: Causes severe skin burns and eye damage	Negligible risk	Very high risk: H400: Very toxic to aquatic life
Methylamine- hydrochloride c=0.025 mol/L	593-51-1	Negligible risk	Medium risk: H335: May cause respiratory irritation	Low risk: H315: Causes skin irritation, H319: Causes serious eye irritation	Medium risk: H302: Harmful if swallowed	Negligible risk

Today, Woehlk test and Fearon's test are hardly used in laboratory work anymore; modern methods that primarily use chromatography are faster and more accurate.

Nevertheless, both tests have a very special appeal due to their splendid coloring (Figure 1). In addition, it is highly informative for school chemistry classes because they allow the semi-quantitative analysis of different dairy products (Figure 10) for their lactose content. Through these tests it can be visualized and explained to students that, obviously, lactose-free milk differs in lactose content from whole milk, but that both milk types also differ from kefir, milk-based creamer, and other dairy products as they all have different lactose contents. Students with a family history of lactose intolerance are assumed to be very interested in testing their own preferred dairy products, while for other students, the test also remains an interesting experiment.

Figure 10: Variety of dairy products, from left to right: yogurt, sour cream, whole milk, heavy whipping cream, cream cheese, lactose-free milk, half and half cream and milk, mascarpone, ricotta cream. In European countries, this product range can be expanded to include kefir, buttermilk, creme fraiche, etc. (Photo: Jeff Nordine).

From the test results (Figure 11), students can easily deduce that the dairy products tested contain different amounts of lactose (or none, if the resulting solution is yellow). The reason for this is the presence of different enzymes: such as β -Galactosidase in test tube no. 2, which hydrolyzes lactose into galactose and glucose. Thus the number of carbohydrate molecules in lactose-free milk is double the number of those molecules in regular whole milk. Students having trailed the teaching module shouted enthusiastically "Take one – make two! That is why lactose-free milk tastes sweeter than regular whole milk!" All students can classify the dairy products tested according to the amount of lactose present. Those students dealing with lactose intolerance may – in preparation for this experiment – have been asked to bring their own commonly-consumed milk products to analyze those products as well.

Figure 11: Result of the Woehlk test with chosen dairy products (Photo: Klaus Ruppersberg).

Application in different teaching and learning approaches

This experiment (see Worksheet in supplementary material) is quite suitable for both inquiry-based learning and context-based learning. In the former approach, students simulate scientific research processes by investigating a research question and performing reasoning processes to derive at answers (among others, cf. NGSS Lead States, 2013). For this experiment, students could for example formulate the research question “Why does lactose-free milk taste different from whole milk?” They could then perform Fearon’s test to find out that the lactose content in different milk types is indeed different, i.e. no lactose is present in lactose-free milk while a considerable amount is present in whole milk. Students could then research what happens in the process of removing the lactose from regular milk in dairy plants and would find out that enzymes are involved breaking up the lactose molecules into two shorter sugar molecules. Then students would reason, as described above, that lactose-free milk must taste sweeter as it contains more sugar molecules.

In the context-based approach, a certain every-day phenomenon or product provides the context in which particular aspects of the scientific discipline are dealt with (among others, cf. Parchmann et al., 2006). The chosen context is the common theme underlying a teaching unit and is revisited at different steps in the teaching process. This experiment could well fit into larger contexts such as *milk* or *illnesses*. In the former context, the experiment could be used to differentiate different milk types and could be followed by an analysis of other classes of substances found in milk products besides sugars. In the latter context, the experiment would be one example of how different illnesses can be distinguished through chemical analyzing methods.

A third approach in which this experiment could appropriately be used, is the historically problem-oriented or historical approach. This approach primarily addresses emotional and social aspects of chemistry by focusing on historically important scientists and the influences of the knowledge present at those times and societal constraints on their thinking; at the same time, it allows students to relate to how concepts were being generated and refuted, and thus understand the development of scientific ideas, knowledge and concepts (Jansen, Ulses, Matuschek, Fickenfrerich, & Peper, 1986; Mamlok-Naaman et al., 2005). If this approach is to be used, students could be introduced to the motivation of chemist and pharmacist Alfred Woehlk who, without a direct intention for the test’s application, tried to find a test to differentiate lactose from glucose, or in other words, a disaccharide from a monosaccharide. Through so-called history lifts, i.e. authentic historical texts or pictures, students could be guided toward the work of Austrian urologist Hans Malfatti who only 1 year later found a practical use for Woehlk’s test: he stated, for example, that children whose mothers had had glucose in their urine during pregnancy could develop severe health problems, which did not appear if their mothers only had had lactose present in their urine. Malfatti knew that some of these women could be suffering from gestational diabetes, a symptom of which is the presence of glucose in their urine. Therefore, he wanted to differentiate the two sugars and he was glad to read of Woehlk’s fundamental research in Copenhagen. Students then need to form a hypothesis as to why glucose can be differentiated from lactose in this experiment. As the Woehlk test works with hazardous chemicals, the experiment might not (or should not, depending on state or school safety regulations) be performed but only considered theoretically. At precisely this aspect, the ideas of William Robert Fearon (Dublin, Ireland) can be shown to students as he developed a comparable test with less hazardous chemicals about 40 years after Malfatti’s practical application of Woehlk’s test. By recreating this historical process, students can relate to one driving force in scientific development: the idea of doing fundamental research, finding a practical application for this research and delineating the desire or necessity to substitute hazardous with less dangerous chemicals while still arriving at the same analytical result.

Conclusions

Woehlk test and Fearon's test are two traditional chemical detection methods that are quite suitable for experimental chemistry classes: They provide a visual and therefore motivational approach and explanatory power to an investigation and detection of carbohydrates. As both reaction mechanisms are not completely explained as of today, they also pose an interesting possibility for discussing the generation and development of research in chemistry. The historical approach can be based on the tests' relevance in chemical and medical developments: being discovered by chance in Copenhagen, Denmark, and having been applied to the urology lab a short time later in Innsbruck, Austria.

A further aspect is worth discussing with students: Substituting ammonia with methylamine allows for a safer experiment and a more sustainable use in school chemistry while the original functionality of the experiment remains the same. As both tests were replaced by chromatographic methods in the 1960s, students could also explore the usage and function of newer detection methods. Last but not least, other topics can be linked to these tests such as: "Is sugar healthy or unhealthy?" Maillard reaction (food chemistry), human genetics ("How digestible is lactose?") and anthropology (milk and dairy products since the Neolithic Revolution).

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