100 Years "Schlüssel-Schloss-Prinzip": What Made Emil Fischer Use this Analogy?**

Frieder W. Lichtenthaler*

Emil Fischer's famous lock-and-key analogy (Schlüssel-Schloss-Prinzip) for the specifity of enzyme action has provided successive generations of scientists with a mental picture of molecular recognition processes, and thus has shaped to a marked degree the development not only of organic chemistry, but, through its extension to basic live processes, that of biology and medicine as well. The hundredth anniversary of the first use of this most fertile metaphor provides a welcome opportunity not only for highlighting its paramount importance, but for gaining an understanding and appreciation of the creative processes involved, of the constructive reasoning and the thought patterns underlying the fundamental insight. Accordingly, this account attempts to trace how Fischer was led to the lock-and-key analogy, based on the state of knowledge and the views prevailing at the time. It reveals that Fischer, who had a pronounced tendency against any sort of theoretical speculation, refrained from taking this metaphor any further, that is to the obvious extensions of what turns the key, and what kind of doors are then opened. Except for a small refinement -the differentiation of main key and special keys to account for the fact that some yeasts can ferment a larger number of hexoses than others—he rather expounded on the scope of the lock-and-key picture: "I am far from placing this hypothesis side by side to the established theories of our science, and readily admit, that it can only be thoroughly tested, when we are able to isolate the enzymes in a pure state and thus investigate their configuration." Others, most notably P. Ehrlich und F. Lillie, by introduction of the concept of stereocomplementarity into medicine and biology, induced the lock-and-key analogy to become something of a dogma for explaining principal life processes.

Ich halte Lehre und Studium der historischen Entwicklung der Wissenschaft für unentbehrlich.... Unsere Lehrbücher versagen darin.
Richard Willstätter[1]

Emil Fischer's famous lock-and-key analogy for the specifity of enzyme action has provided successive generations of scientists with their mental picture of molecular recognition processes, and, thus has shaped to a marked degree the development not only of organic chemistry, but, by extension to basic life processes, that of biology and medicine as well.

Fischer's seminal paper in which he first used the lock-and-key metaphor appeared in *Berichte der Deutschen Chemischen Gesellschaft* of 1894.[2] Thus, a century has passed away since and accordingly, this provides a unique opportunity to commemorate the 100th anniversary of this most fertile hypothesis—not only for historical purposes or for keeping pivotal facts from oblivion, but for gaining an understanding and appreciation of the creative processes involved, of the thought patterns underlying the fundamental insight, and the constructive reasoning that eventually led to it. A comprehension of these factors appears to be required to get a true measure of the magnitude and significance of Fischer's basic contribution.

Any attempt—after a 100 years—to trace what led Fischer to the lock-and-key analogy, must go back to the state of knowledge and the views pevailing at the time, that is around 1890, and to the scientific school from which Fischer emerged. In 1871, he had entered the University of Bonn, where he attended lectures by A. Kekulé and R. Clausius, yet, in the following year transferred to the University of Strassburg to study with Adolf Baeyer, earning his doctorate with him in 1874 at the age of 22. A year later, while working already independently in Baeyer's laboratory, he accidentally discovered phenylhydrazine[3] which was to become the key reagent for his exploration of the sugars, when, ten years later, he finally applied it to the then existing sugars.[4]

The research school of Adolf Baeyer (1835–1917[5]), from which Fischer emerged—first in Strassburg, and then for 40 years after 1875 at the University of Munich—was a major "forge" of talent. A group photograph[6] of 1878 (Fig. 1)

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Fig. 1. Photograph of the Baeyer group in early 1878 at the laboratory of the University of Munich (room for combustion analysis), with inscriptions from Fischer's hand [6].

attests to that almost literally: the unusually wide hood in the background is certainly more reminiscent of a forge than of a laboratory. In the center Adolf Baeyer, wearing a prominent hat; since several others also wear headgear, we may deduce that in the winter of 1878 the heating was deficient in that laboratory. To the right of Baeyer the 25-year-old Emil Fischer, in a peaked cap and strikingly self-confident three years after his Ph.D.; to the left Jacob Volhard (1834–1910), who was in charge of the analytical division in Baeyer's institute, and whose successor Fischer was to become in Munich a year later (1879), and at the University of Erlangen in 1882.

Fischer, at Munich, pursued several classical organic research topics: the phenylhydrazones of acetaldehyde, benzaldehyde, and furfural were unequivocally characterized and structurally

Frieder W. Lichtenthaler, born 1932 in Heidelberg, studied chemistry at the University of Heidelberg from 1952–1956 and received his doctorate there in 1959 under F. Cramer for research on enol phosphates. The following three years he spent as a postdoctoral fellow at the University of California, Berkeley, with Hermann O. L. Fischer—the only of Emil Fischer's sons who survived the first World War. He subsequently worked as an assistant at the Technische Hochschule Darmstadt, where he acquired his "Habilitation" in 1963, was appointed associate professor in 1968, and was promoted to full professor in 1972. His research activities center on the generation of enantiopure building blocks from sugars, their utilization in the synthesis of oligosaccharides and complex non-carbohydrate natural products, the computer simulation of chemical and biological properties of sugars, and studies towards the utilization of carbohydrates as organic raw materials.

secured;[7] over a number of years (1876–1880) he did extensive investigations on rosaniline dyes with his cousin Otto Fischer (Fig. 1, far left, sitting),[8] and in 1881 he started work on purines, investigating the structure of caffeine,[9] research that eventually led to his classification of the purines. In 1882 at the age of 30, he moved from Munich to Erlangen, accepting the chair of chemistry at that university, and there he was intensely occupied with the conversion of phenylhydrazine into N-heterocycles,[10] which led to the Fischer indole synthesis.[11] It was in Erlangen in 1884, that is after having left Baeyer's sphere of influence for over two years, that he began his studies on sugars, by reaction of those that were known at the time (glucose, fructose, galactose, maltose, sucrose, and lactose) with phenylhydrazine.[3, 12] The hydrazones and osazones obtained thereby have not only rendered invaluable service for the identification and isolation of the then existing sugars, but also have been instrumental in the preparation of new ones. In 1888 Fischer had moved to the University of Würzburg by then—he discovered a new hexose in this way:[13] gentle oxidation of mannitol with nitric acid gave a mixture which could not be characterized as such, but on exposure to phenylhydrazine afforded a crystalline phenylhydrazone, isomeric with the one generated from glucose (Fig. 2). By the acid hydrolysis of this product, an as yet unknown hexose was obtained, which he named mannose.

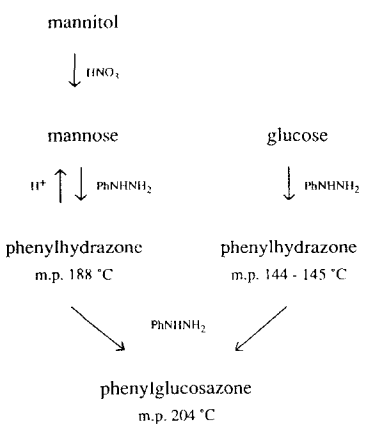


Fig. 2. Synthesis of mannose from mannitol in 1888 [13] soon thereafter to be discovered in nature [16].

It is in this stage of Fischer's purely chemical-synthetic studies of sugars, in the first of four papers with Hirschberger on mannose,[13–15] that we find, rather unpreparedly, the lapidary sentence:[13] "Mannose is avidly fermented by beer yeast at room temperature even in strongly diluted aqueous solution." For Fischer, however, it was not a peculiar, remote thing to incorporate yeast into his investigations, since he had developed a curiosity in yeast fermentation as a youth already – sparked by the entrepreneurship of his father. Laurens Fischer was a successful businessman, and in 1870—Emil was 18 by then—he invested a large amount of money in the foundation of a beer brewery in Dortmund, an enterprise that was later turned into a stock company, the "Dortmunder Aktienbrauerei" of today; Laurens Fischer was chairman of the board for several decades.

In the winter of 1876/1877, Emil Fischer spent three months at the University of Strassburg – on Baeyer's suggestion obviously, since he held the position of an assistant at his Munich institute – to acquire more expertise in quantitative analysis in the laboratory of Prof. Rose. A delightful passage of Fischer's autobiography elaborates on his encounter with yeast there:[17]

"During the winter semester of 1876/1877 I again was in Strassburg, and there, through Dr. Albert Fitz, a wealthy winegrower from the Palatinate, was introduced to the book of Pasteur "Etudes sur la bière", that had just appeared. Therein, this ingenious researcher had laid down his experiences on the contamination of beer-yeast by other microorganisms and their harmful effect on the quality of the beer. When I reported on this to my father, he urged me to study this subject very thoroughly, which I gladly did since it interested me scientifically. A fine microscope was immediately acquired, and with the help of Dr. Fitz and the botanist Prof. de Bary I made studies on moulds, sprouts, and yeasts, from which I later profited immensely in my investigations of the sugars. For the time being, however, I had to make practical use of this new knowledge.

Accordingly, I moved with my microscope to Dortmund for several weeks, to train the workers of the brewery in the new identification procedures. Presumably, I was the first chemist in Germany who attempted this, and have to admit, that I was met with substantial distrust by the men. They made every effort to lead me astray with false statements on the origin and the quality of the yeast under examination. They became more serious-minded though after I could find out, with the help of the microscope, those yeast types that were spoiled. Yet, I did not succeed in instructing any of the men in the correct use of the microscope."

Through these activities, Fischer obviously had developed a keen interest in the subject, because he remarks: "The chemistry of yeasts interested me so highly, that I certainly would have done own research in this field had I stayed longer in Strassburg."[17]

Seen in this context, it was a quite obvious move for Fischer (Fig. 3) to test whether the newly prepared hexose, of which the

Fig. 3. Emil Fischer (1852–1919) in 1889 [18].

set of reactions summarized in Figure 2 had shown it to be the 2-epimer of glucose, would also be fermented by yeast. Similarly, when racemic sugars became available by his investigations of the formose reaction, it became standard practice to expose them to "ordinary beer yeast" for evaluation of their fermentability. Thus, besides proving that D-mannose indeed formed ethanol on yeast fermentation (Fig. 4),[15] it was estab-

D-mannose	⟶	CO_2 + ethanol	[13,15]
D,L-fructose	⟶	L-fructose	[19,20]
D,L-mannose	⟶	L-mannose	[20]
D,L-glucose	⟶	L-glucose	[21]
D,L-galactose	⟶	L-galactose	[22]
D-gulose	—X⟶		[23]
L-gulose	—X⟶		[24]
D-*manno*-heptose	—X⟶		[25]
D-*gluco*-heptose	—X⟶		[26]

Fig. 4. Fischer's early observations (1888–1892) on the fermentation of sugars with beer yeast [27].

lished that in the case of racemic glucose, mannose, galactose, and fructose, only the D component was devored, allowing the isolation and characterization (as hydrazones and osazones) of the corresponding L-sugars.

The study of the fermentation of these sugars was a by-product of his synthetic work, until, at the end of 1891, he had proceeded so far as to have reached the goal: the relative configurations of the sugars had been unravelled. This proof not only put carbohydrate chemistry on a rational basis but—more importantly for that time—provided unequivocal proof for the validity of the Le Bel–van't Hoff theory of stereoisomerism.[28] It became the basis for the sugar family tree (Fig. 5) as it is—100 years later—in our textbooks today.

The completion of this most remarkable, classic piece of work, accomplished by ingeniously planned organic syntheses and brilliant mathematical reasoning had brought order and clarity to the field. To Fischer it was the incentive for now venturing into topics of much higher complexity, that is into biological phenomena:[29]

> "After the classification of the monosaccharides has essentially been concluded by the establishment of their configurational formulae, it is now obvious to utilize the experiences, which have led to this goal, for the purposes of biological research."

Following the early observations on the fermentability of sugars (Fig. 4), which had more the character of orientative tests than carefully planned experiments, Fischer apparently realized that the ordinary brewer's yeast ("gewöhnliche Brauereihefe") he had been using was not pure, and that therefore the results could be misleading. So he made, together with Hans Thierfelder,[30] a comparative study of natural and synthetic monosaccharides with respect to their behavior towards various families of yeast. This resulted in a landmark paper in the "*Berichte*" of 1894.[31] Fischer, thereby, was in the fortunate position, that his sugar studies had left him with a rich stock of rare sugars—nowhere else in chemistry was such a fine inventory of isomers available—yet some of these were only accessible in small amounts.[31]

> "Since the preparation of the artifical sugars is in part quite laborious and the experiments had to be varied frequently we have used a small fermentation tube of the form shown below to save material" (Fig. 6).

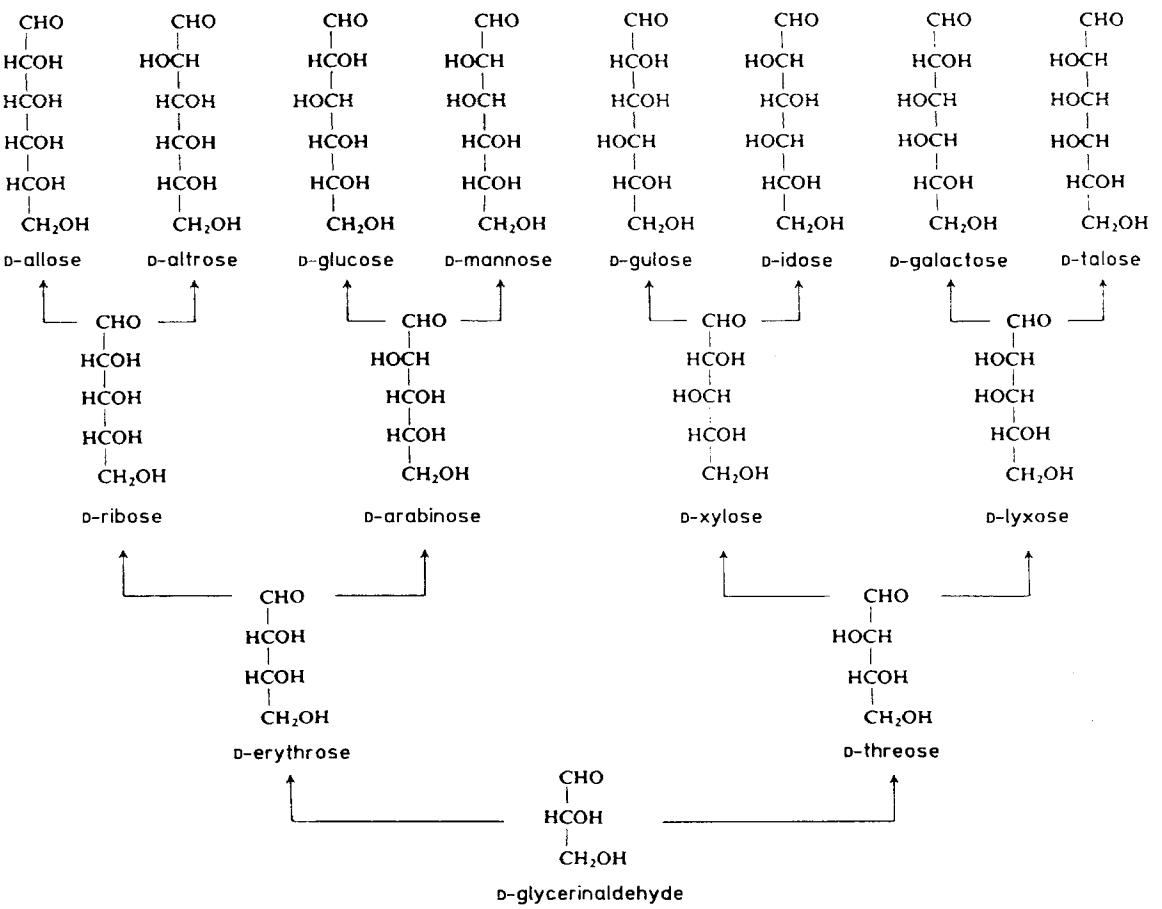


Fig. 5. The sugar family tree of D-aldoses.

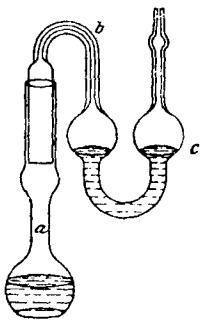


Fig. 6. Semimicro scale assay for the fermentation of sugars by yeasts [31] in original size. a = Fermentation flask, b = S-trap for CO_2 generated, c = aqueous $Ba(OH)_2$. Example: 70 mg sugar in 0.35 mL H_2O, 0.35 mL aqueous yeast abstract; sterilization, addition of 13 mg of a pure yeast species; 3–10 d at 24–28 °C.

This microscale fermentation assay was quite elaborate for the time, allowing one to work with 70 mg of sugar; the bulb holding the sample has a volume of about 1 mL only. It is interesting to perceive today Fischer's keen sense for meticulous observations:[31]

"In all cases, even when the sugar is not fermented, a small amount of carbon dioxide evolves, which covers the surface of the barium hydroxide with a thin layer of carbonate. Since this phenomenon occurs even when no sugar has been added to the solution, it is obviously caused by the small amount of carbohydrate present in the yeast itself or the extract.

The situation is quite different, when the material is readily fermentable: the barium hydroxide is not only becoming strongly turbid, but is neutralized.

Intermediate cases are these, where material has to be brought into a fermentable state first, as with the glucosides; fermentation proceeds slowly ...., yet here too, the amount of carbon dioxide developed is always large enough, that one cannot be in doubt about the real occurrence of fermentation."

Observations of this sort led to the data collected in Scheme 1, a reproduction from the first[31] of four papers to appear on the subject in the second half of 1894:[2, 29, 31, 32] *d*-mannose, *d*-fructose, and, to a lesser extent *d*-galactose resemble *d*-glucose, as does sucrose ("Rohrzucker") and maltose, whilst one of the yeasts ("Milchzuckerhefe") fermented sucrose and lactose ("Milchzucker"), yet left maltose untouched. All of the yeasts were indifferent towards a variety of synthetic sugars.

Fischer seemed to be particularly intrigued by the fact, that *d*-talose, the 2-epimer of galactose, was not fermented (Fig. 7), since he notes:[31]

"*d*-Talose relates configurationally to *d*-galactose as does *d*-mannose to *d*-glucose. As *d*-galactose already ferments less readily than the two others, any further small change in geometry eliminates fermentability altogether."

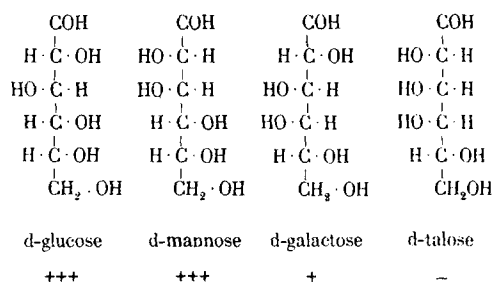


Fig. 7. Fermentability of hexoses by yeast [31].

Considerations such as these led to the cautious rationalization, "that the yeast cells with their asymmetrically formed agent are capable of attacking only those sugars of which the geometrical form does not differ too widely from that of *d*-glucose."[31]

On extending this inquiry to natural and artificial glucosides, Fischer found that these materials arrange themselves into distinct groups with respect to their behavior towards air-dried yeast extract and the aqueous extract of bitter almonds ("invertin" and "emulsin", respectively). Although both were later shown to be crude mixtures of enzymes, the former only cleaved α-glucosidic linkages, whereas the other, just as specifically, only hydrolyzed β-glucosides (Table 1).

The second of these four 1894 papers on yeast fermentation carries the unassuming title "influence of the configuration on

	d-Mannose	*d*-Fructose	*d*-Galactose	*d*-Talose	*l*-Mannose	*l*-Gulose	Sorbose	*l*-Arabinose	Rhamnose	α-Glucoheptose	α-Glucooctose	Rohrzucker	Maltose	Milchzucker
S. Pastorianus I	†††	†††	†††	–	–	–	–	–	–	–	–	†††	†††	–
S. Pastorianus II	†††	†††	††	–	–		–	–	–	–	–	†††	†††	–
S. Pastorianus III	†††	†††	†††	–	–		–	–	–	–	–	†††	†††	–
S. cerevisiae I	†††	†††	†††	–	–		–	–	–	–	–	†††	†††	–
S. ellipsoideus I	†††	†††	††	–	–		–	–	–	–	–	†††	†††	–
S. ellipsoideus II	†††	†††	†	–	–		–	–	–	–	–	†††	†††	–
S. Marxianus	†††	†††	†††	–	–		–	–	–	–	–	†††	†††	–
S. membranaefaciens	–	–	–	–	–		–	–	–	–	–	–	–	–
Brauereihefe	†††	†††	†††	–	–	–	–	–	–	–	–	†††	†††	–
Brennereihefe	†††	†††	†	–	–	–	–	–	–	–	–	†††	†††	–
S. productivus	†††	†††	–	–	–	–	–	–	–		–	†	†††	–
Milchzuckerhefe	††	†††	†	–	–		–	–	–	–	–	†††	–	†††

††† bedeutet keine Reduktion der Fehlingschen Lösung nach 8 Tagen, also vollständige Vergärung.
†† „ eine ganz schwache Reduktion nach 8 Tagen, also fast vollständige Vergärung.
† „ deutliche Reduktion nach 8 Tagen, aber unzweifelhafte Gärung.
– „ keine Gärung.

Scheme 1. Behavior of sugars towards pure yeasts (from ref. [31]). ††† denotes no reduction of the Fehling's solution after 8 days, thus complete fermentation. †† denotes very weak reduction after 8 days, thus almost complete fermentation. † denotes significant reduction after 8 days, but undoubted fermentation. – denotes no fermentation.

Table 1. Fermentability of glycosides [2, 32].

Glycoside	Yeast enzyme (invertin) [a]	Emulsin [b]
methyl-α-D-glucoside	+	–
ethyl-α-D-glucoside	+	–
saccharose	+	–
maltose	+	–
methyl-α-L-glucoside	–	–
methyl-α-D-mannoside	–	–
methyl-α-D-galactoside	–	–
ethyl-α-D-galactoside	–	–
methyl-β-D-glucoside	–	+
phenyl-β-D-glucoside	–	+
methyl-β-D-galactoside	–	+
lactose	–	+

[a] Aqueous extract of air-dried beer yeast (*Saccharomyces cerevisiae*, type Frohberg). [b] Aqueous extract of bitter almonds.

545. Emil Fischer: Einfluss der Configuration auf die Wirkung der Enzyme.

[Aus dem I. Berliner Universitäts-Laboratorium.]
(Vorgetragen in der Sitzung vom Verfasser.)

Das verschiedene Verhalten der stereoisomeren Hexosen gegen Hefe hat Thierfelder und mich zu der Hypothese geführt, dass die activen chemischen Agentien der Hefezelle nur in diejenigen Zucker eingreifen können, mit denen sie eine verwandte Configuration besitzen[1]).

Diese stereochemische Auffassung des Gährprocesses musste an Wahrscheinlichkeit gewinnen, wenn es möglich war, ähnliche Verschiedenheiten auch bei den vom Organismus abtrennbaren Fermenten, den sogenannten Enzymen, festzustellen.

Das ist mir nun in unzweideutiger Weise zunächst für zwei glucosidspaltende Enzyme, das Invertin und Emulsin, gelungen. Das Mittel dazu boten die künstlichen Glucoside, welche nach dem von mir aufgefundenen Verfahren aus den verschiedenen Zuckern und den Alkoholen in grosser Zahl bereitet werden können[1]). Zum Vergleich wurden aber auch mehrere natürliche Producte der aromatischen Reihe und ebenso einige Polysaccharide, welche ich als die Glucoside der Zucker selbst betrachte, in den Kreis der Untersuchung gezogen. Das Ergebniss derselben lässt sich in den Satz zusammenfassen, dass die Wirkung der beiden Enzyme in auffallender Weise von der Configuration des Glucosidmoleküls abhängig ist.

Versuche mit Invertin.

Das Enzym lässt sich bekanntlich aus der Bierhefe mit Wasser auslaugen und soll aus der Lösung durch Alkohol unverändert gefällt werden. Aus den später angeführten Gründen habe ich auf die Isolirung desselben verzichtet. Die nachfolgenden Versuche sind vielmehr direct mit einer klar filtrirten Lösung angestellt, welche durch 15stündige Digestion von 1 Theil lufttrockener Bierhefe (Saccharomyces cerevisiae, Typus Frohberg, Reincultur) mit 15 Theilen Wasser bei 30—35° bereitet war.

[1]) Diese Berichte **27**, 2036.

Fig. 8. Title page of the second [2] of Fischer's four landmark papers in 1894 on the influence of the configuration on the action of enzymes.

the action of enzymes" (Fig. 8), reporting some of these results in a very sober, purely scientific diction.[2] Towards the end—as usually found in the majority of Fischer's publications—he gives clear indications on what he is to do next: incorporation of other enzymes into the study, such as glucase, ptyalin, myrosin, and the ferments of pancreas, and their extension to the rare oligosaccharides, as for example isomaltose, turanose, melibiose and melitriose (Fig. 9). Then, very much towards the end of this paper, in the coda quasi, in musical terms, Fischer tries to sum up and rationalize the observations available. The resulting section contains the crucial metaphor:

> "The restricted action of the enzymes on glucosides may therefore be explained by the assumption that only in the case of similar geometrical structure can the molecules so closely approach each other as to initiate a chemical action. To use a picture I would like to say that enzyme and glucoside have to fit together like lock and key in order to exert a chemical effect on each other. The finding that the activity of enzymes is limited by molecular geometry to so marked a degree, should be of some use in physiological research. Still more important though appears to me the proof, that the previously assumed difference between the chemical activity of a cell and the mode of action of chemical reagents is, factually, non-existent."[2]

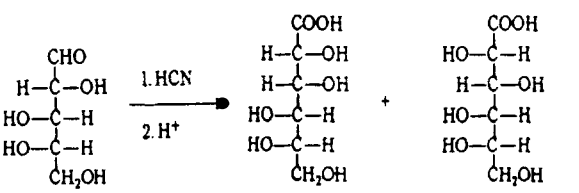
Emil Fischer left many contributions of great brilliance in the annals of science:

- the unravelment of the sugar configurations as the classical piece of exact mathematical reasoning in any experimental science[12]
- the classification of purines[33] and the synthesis of the first nucleosides[34]
- the laying of the chemical and biological foundation of protein chemistry by his extensive work on amino acids, peptides, and proteins,[35]
- the first unifying concept on the structures of the complex natural products depsides and tannins.[36]

But here, an analogy, a metaphor, almost casually thrown in at the end of a paper, develops a life of its own, to become one of the most frequently invoked concepts of the past 100 years. Apparently, the lock-and-key analogy met a conceptual need of the time, for within a very short period it formed an interface between chemistry, biology, and medicine—very much to the surprise of Fischer himself, since he did not expound on it. Particularly, he refrained from going any further—at least in print—although I am sure, in his thoughts, he must have taken this picture to the obvious questions, what turns the key, and what kind of doors are then opened. The only extension to be found in print, in an extensive 43-page review on his investigations on sugars of 1894, is a small refinement:[29]

> "The action of enzymes involves a far-reaching chemical process which takes place readily or not at all. Here, apparently, the geometrical structure exerts such a profound influence on the playing of the chemical affinities, that it appeared legitimate to me to compare the interacting molecules with key and lock.
> If one wants to do justice to the fact, that some yeasts can ferment a larger number of hexoses than others the picture may be completed by the differentiation of main key and special keys."

It was obvious to apply the concept of lock-and-key complementarity to the question of asymmetric synthesis in plants, most notably to the process of assimilation. Along the way of the gradually unfolding interrelationships between the sugars, Fischer, in 1889, had made another key discovery that was to have major bearing on biological questions. He uncovered the phenomenon of asymmetric synthesis:[37] the cyanohydrin extension of natural L-arabinose does not only give L-mannonic acid on hydrolysis, as Kiliani had previously shown,[38] but a second product, the 2-epimeric L-gluconic acid, as evidenced by their distinctly different, well-crystallizing phenylhydrazides:



L-arabinose L-mannonic acid L-gluconic acid

2992

von Leuchtbacterien beweisen will, ist wohl geeignet, ernste Bedenken gegen die Zuverlässigkeit des Resultats zu erwecken. In der That ist denn auch Beyerinck's Ansicht von Schnurmans Stekhoven [1]) sehr bestimmt bestritten worden. Letzterer kommt vielmehr zu dem Schluss, dass das Enzym der Kefirhefe zwar den Rohrzucker und die Raffinose, aber nicht den Milchzucker zerlege. Die Frage, wer hier Recht hat, lässt sich leicht entscheiden, wenn man den Versuch mit reiner Kefirhefe so anstellt, wie er oben für die Kefirkörner beschrieben ist. Ich werde denselben ausführen, so bald mir eine genügende Menge der Hefe zur Verfügung steht.

Ferner beabsichtige ich, noch einige verwandte Enzyme, wie die Glucase, das Ptyalin, Myrosin und die Pancreasfermente zum Vergleiche heranzuziehen und die Versuche auch auf die selteneren Polysaccharide, wie Isomaltose, Turanose, Melibiose, Melitriose, Trehalose, Melezitose, die künstlichen Dextrine etc. zu übertragen. Zweifellos werden sich dann noch mehr solcher Gegensätze zeigen, wie sie zwischen dem Invertin und Emulsin jetzt festgestellt sind.

Aber schon genügen die Beobachtungen, um principiell zu beweisen, dass die Enzyme bezüglich der Configuration ihrer Angriffsobjecte ebenso wählerisch sind, wie die Hefe und andere Mikroorganismen. Die Analogie beider Phänomene erscheint in diesem Punkte so vollkommen, dass man für sie die gleiche Ursache annehmen darf, und damit kehre ich zu der vorher erwähnten Hypothese von Thierfelder und mir zurück. Invertin und Emulsin haben bekanntlich manche Aehnlichkeit mit den Proteïnstoffen und besitzen wie jene unzweifelhaft ein asymmetrisch gebautes Molekül. Ihre beschränkte Wirkung auf die Glucoside liesse sich also auch durch die Annahme erklären, dass nur bei ähnlichem geometrischem Bau diejenige Annäherung der Moleküle stattfinden kann, welche zur Auslösung des chemischen Vorganges erforderlich ist. Um ein Bild zu gebrauchen, will ich sagen, dass Enzym und Glucosid wie Schloss und Schlüssel zu einander passen müssen, um eine chemische Wirkung auf einander ausüben zu können. Diese Vorstellung hat jedenfalls an Wahrscheinlichkeit und an Werth für die stereochemische Forschung gewonnen, nachdem die Erscheinung selbst aus dem biologischen auf das rein chemische Gebiet verlegt ist. Sie bildet eine Erweiterung der Theorie der Asymmetrie, ohne aber eine directe Consequenz derselben zu sein; denn die Ueberzeugung, dass der geometrische Bau des Moleküls selbst bei Spiegelbildformen einen so grossen Einfluss auf das Spiel der chemischen Affinitäten ausübe, konnte meiner Ansicht nach nur durch neue thatsächliche Beobachtungen gewonnen werden. Die bis-

[1]) Koch's Jahresbericht über Gährungsorganismen 1891, 136.

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herige Erfahrung, dass die aus zwei asymmetrischen Componenten gebildeten Salze sich durch Löslichkeit und Schmelzpunkt unterscheiden können, genügte dafür sicher nicht. Dass man die zunächst nur für die complicirten Enzyme festgestellte Thatsache bald auch bei einfacheren asymmetrischen Agentien finden wird, bezweifle ich ebensowenig wie die Brauchbarkeit der Enzyme für die Ermittlung der Configuration asymmetrischer Substanzen.

Die Erfahrung, dass die Wirksamkeit der Enzyme in so hohem Grade durch die moleculare Geometrie beschränkt ist, dürfte auch der physiologischen Forschung einigen Nutzen bringen. Noch wichtiger für dieselbe aber scheint mir der Nachweis zu sein, dass der früher vielfach angenommene Unterschied zwischen der chemischen Thätigkeit der lebenden Zelle und der Wirkung der chemischen Agentien in Bezug auf moleculare Asymmetrie thatsächlich nicht besteht. Dadurch wird insbesondere die von Berzelius, Liebig u. A. so häufig betonte Analogie der »lebenden und leblosen Fermente« in einem nicht unwesentlichen Punkte wieder hergestellt. —

Bei der Ausführung obiger Versuche habe ich mich der eifrigen und geschickten Beihülfe des Hrn. Dr. Paul Rehländer erfreut. Ferner bin ich für die Ueberlassung von reingezüchteten Hefen den HH. Dr. H. Thierfelder, Prof. M. Delbrück und Dr. O. Reinke zu grossem Danke verpflichtet.

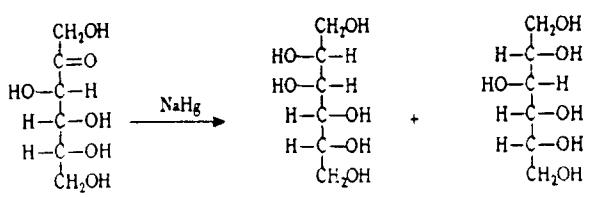
Fig. 9. Final section of Fischer's seminal 1894 paper [2] in which the lock-and-key metaphor was first used.

As it turned out, this is the first example of an asymmetric synthesis recorded in the literature, on which Fischer commented in the following way:[39]

> "The simultaneous formation of the two stereoisomeric products on the addition of hydrogen cyanide to aldehydes, which was observed here for the first time, is quite remarkable in theory as well as in practice."

This first example of an asymmetric synthesis was soon to be followed by a second case, since sodium amalgam reduction of D-fructose gave rise to two stereoisomeric products, namely D-mannitol and D-sorbitol.[40] Fischer clearly realized the basic importance of this result:

> "The reduction of fructose is the second reaction in the sugar group, which generates two stereoisomeric products due to the formation of an asymmetric carbon atom. The same phenomenon will undoubtedly be observed much more frequently in the future, and most probably will be generally found with all compounds that are asymmetric a priori."



D-fructose D-mannitol D-sorbitol

Four years later, in one of these 1894 papers,[29] the biological significance of these sober chemical findings had been fully realized and applied to assimilation by invoking the lock-and-key concept:

> "It seems to me that this concept offers a simple solution for the enigma of natural asymmetric synthesis. According to the plant physiologists, carbohydrate formation takes place in the chlorophyll granule, which itself consists entirely of optically active substances. I can imagine that the formation of carbohydrates is preceeded by the generation of a compound

of carbonic acid or formaldehyde with those substances; and that then, due to the already existing asymmetry of the entire molecule, the condensation to the sugars takes place in an asymmetric fashion too.
Their asymmetry can thus be readily explained by the nature of the material from which they were produced. Of course, they also provide the material for new chlorophyll granules which, in turn, produce active sugar. In this manner, the optical activity propagates from molecule to molecule, as life itself does from cell to cell. Hence, it is not necessary to attribute the formation of optically active substances in the plant to asymmetric forces outside the organism, as Pasteur had supposed. The origin rather lies in the structure of the chlorophyll granule that generates the sugar, and with this conception the difference between natural and artificial synthesis is completely eliminated."

In a lecture of 1894 on "The chemistry of carbohydrates and their importance for physiology" he again advocated this view in more general form:[41]

"Whoever wants to conclusively elucidate the process of assimilation, will have to tackle the more special question, why the plant exclusively generates optically active sugar whilst chemical synthesis leads to the inactive products. This contrast appeared so fundamental to Pasteur, who created the precept of molecular asymmetry, that he considered the generation of active substances to be a privilege of the organism. The progress of science has deprived the highly respected lifeforce of even this last hiding-place; for we are now in a position to artificially prepare such active substances without any assistance from a living organism."

With these words, Fischer had clearly repudiated the accepted view of the time—asserted by Pasteur—that fermentation is inextricably tied to living cells, wherein a "vis vitalis" was supposedly operating. Eduard Buchner is usually credited to have demonstrated in 1897[42] that fermentation can occur outside living cells, thus unequivocally refuting Pasteur's view. The above passage of Fischer in 1894 proves, that he had arrived at this conclusion already three years earlier.

In 1894, when Fischer first used the lock-and-key analogy to illustrate enzyme specifity, he was 42. He lived for another 25 years, during which time he published the imposing number of over 400 further papers.[43] However, he referred to the lock-and-key concept only in another three: in his Nobel lecture in 1902, rather incidentally,[44] in his Faraday lecture at the University of London in 1907,[45] also quite cursorily, and, at the end of an extensive, 28-page review of 1898, with the momentous title "Significance of Stereochemistry for Physiology." Therein,[46] Fischer apparently felt that he had to state the scope of the analogy he had proposed, because others were taking it too far:

"The reasons for these phenomena are in all probability to be found in the asymmetric structure of the enzyme molecule. Although one does not know these substances in a pure state, their similarity with proteins is so close and their generation from these so probable, that they have undoubtedly to be considered as optically active, and, hence, asymmetric molecular forms.
This had led to the hypothesis, that there must be a similarity in the molecular configuration between the enzymes and their object of attack, if reaction is to take place. To make this thought more perspicuous, I have used the picture of lock and key.
I am far from placing this hypothesis side by side to the established theories of our science, and readily admit, that it can only be thoroughly tested, when we are able to isolate the enzymes in a pure state and thus investigate their configuration."[46]

Paul Ehrlich, for example, from 1897 on, introduced the lock-and-key complementarity into the then young discipline of immunology through his so-called "side chain theory of immunity", as illustrated in a publication from 1900[47] (Fig. 10): each cell possesses a number of side chains, which bind toxins in a lock-and-key type manner. The binding of such toxins causes the overproliferation of that particular side chain some of which are set free from the cells as antibodies. In the case of diseases that leave immunity there are so many free "side chains" (antibodies) in the blood that appreciable fixations at the cell cannot occur.

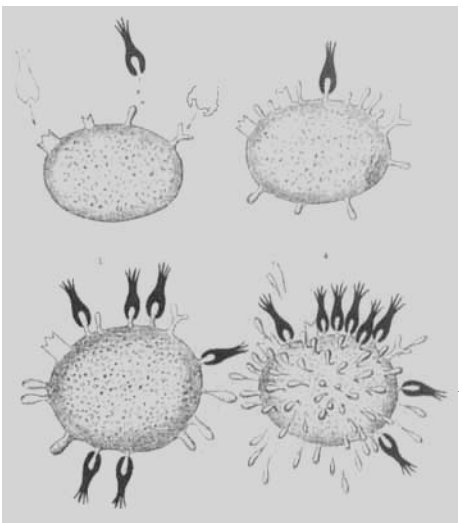


Fig. 10. Ehrlich's side chain theory of immunity as illustrated in 1900 [47].

The lock-and-key complementarity also gained headway in embryology, particularly from 1914 on, when Lillie, at the University of Chicago, invoked it to describe recognition between sperm and cells.[48] He crystallized his ideas on the interaction of the components involved into an explicit lock-and-key diagram (Fig. 11), a dangerously elaborate concept in view of the few secure experimental data available then.

Accordingly, Ehrlich had brought stereocomplementarity from the realm of chemical reactions in solution to reactions on the cell surface, whilst Lillie and others[49] extended it to cell–cell interactions. So, the first two decades following Fischer's use of the lock-and-key analogy saw a rather free, uncontrolled proliferation of the concept from chemistry into medicine and biology—and, along the way, its use became more and more speculative.

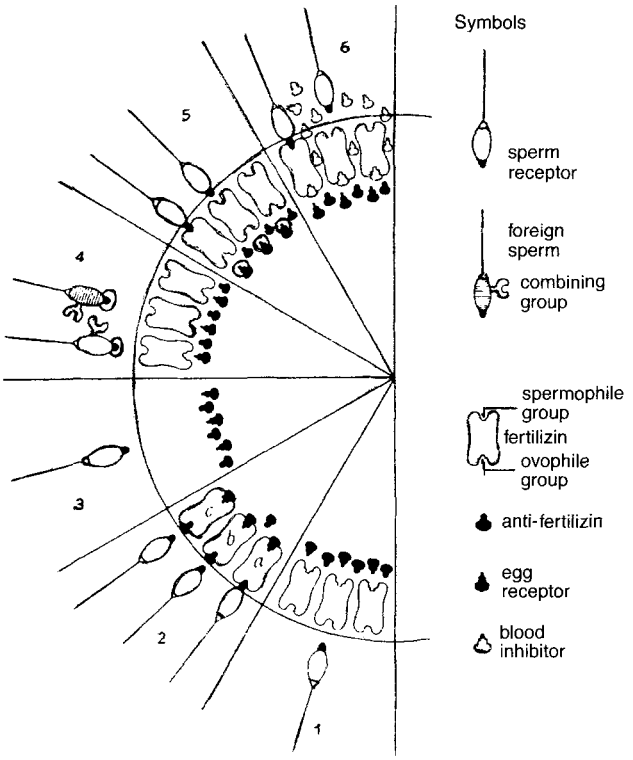


Fig. 11. Lillie's theory of fertilization as diagrammed in 1914 [48]: segment 1 shows the situation before fertilization; in segment 2, the sperm receptor binds to the spermophile group of "fertilizin", activating the ovophile group to bind to the egg receptor. Molecules of antifertilizin combine competitively with this site on other fertilizin molecules to forbid the binding of other sperm. The other segments refer to experiments not relevant to the discussion here.

The passage of time corrects many a distortion of perspective. Both theories survived only insofar as they allowed recognition of the complex relationship in very general terms, and hence, had a favorable influence on the concretization of research activities. Wherever they were used for too detailed analyses, they failed, obviously because the ground of scientifically established facts was left too far behind in the quest to explain phenomena much too complex as to yield to rationalization or comprehension at the time.

Unlike these theories from Ehrlich, Lillie, and others along similar veins,[49] Fischer's lock-and-key analogy still stands in the annals of science—a 100 years later—as a most fertile concept. Maybe, because it was unspecified in its details, thus leaving ample room for the imagination of chemists, biologists, and medical researchers alike.

Fischer had an unfailing intuitive perception for identifying important areas of research in organic chemistry and brought unsurpassed creativity to the conception of experiments and their skilful execution. The most striking feature of Fischer's scientific personality may be found in his pronounced tendency against any sort of theoretical speculation. Two instances may document this attitude further, one concerning the Walden inversion, to which Fischer had contributed[50] and which was controversially discussed around 1912. In a letter to T. W. Richards,[51] Fischer writes:

"I do not derive much pleasure from theoretical things. The occupation with the Walden inversion was rather a digression and recuperation from the extensive work on proteins. Moreover, so many limited heads have now jumped on this question, that the delight thereon is spoiled completely."

The second example refers to the question still open around 1914 on the ring sizes of the fructose and glucose portions of sucrose (formulations see Fig. 12), and how these sugars are

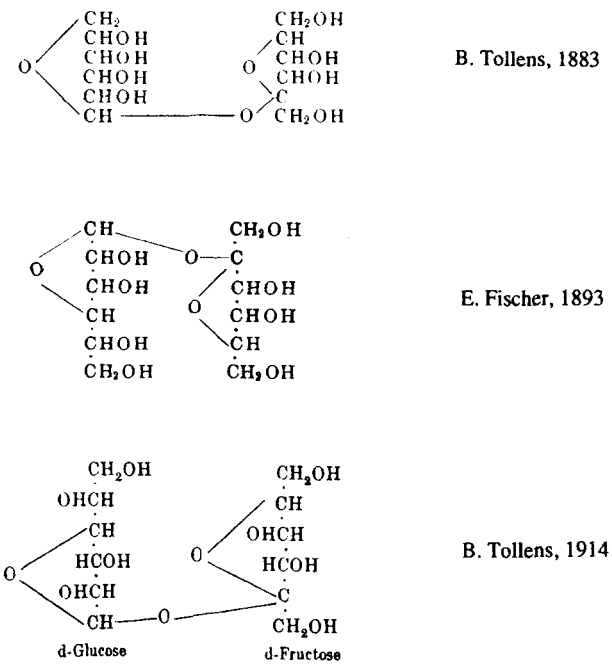


Fig. 12. Structural representations of sucrose by Tollens [53, 55] and Fischer [54].

linked. Fischer clearly states his position,[52] which may rightfully be extended to the lock-and-key picture:

"We know nothing definite on the mode, how the fructose residue is linked in cane sugar, thus leaving huge room for speculation. I, however, gladly renounce to use it."

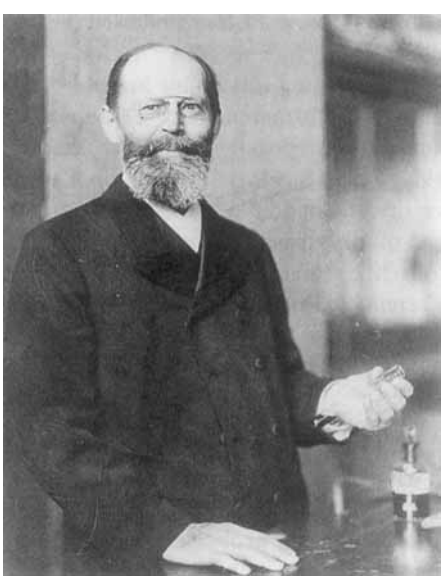


Fig. 13. Emil Fischer around the turn of the century.

In concluding this centennial tribute to one of the really great figures of our science (Figs. 13, 14) and to the lock-and-key concept with which he had a major influence on interrelating chemistry, biology, and medicine, I would like to cite a passage from his 1907 Faraday lecture at the University of London, entitled "Synthetic Chemistry in Relation to Biology", in which he clearly states his conviction to give in one's theoretical reasonings expression only to observed facts:[45]

> "The separation of organic chemistry from biology was necessary during the past century while experimental methods were being elaborated; now, that our science is provided with a powerful armory of analytical and synthetical weapons, chemists can once more renew the alliance both to its own honor and to the advantage of biology. The prospect of obtaining a clearer insight into the wondrous series of processes which constitute animal and vegetable life may well lead organic chemistry and biology to work with definite purpose to a common end.
> In order, as far as possible, to avoid mistakes in this difficult task and to shield ourselves from the disappointment which is the inevitable consequence of exaggerated hopes, we cannot do better than strive to imitate the great example of Faraday, who always, with rare acumen, directed his attention to actual phenomena without allowing himself to be influenced by preconceived opinion, and who in his theoretical conceptions gave expression only to observed facts."

This attitude with respect to the interpretation of experimental results applies to our science today as much as it did a 100 years ago. Especially in the field of molecular recognition which is in a very active phase of its development, we should comply with it most rigorously, as it gives us an unfailing measure of how far we should go with our interpretations today, and what we should leave for the next 100 years.

Fig. 14. Emil Fischer around 1910 in his laboratory at the University of Berlin [6].

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German version: *Angew. Chem.* **1994**, *106*, 2456

[1] R. Willstätter, *Aus meinem Leben*, Verlag Chemie, Weinheim, 1973, p. 324. "I consider the teaching and study of the historical development of science as essential. Our textbooks fail in this respect."
[2] E. Fischer, *Ber. Dtsch. Chem. Ges.* **1894**, *27*, 2985–2993.
[3] E. Fischer, *Ber. Dtsch. Chem. Ges.* **1875**, *8*, 589–599; G. B. Kauffman, R. P. Ciula, *J. Chem. Educ.* **1977**, *54*, 295.
[4] E. Fischer, *Ber. Dtsch. Chem. Ges.* **1884**, *17*, 579–584.
[5] R. Huisgen, *Angew. Chem.* **1986**, *98*, 297–311; *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 297–311.
[6] a) The original and numerous other photographs are contained in the Collection of Emil Fischer Papers, housed in the Bancroft Library at the University of California, Berkeley. This scientific bequest consists of 34 boxes of material: lab notes, manuscripts, drafts of his autobiography "Aus meinem Leben", and about 5000 letters, that reached Fischer during his 40-year scientific career; for example, 157 letters during 1889–1915 from his teacher A. von Baeyer, others from F. Beilstein (11), A. Hantsch (56), F. Haber (35), J. H. van't Hoff (31), A. W. Hofmann (5), A. von Kekulé (6), H. Kiliani (16), V. Meyer (46), W. Nernst (67), B. Tollens (20), P. Walden (13), O. Wallach (46), and R. Willstätter (33); Fischer's intense interaction with industry is documented by 181 letters from C. Duisberg (from 1895–1919), from Bayer (271), BASF (54), Boehringer Mannheim (116), Hoechst (50), and Merck (33). The papers eventually reached Berkeley through Fischer's son, Hermann Otto Laurens Fischer [6b] who carried this collection through all the stations of his unusually cosmopolitan career: Privatdozent in Berlin, Professor of Pharmaceutical Chemistry in Basel, Director of the Banting Institute, Toronto, and Professor of Biochemistry at the University of California Berkeley; b) F. W. Lichtenthaler, *Carbohydr. Res.* **1987**, *164*, 1–22.
[7] E. Fischer, *Ber. Dtsch. Chem. Ges.* **1876**, *9*, 880–891; *Justus Liebigs Ann. Chem.* **1878**, *190*, 184–188.
[8] E. Fischer, O. Fischer, *Ber. Dtsch. Chem. Ges.* **1876**, *9*, 891–900; *Justus Liebigs Ann. Chem.* **1878**, *194*, 242–303; *Ber. Dtsch. Chem. Ges.* **1880**, *13*, 2204–2207.
[9] E. Fischer, *Ber. Dtsch. Chem. Ges.* **1881**, *14*, 637–644, 1905–1915; *ibid.* **1882**, *15*, 29–33, 453–456; *Justus Liebigs Ann. Chem.* **1882**, *215*, 253–320.
[10] E. Fischer, F. Jourdan, *Ber. Dtsch. Chem. Ges.* **1883**, *16*, 2241–2245; E. Fischer, O. Hess, *ibid.* **1884**, *17*, 559–568; E. Fischer, *Justus Liebigs Ann. Chem.* **1886**, *236*, 116–126.
[11] P. A. Roussel, *J. Chem. Educ.* **1953**, *30*, 122–125; B. Robinson, *Chem. Rev.* **1963**, *63*, 373–401.
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[13] E. Fischer, J. Hirschberger, *Ber. Dtsch. Chem. Ges.* **1888**, *21*, 1805–1809.
[14] E. Fischer, J. Hirschberger, *Ber. Dtsch. Chem. Ges.* **1889**, *22*, 365–376, 1155–1156.
[15] E. Fischer, J. Hirschberger, *Ber. Dtsch. Chem. Ges.* **1889**, *22*, 3218–3224.
[16] R. Gans, W. E. Stone, B. Tollens, *Ber. Dtsch. Chem. Ges.* **1888**, *21*, 2148–2152; R. Gans, B. Tollens, *Justus Liebigs Ann. Chem.* **1888**, *249*, 245–257; R. Reiss, *Ber. Dtsch. Chem. Ges.* **1889**, *22*, 609–613.
[17] E. Fischer, *Aus meinem Leben*, Springer, Berlin, **1987**, pp. 18–20 and 70. (Reprint of the original 1922 version, edited by M. Bergmann, with an English commentary by B. Witkop).
[18] Picture from the congratulatory album of photographs presented to August von Kekulé on the occasion of his 60th birthday (September 6, 1889). The original document is in the collection of Kekulé papers, in the Institut für Organische Chemie, Technische Hochschule Darmstadt.
[19] E. Fischer, J. Tafel, *Ber. Dtsch. Chem. Ges.* **1889**, *22*, 97–101.
[20] E. Fischer, *Ber. Dtsch. Chem. Ges.* **1890**, *23*, 370–394.
[21] E. Fischer, *Ber. Dtsch. Chem. Ges.* **1890**, *23*, 2611–2624.
[22] E. Fischer, J. Hertz, *Ber. Dtsch. Chem. Ges.* **1892**, *25*, 1247–1261.
[23] E. Fischer, R. Stahel, *Ber. Dtsch. Chem. Ges.* **1891**, *24*, 528–539.
[24] E. Fischer, O. Piloty, *Ber. Dtsch. Chem. Ges.* **1891**, *24*, 521–527.
[25] E. Fischer, F. Passmore, *Ber. Dtsch. Chem. Ges.* **1890**, *23*, 2226–2239.

[26] E. Fischer, *Justus Liebigs Ann. Chem.* **1892**, *270*, 64–107.
[27] a) In the Figures 4 and 5 the affiliation of the individual sugars to the D- or L-series has already been made. It should be noted though, that Fischer only introduced the symbols *d* and *l* as late as 1890 [27b] (cf. Scheme 1 and Fig. 7), deriving their meaning from the sign of rotation. Rosanoff in 1906 [27c], and Wohl and Freudenberg in 1923 [27d] brought the use of the *d* and *l* symbols on a logical, genetic basis by selecting the enantiomeric glyceraldehydes as points of reference, such that any sugar belongs to the *d*-series, if it can be derived from *d*-glyceraldehyde by successive Kiliani-Fischer syntheses. The present use of the D- and L-notation [27e] started around 1940; b) E. Fischer, *Ber. Dtsch. Chem. Ges.* **1890**, *23*, 370–394; c) M. A. Rosanoff, *J. Am. Chem. Soc.* **1906**, *28*, 114–121; d) A. Wohl, K. Freudenberg, *Ber. Dtsch. Chem. Ges.* **1923**, *56*, 309–313; e) Rules of Carbohydrate Nomenclature, No. 4 and 5 in *The Carbohydrates, Vol. IIB* (Eds.: W. Pigman, D. Horton), Academic Press, New York, **1970**, p. 809ff.
[28] Aside of ref. [12], there are several pertinent accounts of Fischer's conquest of the sugar configurations, for example C. S. Hudson, "Emil Fischer's Discovery of the Configuration of Glucose", *J. Chem. Educ.* **1941**, *18*, 353–357; "Emil Fischer and His Contribution to Carbohydrate Chemistry". K. Freudenberg, *Adv. Carbohydr. Chem.* **1966**, *21*, 1–38.
[29] E. Fischer, *Ber. Dtsch. Chem. Ges.* **1894**, *27*, 3189–3232; quotation from p. 3228.
[30] Hans Thierfelder (1858–1930) earned his doctorate (Dr. med.) at the University of Rostock (1884), then joined Hoppe-Seyler's group at the University of Strassburg, where he habilitated in 1887 with work on the chemistry and metabolism of glucuronic acid. With Hoppe-Seyler he coedited the 6th edition of the "*Handbuch der physiologisch- und pathologisch-chemischen-Analyse*" (1893). In 1891, he accepted a position at the hygienic institute of the University of Berlin, where he came into contact with Emil Fischer, that eventually led to the joint work on the yeasts [31]. From 1909 on, Thierfelder was professor of physiological chemistry at the University of Tübingen. For a biographical memoir, see: E. Klenk, *Z. Physiol. Chem.* **1931**, *203*, 1–9.
[31] E. Fischer, H. Thierfelder, *Ber. Dtsch. Chem. Ges.* **1894**, *27*, 2031–2037.
[32] E. Fischer, *Ber. Dtsch. Chem. Ges.* **1894**, *27*, 3479–3483.
[33] E. Fischer, *Untersuchungen in der Puringruppe (1882–1906)*, Springer, Berlin, **1907**.
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[35] E. Fischer, *Untersuchungen über Aminosäuren, Polypeptide und Proteine I (1899–1906)*, Springer, Berlin, **1906**; *II (1907–1919)* (Ed.: M. Bergmann), Springer, Berlin, **1923**.
[36] E. Fischer, *Untersuchungen über Depside und Gerbstoffe (1908–1919)*, Springer, Berlin, **1921**, 524 pages.
[37] E. Fischer, F. Passmore, *Ber. Dtsch. Chem. Ges.* **1889**, *22*, 2728–2736; E. Fischer, *ibid.* **1890**, *23*, 2611–2624.
[38] H. Kiliani, *Ber. Dtsch. Chem. Ges.* **1885**, *18*, 3068–3072; **1886**, *19*, 211–227.
[39] E. Fischer, *Ber. Dtsch. Chem. Ges.* **1890**, *23*, 2114–2141; quotation from p. 2134.
[40] E. Fischer, *Ber. Dtsch. Chem. Ges.* **1890**, *23*, 3684–3687.
[41] E. Fischer, Lecture given on August 2, 1894 in Berlin, on the occasion of the foundation day of the School of Military Physicians, A. Hirschwald Verlag, Berlin **1894**, 1–36; quotation from p. 30.
[42] E. Buchner, R. Rapp, *Ber. Dtsch. Chem. Ges.* **1897**, *30*, 2668–2678.
[43] a) Fischer, from about 1896 on, shifted his main research efforts to the systematic exploitation of peptides [35], purines [33], and tannins [36]. Yet he retained a keen interest in sugars [43b] and their action with enzymes; his last two papers [43c], received by the editorial office of *Hoppe-Seyler's Zeitschrift für physiologische Chemie* on July 14, 1919, the day of his death, dealt with sugars, the former being entitled "Einfluss der Struktur der β-Glukoside auf die Wirkung des Emulsins." b) E. Fischer, *Untersuchungen über Kohlenhydrate und Fermente II* (1908–1919), Springer, Berlin, **1922**, 543 pages; c) E. Fischer, *Hoppe-Seyler's Z. Physiol. Chem.* **1919**, *107*, 176–202; *ibid.* **1919**, *108*, 3–8.
[44] *Nobel Lectures Including Presentation Speeches and Laureates Biographies, Chemistry, 1901–1921* (Ed.: Nobel Foundation), Elsevier, Amsterdam, **1966**, pp. 15–39.
[45] E. Fischer, Faraday Lecture on "Synthetical Chemistry in its Relation to Biology", *J. Chem. Soc. (London)* **1907**, *91*, 1747–1765.
[46] "Bedeutung der Stereochemie für die Physiologie": E. Fischer, *Hoppe-Seyler's Z. Physiol. Chem.* **1898**, *26*, 60–87; quotation from p. 82/83.
[47] "On Immunity, with Special Reference to Cell Life": P. Ehrlich, *Proc. R. Soc. London*, **1900**, *66*, 424–448; quotation from p. 433.
[48] "The Mechanism of Fertilization in Arbacia": F. R. Lillie, *J. Exp. Zool.* **1914**, *16*, 523–590.
[49] "Intellectual Traditions in the Life Sciences: Stereocomplementarity": S. F. Gilbert, J. P. Greenberg, *Persp. Biol Med.* **1984**, *28*, 18–34.
[50] E. Fischer, *Justus Liebigs Ann. Chem.* **1911**, *381*, 123–141; *ibid.* **1911**, *386*, 374–386; *ibid.* **1912**, 350–362.
[51] Letter dated November 11, 1912, to Theodore William Richards (1868–1928), professor at Harvard University. Richards did postdoctoral work with W. Ostwald in Leipzig, and in 1907 had an exchange professorship at Fischer's institute at the University of Berlin. In the Emil Fischer papers at the Bancroft Library, Berkeley, CA, there are 39 letters by Richards dated 1905–1915 (originals) and the responses by Fischer (copies).
[52] E. Fischer, *Ber. Dtsch. Chem. Ges.* **1914**, *47*, 1980–1989; quotation from p. 1984.
[53] B. Tollens, *Ber. Dtsch. Chem. Ges.* **1883**, *16*, 921–924; quotation from p. 923.
[54] E. Fischer, *Ber. Dtsch. Chem. Ges.* **1893**, *26*, 2400–2412; quotation from p. 2405.
[55] B. Tollens, *Kurzes Handbuch der Kohlenhydrate*, J. A. Barth Verlag, Leipzig, **1914**, p. 363.