

Review

History of Urinalysis

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Abstract

Urine is historically the oldest biological material used for diagnostic purposes. Urine testing dates to the ancient Babylonians and Sumerians. Uroscopy consisted of visual and organoleptic assessment (color, clarity, odor, taste testing) of urine. Its principles did not change until the Age of Enlightenment. In the 16th century, when the first microscope was constructed, uroscopy was enriched with the assessment of urine sediment. As chemical methods have developed, tests for various analytes in urine have been introduced into diagnostic methods. The presence of sugar and protein and excretion of urea, creatinine, uric acid and electrolytes, such as sodium, potassium, chlorides, calcium, and magnesium and phosphates, were assessed. Over the years, the set of tests performed on urine has changed, among others, due to the possibility of performing more diagnostically reliable tests in blood. Although currently the most common material for laboratory tests is blood, a general urine test has not lost its importance, and it is a widely performed screening test.

Keywords: uroscopy; urinalysis; chemical urine test

1. Introduction

The etymology of the word urine is intricate. The term “urine” is derived from the Latin word “*urina*”, which, in turn, is derived from the Greek word “*uron*”. The etymology of the particle *ur* is rooted in Pto-Indo-European, thereby suggesting a connection with water. In Latin, *urinare* is defined as “to immerse in water.”

Urine has long been of interest to both medical practitioners and the general public. The ancient Romans used it for tanning, laundry, and even as a teeth-whitening agent, due to its ammonia content. This bodily fluid was also employed as one of the earliest disinfectants. Gardeners referred to it as “liquid gold” because of its effectiveness as a fertilizer, owing to its nitrogenous compounds and mineral salts. The examination of urine for diagnostic purposes, known as uroscopy, has a history as long as medicine itself. Initially, uroscopy relied on visual inspection and bordered on divination, reaching the height of its popularity in the Middle Ages. Nevertheless, urine remained the primary biological diagnostic material until the mid-20th century. Today, blood is the most commonly analyzed biological fluid; however, advances in technology and diagnostic methods have led to numerous modern laboratory tests being performed on urine as well. The novelty of this review is a detailed and critical description of clinical chemistry urine tests from the golden era of urinalysis, from the mid-19th century to the first half of the 20th century. Information was obtained from source materials such as original textbooks from the times described and referrals or urine test results from archives, museums and the authors’ own collections.



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2. Early Urinalysis

2.1. Ancient Times

The concept of examining body fluids as indicators of changes in internal organs was born in antiquity. It is likely that the first organoleptic analyses of urine took place approximately 6000 years ago [1]. The pioneers in this field were the ancient Sumerians and Babylonians. They were able to identify 20 types of urine and link these characteristics to diseases. Babylonian terms such as “*sinatu pizu*” (white or clear urine), “*sinatu zalmi*” (black urine), “*urpati sinatu*” (clouds, clouds in urine), “*tidu da sinatu*” (silt, mud or sludge in urine) and “*sinatu bursi*” (bright red urine) were indicative of their knowledge in the study of urine [2]. The Babylonians also used an assessment of the appearance of urine to diagnose venereal diseases. They claimed that if a man’s urine looked like donkey urine or beer yeast then he was suffering from gonorrhea [3]. Ancient Hindu medicine was based not only on a diagnosis of disease on the basis of the appearance of urine but also on visual inspection of the patient (evaluation of voice, eyes, skin and pulse) [2]. In Sanskrit texts, urine-related symptoms or diseases (*Prameha*) are categorized into 20 different groups. Thus, for example, the term “*Udakameha*” refers to urine that looks like water (water-light), “*Surameha*” to tea-like urine, and “*Madhumeha*” is the sweet urine of diabetics. Charaka (300 BC) and Sushruta (6th century BC) the founders of Ayurveda, observed (or relied on earlier, oral tradition) that sweet urine attracts black ants and linked it to polyuria [4]. Similar observations were also made in ancient Mesopotamia and Egypt. Examination of urine consisted of pouring it on the ground and observing whether it attracted insects. If it did, the patient was diagnosed with diabetes or boil [5]. The Egyptian Kahun Papyrus mentions red urine, while the Ebers Papyrus (1550 BC) attributes hematuria to “worms in the stomach.” According to Sir Marc Armand Ruffer (1859–1917), an Anglo-German experimental pathologist and bacteriologist who analyzed ancient Egyptian medical records, red urine was evidence of infection with *Schistosoma hematobium*, a blood fluke that lives in the blood vessels of the lower pelvis, particularly of the bladder [3].

Ancient Egypt also has records of pregnancy tests and fetal sex identification. A pregnant woman had to urinate daily into two bags of grain. One contained wheat grains and the other contained barley grains. If the wheat began to sprout first, a girl would be born; if the barley sprouted first, a boy would be born [6]. The reliability of this test was confirmed in 1963 [7].

The ancient Greeks appreciated the value of urine testing. Hippocrates (460–370 BC), the founder of rational-empirical medicine, included organoleptic urine testing in his diagnostic protocol for examining patients, on a par with listening to the patient’s breath and observing the color of their skin [8]. The ancients believed that there were two types of urine. The first type of urine was produced in the brain and flowed down the spinal cord, while the second type came from the liver. Hippocrates described the first type as “urine from blood and night,” understood as the portion filtered or collected by the kidneys, whereas the second type was thought to pass directly from the intestines, liver, or stomach into the bladder [9]. According to Hippocrates, foaming urine indicated kidney disease or chronic illness [10]. He suggested that urine sediment containing blood and pus indicated serious medical conditions. Rufus of Ephesus, on the other hand, associated hematuria (red urine) with the inability of the kidneys to filter blood properly [11]. Consequently, Galen described polyuria as “urinary diarrhea” and drew attention to water balance. He also claimed that the quality of urine depends not on the functioning of the kidneys, but on the condition of the blood, the mixture of juices, food, and the functioning of the liver [12].

2.2. Middle Ages

During the Middle Ages, uroscopy was considered a fundamental examination of great diagnostic and prognostic importance. Between the 4th and 7th centuries, many physicians and authors of medical textbooks, such as Oreibasios, Aetios of Amida, Magnus of Emesa, and Paulus Aeginata, described the principles of this examination. The most famous and highly regarded work was “De Urinis” by Theophylus Protospatharius of Constantinople (probably 610–641), whose writings were considered the gold standard of urine testing for many centuries [13].

The Persian physician Rhazes (Abu Bakr Muhammad ibn Zakariyya ar-Razi; 856–925) introduced a hydrostatic method for measuring the urine specific gravity [14]. Another Persian physician, Avicenna (Abu Ali Husain ibn Abdullah ibn Sina, 980–1037), was the first to analyze liver function on the basis of the color and smell of urine. He noticed that the urine of patients with liver disease was more yellow and had an unpleasant odor [12]. In his work, “Al’-Arjuzat fi’ t-tibb” (Poem on Medicine), wrote that “urine is a great guide to knowledge about disease.” [15]. Around 900 AD, the Jewish physician and philosopher Isaac Israeli ben Solomon (832–932), better known as Isaac Judaeus, developed guidelines for urine testing, which he included in his work “Liber Urinarium”. This work was translated from Arabic into Latin in the 11th century [13].

Christians in the Middle Ages believed that illness was a punishment for sins or the result of witchcraft. A detailed diagnosis of the disease was therefore unnecessary. Despite this, uroscopy was widely practiced. The following four characteristics of urine were determined: appearance, color, sediment, and taste [16]. Constantinus Africanus (1018–1085), one of the founders of the school in Salerno, argued in his work “De instructione Medici”, that urine is a better diagnostic tool than pulse assessment and allows for a better diagnosis of the patient’s ailment. He also describes how a urine test should be performed correctly [17]. According to the Jerusalem Code of 1090, if a doctor did not perform a urine test during a consultation, he could be subjected to public flogging [18]. At the turn of the 12th and 13th centuries, Gilles de Corbeil (c. 1140–1224), a student at the medical school in Salerno, compiled the contemporary knowledge on urine testing in his treatise “Liber de Urinis”. Corbeil also introduced a vessel called a *matula* (from Latin *matula*—pot, chamber pot; urine flask) or *jorden* (*jordan*—chamber pot; likely due to its resemblance to the containers in which holy water from the Jordan River was sold) into the examination process [19], which became a typical attribute of medieval doctors. His successors, such as Bernard of Gordon (1285–1318), a doctor and professor at one of the first universities in Montpellier, also attached great importance to the examination of urine [4]. Thus, uroscopy was recommended by both leading medical centers of the time—in Salerno and Montpellier. In 1300, the Byzantine physician Johannes Zacharias Actuarius (1275–1328) wrote in his treatise on urine “Περί Ουρῶν”, Latin “De Urinis Libri Septem” presented the theory that molecules in the urinary vessel tend to gather in a specific part of it, which in turn indicates the parts of the body affected by the disease. The author also provided instructions on how to collect urine. “Urine should be collected in a large, transparent, and clean bottle, in the shape of a bladder [...] The container must be large enough to hold 24 hours’ worth of urine [...] The [collected] urine should be protected from heat, cold, and sunlight.” [14]. The color of urine was considered an important diagnostic indicator for differentiating between diseases. In the Middle Ages, doctors distinguished between more than 20 colors of urine, from crystal clear, camel snow-white (*ut vellus cameli*—like camel hair) to raspberry red, lush green, and even black (*ut cornu*—like horn) [20].

In the Middle Ages, there was also a pregnancy test that involved observing a needle dipped in a woman’s urine. If the needle turned black or rusty, it indicated that the woman was expecting [21].

At the turn of the 15th and 16th centuries, textbooks included tables for differentiating urine and facilitating diagnosis based on the examination of this material. For example, in the work of Uldaryk Binder (Pinder), a city physician from Nuremberg, (14??-1519?) entitled “*Epiphanie Medicorum. Speculum videndi urinas hominum*”, (Epiphany of Doctors. A mirror in which human urine can be seen) published in 1506 [22,23] (Figure 1). Due to numerous translations of texts on uroscopy into native languages, this science became accessible not only to academic physicians but also to numerous “charlatan doctors.” Uromancy, or divination from urine, began to be practiced by uneducated men, mainly in Germany, referred to as water observers, urine prophets, fountain observers, urine observers, or urinary roosters. Uromancy and, to some extent, the excessive importance attached to uroscopy were criticized during the Enlightenment. In a letter to the Marquis de Florian dated 3 January 1774, Voltaire wrote, “The absurd charlatanism of divining diseases and temperament from urine brings shame to medicine and reason.” [9]. Nevertheless, the methods of urine testing developed in the Middle Ages remained virtually unchanged until modern chemists developed chemical methods for testing the components of this excretion [20].

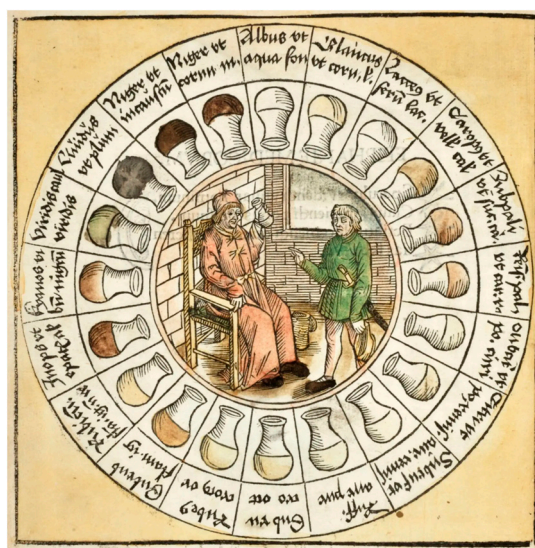


Figure 1. Urine color chart. [public domain; reproduced from Ref. [22]].

2.3. Early Modern Period

In 1690, French clergyman, novelist, and lexicographer Antoine Furetière (1619–1688) in his work “*Dictionnaire universel*” (Universal Dictionary), published in Amsterdam, wrote that “good doctors assess diseases by urine. Urine passed after the first awakening into a clear and transparent vessel is suitable for examination [24].” The color (white, orange, golden, saffron, red, wine-colored, green, or blue) was evaluated first and foremost. Any unpleasant odor that could not be explained by food consumption was considered a symptom of disease. The “examination” also included an assessment of the clarity of the urine, any cloudiness that arose after shaking the vessel, and the thickness of the layer of *hypostasis* (sediment) [25]. In the 17th century, Dutch physician and humanist Herman Boerhaave (1668–1738) introduced the measurement of specific gravity into urine testing. The residue of distilled urine was weighed to calculate its density. This was a complicated and time-consuming procedure. The test was greatly simplified by the later introduction of urinometers. A breakthrough came in 1849 when Austrian chemist Johann Florian Heller (1813–1871) constructed and used a floating urinometer (urine densimeter) (Figure 2) made of glass and consisting of a weight, a float, and a calibrated tube [26].



Figure 2. Urinometer. *Note:* Photo by P. Tomasik; reproduced with permission.

In the 16th and 17th centuries, attempts were made to supplement uroscopy with physical and chemical tests. Flemish physician and chemist Jean Baptiste van Helmont (1580–1644), a student of Paracelsus, was the first to introduce weight analysis into urine testing. He weighed urine samples taken at different times of the day, but unfortunately failed to draw any constructive conclusions [5]. In 1674, English physician Thomas Willis (1621–1675) rediscovered the link between the characteristic sweet taste of urine and diabetes, and demonstrated the usefulness of this test in the differential diagnosis of diabetes from diabetes insipidus [27]. Furthermore, Willis was the first to state that changes observed in urine reflect those occurring in blood [28]. Twenty years later, in 1694, Dutchman Federik Dekkers (1644–1720) observed that urine containing protein forms a precipitate when boiled with acetic acid [27].

The examination of urinary sediment dates back to the 17th century, when the first practically useful laboratory microscopes began to be constructed (Hans (father) and Zacharias (son) Janssen from Middleburg in the Netherlands are considered to be the inventors of the microscope. One of the first designers of a microscope useful in the laboratory was Antoni van Leeuwenhoek, which was made famous by P. de Kruif's book *Microbe Hunters*). The first examination of urine using a microscope dates back to 1630, when the French humanist, astronomer, and antiquarian Nicolas-Claude Fabri de Peiresc (1580–1637) described crystals found in urine as resembling “a pile of square bricks.” The English naturalist and architect Robert Hooke (1635–1703) showed that the crystals in urine are likely to be uric acid and calcium oxalate [29]. In 1775, J.W. Tichy initiated routine testing of patients' urine sediment [30].

The origins of chemical urine analysis date back to the 18th century. In 1776, English physician Matthew Dobson (1732–1784) noticed that the urine of a diabetic patient, when fermented and then evaporated, tasted like caramel. In 1780, Scottish physician Francis Home (1719–1813) used a fermentation test with yeast to detect the presence of sugars in urine. The presence of gas (carbon dioxide) indicated the presence of sugar in the urine (positive result). At the turn of the 18th and 19th centuries, a British physician and chemist William Hyde Wollaston (1766–1828) demonstrated the presence of uric acid, calcium, ammonium salts, and cystine in kidney stones [31].

3. Clinical Laboratory Urinalysis

3.1. 19th Century

Since the publication of Johannes Peter Müller's (1801–1858) physiology textbook in 1833, in which the author described a method for qualitative urine testing developed in 1809 by Jöns Jacob Berzelius (1779–1848), there has been enormous interest in researching the chemical composition of urine in health and disease. In the early 19th century, the practice of routinely conducting chemical analyses of urine emerged, with some of these tests overseen by Ernst Felix Immanuel Hoppe-Seyler in Tübingen, among others [32].

In the 1840s, microscopic examination of urine sediment was also introduced into clinical practice, allowing for the identification of erythrocytes, pus, and various types of crystals in preparations. Herman Reider and Auguste Sheridan Delépine published the *Atlas of Urine Sediments* in 1899, which contributed to the spread of this type of testing.

At the end of the 19th century, it was discovered that the shape of crystals in urine sediment depends on the chemical compounds that form them. Ammonium phosphate can occur in the form of a prism or fern leaf and often accompanies urinary tract infections, whereas uric acid crystals can be rhombus-shaped and are of little clinical significance. Calcium oxalates take on octagonal or “envelope” shapes and are normal components of urine. The cystine crystals are colorless hexagonal plates and are rare. Crystals of other amino acids, such as tyrosine and leucine, observed in urine sediments may be associated with liver disease [33].

In 1841, Karl August Trommer (1806–1879) developed a chemical method for detecting sugar in urine. In this method, sugars reduce copper cations Cu^{2+} by oxidizing themselves to aldonic acids (e.g., glucose oxidizes to gluconic acid). However, it was not as widely used as its modification, which was popularized in 1849 by the German chemist Hermann Chrystian von Fehling (1811–1885) [34]. It is performed using Fehling's reagent, which contains copper (II) cations complexed with tartrate anions. The appearance of a red copper (I) oxide precipitate indicates a positive test result [35].

In 1844, Johann Florian Heller described a test for detecting the presence of albumin in urine [14]. When concentrated nitric acid is added to urine, a white ring forms at the interface between the liquids, indicating the presence of albumin in the test material [36]. This test was named after its discoverer, the Heller ring test.

In 1847, doctors MacIntyre and Watson noticed opalescence in the urine of one of their patients. When heated to 60 °C, a precipitate formed in the urine sample, which dissolved when heated further, and when the urine was cooled to 60 °C, the precipitate reappeared. Unable to identify the protein, the doctors sent the urine sample to physician and chemist Henry Bence-Jones (1813–1873), who verified and published their discovery, and the protein (light immunoglobulin chains) was eventually named after him [37].

In 1868, Max Jaffè (1841–1911) noticed that when zinc was added to the urine of feverish patients, it exhibited a strong green fluorescence [38]. The compound that reacted in this way was named urobilin. Jaffè observed that it was also present in the urine of healthy patients but at lower concentrations. This observation led to attempts to determine the normal limits for certain components physiologically present in urine [39]. In 1886, Jaffè developed a method for estimating the creatinine concentration using picrate. The reaction of picrates with creatinine in an alkaline environment produces a yellow-red derivative of 2,4,6-trinitrocyclohexadiene. The intensity of color is directly proportional to the creatinine concentration [40]. In 1844, Max Joseph von Pettenkofer (1818–1901) was the first to describe a method for detecting bile acids in blood serum and urine [41]. The test, named after its discoverer, involves the reaction of bile acids with hydroxymethylfurfural, which is formed when concentrated sulfuric acid acts on sucrose [42,43]. The appearance of a red or purple color indicated the presence of bile acids in the sample [44,45].

The first two tests for detecting bilirubin in urine involved oxidizing it into the green and blue pigments biliverdin and bilicyanin. Alphonso Dumontpallier (1826–1898), a French physician, used diluted iodine tincture as an oxidizing agent, which, when carefully added to urine, formed a layer on the surface. The test was considered positive when a green ring formed at the interface. Leopold Gmelin (1788–1853), a German chemist and professor of medicine and chemistry in Heidelberg, proposed the use of nitric acid instead of iodine, in the presence of which bilirubin formed a green, blue, or purple ring at the phase boundary [31]. Another method for detecting bilirubin in reaction with p-diazobenzenesulfonic acid was published in 1884 by the German bacteriologist Paul Ehrlich (1854–1915) [46].

In the second half of the 19th century, urea in urine was measured using the Liebig's method or Regnard's apparatus, and nitrogen in urine using the Seegen's method [47], creatinine using Maly's method and Neubauer's method [48], uric acid using the Salkowski's method [49], indican using the Hoppe-Seyler's method, and the Jaffe's method [50]. Chlorides in urine using the Mohr's method, phosphoric acid using the Neubauer's method [51], glucose using the Fehling's method and the Böttger test. Blood in the urine was also detected using the Heller's method [52].

In Poland during the partitions, as in the partitioning countries and throughout Europe at the turn of the 19th and 20th centuries, efforts were made to standardize the performance of general urine tests. Properties such as urine volume, clarity (transparency), specific gravity (specific weight), interaction (pH), odor, and color were described. The colors and odors most commonly used in the descriptions of the test results and the possible causes recognized at the time are listed in Tables 1 and 2.

Table 1. Urine color. Adapted from Ref. [53].

Urine Color	Cause	Pathological Condition
Straw (urine almost colorless)	Lack of proper pigments or significant fluid intake (<i>Urina potus</i>).	Anemia Diabetes Kidney diseases
Pale yellow, amber	Normal urine	
Dark yellow	Elevated levels of urinary pigments or insufficient breakdown of blood pigments. After consuming santonin, rhubarb, or senna.	Acute kidney diseases
Yellowish-milky	Presence of fat or pus globules.	Chyluria * Acute nephritis (Nephritis acuta)
Greenish, brownish-green	Presence of bile pigments.	Jaundice (<i>Icterus</i>) Liver diseases
Greenish-yellow, turning brown after a while and then black	Due to breakdown of blood pigments (methemoglobin). After ingesting carbolic acid, urine may turn almost black, but this coloration is temporary.	Acute kidney inflammation (hyperemia)
Reddish-pink	Presence of unchanged blood pigments. The coloration may also result from plant pigments, e.g., madder, campeche logwood.	Injuries (wounds) of the urinary tract
Bluish	Presence of indigo dyes. After some time, a bluish film forms on the surface of the urine, and indigo dyes settle at the bottom; the urine pH is alkaline.	Cholera Typhoid fever

* Chyluria: presence of lymph in urine, causing milky appearance.

Table 2. Urine odor. Adapted from Ref. [54].

The Odor of Urine	Cause
Ammonia	During ammoniacal fermentation
Hydrogen sulfide	Cystitis, bact. Coli com., (<i>E. coli</i> infection)
Fecal odor	Festering tumors or intestinal fistula
Acetic acid-acetone	Diabetes (especially during diabetic coma) and certain febrile states
violet-like	After using turpentine
Aroma of roots	After consumption of saffron, cubeb pepper, or Chopart's balsamic mixture [○] [55] or after ingestion of Peruvian balsam
Mint-like	After consumption of mint or menthol.
Sharp, unpleasant	After consumption of garlic or asparagus (methyl mercaptan).

[○] a mixture with a taste considered unpleasant by modern standards, intended for the treatment of persistent gonorrhea in the late 19th century. Composition: an alcoholic solution of copaiba resin with added Tolu balsamic syrup, peppermint, and orange blossom.

In the microscopic examination of urine sediment, attention was given to inorganic crystalline (crystals) and amorphous components. The most commonly described organic elements were epithelia and their types, erythrocytes and leukocytes, mucus, and renal casts, as well as parasites (tapeworm eggs (*Echinococcus*), pinworms (*Oxyuris*), trichomonads (*Trichomonas*), and lamblia (*Cercomonas*)) [56].

Many Polish scientists have focused on the study of urine, which has resulted in numerous publications. These include the already mentioned “Podręcznik do rozbioru moczu dla studentów i farmaceutów” by Alfons Bukowski, published in Warsaw in 1888 [53]; “Podręcznik do rozbioru moczu: dla lekarzy i uczniów medycyny” by Józef Wiczowski (Kraków, 1889) [57]; “Zarys semiotyki moczu” by Stanisław Serkowski (Łódź, 1904) [54]; and “Podręcznik do badań fizjologiczno-chemicznych: metody fizyczne, ogólnchemiczne i analiza moczu” by Leon Marchlewski (Kraków, 1916) [58]. The second, expanded edition of the latter handbook was also republished in independent Poland in 1924.

3.2. 20th Century

At the beginning of the 20th century, colorimetric methods were intensively developed, allowing for quick and relatively easy determination of an increasing number of components in blood, urine, and other body fluids [59]. Otto Knut Folin (1867–1934), a Swedish professor of biochemistry, developed quantitative analytical methods for urea, ammonia, creatinine, uric acid, nitrogen, phosphorus, and chlorides between 1904 and 1922. This scientist introduced and popularized the Jaffe method for estimating creatinine in urine. In 1906, Richard Bauer introduced the galactose tolerance test with oral administration. He reported that galactose appears in urine approximately 5 h after administration [60]. The patient's urine was tested for sugar using the qualitative methods of Fehling, Benedict, and Nylander. If sugar was detected, it had to be quantified using a polarimeter [61]. In 1901, Paul Ehrlich introduced a method using p-dimethylaminobenzaldehyde in the presence of hydrochloric acid, which produces a red color in the presence of elevated urobilinogen in urine [62].

At the beginning of the 20th century, the following organic compounds were detected in urine: urea, uric acid, creatine, creatinine, xanthine, hippuric acid, oxalic acid, oxalic acid, urinary pigments, protein, peptone, grape sugar (glucose), inositol, bile acids and pigments, and blood pigments [53]. Among the minerals tested in urine were chlorides, phosphates, alkaline and earth sulfates, carbonic acid, nitrogen and nitrates, oxygen, and iron.

In 1938, Michael (Mihály) Somogyi (1883–1971) developed a method for determining amylase in serum and urine. The test is based on measuring the time needed for starch absorbed on the surface with iodine (blue color of the solution) to be broken down by diastase (amylase) present in the biological material being tested into low-molecular-weight

dextrins (colorless solution) [63]. In 1938, Shaw isolated catecholamines from biological material using aluminum oxide and described the presence of these substances in human blood and urine. In the same year, Nancy Helen Callow and Robert Kenneth Callow described the presence of 17-ketosteroids in urine [64], which was confirmed two years later by A. F. Holtroff and F. C. Koch. Scientists used Zimmerman's reaction, first described in 1935, in which 17-ketosteroids react with m-dinitrobenzene in an alkaline alcohol solution, producing a red color. 17-hydroxycorticosteroids were not measured in laboratories until 1950, when Curt C. Porter and Robert H. Silber described a colorimetric reaction for their determination using phenylhydrazine [65].

With the technical progress that has been occurring steadily since the 1950s, efforts have been made to perform laboratory tests, including urine analysis, which are increasingly simple to perform and accessible to patients in home settings. Over time, urine test strips have begun to be used, allowing for a faster assessment of the general physicochemical properties of urine. A urine test strip enables the estimation of parameters such as glucose, pH, blood, ketone bodies, protein, nitrites, specific gravity, bilirubin, and urobilinogen. At the same time, routine evaluation of certain physiologically or pathologically occurring urine properties and analytes has gradually been abandoned. In addition to the taste test mentioned earlier, the assessment of urine odor and foam was discontinued. However, notably, the characteristic smell of urine (described as "mousy" or "barn-like") led to the discovery of phenylketonuria.

4. Obsolete and Modernized Urinalysis Parameters: Summary and Chronology

This section outlines urinary parameters that are no longer routinely measured or are now assessed under different names or using modernized methods. The information presented is based on historical urine test requests, laboratory results, and physicians' notes from 1850 to 1970 preserved in the authors' collections. The detailed methods of determination are described in Supplementary Materials.

4.1. Parameters Currently Not Analyzed

Mucin

A glycoprotein normally present in urine in minimal amounts. In larger quantities, it is secreted in many diseases, such as cystitis (bladder inflammation), acute and chronic gonorrhea, which appear as threads, and nephritis, which appears as casts. Additionally, it may be present in cases of diphtheria, scarlet fever, or the use of irritant drugs, cantharidin, or turpentine oil [53].

Pus

Pyuria may result from urethritis, cystitis, or pyelitis (pyelonephritis), or may be a contaminant from the genital tract. Pus consists of leukocytes fighting pathogens, as well as bacteria or fungi. In strongly acidic urine, pus often indicates pyelonephritis, whereas in neutral or alkaline urine, it suggests cystitis. Sometimes pus-containing urine is turbid with a foul odor, as occurs with vesicointestinal fistulas caused by tuberculosis, syphilitic lesions, or tumors. Large amounts of pus are observed in the urine of patients with renal pelvis tuberculosis. In chronic urinary tract infections, pus forms a grayish-white sediment [54].

Both above were replaced by more convenient microscopy sediment analysis.

Albumose

Albumoses are intermediate products of protein hydrolysis that do not precipitate upon heating but may precipitate with salts such as ammonium sulfate or zinc sulfate. Albumose is absent or present only in minimal amounts in normal urine (approx. 55 mg/L).

In kidney diseases, it is produced simultaneously with protein, sometimes even before proteinuria develops or after it subsides. In the first case, it may serve as a precursor to proteinuria; in the second case, it may indicate incomplete recovery of kidney function. Albumose may also appear in urine during phosphorus poisoning, after the ingestion of larger amounts of guaiacol or antipyrine, the injection of old tuberculin, or iodine tincture [58]. This parameter was replaced by total protein and/or urinary albumin.

Indican

Indican (3-indoxyl sulfate or indoxyl glycoside) was considered as an indicator of the intensity of protein putrefaction in the large intestine. It is absent in newborns and breast-fed infants and is a normal urinary constituent in adults. Higher urinary levels occur in people on a meat-rich diet than in those on with a plant-based diet. Increased indican excretion often coincides with increased excretion of oxalic acid and phenol; decreased excretion is observed in renal failure. Indoxyluria may also be observed in peritonitis, appendicitis, typhoid fever, cholera, and gastritis/duodenitis [53]. The diagnostic value of this test was disputable. In the nowadays for dysbiosis the breath tests are used.

Russo's reaction

Russo's 1905 test was proposed as a diagnostic method for typhoid fever [64,65].

Weisz (Weiss) urochromogen reaction

A nonspecific test for reducing substances in urine, historically used to assess the prognosis of severely ill patients and to help detect pulmonary tuberculosis [58].

The diagnostic value and clinical usefulness of both of above tests were discredited by clinicians at that time. Finally they were abandoned soon.

4.2. Parameters That Are No Longer Analyzed as Part of the Routine Urinalysis Panel and Are Now Considered Specialized Examinations Carried out Using Different Methods

Urea

Elevated urea levels are observed in fever, diabetes, anemia, or after the ingestion of chemicals and drugs such as table salt, borax, ammonium chloride, iron, Glauber's salt, caffeine, quinine, or salicylates. Reduced urea levels occur in some forms of nephritis, especially acute nephritis, during uremic attacks, degenerative liver diseases, phosphorus poisoning, as well as cirrhosis or hepatocellular carcinoma [53].

Creatinine

Elevated creatinine occurs during fever, after physical exertion, and with a meat-rich diet. It also increases in acute and chronic renal failure and in heart failure. Reduced levels are observed in states of weakness and starvation, muscle atrophy, during pregnancy, or after the use of certain anti-inflammatory, anti-allergic, diuretic, or steroid medications.

Uric Acid

Increased uric acid levels are observed after excessive physical exertion, fatigue, febrile illnesses, arthritis, leukemia, and liver cirrhosis. Decreased levels occur in pyuria, after the ingestion of certain drugs such as quinine sulfate or lithium carbonate, and in lead poisoning [57].

Chlorides

Reduced chloride excretion is observed in effusions, diarrhea, febrile states, and tuberculosis. Increased excretion occurs in typhoid fever, after bathing, or during resorption of effusions [53].

Sulfates

Sulfates enter the urine from the blood, either through dietary intake or via the lymphatic system as a result of the breakdown of sulfur-containing proteins. The main factors affecting urinary sulfate excretion are diet and the rates of tissue breakdown and renewal. Urinary sulfate excretion is higher in individuals on a meat-rich diet than in those who are fasting. Reduced excretion is observed in anemia and in gastrointestinal diseases. Elevated excretion occurs in the early stages of acute diseases accompanied by fever, typically with reduced urine volume and increased specific gravity, as well as in conditions associated with tissue breakdown and elevated urea concentration [66].

Phosphates

Increased phosphate excretion is observed in diseases accompanied by fever, encephalitis, malaria, rickets, after the ingestion of mineral water containing phosphates, and with medications containing phosphorus compounds. Decreased phosphate excretion occurs in kidney, heart, and nervous system diseases; in rheumatism; and in gout. Low phosphate concentrations are also observed in urine with low specific gravity, such as that of *Urina potus* and *Urina spastica* [67].

4.3. Parameters Currently Analyzed Under Different Names and Using Different Methods

Acetoacetic Acid, Acetone, β -Hydroxybutyric Acid (Ketone Bodies)

Acetoacetic acid and acetone are associated with diabetes and produce the characteristic odor of urine, which was historically considered a *signum mali omnis*, although many cases of diabetes occur without acetone excretion. Excretion of acetoacetic acid and acetone also occurs in gastric and intestinal cancers, during acute infectious diseases, after anesthesia, and during poisoning with sulfuric acid or morphine. β -Hydroxybutyric acid is excreted in more severe cases of diabetes, alongside acetoacetic acid and acetone. The accumulation of β -hydroxybutyric and acetoacetic acids in the body has historically been used to explain diabetic coma [54].

Blood Pigments (Hematuria)

The presence of blood in urine is always considered a pathological sign, except in cases where it is accidental, such as contamination from hemorrhoids or menstruation, or when it occurs after physical exertion [62].

Bile Pigments (Bilirubin and Urobilinogen)

Elevated bile pigment concentrations appear in urine as a result of liver dysfunction. This occurs in all forms of jaundice regardless of the cause (including obstructive jaundice caused by bile stasis and hemolytic jaundice caused by excessive breakdown of red blood cells), liver cirrhosis, liver tumors, acute hepatic atrophy, as well as uncompensated heart defects [54,68].

Figure 3 presents the chronology of routine measurements of specific urinary analytes. The figure was prepared on the basis of urine test requests and results, as well as physicians' records from 1850 to 1970, which are held in the authors' collections.

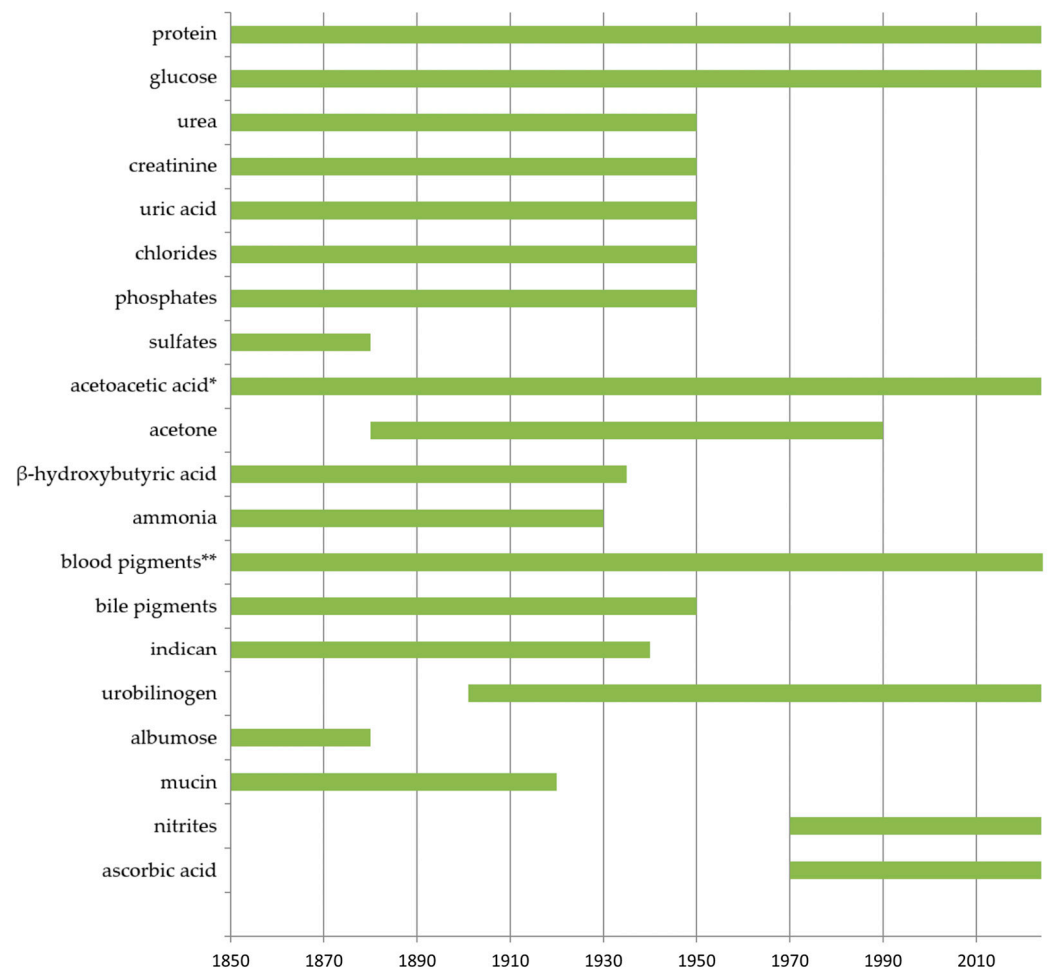


Figure 3. Chronology of the measurement of selected chemical parameters in routine general urinalysis. * currently called ketone bodies or ketones; ** currently called blood/erythrocytes.

In addition to chemical analysis of urine components, estimation of the solid fraction was popular until the 1930s. This was performed approximately by multiplying the last two digits of the urine's specific gravity by a factor of 2.237. For example, if the specific gravity of urine was 1.018, multiplying 18 by 2.237 yielded 40.28 g of solids per liter of urine. If the daily urine volume was 1500 mL (1.5 L), the total solids amount was 64.4 g.

5. Present Day and Future Perspectives

Urine assessment has played an important role in medical practice since ancient times. As a readily accessible biological material, urine was frequently studied. In antiquity, examination had been limited to organoleptic evaluation of quantity, color, odor, and clarity, and later also of sediment. Urinalysis was considered so essential that a medieval physician could be punished for failing to perform it.

With the development of chemistry, urinalysis has gained new attention. New and modified chemical methods allowed results to be obtained not only qualitatively but also quantitatively, significantly facilitating diagnosis and monitoring of treatment. Initially, these analyses were time-consuming and sometimes required large urine volumes; however, with technological progress and laboratory automation, simpler methods gradually replaced the older methods, often still on the basis of reaction principles developed by the original discoverers.

By the 1950s, medical laboratories began using urine test strips, which greatly shortened the time needed for physicochemical analysis. Up until the 1970s, urinalysis remained

a standard laboratory test. Later, with the development of methods to assess various blood compounds, it became a supplementary test, although still widely performed. The advent of immunochemical methods at the turn of the 20th and 21st centuries enriched urinalysis with new markers of acute and chronic kidney like neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule 1 (KIM-1), liver-type fatty acid binding protein (L-FABP), cystatin C (CyC), soluble urokinase plasminogen activator receptor (suPAR0), DKK3 (dickkopf-3), MCP-1 (monocyte chemotactic protein 1), and markers associated with fibrosis. Many new tests, primarily genetic, have also been developed for the early detection of urinary tract cancers, such as mutations in the TERT, FGFR3, and PIK3CA promoter genes in bladder cancer, or a panel of RNA biomarkers (EPCAM, TTC3, H4C5) in prostate cancer. Much research is being conducted on early urinary markers for the detection of cancers in other organs. Improved urine tests are available for the diagnosis and treatment of endocrine and metabolic diseases, as well as poisoning, utilizing various modifications of mass spectrometry [69].

Non-invasively collected urine is very convenient for point-of-care testing (POCT) and self-diagnosis. It can be assumed that widespread, one-time screening of substances excreted in urine will be helpful in assessing people's health, treating diseases, and managing a healthy diet and even lifestyle in the near future. This may become possible without the need for urine collection, but with the use of smart toilets [70].

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/app16010175/s1>.

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