Reflexiones

Frédéric Alphonse Musculus

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Abstract

Frédéric Alphonse Musculus (1828-1888) was a French military pharmacist who worked mainly on starch, its reactions, and derivatives, and urease. His found that dextrin was not the product of a hydration alone but the result of the decomposition of the starchy matter. Dextrin and glucose appeared always in the same 2:1 ratio. The same results were obtained using diluted sulfuric acid or diastase as the reaction agent. He showed that the results of Payen on the subject were wrong because he had used an impure dextrin. Together with David Gruber, a major brewer producer, synthesized many starch derivatives, among them, erythrodextrin, achroodextrin *a, b, g*, and soluble starch, and concluded that starch was a polysaccharide of formula $n(C_{12}H_{20}O_{10})$, where n was larger than 5 or 6. With Méring he studied the action of saliva and pancreatic juice upon glucose and glycogen. Musculus separated from putrefying urine a ferment, which eventually Miquel would name urease.

Keywords

Dextrin; ferments; glucose; glycogen; soluble dextrin; urine

Resumen

Frédéric Alphonse Musculus (1828-1888) fue un farmacéutico militar francés que investigó principalmente el almidón, sus reacciones y derivados, y la ureasa. Encontró que la dextrina no era el resultado de solo una hidratación, pero el efecto de la descomposición de la sustancia. La dextrina y la glucosa aparecían siempre en la razón 2:1. Los mismos resultados eran producidos cuando el agente activo era el ácido sulfúrico diluido o la diastasa. Demostró que los resultados de Payen eran erróneos porque había usado una dextrina impura. Junto con David Gruber, un importante fabricante de cerveza, sintetizaron una variedad de derivados del almidón, entre ellos, la eritrodextrina, las acroodextrinas *a, b, g*, y el almidón soluble, y concluyeron que el almidón era un polisacárido de fórmula $n(C_{12}H_{20}O_{10})$, en que n era mayor de 5 ó 6. Junto con Méring estudiaron la acción de la saliva y la secreción pancreática sobre la glucosa y el glicógeno. Musculus separó de la orina putrefacta un fermento que eventualmente Miquel llamó ureasa.

Palabras clave

Dextrina; dextrina soluble; fermentos; glicógeno; glucosa; orina

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Life and career (Balland, 1882; Anonymous, 1888; La Bel, 1889-1890)

Frédéric-Alphonse Musculus was born on July 16, 1828, in Soultz-sous-Forêts (Bas-Rhin), the son of a third-generation pharmacist and owner of the pharmacy Musculus. He received his diploma of pharmacist in 1854 and the following year entered the Parisian military hospital Val-de Grâce as trainee. In 1856 he was sent to Constantine (Algiers) where he remained for two years. In 1859 he participated in the Italian Campaign against the Austrian Empire, which laid the foundation of the Kingdom of Italy. After serving in different garrisons in Corsica and France he was promoted to pharmacien-major in 1866. While at the military hospital of Vincennes, in the eastern suburbs of Paris, he frequented the laboratory of Jean-Baptiste Boussingault (1802-1887) and began his research on starch, which would be his main project for many years. In 1870 he participated in the Franco-Prussian war and was decorated for his many services. At the death of his father (1872) he resigned his military position to take charge of the family pharmacy. In 1874 he was appointed chief pharmacist of the Strasbourg hospital (Balland, 1882; Anonymous, 1888; La Bel, 1889-1890).

Frédéric Musculus passed away on May 26, 1888, and was buried in the St. Gallen Cemetery in Strasbourg-Königshöfen.

Musculus participated actively in public and academic activities and was rewarded accordingly. He was President of the Society of Science, Agriculture and Arts of Lower Alsace, inspector of pharmacies, examiner of pharmacists at the Université se Strasbourg, member of the Conseil d'Hygiène of the city of Strasbourg, and chevalier de la Legion d'Honneur (1871).

Scientific work

Musculus wrote about 30 papers in the areas of analytical, inorganic, and organic chemistry, physiology, and toxicology. In addition to the subjects discussed below, Musculus developed a new method for detecting spots of washed blood (Choulette & Musculus, 1857-1858) and an alcoholmeter based on capillarity to determine the alcoholic richness of wines and the strength of acetic acid (Musculus, 1863), made a detailed study of the stannic hydrates (stannic and metastannic acids) (Musculus, 1867, 1868), discovered a new compound formed in the urine after digestion of chloral hydrate (Musculus & de Méring, 1875); developed a qualitative and quantitative method for determining albumen in urine (Musculus, 1880); analyzed a larger number of wines of 1879 (Musculus & Amthor, 1882); etc.

Starch and derivatives

Most of Musculus' research was devoted to the study of starch, its reactions, and derivatives (Musculus, 1860, 1862, 1865, 1869, 1870, 1872, 1874b; Musculus & Gruber, 1878; Musculus & Méring, 1879; Musculus & Meyer, 1881).

Starch

Musculus wrote that most scientists agreed that starch, before it was converted by dilute acids into glucose, went through the intermediate stage of dextrin, and that dextrin was simply a

molecular modification of these compounds, which would become glucose by addition of four equivalents of water (Musculus, 1860). Musculus found that his experimental results contradicted these beliefs: dextrin was actually the result of the *decomposition* of the starchy material and not of its *hydration*. This conclusion was based on the following facts: (1) diastase did not react with dextrin; (2) glucose and dextrin appeared simultaneously and always in the same ratio; (3) diluted sulfuric acid acted initially like diastase but continued to react after the disappearance of the starch, although at a much slower rate; and (4) the simultaneous appearance of dextrin and glucose manifested itself with the acid as with the diastase and always in the same ratio (Musculus, 1860).

Musculus digested starch with a solution of diastase, at 70° to 75 °C, and noticed that the amount of glucose formed increased until the liquid ceased to be colored blue or red by the iodine tincture. At this moment the reaction ended although a large amount of dextrin was still present, as easily proven by boiling the solution with water containing 1% of sulfuric acid. Addition of more starch resulted in re-initiation of the reaction and its stoppage when the iodine tincture signaled the disappearance of the glucose. If the weight of glucose added was the same as the initial one, the glucose formed was double that of the first stage. If the reaction was stopped before its completion and the unreacted starch removed by filtration, the filtrate was found not to be colored blue by the iodine tincture and to contain a mixture of two equivalents of dextrin and one of glucose. This ratio was always the same, independent of the time of reaction. The same ratio was found when using water acidulated with 1% of sulfuric acid. In this case, the rate of reaction decreased until it became very low. This result was clearly inconsistent with the assumption that the reaction was a simple hydration Musculus wrote that his intention was to bring his results to the attention of chemists, without trying to change the formula of starch; he expected that they would allow explaining the phenomenon from an different angle than the *action by presence* (catalysis) exerted by acids very avid of water (Musculus, 1860).

Musculus concluded as follows: (1) In the manufacture of glucose, where the end of the reaction is assumed to be the point where the iodine tincture ceases to turn blue the liquid, and alcohol does not cause precipitation, a large amount of dextrin remains mixed with the sugar. Since this substance does not ferment with yeast, it causes a substantial harm to the consumer. Hence, it is recommended that manufacturers carry the reaction in a closed flask, at a much higher temperature and for a longer time; (2) the strong resistance of dextrin to the action of diluted sulfuric acid allows an easy analytical procedure of the mixture of sugar and dextrin. It is enough to boil de mixture for one minute to modify all the sugar and allow its determination by means of the Fehling liquor. Boiling does not modify the dextrin; any starch present can be easily destroyed by the diastase, which does not act upon sugar and dextrin; (3) the extremely large amount of barley that brewers must use to produce a liquid containing a little of alcohol can be explained by the way of action of diastase: two thirds of the starch go into the beer as dextrin, which communicates to the drink the slightly gummy consistency that the consumers appreciate; and (4) the fabrication of eau-de-vie from grains, where the sugar is manufactured from barley germ, presents an unavoidable two-thirds loss (Musculus, 1860).

A following paper by Anselm Payen (1795-1871) provided a general analysis of the transformation of starch into sugar and dextrin. Many scientists had studied the progress of the reaction between starch and sulfuric acid or with diastase, without agreeing on the ratio of the glucose and dextrin formed. In general, it was accepted that the first stage of the reaction yielded a maximum of dextrin and a minimum of glucose and that the amount of the latter increased as the reaction proceeded, without being able to establish the best conditions for this

transformation and the influence limit of the agents that activated the reaction. The recent results reported by Musculus established a ratio dextrin: glucose = 2: 1, which led naturally to suggest carrying the reaction with sulfuric acid in closed flasks so as to elevate the pressure to a value corresponding to the temperature 108 ℃. In addition, the reaction of diastase with starch was unable of saccharifying more than one third of the amylaceous material. Payen severely criticized the second recommendation; he thought that Musculus' theoretical conclusions were, perhaps, not sufficiently justified. The different state of aggregation of the starchy particles offered in each grain of fecula, and each of the ten to fifteen layers concentrically nested elements that composed it, alternatively a minimum and a maximum of cohesion from the center to the periphery. These diverse states would be enough to explain the rapid transformation of the amylaceous substance into two products: one resulting from the dissolution followed by almost immediate saccarification, the other stopping after the more difficult dissolution of the more strongly aggregated portions. This resistance was, perhaps, increased by the presence of the glucose produced, resulting in partial overcoming of these obstacles. Payen justified this claim by indicating that Roch Théogène Guérin-Varry (1800-1852) had reported the total saccharification of dextrin by diastase by repeating the reaction many times after removing every time the glucose formed with alcohol (Payen, 1861; Guérin-Varry, 1835).

In a following note, Musculus rejected Payen's criticism by stating again that treating starch with a solution of diastase resulted in the formation of an increasing amount of glucose until all the starch had disappeared (Musculus, 1862). Afterwards, no additional sugar was formed, no matter how long the mixture was heated. Addition of a new amount of starch resulted in the re-initiation of the reaction until it stopped again, as in the first run. This result could not explain under the assumption that starch transformed first into dextrin and then into sugar; it required accepting that diastase was more active for one part of the dextrin than for the other, an impossible fact. Carrying the reaction with diluted sulfuric acid produced the same results, except that the reaction continued after the starch had been exhausted, although at a much slower rate. Payen had admitted that in order to obtain the maximum amount of glucose he had to heat the liquid for an additional five hours. Musculus had found that at atmospheric pressure the rate of reaction increased with an increase in temperature. This finding had led him to recommend that the reaction be carried in a closed vessel to avoid obtaining the brown colored liquid produced by long boiling at atmospheric pressure. It was perfectly admissible to accept that under the influence of sulfuric acid, starch, the same as fatty acids, split into dextrin and glucose, with fixation of water, in the same manner that fatty acids under the action of the same acids split into a fatty acid and glycerin with fixation of water. Other reactions were known to occur in a similar manner: i.e., salicin split into glucose and saligenin, phlorizin into glucose and phloretin, tannin into glucose and gallic acid, etc. (Musculus, 1862).

In another paper Musculus described his attempts to determine the chemical constitution of the starchy material (Musculus, 1869). According to Payen's observations it appeared that the formation of dextrin and glucose were simultaneous phenomena and according to the pertinent theory developed by Marcelin Berthelot (1827-1907), it seemed that the dextrin colorable violet by iodine was the first stage of a split, where the sugar produced by diastase corresponded at least to two equivalents (Payen, 1861; Berthelot, 1861, Musculus, 1869). Musculus tried unsuccessfully to stop the reaction at the point were the liquid was tinted violet by iodine. Afterwards he tried to isolate dextrin in order to compare its metamorphosis with those of starch. He found that when dextrin no colorable by iodine was dissolved in water together with glucose and soluble starch, it was impossible to separate the three components. This negative result led him to obtain dextrin

in a state insoluble in water. It was known that heating starch with glacial acetic acid in a closed vessel at 100 ºC resulted in the formation of soluble starch. A different result was obtained when the process was conducted with acetic acid containing 10% wt. of water. This resulted in the formation of glucose and a residue insoluble in water that at first look seemed to be the same as starch. A microscopic examination showed that the grains of starch had more or less kept their shape but instead of being colored blue by iodine they presented a reddish tint. Boiled in water they dissolved partially without forming a paste and the liquid being colored violet by iodine. The residue, after being washed with cold water to eliminate all the soluble substances and dried, was found to be composed mostly by insoluble dextrin. In contact with diastase, it produced a material containing no more than 23% wt. sugar. Starch treated in the same manner provided 33% wt. sugar, a result that indicated that the dextrin colored violet by iodine was not a metameric variation of the starchy material but identical with it, less the sugar that had been detached by the acetic acid and present in the solution. Musculus remarked that the insoluble dextrin was always accompanied by a certain number of non-attacked grains, so that the number 23% wt. was larger than the true value. In addition, the split produced by the acetic acid was not neat, it always produced dextrin and glucose accompanied by a certain amount of soluble starch. Hence it was impossible to use the results to determine a rational formula for the starchy material. Musculus added that the dissolved diastase seemed to be identical with glycogen (Musculus, 1869).

Musculus wrote that chemists did not agree on the meaning of the term *soluble starch* (Musculus, 1874b). Some considered it to be the matter colorable blue by iodine, which could be eliminated by means of water and that Carl Nägeli had named granulose (Nägeli, 1865). To others, it was the substance colorable violet by iodine that Antoine Béchamp (1816-1908) had obtained by treating starch with sulfuric acid (Béchamp, 1855). Nägeli believed that granulose was finely mechanically divided starch, able to pass through a filter, without being actually soluble in water. Most scientists believed that granulose were actually soluble in water but did not agree on its properties. This had led to assigning to it a variety of names, i.e., amyduline, amidine, amylagine, etc. To Musculus, granulose was actually a mixture of substances, among them a starch portion colorable blue by iodine, soluble starch, dextrin, glucose, etc. In a previous paper (Musculus, 1870) he had given the name globulized dextrin to the body obtained by dissolving the starch in boiling acidulated water, followed by saturation of the acid with calcium carbonate, filtration, and evaporation to a syrupy state. This resulted in the precipitation of abundant granules insoluble in cold water and soluble in water at 50 ºC. Upon drying, the resulting soluble white starch was found to be composed of disorganized grains. Alcohol precipitated it from its warm solution in water as insoluble starch. Mixed with dextrin and glucose (as in its mother liquor) resulted in the loss of all its particular properties.

Musculus added that commercial dextrin exhibited a similar behavior. With alcohol it was possible to extract grains of soluble starch. The artificial grains of starch gave with iodine the same gamma of colors given by the natural grains, in addition to those furnished by dextrin. Thus, a diluted solution turned red and concentrated by saturation it turned violet. Diastase split soluble starch in the same manner as natural starch, although faster and neater. Musculus was able to prepare dextrin from cellulose and from glucose (Musculus, 1874b).

Musculus, in collaboration with David Gruber, a brewer from Strasbourg, carried an extensive research on the many starch derivatives that could be prepared under the action of diastase or boiling diluted sulfuric acid (Musculus & Gruber, 1878).

1. Soluble starch, prepared in a pure state by Musculus (Musculus, 1869, 1874b). Insoluble in cold water and soluble in hot water at 50^0-60 ºC. Colored blue by iodine when in the solid state and wine red when in aqueous solution. Rotatory power [a] = 268º and reducing power 6.

2. Erythrodextrin, named so by Ernst Brücke (1819-1892) because of the red color assumed in the presence of iodine. It differed from the soluble starch in being always soluble in water and constituted the largest part of commercial dextrin. Easily attacked by diastase, even in minimum amounts.

3. Achroodextrin *a*, not colored by iodine; rotatory power [a] = 210º and reducing power 12. Partially saccharified by diastase, although less easily than soluble starch and erythrodextrin. Found in brown beer obtained from malt strongly roasted at 110º to 120 ºC. At this temperature the diastase was partially altered and its action weakened. Also obtained from the malt of weak in alcohol and rich in extractive matters.

4. Achroodextrin *b*, rotatory power [a] = 190º and reducing power 12. Not attacked by diastase, at least during 24 hours. Found in pale beer.

5. Achroodextrin *g*, rotatory power [a] = 150º and reducing power 28. Not attacked by diastase, even during one year. Transformed by boiling dilute sulfuric acid into glucose, after many hours of boiling. Obtained by boiling starch with sulfuric acid of 2.5% wt. until the liquid was not precipitated by alcohol, followed by destruction of the sugar formed by fermentation.

6. Maltose, rotatory power [a] = 150º and reducing power 66. Hardly attacked by fermentable diastase under ordinary conditions.

7. Glucose, rotatory power [a] = 56º and reducing power 100; fermentable.

Musculus and Gruber stressed that the reported values of the rotatory and reducing powers for the above substances were only approximate because these substances were non-crystallizable and did not form stable combinations. In addition, they wrote that the dextrins isolated by Lucien Bondonneau were not exactly identical with the ones they had prepared; anyhow, Bondonneau had also found that the rotatory power diminished with the process of saccharification while the solubility in alcohol increased (Bondonneau, 1874). Interpretation of these results with the old theory required that starch go through five consecutive isomeric transformations before becoming glucose, an idea hard to accept. The existence of five dextrins non-attackable by diastase made this theory unacceptable. Consequently, Musculus and Gruber assumed that starch was a polysaccharide of formula $n(C_{12}H_{20}O_{10})$ where the exact value of *n* should not be less than 5 or 6. Under the influence of diastase ferments or diluted acid, this carbohydrate went through a series of successive hydrations and splits. Each split produced maltose and a new dextrin of lower molecular mass while the value of *n* became smaller and smaller up to the production of achroodextrin *g*, which transformed into maltose by simple hydration. Maltose hydrated afterwards and split into two molecules of glucose (Musculus & Gruber, 1878):

$$C_{12}H_{22}O_{11} + H_2O = 2C_6H_{12}O_6$$
maltose   glucose
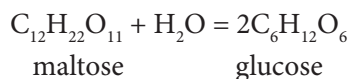
Musculus and Friedrich Joseph von Méring extended the study to the action of saliva and pancreatic juice upon starch and glycogen (Musculus & Méring, 1879). Their memoir began with a short historical review, starting from the 1811 finding of Gottlieb Kirchhoff (1764-1833) that diluted acids decomposed starch into glucose and that the intermediate products belonged

to the dextrin family (Kirchhoff, 1811), and ending with the discovery and isolation of maltose by Augustin Pierre Dubrunfaut (1797-1881) and Cornelius O'Sullivan (1841-1907) (Dubrunfaut, 1847; O'Sullivan, 1872). Musculus and Méring wrote that the production of maltose by the action of diastase on starch made very probable the opinion that the same sugar originated under the influence of the diastase ferments (such as saliva and pancreatic juice) in the organism. The results of their experiments proved this assumption to be true. For example, a paste made of 100 g of dry starch and 1,200 cm^3 of water was mixed with 500 cm^3 of filtered saliva and digested for six hours at 40 °C. The resulting product, having a reducing power of 52, was evaporated to a syrupy state and then treated with pure alcohol. The resulting precipitate was dissolved in water and treated with beer yeast, after fermentation the solution was found to contain a substantial amount of dextrin, highly dextrorotatory and reducing. The liquid separated from the first precipitate was found to contain maltose pure. A detailed analysis of the different streams showed that the reaction of saliva with starch produced maltose and glucose. Similar experiments were conducted with saliva, diastase, and glycogen, and the pancreatic juice and starch. The results of the these experiments led Musculus and Méring to conclude as follows (Musculus & Méring, 1879): (1) Saliva and pancreatic juice yielded with starch the same products as the those of the split with diastase, that is, the reducing dextrins maltose and glucose; (2) glycogen, under the influence of saliva and diastase, yielded maltose and glucose; (3) the dextrins produced by glycogen differed from those from starch in being less hygroscopic and having less reducing power. In addition, the saccharification of glycogen yielded a dextrin that was not attacked by diastase and saliva when the reducing power had reached the value 37 (this occurs with starch when the reducing power has reached 50). Diastase acted upon glycogen weaker than saliva; (4) there was only one glycogen, whether the animal from which it came had been fed exclusively with carbohydrates, or with albuminoidal substances; and (5) the existence of reducing dextrins of variable reducing power that accompanied maltose and glucose, demonstrated the need to resort to fermentation for the determination of sugar in the liquids of the animal economy, and explained the differences that existed between the results obtained by the different researchers, according to whether they used the method of fermentation or that the reduction with the blue liquor (Fehling). Méring had indeed found that the reducing power of the alcoholic extract of the blood of the portal vein, after a starchy food, increased by 25% when heated with diluted sulfuric acid (Méring, 1877). Was this increase due to the presence of maltose or dextrin or both at the same time? Musculus and Méring hoped to answer this question in another study using fermentation (Musculus & Méring, 1879).

Dextrin

According to Musculus, many commercial products sold under the name dextrin behaved totally different with certain reagents. For example, commercial gommelin (another name for dextrin) was strongly colored by iodine while the substance present in a malt infusion, previous to fermentation, was not colored by this compound. This confusion probably explained the variety of results reported for the splitting of starch into dextrin and glucose. Most probably, the product sold in the commerce under the name dextrin was a mixture of starch, dextrin, and glucose. Hence the need to clearly define the real substance named dextrin (Musculus, 1865).

Dextrin was usually prepared by boiling starch with diluted sulfuric acid until the moment when the liquid did not react with iodine. The resulting liquid was now mixed with well-washed beer yeast and let to ferment completely. The filtrate was then evaporated to dryness and extracted several times with boiling alcohol. The resulting dextrin appeared as a gummy substance, highly

hygroscopic and very soluble in water. Alcohol precipitated it from the aqueous solution. The solution was not precipitated by lead sub-acetate, was not colored by iodine, and did not reduce the Fehling liquor. Dextrin was dextrorotatory with a rotatory power about three times that of glucose. This result explained why maltose, a mixture of glucose and dextrin, had a rotatory power of 2/3 that of glucose. Dextrin, boiled with water acidulated with sulfuric acid, transformed slowly into glucose. This reaction went faster in water heated to 108 °C (a temperature easily obtainable in a bath containing a saturated solution of sodium chloride). Even so, complete saccharification required four hours of heating, a result that explained why heating for less time yielded less glucose than the expected value. Payen had reported that boiling a starch solution at atmospheric pressure for 5.5. hours with a 2% aqueous solution of sulfuric acid was enough for obtaining complete saccharification. The best proof that he was wrong was the fact that this procedure yielded a product containing 83.6% glucose, while replacing the sulfuric acid by HCl the sugar content increased to 85,5% (Payen 1861; Musculus, 1862).

Diastase had no effect on dextrin, as reported in a previous publication (Musculus, 1860), a result that proved that this compound acted only upon substances able to form a blue or red combination with iodine. It also proved that without taking into consideration the coloration with iodine, that is, stopping the reaction while the reaction with iodine was still possible, resulted in producing an amount of glucose smaller than the maximum one achievable with diastase. This fact explained why Payen had obtained, after 17 hours of reaction, only 52% of the maximum amount of glucose attainable. Payen claimed that glucose prevented the action of diastase upon dextrin. In order to test this claim, Musculus reacted pure dextrin with a solution of diastase containing a very small amount of glucose (less than 0.5 g glucose per 100 cm^3) and found that no additional glucose had formed. The same result was obtained reacting pure dextrin with pure diastase. Musculus also treated a mixture of glucose and dextrin with well-washed beer yeast; no reaction took place during the first eight days but upon addition of malt infusion resulted in the dextrin fermenting equally (Musculus, 1862).

Musculus conducted similar experiments with commercial dextrin and the results indicated clearly that all of them contained glucose. This fact indicated that Payen had used gommelin for his experiments and assumed, erroneously, that it was pure dextrin. It also explained why Payen believed that Musculus' results were inadmissible (Musculus, 1862).

In a following paper Musculus described in detail the dextrin soluble in water that he had synthesized by heating starch with glacial acetic acid (Musculus, 1870). This dextrin was composed of grains of starch that had kept their original organization in spite of the chemical modification they had experimented. Musculus believed that this fact explained its insolubility in water. Heated in water at 100 °C for 10 to 12 hours turned soluble again. Upon dried, they become a yellow powder similar to ordinary dextrin. Evaporation to a syrupy state resulted in turbidity and transformation into an insoluble matter that remained in suspension in the liquid, but could be precipitated by addition of a large amount of water. Separation by filtration, followed by drying, produced a white power composed of fragments of starch grains, lacking any definite structure. This material was insoluble in cold water but dissolved promptly in water heated to 50 °C. Evaporated to dryness it turned again insoluble in water (Musculus, 1870).

Musculus wrote that this insoluble dextrin could also be prepared by direct saccharification of starch with diastase and stopping the reaction once the liquid became colored red violet by iodine. Saccharification with sulfuric acid produced the same result but the product had some new proprieties. In this case the solid did not remain in suspension but precipitated. Microscopic

examination showed that it was formed of small round grains (which he named *globulized dextrin*), which grew with time from a diameter of 0.001 mm to 0.030 mm at the end of three months. These grains were insoluble in cold water and dissolved abruptly in water heated to 50 ^{0}C. Iodine did not color the grains of dextrin but caused their separation, particular if used in great excess. Musculus reported that both insoluble dextrins had the same specific rotatory power, [a] = +208°, substantially higher that of ordinary dextrin, [a] = +138.7° (Musculus, 1870).

In 1872 Musculus reported that addition of alcohol to a solution of glucose in concentrated sulfuric acid resulted in the appearance of a new substance that he considered was a dextrin (Musculus, 1872). Two years later Armand Gautier (1837-1924) reported the preparation of a similar substance by passing a stream of dry HCl gas through a solution of pure glucose in alcohol. He assigned it the formula $C_{12}H_{22}O_{11}$ and ranged it among the isomers of saccharose (Gautier, 1873). This information led Musculus and A. Meyer to extend the work of Musculus to see verify if the substance he had prepared should really be ranged among the dextrins (Musculus & Meyer, 1881). To do so they melted 30 g of very pure dextrose-glucose in a hot bath of calcium chloride. After cooling they added little by little 30 g of concentrated sulfuric acid while the mixture was heated to about 60 °C and assumed a brown color. Afterwards they added 800 g of anhydrous alcohol, filtered the mixture, and left it alone for eight days. The resulting precipitate was filtered, washed first repeatedly with cold anhydrous alcohol, then with 300 g of boiling anhydrous alcohol, and finally dried. This produced 10 g of an amorphous white powder (corresponding to about one-half of the glucose employed that was hydroscopic but not deliquescent. The mother liquor retained the other half of the glucose, probably as a compound of glucose and sulfuric acid. The solid was dissolved in water distilled to eliminate the retained alcohol and purified further. The final product was a white powder, extremely hygroscopic and deliquescent. Elemental analysis indicated that it contained, by weight, 44.69% carbon, 6.50% hydrogen, and 48.81% oxygen, corresponding to the formula $C_6H_{10}O_5$, the empirical formula of dextrins. Musculus & Meyer reported that the hydrated substance presented all the properties of dextrin. It was amorphous, pale yellow, very soluble in water and had a slight sweet taste. It was not colored by iodine, it reduced the Fehling liquor, was dextrorotatory and had rotatory power [a] =131 to 134°. It was not fermented by beer yeast and not saccharified by diastase. Boiling with diluted sulfuric acid converted it totally into glucose (Musculus & Meyer, 1881).

Urine, urea, and urease

Musculus wrote that the rapid transformation of urea into ammonium carbonate was due to the particular ferment that appeared during the fermentation of urine, described by Louis Pasteur (1822-1895) and Philippe Edouard van Thiegem (1839-1914) (Musculus, 1874a). Pasteur had used this phenomenon as one of his many experiments to demonstrate the impossibility of spontaneous generation (Pasteur, 1860). In a necked vessel he had put 100 cm³ of fresh urine and boiled it for 2-3 minutes while taking all the necessary measures to sterilize the air. He had let the contents to cool, closed the neck hermetically by means of an alcohol lamp, and left the flask in a stove at 30 °C during six weeks. Upon opening, no alteration of the contents was observed. Pasteur now added to the contents a piece of asbestos charged with air dust, sealed it and returned it to the stove. To assure the validity of his experiments he prepared a second flask, as before, and introduced in it a similar sample of asbestos, previously calcined for a short time. At the end of the test time, no changes were observed in the liquid of the second flask. After 36 hours, the first flask with the urine that had been in contact with the air dust was full of organized organisms

or infusoria. Among the latter Pasteur recognized mainly the presence of bacteria of the same nature that appeared when urine was left in contact with atmospheric air at 30 ºC. During the following days Pasteur observed an abundant precipitation of crystals of ammonium-magnesium phosphate and crystals of alkaline urate. The urine became more and more ammoniacal, while its urea content decreased under the action of the organized ferment. Pasteur added that the ferment was not a dead entity void of specific properties. It was a living organism that came from the air and was not an albuminous matter altered by the air. The presence of albuminous substances was a basic requisite because the ferment needed them to live; it was its food. Eventually Pasteur found the micrococcus responsible and named it *Torula ammoniacale* (Pasteur, 1860). Twelve years later, Philippe Edouard van Thiegem (1839-1914) repeated these experiments and added that in the exceptional cases where the torulaceous ferment developed alone, the liquid remained limpid and the deposit formed at the bottom of the flask was composed of strings and clusters of spherical globules of diameter about 0.0015 mm, mixed with crystals of urates and ammonium-magnesium phosphate (van Thiegem, 1864). In the more general case where the torulaceous ferment was accompanied by infusoria, the fermentation was slower. In addition to the infusoria, the bulk and surface of the liquid contained "vegetables", the torulaceous ferment developed with difficulty, and the liquid remained acid or neutral for months. Upon addition of a good ferment (i.e. beer yeast), all these difficulties disappeared and the urea vanished completely within one or two days. Hence, the transformation of the urea in the urine was correlative with the life and development of an organized vegetable ferment (van Thiegem, 1864).

Musculus tried and succeeded in collecting these globules and drying them at a moderate heat, with the purpose of keeping them in this state and reviving them in water when required (Musculus, 1874a). The urine he used was already in full alkaline fermentation. The filtrate passed very fast until the globules retained in the pores of the paper began to obstruct the operation. He washed the filter with distilled water until the passing liquid was no more alkaline and then dried the paper at 35º to 40 ºC. He realized that this paper was a very sensitive reagent for urea; it was enough to submerge it into a dilute solution of urea for 15 to 20 minutes to charge the liquor with ammonium carbonate, a chemical easy to detect. Although he did not know yet the shell life of the paper, he found that it kept its activity during at least one month. A microscopic examination showed the presence of many globules and that the monads were seen as moving points as soon as they came in contact with water. The best manner to use the paper was to color it with turmeric and recommended keeping it in a corked bottle, far from humidity (Musculus, 1874a). Also, the liquid to be tested should be neutralized before checking it for urea. If it contained an alkaline carbonate, it had to be treated first with an acid to decompose the bicarbonates present. These salts could induce to error because they did not color brown the turmeric paper immediately, but after some time. Neutral salts did not affect ferment; they seemed, on the contrary, to activate it. The paper could also be used for the quantitative analysis of urea: to do so the paper was moistened with a little of tournesol, then with diluted sulfuric acid so as to generate a red color, and then left alone for five to six hours at 25 to 30 ºC to complete the fermentation. The ammonia formed was then titrated with a dosed acid. Musculus added that the ferment did not act on albuminous matter, uric acid, and other bodies such as xanthine, hypoxanthine, etc. (Musculus, 1874a).

A microscopic examination of the dry ferment showed no cellules like those seen in the urine deposits, which were assumed to transform the urea into ammonium carbonate (Musculus, 1876). Anyhow, it was accepted that the mucus of the bladder also acted as ferment, as best shown by the solubility of the ferment in water. Musculus demonstrated this property by filtering a mixture of pulverized mucus with water. Urea was added and the total put in a stove at 35 to

40 ºC; after one hour, a substantial amount of ammonium carbonate had precipitated and after twelve hours the fermentation was completed. The soluble matter behaved like mucin, it was precipitated by alcohol and acetic acid. The dry precipitate appeared as a bright brown amorphous matter, soluble in water, and, particularly, in aqueous sodium chloride. The ferment was strongly attacked by acids such as HCl, sulfuric, acetic, tartaric, salicylic, etc. Alkalis hindered the ferment but did not destroy it; heat destroyed it easily, already at 80 ºC. These results indicated that the properties of the ferment of urea were different from those of organized ferments; actually they were similar to those of soluble ferments, such as diastase, saliva, and pancreatic juice, although saliva and pancreatic juice were less sensitive to the action of acids (Musculus, 1876).

In 1885, Arthur Lea cultivated *Micrococcus Ureæ* in large scale and confirmed the results of Musculus (Lea, 1885). In 1890 Pierre Miquel also confirmed the results of Musculus and reported that he had found in nature a large variety of microorganisms gifted with the same property. He believed that all this microbes of the ammoniacal fermentation secreted a soluble ferment (urease) when grown in a culture devoid of urea, so that the destruction of the urea at room temperature took place by the intermediate action of this soluble ferment (Miquel, 1890).

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