# General Laws for the Action of Diastases

Victor Henri
Doctor of Philosophy (University of Göttingen),
Doctor of Science and *Préparateur* at the Sorbonne,

To my dear Master, Monsieur Dastre
Professor at the Sorbonne

*Homage of recognition and devotion*

published in 1903 by Librairie Scientifique A. Hermann, Paris, as

*Lois générales de l'action des diastases*

Translated by Athel Cornish-Bowden

with corrections by Jean-Pierre Mazat.

# Contents

# Introduction to the translation

### Translation note

The translation is by Athel Cornish-Bowden, checked for errors in understanding the French original by Jean-Pierre Mazat. Where Henri included footnotes (mainly, and somewhat haphazardly, for literature references) these are given as footnotes. Where no references are given they were not included in the original. In a few important cases they have been added in the margin, and all other editors' notes are also given in the margin.

In general I have replaced the units used by Henri with the units used today, in particular normal, n or N by M. Henri appears to have varied arbitrarily between representing logarithms with ln or with log: my impression is that these are not intended to be different, and both correspond to what would be written as ln in modern biochemistry. However, it may be that he preferred ln when integrating differential equations and log for calculations. In order not to modify too much the "flavour" of Henri's presentation I have retained some of his terminology:

| Henri's term | English equivalent | Modern term |
|---|---|---|
| *invertine* | invertin | invertase |
| *diastase* | diastase | enzyme |
| *ferment* | ferment | enzyme |
| *lévulose* | laevulose | fructose |
| *autocatalysis négative* | negative autocatalysis | inhibition |
| *soda* | soda | sodium hydroxide |
| *molécule gramme* | gram molecule | mole |

### Henri's origins

The conventional account (repeated, for example, in my book *Fundamentals of Enzyme Kinetics*, 2012) is that Victor Henri was born in Marseilles, orphaned when very young, adopted by Russian aristocrats and taken to St Petersburg. The real history is more complicated, and more interesting.

He was indeed born in Marseilles, but he was not an orphan: he was the illegitimate son of Aleksandra Viktorovna Lyapunova and her brother-in-law Nikolai Aleksandrovich Krilov, who came to Marseilles in order for their son to be born in France, where he would have all the rights of a French citizen, whereas if born in Russia his legal status would have been very difficult. After he was born he was adopted by Nikolai and his wife Sofia Viktorovna Lyapunova. The family name of "Henri" appears to have been assigned by the registrar, according to French custom at the time.

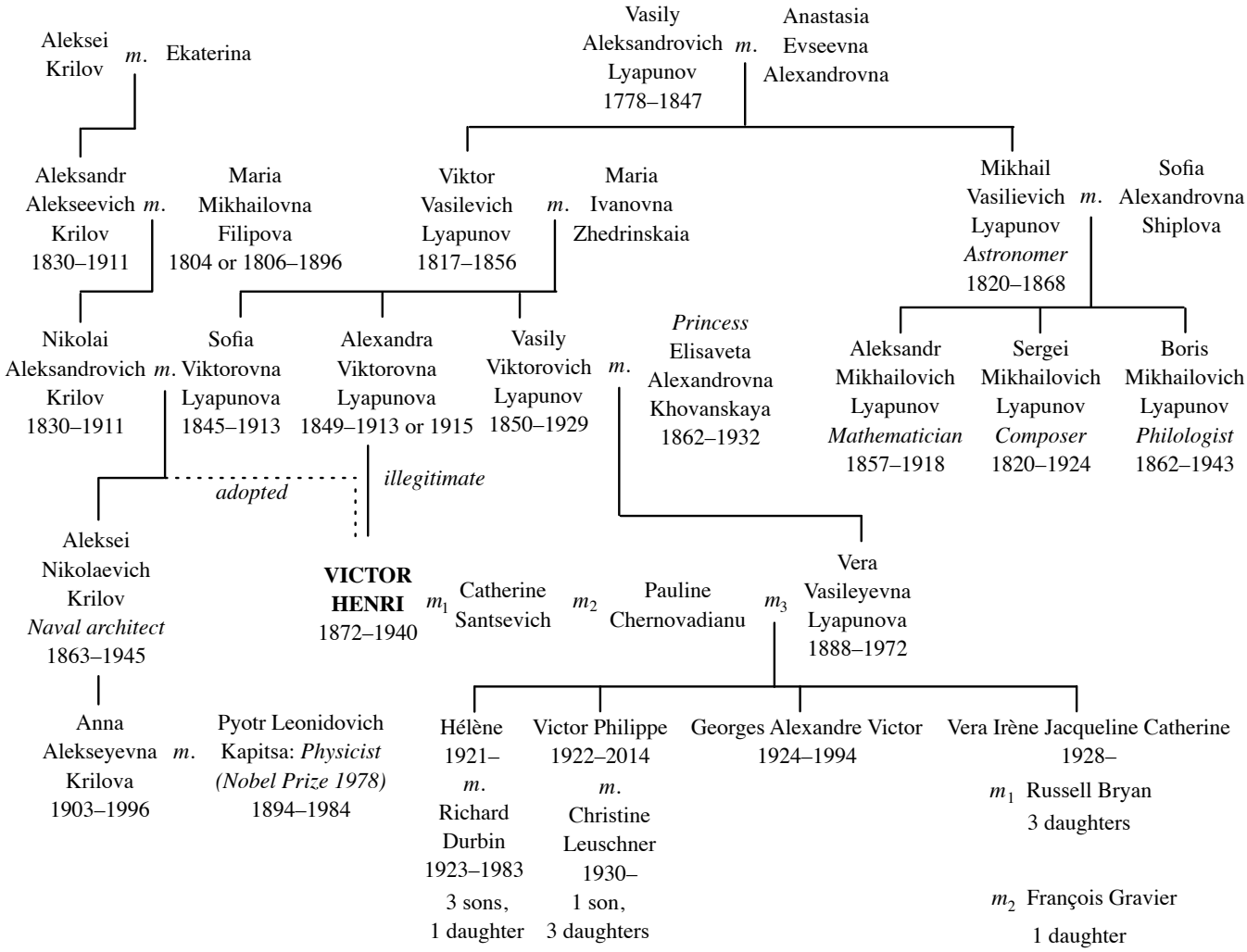
There is no universally accepted and understood system for representing Russian names in English, and I have followed the spellings used by Janine Henri. In French sources *Lyapunov* is commonly written as *Lyapounov* or *Lyapounoff*.

Henri's mothers came from one of the most distinguished scientific families of Russia, the Lyapunovs. Their uncle Mikhail was a well known astronomer, and his three sons were all highly distinguished in their different fields. In particular, Aleksander was a mathematician whose work (often alluded to in "Lyapunov exponents") remains very well known today.

Henri's half-brother Aleksei was a naval architect who made a major con-

tribution to the organization of the Russian and Soviet navies. His daughter Anna married Peter (Pyotr) Leonidovich Kapitsa, who was a pioneer of low-temperature physics and was awarded the Nobel prize in 1978. Their son Andrei, himself a distinguished geologist, was the original source of the information about Henri's connection with the Lyapunov family, corrected and expanded here on the basis of information supplied by Janine Henri, Henri's granddaughter, to whom I am most grateful.

http://flot.com/publications/books/shelf/memories/1.htm (in Russian). See below for an English translation.




## Education and early career

After primary and secondary education in St. Petersburg Henri studied in Paris, and became the first collaborator of Alfred Binet, the pioneer in intelligence testing, with whom he studied hysterical patients from Charcot's department at the Salpétrière hospital. After a long period of time in Germany, particularly in Leipzig and Göttingen, he obtained his first doctorate in psychology in 1897 at Göttingen; his thesis concerned tactile sensations. Afterwards he came back to Paris and performed new experiments with Binet on intellectual fatigue that led him to the physiology laboratory in the Sorbonne to perform some chemical analysis to study nutritional exchanges during intellectual work. He obtained a position of *préparateur* (a sort of lecturer) in this laboratory directed by Albert

The expression *nutritional exchanges* is the literal translation of the words *échanges nutritifs* that Henri used many times in his paper. It refers to the differences produced by different degrees of intellectual effort in the composition of the excreta of subjects following a controlled diet and controlled muscular activity.

Dastre, a pupil of the great physiologist Claude Bernard, where he began the research on diastases that forms the subject of his thesis. He received his second doctorate from the Sorbonne in 1903 on the basis of the thesis that is given here in translation. In 1907 he was nominated Professor of Physiology in Paris. He then moved to Russia where he was responsible for the organization of the chemical industry for defence. On his return to Paris he presented, in April 1920, Einstein's principle of relativity to philosophers and psychologists, with its philosophical consequences. In 1920 he was nominated at Zürich University where he remained for ten years. After a brief period at Berre-L'Étang (near Marseilles), where he was to be in charge of a planned great institute of petrochemistry, he was nominated at the Science Faculty in Liège (Belgium).

## Andrei Petrovich Kapitsa's text

**translated by Stefan Schuster**

In the book, nothing is said about the reasons of the journey of the Krilov (Крылов) family to Marseilles in 1872. Today, 130 years later, one can finally speak about what was considered a family secret for a long time. The reason was that the sister of the mother of Aleksei Nikolaevich (Алексей Николаевич), Aleksandra Viktorovna Lyapunova (Александра Викторовна Ляпунова), was about to give birth. The father of the child was Nikolai Aleksandrovich Krilov (Николаи Александрович Крылов). If the child had been born in Russia, then it would have been as an illegitimate child, and a very unfortunate fate would await it. Therefore, the decision was taken by the entire family to travel to Marseilles, because in France such children have all the rights of a normal citizen. The child born there received the name Victor and his godfather was Aleksei Nikolaevich, from whom he received his father's name. The family name Henri (in Russian Анри, Anri) was given to him (Victor Henri, 1872-1940). Often, another Russian transliteration is found: Виктор Генри (Viktor Genri).

The book referred to (the main subject of the web page) is *Memoirs of Academician Aleksei Nikolaevich Krilov (Мемуары академика Алексея Николаевича Крылова).*

"His father's name" means the patronymic Alekseevich (Алексеевич), which is used in Russian but not in Western publications. Victor Henri's own scientific publications never included it.

As a French citizen, he travelled to Russia, where he attended a German secondary school. Later he returned to Paris, where he lived with the mother from the age of 14 years on. In 1891, he entered the Sorbonne, where he started to receive an education in mathematics and, later, in Natural Sciences. After finishing university, he became intrigued by philosophy and psychology. In 1897, he defended a doctoral thesis at the University of Göttingen on the topic "Localization of gustatory sensations". Consequently, he published a number of papers in the field of psychology. In 1902, he defended, at the Sorbonne, a doctoral thesis in the field of physico-chemical biology. His interests are unusually wide. During World War I, he was a French attaché at the embassy to Russia. He dealt with organizing the chemical industry for defence purposes.

Victor Henri is known by his work in the field of photochemistry. In 1930, he was appointed head of the chair of Physical Chemistry at Liège University, where he worked until 1940. Many of his papers became the basis of outstanding new directions in science. When the war against Germany started, he worked with Langevin on military problems.

He had two sons, Victor and Aleksei, and two daughters, Hélène (Елена) and Vera (Вера). With the latter, I got acquainted by coincidence at a conference in Tokyo in 1964. There, she came to me and, looking at my conference badge, said to me: "It seems that I am your aunt." Thus, I met Vera Henri, and, later in Moscow, my mother told me about the French branch of our family. My father became a good friend of his half-brother Victor. Many letters of his with Aleksei Nikolaevich have been preserved. Some of the descendants of Victor went to the U.S., and I met with them in the 1980s. Only now have I decided to publicize this story. Still until lately, having relatives abroad could imply a risk [in the Soviet Union].

Victor Alekseevich Henri was married to Vera Vasilevna Lyapunova (Вера

Василевна Ляпунова), the daughter of the princess Elisaveta Khovanskaya (Елизавета Хованская) and of Vasily Lyapunov (Васили Ляпунов). This marriage gave rise to a brilliant branch of the Henris (Krilovs) in France. He himself became a well-known and well-educated physiologist, professor and prize winner of the Institut de France.

### Later career

Henri's later work was mainly in physical chemistry, where he studied absorption spectra, from which he obtained a wealth of information on molecular structures, such as those of naphthalene and phosgene. His last publication recorded in Web of Science concerned the use of ultraviolet spectroscopy for detection of aromatic compounds in mineral oil, and was a collaboration with Chaim Weizmann, later the first President of Israel. He died in La Rochelle after a pulmonary congestion contracted during the 1940 retreat that followed the German invasion of France. Edgar Morin relates the arrival in Toulouse, where he was living, of Henri's wife and her four sons in abject poverty after his death. Fuller accounts of Henri's career are given by Nicolas [S. Nicolas (1994) Qui était Victor Henri?, *L'Année Psychologique* **94**, 385–402], emphasizing his work in experimental psychology, and by Boyde [T. R. C. Boyde (1980) *Foundation Stones of Biochemistry*, Voile et Aviron, Hong Kong].

This biographical sketch is based on the one given in a recent article [U. Deichmann, S. Schuster, J.-P. Mazat and A. Cornish-Bowden (2014) "Commemorating the 1913 Michaelis–Menten paper *Die Kinetik der Invertinwirkung*: three perspectives" *FEBS Journal* **281**, 435–463], which sets out to place Henri's contribution to the early development of enzyme kinetics in the context of that of Michaelis and Menten and other pioneers.

### Henri's thesis

The thesis that is given here in translation was published by the *Libraire Scientifique A. Hermann*, of Paris. Its price is not given on my copy, but advertisements for other books at the back include one for a book of xii+190 pages on *Théorie des Erreurs d'Observation* by P. S. E. Goedseels, offered in an unbound state at 7.50 francs, a substantial sum for that time, equivalent to the price of about 2.2 g of gold.

### References

In accordance with the practice of his time, Henri expressed his literature citations in a highly abbreviated way. Here I have added information in square brackets, as well as giving full journal names. Not all were easy to find, so some details are missing. In addition, *Web of Science* has the deplorable practice of translating original titles into English, often rather incompetently, and omitting the original titles in German or French, so titles obtained from this source are in a sort of approximate English.

# Preface

When a theory seeks to explain a collection of phenomena by invoking the existence of completely new forces or forms of energy, two different cases arise: either these new factors whose existence is postulated are of such a nature that they can be analysed experimentally, thereby allowing a deeper penetration of the studied phenomena, or on the other hand these factors cannot be studied experimentally. In the first case the theory will necessarily lead to the discovery of new facts, it will generate a whole series of experimental research, in consequence true or false, and it will contribute to the development of science. In the second case, in contrast, it will bring purely speculative answers that will easily become dogmatic, and will then be able to bring experimental research to a halt.

Two groups of theories have been proposed for studying the general phenomena of the life of organisms: some consider that vital manifestations are due to physico-chemical actions; the others deny this reduction and admit the existence of new forces that are in themselves "vital", as they are called. As experimentation is based on the methods and data of chemistry and physics, vitalist theories renounce any possibility of experimenting on this vital force; they constitute a sort of brake on experimental research, that is to say on scientific research, and remove the discussion from the domain of experimentation to that of speculation. The action of such theories is thus harmful, as the usefulness of any theory is measured by the number and importance of new facts that it leads one to discover.

Throughout his thesis Henri writes *diastatic* or *diastase* where today we would write *enzymic* or *enzyme*. Here I retain his terminology, but note that it would not be used so broadly today.

Among the phenomena that occur in the living organism, the processes of nutrition, reabsorption, secretion, and defence of the cells of the organism, in sum all the metabolism of the living being, can be considered as reducible almost completely in terms of diastatic activity; in effect, as the numbers of experimental studies increases, the number of activities previously considered as intimately linked to the vitality of certain cells decreases; the studies of Buchner, E. Fischer and others have led to examples of this that are as striking as they are suggestive. As a result the study of the laws governing diastatic behaviour acquire a more than average interest.

Henri does not cite specific references, but we may assume he means E. Buchner (1897) "Alkoholische Gährung ohne Hefezellen" *Berichte der Deutschen Chemischen Gesellschaft* **30,** 117–124; E. Fischer (1894) "Einfluss der Configuration auf die Wirkung der Enzyme" *Berichte der Deutschen Chemischen Gesellschaft* **27**, 2985–2993

The explanation of these diastatic activities has brought about a great number of discussions and theories that can again be distributed in two groups: those that renounce explanations of diastatic activities in terms of the actions of general chemistry, making a special group with their own laws; the others, in contrast, try to bring diastatic activities under the laws of general chemistry, and in this way these theories contribute in some way to theories that explain vital behaviour as chemical activities.

The present work has as its subject the study of the general laws of diastatic activity. The aim is to study the activities of diastases by using the methods and results of physical chemistry; these methods are briefly summarized in the Introduction.

Only three diastases have been considered in this work, invertin, emulsin and amylase; these diastases have the advantage of allowing a quantitative study of their behaviour more easily than for the majority of other diastases. The present work only contains the first part of the experimental study of diastatic activities; thus the influence of the temperature and other conditions of the medium is not

Henri uses the term *invertin* for the enzyme usually called invertase today (or more explicitly $\beta$-fructofuranosidase, EC 3.2.1.26). I shall retain Henri's terminology.

included. In spite of that, it has seemed possible to us to present a general theory of diastatic action, which brings these activities completely under the laws of physical chemistry.

The experiments were done in the laboratory of experimental physiology of the Sorbonne; it is thanks to the encouragement and continual advice of my dear Master, Mr Dastre, that I have been able to familiarize myself with the methods of physical chemistry, and to bring to a conclusion experiments of which some required some precision. I am happy to express to him my sincere gratitude.

I carried out a certain number of experiments on diastases in the laboratory of physical chemistry of Professor Ostwald in Leipzig; I take advantage of this occasion to expression my thanks to Mr. Ostwald for his generous hospitality and for the valuable advice that I found in his laboratory.

*Paris, 2nd January 1903.*

Albert Dastre (1844–1917) was a French physiologist, a former student of Claude Bernard, and known for his work on glycosuria and diabetes.

Wilhelm Ostwald (1853– 1932), a German (originally Latvian) chemist, was one of the founders of physical chemistry. He received the Nobel Prize in Chemistry in 1909.

# Introduction

## Current state of knowledge of catalysis

**1. Definition of catalytic activity according to Berzelius.** By introducing the term *catalytic action* in 1836, *Berzelius* brought together a very varied collection of chemical activities, among which we find: the transformation by sulphuric acid of starch into sugar, observed by Kirchhoff in 1811, and the sweetening of starch by malt extract, likewise described by Kirchhoff, in 1814; the experiments of Thenard on the decomposition of hydrogen peroxide by platinum; Davy's observations of the action of platinum on the mixture of oxygen and hydrogen; and finally Mitscherlich's experiments on the action of sulphuric acid on alcohol, which is converted to ether, etc.

All these reactions, among which we already meet the beginnings of the diastatic reactions, have a certain number of characteristics in common that Berzelius thought it helpful to highlight and to represent under the generic term of *catalytic reactions*.

The catalytic force, says Berzelius, consists in that fact that certain substances can, by their presence alone and not by virtue of their reactivity, arouse "sleeping" reactivities of other substances and thus produce a reaction between them. The action of catalysts is thus analogous to that of heating, and Berzelius says plainly that these substances play no direct part in the reaction. According to this definition the very important experiment of Clément and Désormes (*Annales de Chimie*, **59**, 329, 1806) on the action of saltpeter to favour the oxidation of sulphurous acid by the oxygen in the air should not be included among the catalytic reactions; in fact, these authors explained this activating effect by the formation of oxides of nitrogen as intermediates that brought the oxygen of the air to the sulphurous acid.

We see therefore that since the first studies of the action of substances that accelerate a chemical reaction, and which, finally, are found unchanged in the mixture, these appear in two different kinds: those where chemical intermediates are formed and those in which there are no intermediates; it is to this second category that Berzelius called attention, and the diastatic activities appear there.

We shall not pause to describe the historical development of the question of catalytic activities, noting only that the preceding duality continues today; a great number of studies have been made to defend one or other of the preceding hypotheses and to extend it to more examples.

**2. The difficulty of giving a simple definition of catalytic activity.** We shall begin by summarizing the current state of our knowledge of the laws of catalytic activity, seeing that, as we shall show later, diastatic activity should appear as a particular case of this group of reactions.

The definition of catalytic activity seemed simple at the outset; it was said that a substance that accelerates a reaction without appearing among the products of this reaction, and which, moreover, is found unchanged at the end, has a catalytic activity. However, in studying more closely some reactions that seemed to form

Jöns Jacob Berzelius (1779–1848), a Swedish chemist, is noted in particular for his studies of catalysis.

Gottlieb Sigismund Constantin Kirchhoff (1764–1833) was a Russian chemist.

Louis Jacques Thenard (1777–1857), the son of a poor peasant, was a French chemist, and the discoverer of boron.

Humphrey Davy (1778–1829) was an English chemist, discoverer of sodium and other metals. Michael Fraday worked as his assistant.

Eihard Mischerlich (1794–1863), a German chemist, introduced the law of isomorphism.

J. J. Berzelius (1836) "Quelques idées sur une nouvelle force agissant dans les combinaisons des corps organiques" *Annales de Chimie et de Physique* **61,** 146–151 (reference not given by Henri).

Nicolas Clément (1779–1841) and his father-in-law Charles-Bernard Désormes (1777–1862) were French chemists known in particular for their work on the formation of sulphuric acid. Full reference: N. Clément and C.-B. Désormes (1806) "Théorie sur la fabrication de l'acide sulphurique" *Annales de Chimie* **59**, 329– 339.

part of this group it was seen that there were complications that required some reservation, or to modify the preceding definition. In fact in some cases the substance was not found unchanged at the end, or was not found in its original state, so that its catalytic effect was modified, which was expressed by saying that there was positive or negative "autocatalysis". Finally, cases were reported in which the substance was indeed found unchanged at the end, but analysis of the mixture during the course of the reaction revealed it in combination with other substances. It had to be asked, therefore, if all these exceptions needed to be removed from the class of catalytic processes or if it was the preceding definition that needed to be changed.

In modern usage *autocatalysis* refers to catalysis by the product(s) of a reaction, and *catalysis* is always positive: we do not refer to a substance that impedes a reaction as a negative catalyst.

The current ideas, such as result from numerous studies made under Ostwald's direction in the laboratory of physical chemistry of Leipzig, seem to lead to a modification of the definition of *catalysis*. Let us begin by detailing the most important characteristics of the reactions that have been considered to be catalytic.

**3. Disproportion between the quantity of catalyst and the mass of reactants transformed.** The first essential characteristic that is common to all catalytic reactions is the fact, already observed by Clément and Désormes in 1806, that a given quantity of catalyst can suffice to bring about the transformation of very considerable amounts of the reactants of the catalysed reaction. There is no fixed proportion between the amount of catalyst and the amount of reactants converted. This result is well covered by the condition referred to earlier, according to which the catalyst is found at the end of the reaction in its original form, and it can therefore serve again for another reactions. Let us first give some examples.

Sucrose is converted in aqueous solution in the presence of any acid into glucose and laevulose, and it is known that in varying the acidity by a chemical or physical method it remains constant during the course of the reaction, in such a way that at the end, when all the sucrose has been inverted, the acid is found unchanged. If more sucrose is added it will be inverted in its turn in the presence of this acid. It is clear that there is no relation between the quantity of acid and the quantity of sucrose that can be inverted in its presence.

Laevulose is now usually known as fructose. The mixture of glucose and fructose is called *invert sugar*, and the hydrolysis is known as *inversion.*

In allowing a solution of colloidal platinum prepared by Bredig's method to act on a mixture of hydrogen and oxygen, Ernst[1] showed in one of his experiments that a solution containing 0.04 mg platinum had in fourteen days brought about combination of 10L of the mixture, and that at the end the solution of colloidal platinum was just as active as at the beginning.

Possible reference (not given by Henri): G. Bredig (1901) *Anorganische Fermente*, W. Engelmann, Leipzig. Georg Bredig (1868–1944) was a German chemist who discovered the use of colloidal metals as catalysts.

I have not discovered anything about Carl Ernst, apart from what is here.

However, not all catalytic reactions give rise to unlimited effects as in these previous ones; in some cases the catalyst does not appear at the end of the reaction in the same state as it was at the outset. Thus, for example, if a strip of thoroughly cleaned platinum is brought to a high temperature and placed after cooling in a mixture of hydrogen and oxygen, the combination will occur at a particular rate. If later, after the strip has been left for several hours in the gases mentioned, removed and put again in a mixture of hydrogen and oxygen, the combination then occurs more slowly than in the presence of a freshly heated strip; therefore the catalyst, in this case platinum, does not appear at the end of the reaction in its original state.

There are many examples of this kind, and it is precisely in this class that we must put the diastatic reactions. When invertin produces inversion of sucrose, the diastase is not found to be as active at the end as it was at the beginning; its activity depends not on the duration of the reaction but on the quantity of products that have accumulated, as will be shown later.

[1][C. Ernst "On the catalysis of oxyhydrogen gas through colloidal platinum"], *Zeitschrift für Physikalische Chemie* **37**, 1901, [448–484] (title in English as given by *Web of Knowledge*). Henri referred specifically to page 454.

This modification of the catalyst during the reaction can occur in various different ways, and discussion of the reasons for the change is not always easy. It may happen that the catalyst forms a stable complex with one of the reactants that cannot act on the progress of the reaction; that is what seems to happen in the case of the strip of platinum placed in the mixture of hydrogen and oxygen. It can, on the other hand, happen that among the substances that appear during the reaction one of them plays the role of a catalyst, in such a way that it accelerates or slows down the reaction, as is called an "autocatalysis". The examples are numerous, and here are two types: Ostwald[2] studied the action of hydrogen iodide on bromic acid, this reaction being strongly accelerated by the presence of any acid that plays the role of catalyst. The reaction $HBrO_3 + 6HI = HBr + 3H_2O + 3I_2$ becomes much slower towards the end; this slowing down, which would seem to suggest that the catalyst becomes less active, is due to the appearance of iodine, which acts as a negative catalyst, slowing down the reaction, as Ostwald and afterwards Meyerhoffer[3] showed, by adding some iodine at the start of the reaction.

Henri wrote chemical equations with superscripts where modern practice would use subscripts, for example $HBrO^3$ rather than $HBrO_3$. These have been brought into accord here with modern practice.

Hentschel[4], while studying the decomposition of diacetamide by water, found that the rate of decomposition was faster than expected from the logarithmic law, the curve representing the rate of reaction being a straight line; this acceleration is due to the formation of acetic acid, which is a catalyst, and, as it is formed in greater and greater quantities, it follows that the acceleration brought about by this secondary cause becomes stronger and stronger.

Wilhelm Meyerhoffer (1864–1906) was a German chemist and associate of Jacobus Henricus van 't Hoff.

Finally it can happen that the substance that plays the role of catalyst combines with one of the reactants and the resulting combination constitutes a new catalyst that is stronger than the bare substance, and in this case again the activity of the catalyst will change during the reaction.

Willibald Hentschel (1858–1947) was a German chemist whose work on dyes was completely overshadowed by his later interest in racial purity and Nazi ideology.

It is clear that in some of these cases the secondary activities will be able to influence the bare catalyst, in such a way that the reaction will stop after a certain time: in these cases the catalytic activity can no longer be said to be unlimited, as it will be able to depend directly on the quantity of catalyst and the quantity of substrate. It seems, therefore, that in cases of this kind, which probably includes some diastases, the proposition set out at the beginning of this section will fail.

Perpetual motion of the second kind is motion that violates the second law of thermodynamics.

**4. When the reaction that occurs in the presence of the catalyst reaches an equilibrium, the presence of the catalyst does not change the position of this equilibrium.** This result is exact only in the case where at the end of the reaction the catalyst is found in the same form as at the beginning. In this case, of course, the equilibrium cannot be modified by the presence of the catalyst, without implying that one could have a perpetual motion of the second kind: the catalyst acts therefore only as a *primer*, as an accelerator that must act in the same way on the two opposing rates, of which the equality characterizes the equilibrium. Thus if the reactants A + B combine to give products C + D, as one can represent by the symbols

$$A + B \rightleftarrows C + D$$

and the reaction is not complete at the moment of the equilibrium, according to the hypothesis of Guldberg and Waage there is equality between the rate of combination of A and B and the rate of the reverse reaction, that is to say the combining of C and D. If any catalytic agent increases the first rate it must equally increase the rate of the second reaction.

Henri gave no reference, but he was referring to P. Waage and C. M. Guldberg (1864) "Studier over Affiniteten" *Forhandlinger: Videnskabs- Selskabet i Christiana*, 35–40, 111-120. There is an English translation by H. I. Abrash: http://tinyurl.com/3levsgl

Peter Waage (1833–1900) was a Norwegian chemist who, with his brother-in-law Cato Maximilian Guldberg (1836–1902), a Danish chemist, discovered the law of mass action.

[2][W.] Ostwald ["Studien zur chemischen Dynamik"] *Zeitschrift für physikalische Chemie*, **II**, p. 127-147.

[3]Meyerhoffer, *Zeit. f. phys. Chem.*, **II**. I have not found a more complete reference.

[4][W.] Hentschel, "Ueber Diacetamid" *Berichte der Deutschen Chemischen Gesellschaft*, **23**, 1890, 2394[–2401]

This elementary result has great importance for the study of diastases. In fact some diastases give rise to incomplete reactions, and the reaction stops at a certain point that may be an equilibrium, and then the preceding considerations require that if the quantity of reaction products is increased in the presence of the diastase, the reaction will be seen to proceed in the reverse direction. One could therefore set out as a general rule that if, in the presence of a diastase, a reaction is incomplete and if it arrives at an equilibrium, in the presence of this same diastase, the reverse reaction will be seen when it is put with the products of the reaction. So it is that Hill, Emmeling and E. Fischer have shown that maltase acted both on maltose and also on concentrated glucose, that lactase hydrolysed lactose and brought about combination of glucose with galactose, and that emulsin hydrolysed amygdalin and could also bring about its synthesis. It is certain that the number of this syntheses can be considerably increased; it is easy to establish *a priori* that syntheses of this type should occur the more so as the concentration of the solution is greater.

Hermann Emil Fischer (1852–1919) was a German chemist, awarded the Nobel Prize in Chemistry in 1902. Of his many discoveries in chemistry he is best known in enzymology for his work on stereochemistry and the *lock and key model*.

I know nothing of Hill or Emmeling.

Let us give several examples to show that the presence of a catalyst does not change the state of equilibrium. Hydrogen iodide partially decomposes around 350°, equilibrium being established very slowly at this temperature, needing around 300 hours, according to Lemoine. Now, in the presence of a platinum foam the decomposition of hydrogen iodide at 350° occurs very fast and at the same time combination of hydrogen with iodine is strongly accelerated by platinum foam. The experiments of Hautefeuille and of Lemoine show that the equilibrium is the same when HI dissociates when in the presence of the platinum foam or with catalyst. "Thus, at 350°, working in the presence of platinum foam, M. Hautefeuille has given 0.19 as this limit; working solely under the influence of heating alone I [Lemoine] have found 0.186"[5].

Paul Gabriel Hautefeuille (1836–1902) was a French chemist who worked on aspects of mineralogy and catalysis. Possible reference (not given by Henri): P. Hautefeuille (1867) "Action de la chaleur su l'acide iodhydrique," *Comptes Rendus Hebdomadaires de l'Académie des Sciences* **64**, 608–611.

When studying the polymerization of paraldehyde to aldehyde that occurs at 50.5° in the presence of different catalysts, Turbaba[6] finds that the equilibrium state is the same, whatever catalyst is used in whatever quantity. As catalysts he used $SO_2$, $ZnSO_4$, $(COOH)_2$ and $H_3PO_4$.

Georges Lemoine (1841–1922) was a French chemist noted for the studies that Henri refers to here.

In the same way Kœlichen[7] in a study of the polymerization of acetone,

$$2CH_3COCH_3 \rightleftarrows CH_3COCH_2C(CH_3)_2OH$$

As written this seems not to make sense, as paraldehyde is a trimer of acetaldehyde, and, as it is more stable and easier to handle than acetaldehyde it is used as a convenient way of storing it. Henri probably meant polymerization of acetaldehyde to paraldehyde.

in the presence of different catalysts, finds the same equilibrium state for the different catalysts: soda, triethylamine, piperidine, tetraethylammonium hydroxide and ammonia. The state of equilibrium is also the same regardless of the concentration of the catalyst, as long as it is not too high.

Finally, it is known that the equilibrium between acetic acid, alcohol, ether and water is established faster in the presence of an acid acting as catalyst without altering the position of equilibrium, on condition that the acid is not too strong.

Émile Duclaux (1840–1904) was a French microbiologist and chemist. Henri discusses his work in detail later in the thesis: see §§10–13 in particular.

These results show clearly that the role of the catalyst is not at all of an energetic nature, that it is reduced to being a simple brake or accelerator. One cannot therefore equate the action of a catalyst with an increase in temperature, as Berzelius and other authors (such as Lemoine) did, as we know that the temperature has a clearly defined effect (in relation to the heat of the reaction) on the state of equilibrium. In the same way we must not equate the action of a catalyst with the effect of increasing the pressure or of condensation, as certain authors have done to explain the roles of porous substances; again, the pressure can displace the equilibrium, which is scarcely possible for a catalyst that can be recovered at the end of the reaction in its original state; this does not exclude phenomena of condensation on the surfaces of porous materials.

Karl Oppenheimer: I have discovered nothing about his life. His book *Ferments and their Actions* is available as a modern reprint from BiblioBazaar.

The preceding considerations lead equally to the observation that a catalyst can as easily accelerate an exothermic reaction as an endothermic reaction; this

[5][G.] Lemoine, *Études sur les équil[ibres] chimiques, 1881, [Dounod, Paris], p. 82.*

[6]Turbaba, *Zeit. phys. Chem.*, **38**, 1901, 505 [complete reference not yet found].

[7]Kœlichen, *Zeit. phys. Chem.*, **33**, 1900, 129 [complete reference not yet found].

possibility of catalytic acceleration of endothermic reactions has been denied by several authors, in particular for the diastases by Duclaux (*Microbiologie*, vol. II, p. 16) and by Oppenheimer; this last author arguing at length that diastatic reactions can only be exothermic. The work of van 't Hoff, Gibbs and Le Chatelier has established beyond doubt that such an idea is in conflict with the principles of thermodynamics, and it is not necessary to continue.

Van 't Hoff, Gibbs and Le Chatelier are all too well known to require additional information here.

**5. Effect of catalysts on the rate law of a reaction. Classification of catalytic activities.** If, instead of studying the effect of catalysts on the position of equilibrium of a collection of substances, one directs attention to the rates of catalytic reactions, one arrives at some important results.

1. *Catalysis just due to presence.* When the catalyst exists at every moment in its native state, one can assert that the order of the chemical reaction is not changed by the presence of the catalyst. So, for example, if sucrose is made to be inverted by some acid in aqueous solution, the acidity of the solution remains unchanged during the course of the reaction, which remains always of first order, regardless of which acid is added and regardless of the quantity; in fact, the reaction $C_{12}H_{22}O_{11} + H_2O = C_6H_{12}O_6 + C_6H_{12}O_6$ is certainly of first order in aqueous solution, as the concentration of water can be considered constant. If one defines $a$ as the quantity of sucrose at the start of reaction, $x$ as the quantity inverted after $t$ minutes, $dx$ as the quantity of sucrose inverted during an interval of $dt$, one obtains, according to the law of mass action,

   $$\frac{dx}{dt} = K(a - x)$$

   from which one can deduce, after integration and satisfying the condition that $x = 0$ when $t = 0$, the value of the constant of inversion is

   $$K = \frac{1}{t} \ln \frac{a}{a - x} \qquad (1)$$

   Henri consistently used roman upper-case letters such as K for rate constants. Here I have followed his symbols so far as case is concerned, but have written all algebraic symbols as italic, in accord with modern practice, thus $K$ in this example, and used roman letters for chemical symbols (as did Henri).

   Henri's numbering of equations was unsystematic, and he often gave the same number to different ones on the same page. Here they are numbered systematically, but the original numbers are noted in the margin, in this case I.

   This expression definitely remains constant during the whole course of the inversion, regardless of the concentration of sucrose. Increasing the quantity of acid increases the value of the rate constant for inversion, but the law according to which the inversion occurs remains unchanged.

   This group of catalytic reactions includes the actions produced by colloidal solutions of metals on hydrogen peroxide and on the mixture of hydrogen and oxygen. The noteworthy research carried out by *Bredig* and his students with colloidal metals (platinum, palladium, gold, silver, cadmium, etc.) have shown that the laws of the reactions are not modified by these catalysts. From this point of view there is an essential difference between the activities of these colloidal solutions and the diastatic activities with which they have been classified. In fact, we shall see later that diastases modify the rate laws of the reactions profoundly.

   This case of "pure" catalysis is the simplest that can be found; very often there are different complications that can completely change the law according to which the reaction occurs. As these complications always arise in the diastatic reactions, we must therefore examine in detail the different sorts of complications that can arise in catalytic reactions.

2. *Autocatalysis.* One or more substances that appear during the reaction have a catalytic activity to accelerate or slow down the progress of the reaction; we have given two examples of these autocatalyses in §3. This secondary catalytic activity due to the products of the reaction can in its turn occur in two ways: either the products of the reaction act on the reaction itself, or,

on the other hand, they act on the catalyst and thus change its activity.

Analysis of these reactions is done as follows: let us suppose that we have a first-order reaction whose rate at a time $t$ is represented by $dx/dt = K(a - x)$. If the products of the reaction in a quantity equal to $x$ have a catalytic effect on the reaction, this activity will be proportional with an exponent $n$ to the quantity of these substances and to the concentration of the substance to be transformed, so the rate will be increased by a term $K_1 x^n(a - x)$, and the total rate will be given by the following expression:

$$\frac{dx}{dt} = K(a - x) + K_1 x^n (a - x)$$

If we suppose that $n = 1$, as occurs in most cases, the preceding expression becomes

$$\frac{dx}{dt} = K(a - x) + K_1 x(a - x)$$

or

$$\frac{dx}{dt} = (K + K_1 x)(a - x)$$

Integrating, and defining the constant of integration to give $x = 0$ at $t = 0$, we then have

$$K + aK_1 = \frac{1}{t} \ln \frac{a(K + K_1 x)}{K(a - x)}$$

This expression can be simplified by defining $aK_1/K = \varepsilon$, giving

$$K(1 + \varepsilon) = \frac{1}{t} \ln \frac{a + \varepsilon x}{a - x} \qquad (2)$$

Originally equation II.

This expression contains two constants, $K$ and $\varepsilon$, and once these are determined the reaction should occur according to the law defined by equation 2, regardless of the concentration $a$.

While studying the action of invertin on sucrose in September 1901 I found that the reaction followed a law that did indeed correspond to equation 2 when the rate was followed from start to finish with a specified value of $a$, and in this case the constant $\varepsilon$ was equal to 1. One might have thought therefore that it was an autocatalysis, but this supposition had to be discarded, because the constant $K$ varied with the sucrose concentration $a$.

There are very complete and varied examples of autocatalysis in Paul Henry's work[8] on the action of lactones, and Meyerhoffer's work[9] on the action of hydrogen iodide on bromic acid.

3. *Very rapid formation of intermediate complexes.* Let us suppose again that we have a first-order reaction, as, for example, in the inversion of sucrose. A substance A decomposes in the presence of a catalyst C, and suppose that this catalyst C very rapidly forms an intermediate complex M, so that its formation can be considered as instantaneous. We need to distinguish between the following two cases, according to whether the reaction between the catalyst and A is a complete reaction or whether it is an equilibrium in which only a part of C and of A are combined.

   a) If the reaction is complete, and if the quantity of catalyst is small compared with that of A, the quantity of the intermediate complex M is proportional to the quantity of catalyst C added to the mixture at the

[8] Henry, *Zeit. phys. Chem.*, 1892, **10**, p. 96
[9] Meyerhoffer, *Zeit. phys. Chem.*, **II**

beginning; the intermediate will decompose to give the products of the reaction, regenerating the catalyst C, which will immediately combine with a new portion of A. The rate of transformation will therefore be proportional to the amount of M, and as this is proportional to the amount of catalyst C, which is constant, one can deduce that the rate will be a constant:

$$\frac{dx}{dt} = K, \text{ that is to say } x = Kt$$

and the curve representing such a reaction is a straight line; the value of the constant $K$ is independent of the quantity of A that exists at the beginning. Only when the quantity of A is less than that of the catalyst will the rate change to follow a logarithmic curve.[10]

It is an activity of this type that ought to occur for the diastases, according to Brown's theory, as we shall see later.

Adrian John Brown (1852–1919) was a British chemist with particular interest in malting and brewing, and was first to interpret enzyme saturation in terms of an intermediate complex. Henri discusses his work in detail in §11.

b) If the reaction between C and A is not complete and an equilibrium is established between the quantity of the complex M and the quantities of C and A the discussion becomes a little more complicated. Let $a - x$ be the quantity of A at time $t$, $c$ the quantity of catalyst C added at the start of the reaction, $m$ the quantity of the complex M at time $t$. The quantity of catalyst that is not complexed at that moment will therefore be equal to $c - m$. According to the law of mass action applied to the state of equilibrium between A, C and M, and supposing for more simplicity that

$$\mathrm{A} + \mathrm{C} \rightleftarrows \mathrm{M}$$

then

$$(a - x)(c - m) = K \cdot m$$

from which we can deduce the following value of $m$:

$$m = \frac{c(a - x}{K + a - x})$$

This intermediate complex M decomposes and the rate for its breakdown is, according to the law of mass action, proportional to the value of $m$, and the expression for the rate of reaction is as follows:

$$\frac{dx}{dt} = \frac{K_1 \cdot c(a - x)}{K + a - x}$$

Integration gives

$$K_1 ct = K \ln \frac{a}{a - x} + x \qquad (3)$$

Originally equation III.

This law depends on two constants $K$ and $K_1$. The right-hand side of equation 3 consists of two terms of which one, $K \ln a/(a - x)$, depends only on the ratio $x/a$, that is to say the *proportion* of A transformed at each moment, whereas the other, $x$, is the *absolute* value of the quantity of the substance transformed. Moreover, we see that the constants $K$ and $K_1$, which correspond to the state of equilibrium and to the rate of decomposition of M, enter as isolated terms of the equation 3, and so, if the temperature is varied, as it is probable that this variation in temperature affects the two constants differently, one could in this way analyse the detailed mechanism further, and separately determine the values of $K$ and of $K_1$. No example is known that is developed in this

Marcellin Berthelot (1827–1907) was a French chemist whose view of physical chemistry was very close to Henri's. In particular he strongly opposed Louis Pasteur's adoption of vitalism.

[10] This reasoning can be applied to all the cases of autooxidation explained by the formation of unstable higher oxides, which have been studied by Berthelot.

way, but there is no doubt that one could find several. Thus it seems *a priori* that the activity of iron as transporter of oxygen in the formation of sulphuric acid should be discussed in terms of this formula, given that the ferrous salt does not become completely oxidized, but establishes an equilibrium between oxygen and the ferrous and ferric salts. It is similar for the different phenomena of oxidation studied by various authors.

We shall see later that for the diastases an analogous reasoning will allow us to arrive at a general law for their action.

4. *Slow production of the intermediate complex.* In the catalytic reactions that belong to this group the catalyst forms an intermediate complex with one or more of the substances to be tranformed, and the rate of formation of this complex is low. This intermediate complex then also decomposes slowly, and generates both the products of the reaction and the free catalyst. When discussing the progress of such a reaction it is necessary to take account of the rates of two successive reactions, and the resulting law is more or less complex, following the order of each of the reactions considered.

   Suppose first of all that we are dealing with monomolecular reactions. A substrate A gives complex M with C; this complex M in turn produces B and regenerates the catalyst C. Let $a$ be the quantity of A present at the beginning, and $c$ the quantity of catalyst added at the start of the reaction. After a time of $t$ minutes there will be quantities $a - x - y$ of A, $c - y$ of catalyst, $y$ of M, and $x$ of B.

   The two reactions

   $$\mathrm{A} + \mathrm{C} \rightleftarrows \mathrm{M} \text{ and } \mathrm{M} \rightleftarrows \mathrm{B} + \mathrm{C}$$

   will occur at time $t$ with the following rates:

   $$\frac{dy}{dt} = K \cdot (a - x - y)(c - y) \tag{4}$$ Originally equation 1.

   for the first, and

   $$\frac{dx}{dt} = K_1 y \tag{5}$$ Originally equation 2.

   for the second.

   These two differential equations allow a relation between $x$, $t$ and the two constants $K$ and $K_1$ to be obtained, but we shall not give here the complete development of this solution, which leads to rather complicated equations.

   Let us give an example of catalytic activity that belongs to this group, namely the one studied by *Federlin*[11]. The reaction between potassium persulphate and phosphorous acid is extremely slow; it has as chemical formula

   I have not discovered anything about Wilhelm Federlin.

   $$\mathrm{H_3PO_3} + \mathrm{K_2S_2O_8} + \mathrm{H_2O} = \mathrm{K_2SO_4} + \mathrm{H_2SO_4} + \mathrm{H_3PO_4} \tag{6}$$ Originally equation 1.

   This is theoretically a second-order reaction, as one molecule of phosphorous acid reacts with one molecule of potassium persulphate.

   The reaction occurs at an easily measurable rate if one adds to the mixture a little hydrogen iodide, which acts as catalyst, as it does not appear as a definitive product of the reaction, and a given quantity of hydrogen iodide can transform any amounts of phosphorous acid and of persulphate.

[11] Federlin, *Zeitsch. f. phys. Chem.* **41**, 1902, 565

Federlin was able to analyse the action of hydrogen iodide completely. In fact, if one allows hydrogen iodide to act on the potassium persulphate potassium sulphate is formed together with sulphuric acid and free iodine:

$$K_2S_2O_8 + 2HI = K_2SO_4 + H_2SO_4 + I_2 \quad (7)$$

Originally equation 2.

This reaction occurs at an easily measurable rate, and Price's research[12] has shown that the preceding reaction is of second order.

I have discovered nothing further about Price.

The liberated iodine oxidizes the phosphorous acid according to the following equation:

$$H_3PO_3 + I_2 + H_2O = H_3PO_4 + 2HI \quad (8)$$

Originally equation 3.

regenerating hydrogen iodide. This reaction between iodine and phosphorous acid likewise proceeds at a measurable rate, and the reaction is of second order.

In summary, therefore, the transformation of phosphorous acid and potassium persulphate in the presence of hydrogen iodide occurs as a result of two successive reactions that are inverses of one another, transforming hydrogen iodide into iodine and iodine into hydrogen iodide. Application of the law of mass action to each of these reactions gives a complicated formula that expresses the rate of transformation of persulphate and phosphorous acid. This formula does not at all correspond to the second-order rate law, if the reaction 6 were accelerated by a catalyst that did not form an intermediate complex. As, on the other hand, the rates of the two partial reactions 7 and 8 can be determined in isolation one has the possibility to check whether these hypotheses on the way hydrogen iodide acts as catalyst are correct or not. Federlin examined the experimental results and those calculated from theoretical considerations of the law of mass action, and found an excellent agreement.

It is certain that examples of this kind are not rare and can be found among the diastatic reactions.

5. *Action of a catalyst on a series of successive reactions.* In some cases the reaction provoked by a catalyst is not simple, but is followed by a second reaction between the products of the reaction, in such a way that the reaction proceeds in several stages. The catalyst can, in these cases, act differently on each of these stages and thus modify not only the rate curve but also the nature of the products obtained. Let us cite two examples that have recently been studied by *Fanatar*[13]

I have discovered nothing further about Fanatar.

   a) Hydroxylamine decomposes at high temperature to ammonia, nitrogen and water:

$$3NH_3O = NH_3 + N_2 + 3H_2O \quad (9)$$

Originally equation 1. In this series of reactions Henri used the symbol Az (azote) rather than N for nitrogen.

   and this decomposition is accelerated by platinum black, which acts as catalyst, but in this case one has $N_2O$ instead of nitrogen, and the reaction proceeds as follows:

$$4NH_3O = 2NH_3 + N_2O + 3H_2O \quad (10)$$

Originally equation 2.

   b) Hydrazine sulphate decomposes in the presence of platinum black to give ammonia and nitrogen:

$$3N_2H_4 = 4NH_3 + N_2 \quad (11)$$

Originally equation 1.

   The free hydrazine decomposes on contact with platinum black and gives more nitrogen, as well as hydrogen:

$$2N_2H_4 = 2NH_3 + N_2 + H_2 \quad (12)$$

Originally equation 2.

   Finally, in the presence of soda there is a third way in which hydrazine

[12] Price, *Zeit. physik. Chem.* **27**.
[13] Fanatar, *Zeit. ph. Chem* **40**, 1902, 423, and **41**, 1902 37.

decomposes:

$$3N_2H_4 = 2NH_3 + 2N_2 + 3H_2 \quad (13)$$

Originally equation 3.

The author did not give a complete explanation of these differences, but that they must certainly be treated in terms of the hypothesis of successive reactions with formation of intermediate complexes.

This group of catalytic reactions has great interest for the study of diastatic activities, which very often lead to a whole series of stages in succession: pepsin- and trypsin-catalysed digestion, and the saccharification of starch and glycogen are typical examples. One glimpses very well that certain ferments, as for example pepsin, will above all provoke the initial transformations, for example the albumin stage to the peptone stage, and will act only weakly on the later transformation, whereas other diastases, such as trypsin, will act equally well on the transformation of the peptone.

There seems to be no obvious reason why Henri referred to pepsin as a ferment, but trypsin as a diastase. Although the word *diastase* remains in use for enzymes such as maltase, i.e. in a much more restricted sense than used by Henri, *ferment* as a noun has largely disappeared from everyday use in chemistry. So far as the thesis is concerned, the two terms can be regarded as synonyms of one another and of *enzyme*.

It is the same for the activity of amylase, which can, according to the conditions, act almost solely on the transformation of starch into dextrin, or, on the other hand, on the transformation of dextrin to maltose. The law for the total activity will be modified according to whether we have one or the other of these cases, and we shall see later how studying the rate of the whole reaction allows certain conclusions to be drawn about the intermediate reactions through which the reactants pass before arriving at the final state.

**6. Summary of the study of catalytic activities.** From the preceding study we can deduce two essential conclusions.

1. The presence of a catalyst can completely change the rate curve of a reaction, without implying that such a change contradicts the laws of general chemistry: this result is important for studying the diastases. In fact we shall see later that some authors (such as Duclaux), after finding the the inversion of sucrose by invertin follows a different law from that of the acids, have deduced from it that the general laws of chemistry do not apply to the diasases, a conclusion that is not justified, as seen from the preceding paragraph. We have seen not only that the rate curve of a reaction can be changed completely by the presence of a catalyst, but that the products of the reaction can be different according to whether one has this or that catalyst.

2. The second conclusion that follows immediately is that research on the rate laws of catalysed reactions is useful. If one studies the law according to which a catalytic reaction occurs, discussion of the law will allow classification of the reaction studied into one of the preceding groups, and in consequence this study will give important indications about the detailed mechanism responsible for the catalysis. Sometimes the response will not be absolutely unambiguous, but one will be able, in contrast, to affirm with certainty that a whole series of hypotheses about the explanation for the catalytic activity can be ruled out. Thus, for example, we can state after studying the rates of diastatic reactions that Arthus's physical theory is unacceptable.

Nicolas Maurice Arthus (1862–1945) was a French immunologist and physiologist, best known for his work on anaphylaxis. Henri is probably referring to his book *Nature des Enzymes*, published in 1896.

In consequence, the kinetic study of catalytic reactions will always teach us something new about the nature of these reactions. That is why it is necessary to resume the study of the rates of all the diastatic reactions, as it is only in that way that we can arrive at an understanding of their mechanisms.

One may ask, therefore, what are the general rules to be followed in these studies of rates, and how one ought to discuss the results. Although these are

questions of a general nature that are discussed at length in the classic works of van 't Hoff, Ostwald and Nernst, we shall recall here the main points in a few lines.

**7. Main points in the study of the rates of chemical reactions.** The first question that arises in a study of the rates of reactions is determining the order of reaction, and in consequence the law that determines how the reaction occurs.

As the law of mass action predicts that the rate of a reaction is proportional to the product of the active masses of the substances that participate in the reaction, it follows that one will distinguish different orders of reaction according to the numbers of molecules that enter in the reaction. If the chemical reaction occurs in a homogeneous medium according to the equation specifying that $m$A + $p$B + $q$C gives a particular set of products, if $a - x$, $b - x$, $c - x$ are the quantities of A, B, C at a time $t$ and finally $v$ is the volume occupied by the system, the rate of reaction, that is to say $dx/dv$ (expressed in gram molecules) of A, B, C that combine by unit volume during the interval $dt$, will be equal to

$$K \cdot \left(\frac{a-x}{v}\right)^m \left(\frac{b-x}{v}\right)^p \left(\frac{c-x}{v}\right)^q$$

and so one will have the differential equation

$$\frac{dx}{v \cdot dt} = K \left(\frac{a-x}{v}\right)^m \left(\frac{b-x}{v}\right)^p \left(\frac{c-x}{v}\right)^q$$

Integrating, and determining the constant of integration from the initial condition, that is to say $x = 0$ for $t = 0$, one will obtain a certain relationships between the magnitudes $t$, $a$, $b$, $c$, $x$, $v$ and $K$. If one determines the values of $x$ experimentally for particular intervals of time, and substitutes these in the equation, one will be able to study whether the resulting $K$ values are really constant. In practice there are three cases to consider:

1. *First-order reactions.* A single substance A decomposes at a certain rate. In this case $m = 1$ and the rate is equal to

$$\frac{dx}{v \cdot dt} = K \left(\frac{a-x}{v}\right)$$

where $a$ is the quantity of A at the beginning. Integration gives $-\ln(a - x) = Kt + \text{constant}$; for $t = 0$ we must have $x = 0$, so the constant is $-\ln a$, and the law that defines the progress of a first-order reaction has the following form:

$$Kt = \ln\left(\frac{a}{a-x}\right) \tag{14}$$

Originally equation I.

Notice that $v$, the volume occupied by the system, does not appear in this relationship.

A first-order reaction, as for example the inversion of sucrose in the presence of acids, is characterized above all by the logarithmic curve that relates the time and the quantity of substance transformed, and by the independence of the volume occupied. Thus when inversion of sucrose is brought about by an acid in solutions of different concentrations containing 10, 20 and 30 g/L of sugar, the same *proportion* of sugar will be inverted in these solutions at the end of the same time. For example, after an hour 1 g of sugar will be inverted in the first case, 2 g in the second, 3 g in the third, etc.

In consequence, to study whether a reaction is of first order, one must determine a series of value of $x$ corresponding to the different times, then if

the expression $K = \frac{1}{t} \ln \frac{a}{a-x}$ is calculated and found to remain constant, then the rate curve does correspond to a first-order reaction. However, one cannot yet deduce that the reaction is truly of first order: this would be precisely the error made by O'Sullivan and Tompson in the discussion of the reaction produced by invertin. One must calculate the rates for different values of $a$, that is to say for different concentrations of the substance to be transformed, and if one finds that at the end of the same amount of time the same proportion of this substance is transformed one will be able to state that the reaction is truly of first order.

Cornelius O'Sullivan (1841–1907) was an Irish chemist, and Frederick William Tompson (1859– 1930) was a British chemist, both of them working in the brewing industry.

2. *Second-order reactions.* This type of reaction is represented by the chemical formula A + B = AB. If $a$ and $b$ are the quantities in gram molecules of A and B at the beginning, $x$ the amount of AB formed after $t$ minutes, and $v$ the volume of the mixture, one will have, at time $t$, $(a-x)/v$ per litre of A, $(b-x)/v$ of B, and the rate will be given by the following expression:

$$\frac{dx}{v \cdot dt} = K \cdot \frac{a-x}{v} \cdot \frac{b-x}{v}$$

a) A and B are present in equimolar amounts. Then $a = b$ and the rate expression is

$$\frac{dx}{v \cdot dt} = K \cdot \left(\frac{a-x}{v}\right)^2$$

As written by Henri this equation contained an error, as he cancelled $v$ on the right-hand side while leaving it on the left-hand side.

Integration gives

$$\frac{v}{a-x} = Kt + \text{constant}$$

As $x = 0$ for $t = 0$, the constant must be $v/a$, and so the expression for the law of the reaction is

$$\frac{K \cdot at}{v} = \frac{x}{a-x} \qquad (15)$$

Originally equation II.

We see that the relation between $t$ and $x$ depends on the volume occupied by the mixture, and in consequence on the concentrations of A and B.

b) The quantities $a$ and $b$ are not equal. In this case

$$\frac{dx}{v \cdot dt} = K \cdot \frac{(a-x)(b-x)}{v^2}$$

From which, after integration,

$$\ln \frac{b(a-x)}{a(b-x)} = \frac{(a-b)}{v} \cdot K \cdot t \qquad (16)$$

Originally equation II$^{\text{bis}}$.

Thus one will have a second-order reaction when $x$ and $t$ are related to one another by equation 2a or 2b, and in addition the rate will become smaller as the volume increases. Two-fold dilution of the system will decrease the rate two-fold. So, suppose that we have two reactions of saponification of methyl acetate by soda, the first with 100 cm$^3$ of methyl acetate and 0.1M soda, the second with 100 cm$^3$ of methyl acetate and 0.05M soda. If a quarter of the acetate in the first flask is saponified at the end of 30 min, then only one-eighth of the acetate in the second will be saponified. This is an essential difference from the reactions of first order.

Henri expressed concentrations (here and later) in terms of the obsolete unit "normal", which for the specific substances mentioned is the same as molar.

c) *Third-order reactions.* The type of reaction is A + B + C = ABC, and the rate is expressed by

$$\frac{dx}{v \cdot dt} = K \cdot \frac{a-x}{v} \cdot \frac{b-x}{v} \cdot \frac{c-x}{v}$$

and must therefore depend on the square of the volume of the mixture. The full discussion is quite complicated and will take us away from our subject.

These are the essential points that must be regarded as basic in the study of reaction rates, and it is in relying on these principles that we now undertake the study of diastatic activities.

# Chapter I

## History of research on the laws of action of diastases

**8. O'Sullivan and Tompson's research.** The number of studies that have been made on the diastases is very large, but only a few authors have systematically studied the general laws of action of diastases. I shall pause only for the work that specifically concerns this point, those of O'Sullivan and Tompson, Tammann, Duclaux and Brown.

In 1890 O'Sullivan and Tompson published a very detailed study of invertin[14] in which they studied the law of action of this ferment. A measured quantity of invertin was dissolved in water, brought to the experimental temperature and mixed with a certain volume of sucrose solution. To activate the diastase they added a very small amount of acid. At specified times they removed a certain quantity of the solution and poured it into a glass containing some drops of soda, which is sufficient to stop the invertin action. The dosing with sugar was done in the polarimeter.

Gustav Heinrich Johann Apollon Tammann (1861–1938) was a German chemist of Estonian and German origin. His work was mainly in metallurgy.

They constructed the curve representing the progress of the reaction and compared it with what one would obtain with the action of acid on sucrose. They concluded that the two curves were almost exactly the same, from which they deduced that the action of invertin on sucrose follows the logarithmic law of monomolecular reactions, and in consequence the laws of action of invertin and acids are identical.

The authors drew this conclusion by studying the results obtained for three experiments on the rate of inversion. They noticed that in these three cases there were small discrepancies between the logarithmic curve and the curves observed, and that these discrepancies were in the same direction, but they did not study this point, and did not calculate the constant of inversion $K$ given by the relationship

$$K = \frac{1}{t} \log \frac{a}{a - x}$$

The function log used in this equation is probably not intended to be different from ln used in earlier equations, i.e. it does not represent $\log_{10}$ as it would do in present-day biochemical practice.

When this calculation is made for O'Sullivan and Tompson's results $K$ does not remain constant, but increases regularly from the beginning to the end (see the results on the next page).

It can be seen that the values of $K$ increase in a regular manner, so the law followed during the inversion is not exactly the logarithmic law. However, the main error made by O'Sullivan and Tompson in their equating the action of invertin with those of acids came from the fact that they did not study the effect of the sucrose concentration, which would have revealed immediately to them a profound difference between these two activities. This study was done by Duclaux, who showed O'Sullivan and Tompson's error in their conclusions.

[14] [C.] O'Sullivan and [F. W.] Tompson "Invertase: [a contribution to the history of an enzyme or unorganised ferment]", *Journal of the Chemical Society [(Transactions)]*, 1890, **57**, p. 834–931. Henri gave the volume number as **LVII**, and the final page number as 930.

However, before discussing Duclaux's work we shall examine that of Tammann, which appeared at the same time as O'Sullivan and Tompson's.

| Times (minutes) | Proportions inverted | $K = \frac{1}{t} \log \frac{a}{a-x}$ |
|---|---|---|
| | *First series* | |
| 5 | 0.031 | 0.002736 |
| 15 | 0.098 | 0.002986 |
| 30 | 0.192 | 0.003086 |
| 57 | 0.336 | 0.003120 |
| 90 | 0.458 | 0.002956 |
| 120 | 0.585 | 0.003183 |
| 150 | 0.674 | 0.003245 |
| 182 | 0.745 | 0.003261 |
| 210 | 0.798 | 0.003308 |
| 240 | 0.844 | 0.003362 |
| 270 | 0.873 | 0.003319 |
| 381 | 0.935 | 0.003116 |
| | *Second series* | |
| 4 | 0.049 | 0.005455 |
| 19 | 0.192 | 0.004873 |
| 39 | 0.353 | 0.004049 |
| 59 | 0.495 | 0.005029 |
| 79 | 0.618 | 0.005290 |
| 99 | 0.710 | 0.005430 |
| 132 | 0.819 | 0.005624 |
| 164 | 0.887 | 0.005774 |
| | *Third series* | |
| 5 | 0.031 | 0.002736 |
| 17 | 0.088 | 0.002354 |
| 35 | 0.183 | 0.002508 |
| 55 | 0.277 | 0.002561 |
| 78 | 0.373 | 0.002599 |
| 108 | 0.490 | 0.002708 |
| 139 | 0.592 | 0.002801 |
| 170 | 0.667 | 0.002809 |
| 204 | 0.743 | 0.002892 |
| 235 | 0.796 | 0.002938 |
| 359 | 0.908 | 0.002886 |

**9. Tammann's research.** Tammann devoted three articles[15] to the study of the laws of action of diastases. He reported experiments carried out with emulsin and invertin, and examined the influence of a whole series of different factors.

He first set out to show that the actions of these ferments are not complete. A given quantity of emulsin only transforms a certain fraction of amygdalin, salicin, arbutin or aesculin; likewise a particular quantity of invertin can only transform a part of the sucrose to which it is added. This fraction varies with the temperature, the amount of ferment and the amount of substance to be transformed; it also

These are various glycosides, of which aesculin is also sometimes written as esculin or as æsculin.

[15][G. Tammann [(1889)] *Zeitschrift für Physikalische Chemie* **3**, [27], and [G. Tammann [(1895) "Zur Wirkung ungeformten Fermente"] *Zeitschrift für Physikalische Chemie* **18**, [426–442]; [G. Tammann (1892) "Die Reactionen der ungeformten Fermente"] *Zeitschrift für Physiologische Chemie* **16**,[ 271–328].

depends on the accumulation of products in such a way that if these products are eliminated the reaction proceeds further. This limiting proportion is not, according to Tammann, a state of equilibrium, because when a certain amount of products are added one does not see the limit decrease.

This set of results, which led Tammann to the general law that the diastatic actions are incomplete, was derived from experiments that are not all equally valuable. Aside from some experiments, such as those on the action of emulsin on amygdalin, aesculin and arbutin, that are not directly subject to causes of error, we think that some experiments on salicin, and above all on invertin, must be criticized. In fact the author studied series of reactions that lasted more than 24 hours, but he took no precautions against the development of microorganisms. He said himself (*Zeits. physiol. Chem.*, p. 280) that in an experiment on the inversion of sucrose during 3046 minutes, about 500 minutes after the start the solution began to be turbid, following the development of microorganisms. It is not surprising in these conditions that the reaction did not go to completion.

Likewise in some tables on the action of emulsin on salicin that purport to show that the reaction does not go to completion, the author reported that after a certain time it was impossible to obtain a polarimetric reading as the liquid had become turbid. We now know what an important role microorganisms can play in diastatic reactions, whether by increasing the activity or by eliminating it completely. Therefore for this first part of Tammann's work we must be cautious about the action of invertin on sucrose and that of emulsin on salicin. We shall show later that in our conditions these reactions did go to completion.

In relation to the result announced by Tammann that in the case of the diastatic reactions that did not go to completion there was not a state of equilibrium, we also know that the work of Hill, Emmerling and E. Fisher showed the opposite. For the action of maltase on maltose, of emulsin on amygdalin and of lactase on lactose we definitely reach equilibrium, which allows synthesis of these sugars from their decomposition products.

Tammann afterwards examined the rate law of the diastatic activities and found that this law is not simple, depending on the quantity of ferment added to the solution. Thus, for the action of invertin on sucrose he gave ten series of experiments carried out with the same quantity of sucrose and quantities of invertin varying from 0.920 down to 0.001 g. He drew the curves representing the rates of inversion and saw that the shapes were very different according to the dosage of ferment: for large amounts of ferment the rate of inversion continuously decreased, whereas for low amounts it increased more and more with time. This result led him to state that the reaction of invertin with sucrose follows a very complex law, clearly different from the logarithmic law of the acids.

Henri wrote 0.920 g etc. as 0 gr. 920 etc., but it seems clear that the amounts need to be interpreted as here.

When the tables of Tammann's results are examined more closely it can be seen that for the four first series, with quantities of invertin equal to 0.92, 0.46, 0.23, 0.092 g, the rate decreases progressively from the beginning to the end of the reaction. In this series the dosages made during the first 500 min indicate already transformation of more than half of the sugar. On the other hand for the next ten series, after 500 min the transformation is either very slight, or even not detectable at all, and the author gave values measured for much longer period, as much as 5437 min. It was for these periods exceeding 24 h that one finds the anomaly mentioned earlier whereby the rate increased with the time. As he did not say what precautions were taken to avoid the development of microorganisms, it to be feared that these anomalies resulted from this source of error that arise very easily in experiments with nutritive media as rich as 20%.

We must therefore consider only the first four series, and we see in studying them that the expression $K = \frac{1}{t} \log \frac{a}{a-x}$ calculated from these experiments increases regularly from the beginning of the reaction. Here (p. 23) are the results for the first series. This result is thus well in agreement with that obtained by

O'Sullivan and Tompson.

Finally Tammann examined at length the effect of temperature on the diastases, but as we shall not concern ourselves at all with the effect of temperature we shall leave this aspect of Tammann's research completely on one side.

The general conclusion of the author is therefore that the diastases do not follow the same laws as the catalytic activities in general chemistry, and that it is very difficult to find the laws of diastatic action.

| Time (minutes) | Proportion inverted | $K = \frac{1}{t} \log \frac{a}{a-x}$ |
|---|---|---|
| 14 | 0.054 | 0.00172 |
| 35 | 0.170 | 0.00231 |
| 58 | 0.290 | 0.00247 |
| 83 | 0.450 | 0.00271 |
| 100 | 0.481 | 0.00285 |
| 124 | 0.59 | 0.00300 |
| 160 | 0.68 | 0.00309 |
| 202 | 0.767 | 0.00313 |
| 299 | 0.85 | 0.00274 |

**10. Duclaux's research.** In 1898 Duclaux described, in the *Annales* of the Pasteur Institute and in the II$^{nd}$ volume of his *Microbiologie*, a general trial of great importance on the laws of diastatic activity. He began by criticizing O'Sullivan and Tompson's conclusion about the identity of diastatic activity with the effects of acids. This criticism rests on results obtained with different concentrations of sucrose.

In 1878 Barth[16] and in 1883 Duclaux had seen that if one put the same amount of invertin in the presence of increasing doses of sucrose, the quantities of sucrose inverted at the end of the same time interval did not at all vary in proportion to the concentration of sugar, as is the case with the action of acids; on the contrary, for moderately concentrated solutions, between 5 and 15% in Barth's case and between 10 and 40% in Duclaux's, the same amount of sugar is inverted regardless of the sugar concentration. Here are Barth's results from these experiments:

| Sucrose concentration grams per 100 cm$^3$ | mg sugar inverted after 30 min |
|---|---|
| 0.5 | 20 |
| 1.0 | 43 |
| 2.5 | 65 |
| 5.0 | 100 |
| 7.5 | 100 |
| 10.0 | 104 |
| 15.0 | 104 |
| 20.0 | 83 |

Duclaux noticed that this property is not particular for invertin, but it applies also to other diastases, for example to the amylase of urine, according to Dubourg's work. A second point that Duclaux stressed is the result observed by many authors, that at the beginning of a diastatic reaction the rate is constant, and the quantity of substance transformed is proportional to the time, so the representative curve is thus a straight line; this applies up to the transformation

Henri is probably referring to E. Dubourg (1889) *Recherches sur l'amylase de l'urine*, G. Gounouilhou, Bordeaux

[16][M.] Barth (1878) ["Der Kentniss des Invertins"] *Berichte der Deutschen Chemischen Gesellschaft zu Berlin* [**11**, 474–482], p. 481

of about 20% of all the material present in the mixture. Finally, Duclaux stressed the inhibitory effect exerted by the reaction products.

In the basis of this collection of facts, Duclaux constructed a general theory for the action of diastases. From the beginning of this theory he renounced the laws of general chemistry, in affirming that the action of a diastase on a substance to be transformed is independent of the quantity of the substance and depends only on the duration, in such a way that the rate has for expression $dx/dt = K$: it is constant, so the curve is therefore a straight line.

But this is only the first aspect of the phenomenon, and to it is added a second, the effect of the reaction products, an inhibition that explains why the rate decreases as the reaction proceeds. This inhibitory action of the reaction products on diastase is different, according to Duclaux, from the action exerted by the ferment on the substance to be transformed: it depends on the quantity of reaction products. However, here he allows a process contrary to the law of mass action to occur: in effect, the inhibition would be proportional to the quantity of substrate present at the beginning of the reaction. Thus, if at some moment we have quantities $a - x$ of substrate, $a$ being the quantity at the beginning, and $x$ is the quantity of product, the inhibition will be proportional to the ratio $x/t$. He advanced this hypothesis in a completely arbitrary way, and it does not correspond to any theoretical consideration of the nature of the inhibitory action. The rate of diastatic action is represented by the following equation:

Henri referred to the definitive rate of reaction (*la vitesse définitive de l'action diastasique*), but this does not seem to correspond to any normal meaning of *definitive*, and the word is therefore omitted.

$$\frac{dx}{dt} = K - K_1 \frac{x}{a}$$

or

$$\frac{dx}{dt} = \frac{1}{a}(Ka - K_1 x)$$

Integration gives

$$-\frac{1}{K_1} \ln(Ka - K_1 x) = \frac{t}{a} + \text{constant}$$

Noting that $x = 0$ when $t = 0$ we can evaluate the constant:

$$\text{constant} = -\frac{1}{K_1} \ln Ka$$

so that the definitive equation is

$$t = \frac{a}{K_1} \ln \frac{Ka}{Ka - K_1 x}$$

This equation contains two constants, $K$ and $K_1$. If the observed reaction goes to completion, as happens with invertin, one must have $K = K_1$, and this equation takes the form

$$K = \frac{a}{t} \ln \frac{a}{a - x} \tag{17}$$

Originally equation I.

and it is seen that the expression differs from the law for a first-order process only by the factor $a$.

Equation 17 expresses, therefore, on the one hand that the rate of reaction is defined by a logarithmic curve, as found by O'Sulivan and Tompson, but on the other hand that for different sucrose concentrations the *absolute* amount of sugar inverted at the end of the same time is the same in all the solutions, in agreement with the results of Duclaux, Barth and Dubourg.

Several criticisms need to be made of equation 17. First of all, so far as the form of the curve is concerned, it is found in reality that the curve is more rapid than the logarithmic curve. The values of $K$ calculated according to equation 17 for inversion increase in a regular manner from the beginning to the end.

Secondly, the result on the independence of the sucrose concentration of the quantity transformed is correct only for intermediate concentrations. For dilute

solutions Barth had found that the amount of sugar inverted after a certain time became smaller and smaller as the solution became more dilute. Tammann's experiments (pp. 315 and 316) confirm that it was the same for the action of emulsin on amygdalin and of invertin on sucrose. Finally Brown's results, and our own that will be reported later, show that the result of independence of concentration is true only for solutions at intermediate concentrations.

Finally Duclaux's theory has the fault that it assumes two different rate laws for the effect of the diastase on the substrate and for the effect of the reaction products on the diastase, and neither of these laws corresponds to the law of mass action. The diastases are envisaged as obeying their own laws, constituting therefore a class that must be treated apart from the laws of general chemistry.

**11. A. Brown's research.** Let us now pass to the work recently published by *Adrian J. Brown*[17]. In 1892 he showed that the action of brewer's yeast on sugar during alcoholic fermentation did not follow the logarithmic law of acids, but that the rate was represented by a straight line. He has now taken up the question again for invertin, and shown first of all that in studying the inversion of sucrose by invertin the rate follows a law more rapid than the logarithmic law. The expression

$$K = \frac{1}{t} \log \frac{a}{a - x}$$

increases continuously from the beginning to the end. Here are the numerical values for two of his experiments.

Henri does not give a reference for the paper of 1892: A. J. Brown (1892) "Influence of oxygen and concentration on alcohol fermentation" *Journal of the Chemical Society (Transactions)* **61**, 369–385.

| Times (minutes) | Proportions inverted | $K = \frac{1}{t} \log \frac{a}{a - x}$ |
|---|---|---|
| *First series*: 9.48 g sucrose in 100 cm$^3$ | | |
| 30 | 0.265 | 0.00445 |
| 64 | 0.509 | 0.00483 |
| 120 | 0.794 | 0.00571 |
| 180 | 0.945 | 0.00698 |
| 240 | 0.983 | 0.00737 |
| 300 | 1.003 | 0.00737 |
| *Second series*: 19.28 g sucrose in 100 cm$^3$ | | |
| 30 | 0.130 | 0.00201 |
| 64 | 0.256 | 0.00201 |
| 120 | 0.454 | 0.00219 |
| 180 | 0.619 | 0.00232 |
| 240 | 0.738 | 0.00242 |
| 300 | 0.831 | 0.00257 |
| 360 | 0.890 | 0.00265 |
| 420 | 0.935 | 0.00283 |
| 480 | 0.961 | 0.00293 |
| 540 | 0.983 | 0.00327 |
| 581 | 0.990 | 0.00344 |

Afterwards he showed that for solutions at moderate concentrations (from 5 to 30%) a given quantity of invertin hydrolyses the same amount of sucrose after a given time. Here are some numerical examples:

[17] Adrian J. Brown, Enzyme action, *Journal of the Chemical Society [(Transactions)]*, April 1902, **[81]**, pp. 373–388.

| Sucrose concentration grams per 100 $cm^3$ | mg sugar inverted after 60 minutes |
|---|---|
| 4.89 | 1.230 |
| 9.85 | 1.355 |
| 19.91 | 1.355 |
| 29.96 | 1.235 |
| 40.02 | 1.076 |

This result agrees well with those of Barth, Duclaux and Tammann. In consequence, the author concluded, invertin does not the following the law of mass action in the way that it applies to the action of acids.

A. Brown tried next to give a theory that would explain the action of invertin. He supposed that the ferment forms an intermediate complex with part of the sucrose which persists for a certain time and then decomposes to give invert sugar and the free ferment. Thus the complex does not decompose immediately but persists for a certain period of time, a given amount of ferment being able to give rise to these transformations a limited number of times. In consequence, if the amount of sucrose is large compared with that of the diastase the transformation will be independent of the sucrose concentration. On the other hand, if the amount of sucrose is small the number of transformations per unit of time does not reach its maximum, and then the sucrose concentration affects the rate of inversion. The author thus studied very dilute solutions and found that the experiment agreed well with his theoretical expectations. Here are the remainder of the numerical results:

| Sucrose concentration grams per 100 $cm^3$ | mg sugar inverted after 60 minutes |
|---|---|
| 2.0 | 0.308 |
| 1.0 | 0.249 |
| 0.5 | 0.129 |
| 0.25 | 0.060 |

It is plainly evident that for these dilute solutions the rate of inversion depends on the sucrose concentration.

A. Brown's theory is very incomplete. It supposes first of all that the intermediate complex persists for a minimum time before decomposing, but he does not say whether he accepts that the combination occurs very fast and that the decomposition is also very fast, in such a way that it is absolutely impossible to write equations for the process. General chemistry teaches us that we cannot accept a rapid formation of an intermediate complex that would remain intact for a certain time and then abruptly decompose: chemical phenomena do not exhibit discontinuities of this kind, so either the formation of the intermediate complex is slow, or its decomposition is slow, or both processes are slow. Whatever hypothesis we make we will not arrive at an equation that represents the known fact about the inversion of sucrose, and these hypotheses take no account of the effect of invert sugar on the rate of reaction.

Now invert sugar does slow down the action of the ferment, and Brown himself gave some relevant results, but he took no account of them in his theory. This theory is thus seriously incomplete and in no way does it allow the law of action of invertin on sucrose to be predicted.

**12. H. Brown and Glendinning's research.** Following A. Brown's work Horace T. Brown and Glendinning[18] have published some research on the hydrolysis of

[18][H. T. Brown and T. A. Glendinning] "The velocity of hydrolysis of starch by diastase, with some remarks on enzyme action" *Journal of the Chemical Society [(Transactions)]*, April 1902, **[81]**, pp. 388–400.

starch by amylase and they also established a theory of the action of diastases.

So far as the hydrolysis of starch is concerned they show that the law is the same as for invertin; we shall come back later to these experiments. The theory developed by these authors is different from that of A. Brown, having the advantage over this in being more complete and of not allowing the existence of complexes that persist without decomposiing during a minimum time.

Horace Tabberer Brown (1848–1925) was a British chemist who worked in the brewing industry. T. A. Glendinning (born 1878, died after 1963) was a British (later New Zealand) chemist in the brewing industry.

This theory belongs to the group of catalysed reactions with rapid formation of intermediate complex with slow decomposition. We have previously shown (§5, ¶3a) that in this case, if the amount of catalyst is small in comparison with the amount of substrate, the rate curve will initially be a straight line, becoming logarithmic after a certain time, so that it is clear that the rate is independent of the sucrose concentration at moderate concentrations, but, on the other hand, it is dependent for dilute solutions.

This theory well explains some aspects of the activity of diastases, but it cannot provide a complete representation of the progress of the reaction. In fact, the invert sugar is not taken into account in this theory; the authors claim that for dilute solutions the products of the reaction have no important effect, which disagrees with the experimental facts that will be presented later. Let us give a numerical example to show that the theory is incomplete.

Suppose that we have two sucrose solutions of 5% and 10%, and let us add the same amount of diastase to each: we shall see that after an hour there will be the same number of grams inverted in the two solutions, that is to say 1.2 g in each.

Suppose now that we added at the beginning the same amount of invert sugar, 5 g for example, so that the first solution will contain 5 g sucrose + 5 g invert sugar, and the second will contain 10 g sucrose + 5 g invert sugar. As the amount of sucrose is clearly greater than the amount of invertin added in each case, according to the authors' theory we should again find the same amount of invert sugar after an hour, but experiment shows that that is not at all the case: one may find, for example, 0.8 g sucrose hydrolysed in the first solution after an hour, and 1.0 g in the second. There will be a very clear difference.

The whole set of facts of this kind, together with the results for the action of invertase on sucrose and emulsin on salicin, cannot be explained by these authors' theory.

**13. Herzog's experiments.** We must finally mention the research on the law of action of the diastases published by Medwedew[19] on the oxidases and by Herzog[20] on alcoholic fermentation. Medwedew above all studied the effect of the concentration of the ferment.

Herzog measured the rate of alcoholic fermentation of glucose and of laevulose by Buchner's zymase, by determining the amount of carbonic acid liberated after different times. These experiments, still preliminary, showed that the expression $K = \frac{1}{t} \log \frac{a}{a-x}$ remains approximately constant during a reaction, the rate curve seeming to be a logarithmic curve, but comparing the results for different sugar concentrations one sees that the activity follows the same law as that for invertin or salicin: the value of $K$ changes when one passes from one concentration to another and the product $aK$ (which according to Duclaux's theory should remain constant) increases as the concentration $a$ decreases.

[19] [A. Medvedew "Ueber die Oxydationskraft der Gewebe" *Pflügers Archiv* **65** (1896), p 249[–277]; ["Ueber die oxydativen Leistungen der thierischen Gewebe"] **74** (1899), p. 193[–224], "Ueber die oxydativen Leistungen der thierischen Gewebe"**81** (1900), p. 540[–573]: Henri gives the page number for the last of these as 340.

[20] [R. O. Herzog "Ueber alkoholische Gährung"] *Zeitschrift für Physiologische Chemie*, **37**, 1902, 149[–160].

**14. Summary of the history.** In summary, the preceding historical summary shows us that the experimental study of the laws of action of the diastases is only, to say the least, sketched out. The number of systematic experiments carried out until now is too small for one to be able to use them with the aim of constructing any theory.

One fact comes clearly out of this collection, that the diastatic actions do not apparently occur in the same way as those produced by acids. Therefore, in view of what was said in the Introduction, one must concluded that if the diastatic activities are ranked with the catalytic reactions of general chemistry they belong to a different group from those due to acids.

Two principal problems arise in the study of diastatic activities:

1. It is necessary to undertake systematically the experimental study of the question, varying the conditions in as complete a way as possible, and being guided continuously by the methodology of general chemistry.

2. One this experimental study is finished, one must discuss the results in the context again of the laws of geneal chemistry, and examine whether one can arrive at a general conception of the laws of action of diastases in terms of the law of mass action, and without recourse to *sui generis* properties that would apply only to the diastases and would make of them a separate class of phenomena.

These are the two problems that I decided to study.

# Chapter II

## Experiments on invertin

**15. Method.** The invertin used for the experiments was prepared from brewer's yeast, which I ground up with sand, placed in chloroform-saturated water for two to four days in a water batch at 25°, filtered, precipitated with alcohol and dried the resulting precipitate. Five different preparations have been used for the experiments, prepared with different samples of yeast. In adition, in some experiments I have used commercial invertin from "Merck". All these preparations gave similar results.

The sugars used — sucrose, glucose, laevulose — (obtained from Kahlbaum and Schuckhardt) were checked in the polarimeter, with Fehling's reagent and with osazones. Their purity in relation to saline substances was determined by measuring the electric conductability of the solutions: this conductability was very slight.

The dosages were made by means of the polarimeter, except for very dilute solutions, for which I used at the same time determinations with Fehling"s reagent. The precision of the polarimeter readings was quite sufficient. With the Laurent polarimeter and a 22 cm tube one succeeds, with practice, to read with a maximum deviation of 2 min, and as in the majority of experiments the rotation of the plane of polarization was around 10° it can be seen that the reading errors became negligible.

The experiments were done in the great majority of cases at a temperature of 25°, the variations in the temperature of the water bath never reaching 0.5° during a series of experiments. There is an exception for the series done in July 1901 when the external temperature was 29–30° and the temperature of the water bath 21–33°, and one sees that the results of this series are not as regular as those done in other seasons.

In view of the impossibility of dosing the amount of ferment in such a way as to allow comparison between experiments done of different days, it was necessary to make several series the same day and make comparisons among themselves of experiments done the same day with the same invertin solution. This is a complication that one does not have in experiments with acids, and that forces one to make many experiments the same day according to a plan worked out in advance.

A very important point concerns the way the solutions of ferment are prepared. A certain quantity of dried invertin (about 1 dg) was diluted in a mortar wth water (50 $cm^3$, then filtered four or five times, first with simple filters, then with hardened filters from Schleicher and Schühl. In this way an opalescent solution is obtained. If this solution, which appears homogeneous, is left at rest for 2–3 hours, it is noticed that a very light precipitate is formed, or more exactly the lower layer is less opalescent than the upper layer: it is then necessary to filter it several more times, discarding some $cm^3$ of the base. After this, the solution of ferment is left in the water bath at 25° for one or two hours more, and in the moment of using it it is agitated, for extra precaution.

Fehling's reagent, obtained by mixing solutions of copper sulphate and potassium sodium tartrate, is still used as a test for monosaccharides, for example in diagnosing diabetes. It was developed by H. Fehling (1849) "Die quantitative Bestimmung von Zucker und Stärkmehl mittelst Kupfervitriol" *Annalen der Chemie und Pharmacie* **72** 106–113.

Hermann von Fehling (1812 –1885) was a German chemist. His test for monosaccharides is his best-known work.

I stress these points, which are important, because only after taking account of these precautions can one obtain precise and comparable results. In almost all the experiments I made at least two identical experiments as controls, and these two experiments were launched one at the beginning, the other at the end of the series.

The sugar solutions were prepared several hours before the experiments, boiled and brought to the volume necessary at 25° in calibrated flasks at this temperature, in such a way that a normal solution of sucrose meant a solution containing 342 g sucrose in 1L at 25°.

It is important to take account of microorganisms. A great many series were done with solutions containing 0.5 or 1% of sodium fluoride; many series of duration less than ten hours were done with sodium fluoride, and I made many control experiments that showed that in these conditions the errors that could be brought by the microorganisms were insignificant.

The dose of sodium fluoride chosen does not affect the activity of invertin. Several experiments have shown that for weaker doses there was acceleration, and for weaker does the reaction was slowed down. For the rest, the results of a series with sodium fluoride were never combined with those of experiments without fluoride.

**16. Study of the rate of inversion.** We have seen in the historical part that according to O'Sullivan and Tompson the progress of the inversion of sucrose was represented by the logarithmic law of acids, and most authors have considered this result to be well established. I began by doing experiments with as much care as possible, in such a way as to study whether the progress of an inversion produced by invertin really corresponded to the logarithmic law.

I brought 50cm$^3$ of a solution of sucrose to a temperature of 25°, and at given moment I added 2 to 4 cm$^3$ of an invertin solution, also brought to 25°. Immediately after mixing I poured the solution into the polarimeter tube, warmed to 25°, and I made the first reading. Between the moment of mixing and this first reading, which was taken as the starting point, there was an intervals of 2 to 3 minutes. Then I made polarimeter readings at specified times.

If $\alpha_0$ is the rotation of the plane of polarization in the first reading, $\alpha_1$ the reading when all the sucrose is inverted, and finally if $\alpha$ is the reading made after $t$ minutes after the start of the reaction, the ratio $(\alpha_0 - \alpha)/(\alpha_0 - \alpha_1)$ gives the proportion of sucrose inverted at this moment, and this is thus the value of the ratio designated $x/a$ given by the calculation.

Henri applied the definitions of $\alpha_0$ and $\alpha_1$ given here to $\alpha_1$ and $a_1$ respectively. These are clearly incorrect (in particular, $a_1$ is not mentioned again), and the later analysis makes it clear that $\alpha_0$ is the initial reading and $\alpha_1$ is the reading after $t$ minutes. I have therefore replaced his definitions with ones I believe to be correct.

*Example.* For a solution containing 50 cm$^3$ of 0.5M sucrose with 4 cm$^3$ of ferment solution added, the initial rotation is $\alpha_0 = +22°25'$, when the reaction was finished the rotation became $\alpha_1 = -7°5'$, and 115 min after the start it was $\alpha = +13°5'$, so the proportion of sucrose inverted at that moment was $(22°25' - 13°5')/(22°25 + 7°5') = 0.316$, that is to say 31.6% of the sugar was inverted during the first 115 min: it is this ratio 0.316 that is represented by the fraction $x/a$.

The logarithmic formula such as is obtained by the action of acids on sucrose gives the following expression for the constant of inversion:

$$K = \frac{1}{t} \log \frac{a}{a - x}$$

or

$$K = \frac{1}{t} \log \frac{1}{1 - \frac{x}{a}}$$

When values of this expression are caculated for different measures made during an inversion produced by invertin it is seen that it does not remain constant, but shows a very regular increase. Here are some examples that shows these

variations, with values of $K$ multiplied by $10^5$, so a tabulated value of 105 corresponds to $K = 0.00105$:

| Times (minutes) | Proportions inverted, $x/a$ | $K \cdot 10^5$ |
|---|---|---|
| I. 24 *February* 1901, 0.5M sucrose | | |
| 26 | 0.061 | 105 |
| 115 | 0.316 | 143 |
| 182 | 0.487 | 159 |
| 311 | 0.713 | 174 |
| 373 | 0.789 | 174 |
| 511 | 0.892 | 181 |
| 630 | 0.932 | 185 |
| II. 2 *February* 1901, 0.5M sucrose | | |
| 66 | 0.038 | 25 |
| 177 | 0.097 | 26 |
| 330 | 0.186 | 27 |
| 487 | 0.267 | 28 |
| 698 | 0.372 | 29 |
| 1358 | 0.628 | 32 |
| 1959 | 0.777 | 33 |
| III. 2 *February* 1901, 0.2M sucrose | | |
| 81 | 0.101 | 57 |
| 181 | 0.230 | 63 |
| 344 | 0.427 | 70 |
| 502 | 0.577 | 75 |
| 709 | 0.735 | 81 |
| 1369 | 0.938 | 88 |
| IV. 2 *February* 1901, 0.2M sucrose | | |
| 66 | 0.084 | 58 |
| 168 | 0.220 | 64 |
| 334 | 0.426 | 72 |
| 488 | 0.581 | 77 |
| 696 | 0.746 | 85 |
| 1356 | 0.952 | 97 |

It is clear that the values of $K$ increase from the beginning of the reaction to the end: inversion of sucrose by invertin follows more rapid law than that for inversion by acids. On p. 31, in contrast, is an example of an inversion produced by 0.4M hydrochloric acid.

Once this first result was obtained several questions arose. It was first necessary to look for an empirical formula to represent the progress of a reaction, and then to study if the increase in the value of $K$ did not correspond to changes in the ferment other than those due to effects of the sucrose and the invert sugar. It was necessary to ask if the activity of the ferment changed during the reaction, that is to say if the ferment remained comparable with itself during a reaction.

| Times (minutes) | Proportions inverted, $x/a$ | $K \cdot 10^5$ |
|---|---|---|
| 4 *January* 1901, 1M sucrose + 0.4N HCl | | |
| 91 | 0.220 | 118 |
| 226 | 0.489 | 129 |
| 288 | 0.571 | 128 |
| 385 | 0.675 | 127 |
| 506 | 0.774 | 128 |
| 556 | 0.806 | 128 |
| 866 | 0.923 | 129 |
| 1545 | 0.986 | 120 |

**17. Empirical formula for the progress of an inversion.** To search empirically for the formula, I followed a procedure recommended by Ostwald in his *Traité de chimie générale* (vol. II, 2, p. 265). As the value of $K$ increases as the proportion of sucrose inverted increases, one can replace the expression for the rate of reaction $K$ by $K_1(1 + \varepsilon x/a)$, where $K_1$ and $\varepsilon$ are two constants. The rate of reaction at time $t$ is then

The reference appears to be to a French translation of W. Ostwald (1896) *Lehrbuch der allgemeinen Chemie*, W. Engelmann, Leipzig.

$$\frac{dx}{dt} = K_1 \left(1 + \varepsilon \frac{x}{a}\right)(a - x)$$

Integration and determining the constant of integration such that $x = 0$ when $t = 0$ gives the following relationship:

$$K_1(1 + \varepsilon) = \frac{1}{t}\left[\ln \frac{a}{a - x} + \ln\left(1 + \varepsilon \frac{x}{a}\right)\right] \tag{18}$$

Originally equation I.

If for several series of measurements the values of $\varepsilon$ are calculated that correspond to the condition that $K_1$ remains constant during the whole course of reaction, one finds as values of $\varepsilon$ 1.02, 1.04, 0.98, 1.05, 1.01, etc., that is to say values very close to unity. It is reasonable therefore to replace $\varepsilon$ by unity, and equation 18 becomes simpler and takes the following form:

$$2K_1 = \frac{1}{t} \ln \frac{a + x}{a - x} \tag{19}$$

Originally equation II.

It is this new expression found in a purely empirical way that needed to be calculated for different series.

The results obtained were very satisfactory, the expression given by equation 19 remaining, in fact, constant from the beginning to the end of the reaction, or at least up to inversion of 90% of the sugar.

I give here some examples. The following tables contain values of $2K_1$, multiplied by $10^5$:

I. 25 *February* 1901: — 0.5M sucrose (See §16)

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| $2K_1 \cdot 10^5 =$ | 204 | 247 | 253 | 249 | 249 | 243 | 230 |

II. 2 *February* 1901: — 0.5M sucrose (See §16)

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| $2K_1 \cdot 10^5 =$ | 50 | 50 | 50 | 49 | 49 | 47 | 46 |

III. 2 *February* 1901: — 0.2M sucrose (See §16)

| | | | | | | |
|---|---|---|---|---|---|---|
| $2K_1 \cdot 10^5 =$ | 109 | 113 | 118 | 114 | 115 | 110 |

IV. 2 *February* 1901: — 0.2M sucrose (See §16)

| | | | | | | |
|---|---|---|---|---|---|---|
| $2K_1 \cdot 10^5 =$ | 111 | 115 | 118 | 117 | 120 | 118 |

| Times (minutes) | Proportions inverted, $x/a$ | $2K = \frac{1}{t}\log\frac{a}{a-x}$ | $2K_1 = \frac{1}{t}\log\frac{a+x}{a-x}$ |
|---|---|---|---|
| | V. 11 *January* 1901: 0.5M sucrose | | |
| 69 | 0.042 | 27 | 53 |
| 183 | 0.112 | 28 | 53 |
| 392 | 0.240 | 30 | 53 |
| 504 | 0.305 | 31 | 54 |
| 1136 | 0.630 | 38 | 56 |
| | VI. 25 *January* 1901: 0.5M sucrose | | |
| 21 | 0.012 | 25 | 50 |
| 99 | 0.065 | 29 | 57 |
| 215 | 0.141 | 31 | 57 |
| 299 | 0.199 | 32 | 58 |
| 457 | 0.302 | 34 | 59 |
| 585 | 0.372 | 34 | 58 |
| 1200 | 0.659 | 39 | 57 |

In consequence, when it is a question of representing the progress of an inversion produced by invertin from the beginning to the end, the expression

$$2K_1 = \frac{1}{t}\log\frac{a+x}{a-x}$$

gives a very satisfactorily constant value. This empirical law, which I published at the end of 1901, was verified afterwards by A. Brown and it proved equally satisfactory for his experiments. Here is an example given by him:

| Times (minutes) | Proportions inverted, $x/a$ | $K = \frac{1}{t}\log\frac{a}{a-x}$ | $2K_1 = \frac{1}{t}\log\frac{a+x}{a-x}$ |
|---|---|---|---|
| 30 | 0.130 | 201 | 376 |
| 64 | 0.256 | 201 | 355 |
| 120 | 0.454 | 219 | 356 |
| 180 | 0.619 | 232 | 346 |
| 240 | 0.738 | 242 | 343 |
| 300 | 0.831 | 257 | 353 |
| 360 | 0.890 | 265 | 343 |
| 420 | 0.935 | 283 | 351 |
| 480 | 0.961 | 293 | 354 |
| 540 | 0.983 | 327 | 383 |
| 581 | 0.990 | 344 | 395 |

However, this expression $2K_1$ has the faults, first of being established in a purely empirical way; and secondly of changing when one passes from one sucrose concentration to another, as we shall see later.

**18. Evidence that the ferment remains comparable with itself during the whole course of an inversion.** Some authors have claimed that when an enzyme produced a reaction there was some "wearing out," the ferment becoming less active, and in consequence the beginning of the reaction was not directly comparable with the end. Tammann even studied how a ferment (emulsin) changed with time, and basing it on his own experiments, of which many can be criticized, he tried to establish a general law that would explain the diastatic activity. It was important to take up this question again for invertin.

Two different methods can be used for this purpose. The first general method consists of preparing solutions of sucrose and invert sugar in advance that corres-

pond to different stages of the inversion reaction, and adding the same amount of invertin to each. Thus, for example, one can take two solutions, one containing 0.5M sucrose and the other a mixture of 0.2M sucrose with 0.3M invert sugar, this second solution thus corresponding to the first after inversion of three-fifths of the sugar. After adding to these solutions the same amount of invertin and then following the two reactions one will be able to decide whether or not the ferment has changed its activity during the first reaction. In effect, one will compare the rate of a reaction in which the invertin will have produced the inversion of three-fifths of the sucrose with one where the ferment is added without having acted before.

How can these rates be compared? The method is fully specified by what we have said in the preceding section. In effect, suppose that we have on the one hand, a solution containing $a$ of sucrose at the beginning, and, on the other hand, a solution containing $a_1$ of sucrose and $i$ of invert sugar at the beginning, the sum $a_1 + i$ being equal to $a$.

We have seen that in the first case the rate at time $t$ is

$$\frac{dx}{dt} = K\left(1 + \frac{x}{a}\right)(a - x)$$

or

$$\frac{dx}{2}\left(\frac{1}{a+x} + \frac{1}{a-x}\right) = K_1 dt$$

Integration gives

$$\ln(a+x) - \ln(a-x) = 2K_1 t + \text{constant}$$

The constant is determined by the initial condition that $x = 0$ when $t = 0$, so it is equal to zero and we have

$$2K_1 t = \ln\frac{a+x}{a-x} \tag{20}$$

Originally equation I.

For the second solution we can discuss the problem in supposing that the initial position corresponds to a stage of the reaction of a reaction containing initially $a_1 + i$ sucrose, that is to say $a$ sucrose, and which, after a time $t$, contains $a_1$ sucrose and $i$ invert sugar. It is from this moment that we count the intervals and we find that $t$ min after the beginning of this reaction the solution contains $i + x$ invert sugar and $a_1 - x$, that is to say $a - i - x$ sucrose.

The rate at that moment $t$ has therefore the following expression:

$$\frac{dx}{dt} = K_1\left(1 + \frac{i+x}{a}\right)(a - i - x)$$

or

$$\frac{dx}{2}\left(\frac{1}{a+i+x} + \frac{1}{a-i-x}\right) = K_1 dt$$

Integration gives

$$\ln(a+i+x) - \ln(a-i-x) = 2K_1 t + \text{constant}$$

To evaluate the constant we note that $x = 0$ when $t = 0$, and therefore

$$\text{constant} = \ln(a+i) - \ln(a-i)$$

and substituting this in the preceding equation we have

$$2K_1 t = \ln(a+i+x) - \ln(a-i-x) - \ln(a+i) + \ln(a-i)$$

Finally we can simplify further by replacing $a$ by $a_1 + i$, obtaining thus the following definitive equation:

$$2K_1 = \frac{1}{t}\left[\ln\frac{a_1 + 2i + x}{a - x} - \ln\frac{a_1 + 2i}{a}\right] \quad (21)$$

Originally equation II.

In consequence, if the ferment remains comparable with itself during the reaction the value $2K_1$ calculated for the first reaction according to equation 20 should be the same as the value $2K_1$ calculated for the second reaction according to equation 21.

Henri normally wrote $a$ as the concentration of sucrose in the absence of invert sugar, and $a_1$ for this in its presence. This distinction is maintained here.

A great many series made with this aim by this method have given complete agreement for the values of $2K_1$. Let us give some examples:

11 *January* 1901: 0.5M sucrose, $a = 0.5$

| Times (min) | Proportions inverted, $x/a$ | $2K_1 \cdot 10^5$ |
|---|---|---|
| 69 | 0.042 | 53 |
| 183 | 0.112 | 53 |
| 392 | 0.240 | 53 |
| 504 | 0.305 | 54 |
| 1136 | 0.630 | 56 |

Mean: 53.7

11 *January* 1901: 0.3M sucrose + 0.2M invert sugar, $a_1 = 0.3$, $i = 0.2$

| Times (min) | Proportions inverted, $x/a_1$ | $2K_1 \cdot 10^5$ |
|---|---|---|
| 77 | 0.054 | 44 |
| 187 | 0.137 | 47 |
| 498 | 0.311 | 54 |
| 566 | 0.383 | 54 |
| 1128 | 0.678 | 54 |

Mean: 50.6

11 *January* 1901: 0.2M sucrose + 0.3M invert sugar, $a_1 = 0.2$, $i = 0.3$

| Times (min) | Proportions inverted, $x/a_1$ | $2K_1 \cdot 10^5$ |
|---|---|---|
| 71 | 0.057 | 44 |
| 183 | 0.157 | 50 |
| 393 | 0.355 | 52 |
| 502 | 0.428 | 57 |
| 1128 | 0.719 | 55 |

Mean: 51.3

11 *January* 1901: 0.5M sucrose $a = 0.5$ (as in the first series)

| Times (min) | Proportions inverted, $x/a_1$ | $2K_1 \cdot 10^5$ |
|---|---|---|
| 75 | 0.037 | 43 |
| 186 | 0.103 | 48 |
| 399 | 0.228 | 50 |
| 505 | 0.292 | 51 |
| 557 | 0.322 | 51 |
| 1120 | 0.589 | 52 |
| 1172 | 0.611 | 52 |

Mean: 49.6

25 *February* 1901: 1M sucrose $a = 1.0$)

| Times (min) | Proportions inverted, $x/a_1$ | $2K_1 \cdot 10^5$ |
|---|---|---|
| 108 | 0.122 | 98 |
| 176 | 0.199 | 99 |
| 304 | 0.334 | 99 |
| 361 | 0.389 | 98 |
| 506 | 0.511 | 97 |
| 625 | 0.594 | 95 |
| 1304 | 0.854 | 85 |

Mean: 96

25 *February* 1901: 0.5M sucrose + 0.5M invert sugar, $a_1 = 0.5$, $i = 0.5$

| Times (min) | Proportions inverted, $x/a_1$ | $2K_1 \cdot 10^5$ |
|---|---|---|
| 109 | 0.161 | 91 |
| 177 | 0.256 | 91 |
| 304 | 0.412 | 94 |
| 361 | 0.472 | 95 |
| 506 | 0.604 | 95 |
| 625 | 0.682 | 94 |

Mean: 93

The proportion inverted after 304 min was given as 6.412, but this is assumed to be an error for 0.412.

25 *February* 1901: 0.2M sucrose + 0.8M invert sugar, $a_1 = 0.2$, $i = 0.8$

| Times (min) | Proportions inverted, $x/a_1$ | $2K_1 \cdot 10^5$ |
|---|---|---|
| 40 | 0.075 | 94 |
| 117 | 0.211 | 96 |
| 231 | 0.378 | 96 |
| 303 | 0.480 | 100 |
| 490 | 0.658 | 101 |
| 596 | 0.720 | 98 |

Mean: 97

On page 65 of the thesis the middle column is labelled $a_1/x$: this is assumed to be an error.

25 *February* 1901
0.5M sucrose + 0.2M invert sugar, $a_1 = 0.5$, $i = 0.2$, mean $2K_1 \cdot 10^5 = 162$

25 *February* 1901
0.2M sucrose + 0.5M invert sugar, $a_1 = 0.2$, $i = 0.5$, mean $2K_1 \cdot 10^5 = 168$

18 *January* 1901
0.3M sucrose + 0.2M invert sugar, $a_1 = 0.3$, $i = 0.2$, mean $2K_1 \cdot 10^5 = 19$

18 *January* 1901
0.2M sucrose + 0.3M invert sugar, $a_1 = 0.2$, $i = 0.3$, mean $2K_1 \cdot 10^5 = 18$

18 *January* 1901
0.1M sucrose + 0.4M invert sugar, $a_1 = 0.1$, $i = 0.4$, mean $2K_1 \cdot 10^5 = 16$

These results show that it is legitimate to consider the ferment as remaining comparable with itself during the whole period of inversion: the fact that it has produced an inversion does not affect its activity.

However, there exists a second method that allows the same conclusion to be drawn. This method consists of adding the same amount of sucrose or invert sugar to identical reactions and following the reaction after the addition. Thus, for example, we take three flasks containing the same volume of 0.5M sucrose and we then add the same amount of invertin to each flask and follow the reaction. To the first flask we add a new amount sucrose after 90 min to bring its total concentration to 0.13M; to the second flask we add the same amount of sucrose after 170 min, and finally to the third flask after 420 min. If the ferment has remained comparable with itself during the whole period of inversion the values of $2K_1$ calculated for the three series after the addition of sucrose ought to remain equal among themselves. Experiment shows that this equality applies. Here are some examples:

8 *February* 1901

I: 0.5M sucrose; addition to 0.13M sucrose after 90 min.

| Times (min) | Proportions inverted, $x/a$ | $2K_1 \cdot 10^5$ |
|---|---|---|
| 25 | 0.052 | 183 |
| 86 | 0.197 | 201 |
| | Addition of sucrose | |
| 170 | 0.286 | 155 |
| 257 | 0.421 | 154 |
| 322 | 0.510 | 154 |
| 419 | 0.627 | 154 |
| 492 | 0.698 | 154 |
| 558 | 0.752 | 153 |

Mean: 154

II: 0.5M sucrose; addition to 0.13M sugar after 170 min.

| Times (min) | Proportions inverted, $x/a$ | $2K_1 \cdot 10^5$ |
|---|---|---|
| 26 | 0.052 | 178 |
| 87 | 0.191 | 193 |
| 167 | 0.358 | 194 |
| | Addition of sucrose | |
| 258 | 0.416 | 152 |
| 322 | 0.510 | 156 |
| 420 | 0.627 | 155 |
| 493 | 0.698 | 154 |
| 558 | 0.752 | 154 |

Mean: 154

III: 0.5M sucrose; addition to 0.13M sugar after 420 min.

| Times (min) | Proportions inverted, $x/a$ | $2K_1 \cdot 10^5$ |
|---|---|---|
| 26 | 0.052 | 179 |
| 87 | 0.190 | 192 |
| 166 | 0.357 | 195 |
| 257 | 0.522 | 196 |
| 321 | 0.620 | 196 |
| 416 | 0.734 | 196 |
| | Addition of sucrose | |
| 493 | 0.656 | 149 |
| 558 | 0.718 | 154 |

Mean: 152

IV. 0.2M sucrose, addition to 0.13M sugar after 88 min
Mean $2K_1 \cdot 10^5 = 315$ after addition

V. 0.2M sucrose, addition to 0.13M sugar after 168 min
Mean $2K_1 \cdot 10^5 = 321$ after addition

VI. 0.1M sucrose, addition to 0.13M sugar after 33 min
Mean $2K_1 \cdot 10^5 = 451$ after addition

VII. 0.1M sucrose, addition to 0.13M sugar after 108 min
Mean $2K_1 \cdot 10^5 = 434$ after addition

VIII. 0.1M sucrose, addition to 0.13M sugar after 181 min
Mean $2K_1 \cdot 10^5 = 449$ after addition

It is clearly seen that the values of $2K_1$ are the same for the first three series, for series IV and V, and also for series VI, VII and VIII. The discrepancies are less than experimental error.

In consequence, this set of facts shows that in discussing the law of action of the ferment one can consider this to be comparable with itself from the beginning to the end of the reaction; one will only need to take account of the composition of the mixture to deduce the rate of the reaction.

However, these observations teach us another very important point for discussing the laws of the diastases, the effect of invert sugar.

**19. Effect of reaction products on the rate of inversion.** In the historical part we saw how much importance Duclaux attributed to the effect of invert sugar on the ferment. The authors are not all in agreement among themselves on the effect exerted by the reaction products on the diastase; thus, for example, Effront (*Les enzymes*, p. 98) said that addition of invert sugar does not slow down the reaction produced by invertin.

J. Effront (1899) *Les Enzymes et leurs Applications*, page 98, Carré et Naud, Paris. Jean (originally Isaac) Effront (1856–1931) was a Belgian chemist working in the brewing industry.

When we compare two reactions of which one contains some sucrose at the beginning and the other the same amount of sucrose plus some invert sugar, we see immediately that inversion by invertase proceeds more slowly in the second reaction: addition of invert sugar slows down an inversion produced by invertin. We need to study quantitatively the extend of this slowing down in relation to the amounts of sucrose and added invert sugar.

First of all, here are some numerical results obtained from 17 experiments carried out simultaneously the same day with the same amount of ferment. The following numbers show the proportions of sucrose inverted after a certain time; the first six series all contained the same amount of sucrose at the beginning, at a value of 0.5M.

| | Solutions | Proportions inverted after 110 min |
|---|---|---|
| 1. | 0.5M sucrose | 32% |
| 2. | 0.5M sucrose + 0.1M invert sugar | 28% |
| 3. | 0.5M sucrose + 0.2M invert sugar | 25% |
| 4. | 0.5M sucrose + 0.3M invert sugar | 22% |
| 5. | 0.5M sucrose + 0.4M invert sugar | 18% |
| 6. | 0.5M sucrose + 0.5M invert sugar | 16% |

The seven next series contained 0.2M sucrose.

| | Solutions | Proportions inverted after 45 min |
|---|---|---|
| 7. | 0.2M sucrose | 33% |
| 8. | 0.2M sucrose + 0.1M invert sugar | 26% |
| 9. | 0.2M sucrose + 0.2M invert sugar | 22% |
| 10. | 0.2M sucrose + 0.3M invert sugar | 17% |
| 11. | 0.2M sucrose + 0.4M invert sugar | 14% |
| 12. | 0.2M sucrose + 0.5M invert sugar | 11% |
| 13. | 0.2M sucrose + 0.8M invert sugar | 7.5% |

The last four series contained 0.05M sucrose at the beginning

| | Solutions | Proportions inverted after 35 min |
|---|---|---|
| 14. | 0.05M sucrose | 60% |
| 15. | 0.05M sucrose + 0.5M invert sugar | 48% |
| 16. | 0.05M sucrose + 0.1M invert sugar | 42% |
| 17. | 0.05M sucrose + 0.5M invert sugar | 13% |

There is clearly an error in this table. Maybe 0.5M invert sugar in series 15 should be 0.05M.

Careful attention to these numbers indicates the essential points about the effect of invert sugar. We see that

1. The inhibitory effect exerted by invert sugar increases, for a given amount of sucrose, as the amount of invert sugar is increased

2. The inhibitory effect exerted by invert sugar does not depend only on the amount of sugar; it also depends on the amount of sucrose present in the solution. Thus we see that addition of 0.1M invert sugar lowers the proportion of sucrose hydrolysed from 32 to 28% for solutions containing 0.5M sucrose and from 33 to 26% for 0.4M solutions. Similarly addition of 0.5M invert sugar lowers the proportion inverted from 32 to 16% for 0.5M solutions, from 33 to 11% for 0.2M solutions, and finally from 60 to 13% for 0.05M solutions.

3. The same amount of invert sugar exerts a stronger inhibitory action when the sucrose concentration is lower. This is a very important result for the theoretical discussion of diastase activity.

The same conclusion comes from experiments in which instead of adding the invert sugar at the beginning it is added during a reaction. The following numbers indicate the mean amounts of sucrose inverted in mg/min during a period of 55 min after the adition of sugar.

| Solutions | Mean inversion (mg/min) between the 200th and 255th minutes |
|---|---|
| 0.5M sucrose (8.55 g in 50 $cm^3$) | 16.2 |
| 0.5M sucrose, + 2.56 g sucrose after 200 min | 19.5 |
| 0.5M sucrose, + 5.13 g sucrose after 200 min | 19.8 |
| 0.5M sucrose, + 1.8 g invert sugar after 200 min | 12.1 |
| 0.5M sucrose, + 3.6 g invert sugar after 200 min | 10.9 |

| Solutions | Mean inversion (mg/min) between the 80th and 140th minutes |
|---|---|
| 0.2M sucrose (3.42 g in 50 $cm^3$) | 14.8 |
| 0.2M sucrose, + 2.56 g sucrose after 80 min | 24.5 |
| 0.2M sucrose, + 5.12 g sucrose after 80 min | 26.9 |
| 0.2M sucrose, + 1.8 g invert sugar after 80 min | 9.4 |
| 0.2M sucrose, + 3.6 g invert sugar after 80 min | 6.3 |

First of all, these experiments confirm the previous ones: addition of invert sugar slows down the reaction more as the amount added is greater, and as the amount of sucrose in the solution is less. But they show another result that does not follow directly from the previous experiments. It is seen, in fact that addition of sucrose in the middle of a reaction increases the rate. It is a result for which we should pause for a moment.

If we take the solution of 0.5M sucrose, to which we added, before the reaction had even begun, 2.56 g sucrose, we see that this addition of sucrose *does not influence* the rate at the beginning. Thus during the first 90 minutes for the 0.5M solution 24.6 mg/min sucrose are inverted, and for the 0.5M solution with 5.13 g sucrose added at the beginning, we again find 24.5 mg/min inverted. And exacly the sale result is found for the 0.2M sucrose solutions, with 2.56 or 5.12 g sucrose added at the beginning.

*Addition of sucrose has no effect on the rates of inversion when the addition is made at the beginning of the reaction* (as long as the sucrose solution is not more dilute than 0.1M); *but addition of the same amount of sucrose in the middle of the reaction brings about an increase in the rate of inversion.* Thus when, after 200 min, one adds 5.23 g sucrose to the 0.5M solution of which at that moment 48% of the sucrose has been hydrolysed, one sees that the mean rate becomes 19.8 instead of 16.2.

This result is readily understood when one considers the composition of the mixture at the moment of addition. In effect, the invert sugar that has accumulated in the solution will exert an inhibitory action that becomes weaker as the sucrose concentration is greater: the amount of sucrose thus increases, and this fact alone causes the inhibitory effect of the invert sugar to be diminished, and an increase in rate is the result.

I note finally that these different points contradict Brown's theory that was described earlier ( §11).

**20. The inhibitory effect of invert sugar is due almost entirely to laevulose.** It was interesting to investigate whether the inhibitory effect of invert sugar was produced equally by the two sugars glucose and laevulose that appeared during hydrolysis, or whether there was a difference between these two sugars. Several series of experiments have shown that the effect of laevulose is much stronger than that of glucose. Here are several examples that contain the inverted proportions for different solutions.

| Times (min) | 0.2M sucrose | 0.2M sucrose + 0.2M glucose | 0.2M sucrose + 0.2M laevulose | 0.2M sucrose + 0.2M invert sugar |
|---|---|---|---|---|
| 75 | 0.142 | 0.144 | 0.123 | 0.119 |
| 184 | 0.385 | 0.362 | 0.317 | 0.306 |
| 275 | 0.564 | 0.532 | 0.457 | 0.440 |
| 445 | 0.798 | 0.746 | 0.672 | 0.648 |
| 605 | 0.906 | 0.878 | 0.799 | 0.794 |

We shall return to this result in the theoretical part of this work.

**21. Effect of the sucrose concentration on the rate of inversion.** Until now we have only considered the rate of inversion without worrying about the initial sucrose concentration. Let us now examine this activity, on which the literature offers a large amount of information.

When the rates of inversion of different reactions with different sucrose concentrations are compared, it appears that sometimes the concentration has an effect on the rate, and sometimes it does not. Thus for solutions between 0.1M and 0.5M the rate of inversion at the beginning is almost the same, whatever the sugar concentration. But in solutions more dilute than 0.1M the rate of inversion decreases with the concentration. For the solutions above 0.5M the rate decreases as the concentration increases.

The effect of the amount of sugar is thus very complex, and does not resemble that for inversion produced by acids. Here are some examples. The following numbers express the mean amounts of sucrose inverted per minute at the beginning of each reaction.

1st *May* 1902

| Solutions | Amounts inverted (mg/min) |
|---|---|
| 0.01M | 0.58 |
| 0.025M | 1.41 |
| 0.05M | 2.40 |
| 0.1M | 2.96 |
| 0.25M | 4.65 |
| 0.25M | 4.66 |
| 0.5M | 5.04 |
| 0.5M | 5.04 |
| 1.0M | 4.45 |
| 1.0M | 4.45 |
| 1.5M | 2.82 |
| 2.0M | 1.15 |

The sucrose concentration exerts a clear effect below 0.25M, but the rate is not proportional to the concentration of sugar: it increases less than that. So we see that in passing from the 0.025M solution to the 0.1M solution, which is fourfold higher, the rate only doubles (from 1.41 to 2.96), and even in passing from the 0.025M solution to the 0.25M solution, tenfold more concentrated the rate increases a little more than threefold. The effect is therefore very complicated and no simple relationship seems to follow from examination of numerous experiments.

The decrease in rate at high concentrations is also interesting, but there we are in the presence of anomalies that often arise in concentrated solutions, and it is very difficult to seek interpretations of this decrease in rate. We shall have to confine ourselves to the study of dilute solutions.

**22. Dependence of the rate of inversion on the amount of diastase.** This question has been studied many times for different ferments, and several authors have shown that the rate of inversion was proportional to the amount of invertin. For example, O'Sullivan and Tompson determined the time necessary to invert 74% of sucrose with different amounts of ferment at a temperature of

| | | |
|---|---|---|
| 15.5° | 0.15 g of invertin | 283 min |
| 15.5° | 0.45 g of invertin | 94.8 min |
| 15.5° | 1.5 g of invertin | 30.7 min |

the of the amount of diastase for the period there were 424.5, 426.6 and 460.5. There was therefore proportionality between the rate of reaction and the amount of ferment.

I have made several series to study the effect of the amount of invertin, and the results indicated the same proportionality. Here are some examples:

| Solutions | 2 $cm^3$ invertin | 4 $cm^3$ invertin | 6 $cm^3$ invertin |
|---|---|---|---|
| 0.1M sucrose | 73 | 148 | 226 |
| 0.2M sucrose | 36 | 78 | 114 |
| 0.5M sucrose | 22 (?) | 33 | 48 |

Henri did not explain the "(?)". Presumably he thought the value of 22 was too high.

The preceding numbers are the mean values of $2K_1$; it may be seen that the values are proportional to the amount of ferment.

**23. First theoretical interpretation of the results for the activity of invertin:** *Formula of M. Bodenstein.* M. Bodenstein, an assistant in Ostwald's laboratory in Leipzig, after studying the results of my experiments[21], has proposed a first theoretical explanation of these results. I am happy to express here my thanks to M. Bodenstein for the abundant advice that he has given me in the study of the activity of diastases. The hypothesis that serves as the starting point of Bodenstein's theory is that sucrose decreases the activity of the ferment. When a certain amount $\Phi$ of ferment is added to a mixture containing $a_1$ sucrose and $i$ invert sugar, the activity of the ferment is decreased, first by the sucrose and secondly by the invert sugar.

Max Ernst August Bodenstein (1871–1942) was a German physical chemist known for his work in chemical kinetics, and in particular for the concept of a chain reaction.

This decrease in the activity of the ferment is moreover supposed to be proportional to the amount of sucrose and of invert sugar, in such a way that one can argue as if, instead of the amount $\Phi$ of ferment there is a proportion $\phi/(ma_1 + ni)$, where $m$ and $n$ are constants.

The change here from $\Phi$ to $\phi$ probably has no significance.

As the experiments show that an inversion of sucrose occurs more rapidly than according to the logarithmic law of acids, it can be deduced that the inhibitory action exerted by the invert sugar is weaker than that exerted by the sucrose, and therefore Bodenstein concluded that $m$ must be greater than $n$.

The rate of reaction at time $t$, when a quantity $x$ of sucrose has been hydrolysed, will be given by the following equation:

$$\frac{dx}{dt} = \frac{K_2\Phi}{m(a_1 - x) + n(i + x)} \cdot (a_1 - x)$$

or

$$dx\left[m + \frac{ni}{a_1 - x} + \frac{na_1}{a_1 - x} - n\right] = K_2\Phi \cdot dt$$

Integration gives

$$(m - n)x - n(a_1 + i)\ln(a_1 - x) = K_2\Phi t + \text{constant}$$

To determine the constant we put $x = 0$ at $t = 0$, and then

$$\text{constant} = -n(a_1 + i)\ln a$$

So the definitive formula becomes

$$K_2\Phi = \frac{1}{t}\left[(m - n)x + n(a_1 + i)\ln\frac{a_1}{a_1 - x}\right]$$

or

$$K_2\Phi = \frac{a_1 + i}{t}\left[(m - n)\frac{x}{a_1 + i} + n\ln\frac{a_1}{a_1 - x}\right] \qquad (22)$$

Originally equation I.

The constant $K_2$ calculated according to this equation, once values of $m$ and $n$ have been chosen remains constant, on the one hand, during the course of an inversion, and, on the other hand, when one passes from one concentration of sucrose to another.

If the ferment is put at the beginning in a solution containing sucrose alone in amount $a$, equation 22 is simplified, and becomes

$$K_2\Phi = \frac{a}{t}\left[(m - n)\frac{x}{a} + n\ln\frac{a}{a - x}\right] \qquad (23)$$

Originally equation II.

When searching by trial and error for values of $m$ and $n$ M. Bodenstein arrived at $m = 2$ and $n = 1$, and then Bodenstein's constant take the following simple form:

[21] V. Henri, Ueber das Gesetz der Wirkung des Invertins, *Zeitschrift für Physikalische Chemie*, **39**, 1901, 194–216.

$$K_2\Phi = \frac{a}{t}\left[\frac{x}{a} + \ln\frac{a}{a-x}\right] \qquad (24)$$

Originally equation III.

In this expression it is the natural logarithm that needs to be calculated.

This new constant proved to be very satisfactory for a large number of series. Here are some examples:

| Times (min) | Proportions inverted | $K_2\Phi \cdot 10^6$ |
|---|---|---|
| 25 *February* 1901 | 0.5M sucrose | $a = 0.5$ |
| 26 | 0.061 | 238 |
| 115 | 0.316 | 303 |
| 182 | 0.487 | 321 |
| 311 | 0.713 | 316 |
| 373 | 0.789 | 315 |
| 511 | 0.892 | 312 |
| 630 | 0.932 | 288 |
| | | Mean: 298 |
| 15 *July* 1901 | 0.558M sucrose | $a = 0.5$ |
| 47 | 0.048 | 121 |
| 163 | 0.174 | 131 |
| 245 | 0.264 | 137 |
| 365 | 0.378 | 137 |
| 547 | 0.525 | 136 |
| 1171 | 0.807 | 123 |
| | | Mean: 131 |

Here are two examples for series in which at some moment a given amount of sucrose was added:

| Times (min) | Proportions inverted | $K_2\Phi \cdot 10^6$ |
|---|---|---|
| 8 *February* 1901 | 0.5M sucrose | + 0.13M sucrose after 420 min |
| 26 | 0.052 | 204 |
| 87 | 0.190 | 230 |
| 166 | 0.357 | 241 |
| 257 | 0.522 | 245 |
| 321 | 0.620 | 251 |
| 416 | 0.734 | 247 |
| | | Mean: 236 |
| Sucrose added | | |
| 493 | 0.656 | 238 |
| 558 | 0.718 | 255 |
| | | Mean: 246 |

| Times (min) | Proportions inverted | $K_2\Phi \cdot 10^6$ |
|---|---|---|
| 8 *February* 1901 | 0.5M sucrose | + 0.13M sucrose after 90 min |
| 25 | 0.052 | 212 |
| 86 | 0.197 | 242 |
| | | Mean: 227 |
| Sucrose added | | |
| 170 | 0.286 | 221 |
| 257 | 0.421 | 235 |
| 322 | 0.510 | 239 |
| 419 | 0.627 | 243 |
| 492 | 0.698 | 244 |
| 558 | 0.752 | 243 |
| | | Mean: 237 |

It is evident in these last two series that the mean value of $K_2$ remained the same after addition of sucrose.

Here finally are the mean values of these constants calculated by Bodenstein for the different series of experiments:

| Sucrose | Invert sugar | $a$ or $a_1$ | $i$ | $K_2\Phi \cdot 10^6$ |
|---|---|---|---|---|
| 11 *January* 1901 | | | | |
| 0.5M | | 0.5 | | 662 |
| 0.5M | | 0.5 | | 656 |
| 0.3M | 0.2M | 0.3 | 0.2 | 654 |
| 0.3M | 0.3M | 0.2 | 0.3 | 678 |
| 0.3 | | 0.3 | | 554 |
| 0.2 | | 0.2 | | 567 |
| 0.1M | 0.1M | 0.1 | 0.1 | 611 |
| 0.1M | | 0.1 | | 474 |
| 18 *January* 1901 | | | | |
| 0.3M | | 0.3 | | 266 |
| 0.2M | | 0.2 | | 227 |
| 0.1M | | 0.1 | | 274 |
| 0.05M | | 0.05 | | 233 |
| 0.3M | 0.2M | 0.3 | 0.2 | 253 |
| 0.2M | 0.3M | 0.2 | 0.3 | 233 |
| 0.1M | 0.4M | 0.1 | 0.4 | 203 |
| 25 *January* 1901 | 2 cm$^3$ ferment added to 50 cm$^3$ sugar solution | | | |
| 0.5M | | 0.5 | | 65.8 |
| 0.2M | 0.3M | 0.2 | 0.3 | 54.2 |
| 0.4M | | 0.4 | | 73.5 |
| 0.2M | | 0.2 | | 58.4 |
| 0.1M | | 0.1 | | 58.1 |
| 0.05M | | 0.05 | | 44.1 |

In column 3 of these tables Henri wrote $a = 0,5$ (etc.) for the lines in which no invert sugar was present, and $a_1 = 0,3$ (etc.) for the others.

0.05M was incorrectly shown as 0.5M

[Continuation . . . ]

| Sucrose | Invert sugar | $a$ or $a_1$ | $i$ | $K_2\Phi \cdot 10^6$ |
|---|---|---|---|---|
| 25 *January* 1901 | 4 cm$^3$ ferment added to 50 cm$^3$ sugar solution | | | |
| 1M | | 1 | | 237 |
| 0.5M | 0.5M | 0.5 | 0.5 | 241 |
| 0.2M | 0.8M | 0.2 | 0.8 | 241 |
| 0.5M | 0.4M | 0.5 | 0.4 | 258 |
| 0.8M | | 0.8 | | 267 |
| 0.5M | 0.3M | 0.5 | 0.3 | 275 |
| 0.5M | 0.2M | 0.5 | 0.2 | 289 |
| 0.2M | 0.5M | 0.2 | 0.5 | 292 |
| 0.5M | 0.1M | 0.5 | 0.1 | 305 |
| 0.2M | 0.4M | 0.2 | 0.4 | 320 |
| 0.5M | | 0.5 | | 298 |
| 0.2M | 0.3M | 0.2 | 0.3 | 330 |
| 0.2M | 0.3M | 0.2 | 0.2 | 325 |
| 0.2M | 0.1M | 0.2 | 0.1 | 315 |
| 0.2M | | 0.2 | | 338 |
| 0.05M | 0.1M | 0.05 | 0.1 | 295 |
| 0.05M | 0.05M | 0.05 | 0.1 | 253 |
| 0.05M | | 0.05 | | 201 |
| 0.05M | 0.5M | 0.05 | 0.5 | 275 |

Examination of these numbers shows that the value of $K_2$ is indeed constant for the series done with sucrose concentration above 0.1M. On the other hand it can be seen that for lower concentrations (0.05M) the values of $K_2$ deviate from the mean.

**24. Bodenstein's formula does not apply to dilute solutions.** It was necessary to make some experiments paying attention to dilute solutions. These experiments showed that Bodenstein's constant did not apply to these dilute solutions. Here, in fact, are several examples that show the effect clearly:

1 *May 1902*

0.01M sucrose, $K_2\Phi \cdot 10^4 = 100$
0.025M sucrose, $K_2\Phi \cdot 10^4 = 243$
0.05M sucrose, $K_2\Phi \cdot 10^4 = 358$
0.1M sucrose, $K_2\Phi \cdot 10^4 = 513$
0.25M sucrose, $K_2\Phi \cdot 10^4 = 650$
0.25M sucrose, $K_2\Phi \cdot 10^4 = 666$
0.5M sucrose, $K_2\Phi \cdot 10^4 = 650$
0.5M sucrose, $K_2\Phi \cdot 10^4 = 652$
1M sucrose, $K_2\Phi \cdot 10^4 = 545$
1M sucrose, $K_2\Phi \cdot 10^4 = 545$
1.5M sucrose, $K_2\Phi \cdot 10^4 = 340$
2M sucrose, $K_2\Phi \cdot 10^4 = 140$

8 *May* 1902

0.01M sucrose, $K_2\Phi \cdot 10^4 = 174$
0.01M sucrose, $K_2\Phi \cdot 10^4 = 205$
0.025M sucrose, $K_2\Phi \cdot 10^4 = 280$
0.025M sucrose, $K_2\Phi \cdot 10^4 = 280$
0.05M sucrose, $K_2\Phi \cdot 10^4 = 455$
0.05M sucrose, $K_2\Phi \cdot 10^4 = 445$
0.1M sucrose, $K_2\Phi \cdot 10^4 = 565$
0.1M sucrose, $K_2\Phi \cdot 10^4 = 575$
0.2M sucrose, $K_2\Phi \cdot 10^4 = 600$
0.2M sucrose, $K_2\Phi \cdot 10^4 = 630$
0.2M sucrose, $K_2\Phi \cdot 10^4 = 600$
0.5M sucrose, $K_2\Phi \cdot 10^4 = 660$
1M sucrose, $K_2\Phi \cdot 10^4 = 500$

30 *May* 1902 (temperature 31°)

0.01M sucrose, $K_2\Phi \cdot 10^4 = 188$
0.01M sucrose, $K_2\Phi \cdot 10^4 = 209$
0.025M sucrose, $K_2\Phi \cdot 10^4 = 457$
0.025M sucrose, $K_2\Phi \cdot 10^4 = 302$
0.05M sucrose, $K_2\Phi \cdot 10^4 = 920$
0.05M sucrose, $K_2\Phi \cdot 10^4 = 920$
0.1M sucrose, $K_2\Phi \cdot 10^4 = 840$
0.1M sucrose, $K_2\Phi \cdot 10^4 = 630$
0.2M sucrose, $K_2\Phi \cdot 10^4 = 724$
0.2M sucrose, $K_2\Phi \cdot 10^4 = 662$
0.5M sucrose, $K_2\Phi \cdot 10^4 = 610$
0.5M sucrose, $K_2\Phi \cdot 10^4 = 585$
1M sucrose, $K_2\Phi \cdot 10^4 = 490$

Bodenstein's formula cannot therefore be regarded as definitive, as it fails to describe results for dilute soltions, for which, as we know, the laws of general chemistry apply in general better than those for solutions at moderate concentrations.

Moreover, this formula of Bodenstein is based on the hypothesis that sucrose and invert sugar exert an inhibitory effect on the ferment, but it gives no interpretation of this inhibitory action, which is supposedly proportional to the amount of sugar.

# Chapter III

## Theory for invertin action

**25. Theory for invertin action.** I have taken up the study of invertin action on sucrose in taking as starting point a certain number of hypotheses that can be justified experimentally.

It is well known that *Emil Fischer*[22] showed that there is a strict relationship between the action of a diastase and the stereochemical configuration of its substrate, so that one can predict in advance if a given ferment will be able to hydrolyse a particular substrate or if it will remain without action on this substrate. Conversely, when one finds that a particular substrate is hydrolysed by a ferment one can be sure that the chemical constitution of this substrate has a particular form.

This very important result has been subsequently confirmed by several authors. Thus we know, according to E. Fischer's research, that invertin acts on the sugar that yield laevulose on hydrolysis (sucrose, raffinose, gentiobiose), that emulsin acts on the sugars and glycosides that give galactose, and so on.

Fischer concluded from his research on the diastases that the ferment forms an intermediate chemical complex with the substrate, which then decomposes, finally regenerating the free ferment.

Study of the rate of inversion of sucrose, and above all the effect of the sugar concentration, has shown that the inversion follows complex laws that recall those that we have met when studying catalytic reactions in which there was formation of intermediate complexes.

However, we have seen on the other hand that invert sugar likewise exerts an effect on the rate of reaction, and to explain this we have introduced the hypothesis that a complex between the ferment and invert sugar can exist. One could even say that this intermediate complex is formed between the ferment and the laevulose, as we have seen in §20 that the inhibitory action of invert sugar was due almost solely to the laevulose. In this way the effects of reaction products can be reconciled with E. Fischer's result, according to which invertin hydrolyses the sugars that give laevulose on hydrolysis.

In the general classification of catalytic activities we saw that we could either allow the formation of intermediate complexes or, on the other hand, suppose the formation of incomplete complexes with some state of equilibrium between the catalyst and the substrate.

The hypothesis of complete complexes led to a rate equation expressed by $dx/dt = K$, and the curve in this case would be a straight line.

These theoretical considerations led us to suppose that the intermediate complex that form between the ferment and the sucrose, and also those between the ferment and invert sugar, are incomplete and give rise to states of equilibrium.

[22] E. Fischer, *Zeit. f. physiol. Chem.*, **16**. There is no paper by Fischer in volume 16. Probably **16** is a typographical error for **26**, in which case the reference is as follows: E. Fischer (1898/99) "Bedeutung der Stereochemie für die Physiolologie" *Zeitschrift für Physiologische Chemie* **26**, 60–87.

Finally, if we accept that these complexes are incomplete it follows that some part of the ferment will remain uncomplexed. Two suppositions then come to mind:

1. One could suppose that the uncomplexed part of the ferment acted on the sucrose and transformed it.
2. One could also, on the other hand, suppose that it is the complex between the ferment and the sucrose that decomposed and thus brought about the hydrolysis of the sucrose.

What should be the law of action of the ferment if it obeyed the different hypotheses that we have just mentioned? Such is the question that we must examine now.

Suppose that we have a solution containing the amount $a$ of sucrose and that we add an amount $\Phi$ of the ferment: after $t$ minutes we see that there is $a - x$ sucrose and $x$ invert sugar in the solution.

According to the hypotheses mentioned above, we suppose that the ferment is divided between the sucrose and the invert sugar and that a part remains uncomplexed. Let $X$ be the amount of ferment that is not complexed, $z$ the amount complexed at time $t$ with the sucrose and $y$ the amount complexed with the invert sugar. We assume, in addition, that these complexes exist in states of equilibrium, that is to say they obey the law of mass action.

Let us write the equations that express the equilibrium condition. For the equilibrium between the ferment and the sucrose, of which the quantity is $a - x$, we have

$$X \cdot (a - x) = \frac{1}{m} z \tag{25}$$ Originally equation 1.

and for the equilibrium between the ferment and invert sugar,

$$X \cdot x = \frac{1}{n} \cdot y \tag{26}$$ Originally equation 2.

in which $m$ and $n$ are the two equilibrium constants. Finally we take into account that the total amount of ferment complexed and free is equal to $\Phi$:

$$\Phi = X + y + x \tag{27}$$ Originally equation 3.

These three equations allow calculation of the amount $x$ of ferment not complexed and the amount $z$ of the complex between sucrose and the ferment. This gives

$$X = \frac{\Phi}{1 + m(a - x) + nx} \tag{28}$$ Originally equation 4.

and

$$Z = \frac{m \cdot \Phi \cdot (a - x)}{1 + m(a - x) + nx} \tag{29}$$ Originally equation 5.

In view of this let examine how the rate of inversion at time $t$ should be, assuming that we suppose either that it is the free ferment that acts on the sucrose or alternatively that it is the complex $z$ that decomposes.

1. *1st hypothesis.* The free part of the ferment acts on the sucrose. In this case the rate is proportional to the amount of free ferment and the amount of sucrose, so it will be prportional to $X$ and to $a - x$, and in consequence to the product of these two values. Therefore

   $$\frac{dx}{dt} = K \cdot X \cdot (a - x)$$

   or in replacing $X$ by its value from equation 28, we obtain

   $$\frac{dx}{dt} = \frac{K \cdot \Phi \cdot (a - x)}{1 + m(a - x) + nx} \tag{30}$$ Originally equation 6.

2. *2nd hypothesis.* It is the complex between the ferment and the sucrose that decomposes, the rate of this decomposition then being proportional to the amount of this intermediate complex, that is to say to $z$. One will have $dx/dt = K \cdot z$, and in replacing $z$ by its value given by equation 29, one obtains

$$\frac{dx}{dt} = \frac{K \cdot m \cdot \Phi \cdot (a-x)}{1 + m(a-x) + nx} \tag{31}$$

Originally equation 7.

These two expressions for the rate of reaction, equations 30 and 31, are identical, and in consequence whichever hypothesis one makes the law that the inversion sucrose follows will be the same. This result has an interest from the point of view of general chemistry, but I shall not pause to discuss that.

Equations 30 and 31 are not in fact identical, as equation 31 contains a factor $m$ in the numerator and equation 30 does not. What Henri means is that they are identical in *form*, and thus experimentally indistinguishable.

Therefore the rate of reaction has for expression

$$\frac{dx}{dt} = \frac{K_3 \cdot (a-x)}{1 + m(a-x) + nx} \tag{32}$$

Originally equation I.

in which $K_3$ is a constant proportional to the amount of ferment; $m$ and $n$ are two characteristic constants that will be able to vary according to the mixture and the temperature, but which, once determined, should give the same value for $K_3$ whatever the concentration of sucrose or of invert sugar.

$\Phi$ does not appear in this or the next two equations because Henri subsumed it in the value of $K_3$.

If, at the beginning of the reaction we had a mixture of $a_1$ sucrose and $i$ invert sugar, the rate at time $t$ would have as expression

$$\frac{dx}{dt} = \frac{K_3 \cdot (a_1-x)}{1 + m(a_1-x) + n(x+i)} \tag{33}$$

Originally equation II.

We can discuss the general of the activity of invertin by means of equations 32 and 33. Let us consider the case in which at the beginning we have only sucrose, and let us study the rate at the beginning. This rate is evidently obtained by setting $x$ to zero in equation 32, so

$$\text{Initial rate} = \frac{K_3 a}{1 + ma}$$

Notice that apart from the symbols this is what is today commonly called the Michaelis–Menten equation.

It can be seen that when the sucrose concentration is small, that is to say $a$ is small, $ma$ becomes negligible in comparison with unity, and so the initial rate is proportional to the sucrose concentration. But as $a$ increases the rate initially increases steeply and then more slowly, in such a way that when $ma$ is large compared with unity the initial rate will be approximately equal to $K_3 a/ma$, that is to say to $K_3/m$: it will be constant regardless of the concentration of sucrose.

There are several points to note here. First, Henri is describing the plot commonly known as the "Michaelis–Menten" plot (though it was not actually used by Michaelis and Menten). Secondly, the "$x$ axis" in this description is the vertical axis: it does not correspond to what is often loosely called the $x$-axis, i.e. the abscissa. Finally, the asymptote in question is to the left of the axis, i.e. at negative concentrations of sucrose.

Therefore, for dilute solution the rate of inversion is affected by the amount of sucrose, whereas for more concentrated solutions it is almost independent of it. Graphically, the relationship between the concentration of sucrose and the initial rate follows a hyperbola passing through the origin and having an asymptote parallel to the $x$ axis at a distance equal to $K_3$. This is indeed the form one obtains experimentally, as we have seen earlier.

Suppose now that we have at the beginning an amount $a_1$ of sucrose and $i$ of invert sugar, the initial rate will then be

$$\text{Initial rate} = \frac{K_3 a_1}{1 + ma_1 + ni}$$

This expression gives us a complete representation of the effect of invert sugar. In fact, for the same amount of sucrose $a_1$ the rate will be smaller as $i$ becomes larger (examples in §19).

For the same amount of invert sugar $i$ the inhibition produced by this sugar will be stronger as $a_1$ becomes smaller: the same amount of invert sugar inhibits the rate of inversion of a solution weak in sucrose more than of a a solution concentrated in sucrose (examples in §19).

Henri starts the paragraph with "secondly", but it is not clear what it is the second of.

Let us now derive the equation that will provide the constant $K_3$. For integrating equation 32 we can write it as follows:

$$dx\left[\frac{1+na}{a-x}+m-n\right]=K_3dt$$

and we obtain

$$-(1+na)\ln(a-x)+(m-n)x=K_3t+\text{constant}$$

The constant is determined by the condition that $x=0$ when $t=0$:

$$-(1+na)\ln a=\text{constant}$$

so the relationship becomes

$$(1+na)\ln\frac{a}{a-x}+(m-n)x=K_3t$$

or

$$K_3=\frac{a}{t}\left[(m-n)\frac{x}{a}+n\ln\frac{a}{a-x}\right]+\frac{1}{t}\ln\frac{a}{a-x} \tag{34}$$

Originally equation III.

When there are amounts $a_1$ of sucrose and $i$ of invert sugar at the beginning the same approach yields

$$K_3=\frac{a_1}{t}\left[(m-n)\frac{x}{a_1}+n\left(1+\frac{i}{a_1}\right)\ln\frac{a_1}{a_1-x}\right]+\frac{1}{t}\ln\frac{a_1}{a_1-x} \tag{35}$$

Originally equation IV.

It can be seen that the expression for $K_3$ is the sum of two factors, of which one depends on the *absolute* value of $a$, whereas the other depends only on the *ratio* $x/a$. It is this complex form that expresses the influence exerted by the concentration of sucrose on the rate of reaction.

**26. Experimental verification of the preceding theory.** It was necessary first of all to find the values of $m$ and $n$. By trial and error I found that if one puts $m=30, n=10$ one obtains sufficiently constant values of $K_3$. It is possible that one could get an even better constancy by choosing other values for $m$ and $n$. Equations 34 and 35 thus become

$$K_3=\frac{10\cdot a}{t}\left[2\frac{x}{a}+\ln\frac{a}{a-x}\right]+\frac{1}{t}\ln\frac{a}{a-x}$$

$$K_3=\frac{10\cdot a_1}{t}\left[2\frac{x}{a_1}+\left(1+\frac{i}{a_1}\right)\ln\frac{a_1}{a_1-x}\right]+\frac{1}{t}\ln\frac{a_1}{a_1-x}$$

Henri wrote $a$ rather than $a_1$ in the last term of this equation, but it seems clear that $a_1$ was intended.

These are therefore the formulae that will allow calculation of the values of the constant $K_3$.

Let us give some examples of these calculations:

11 *January* 1901

| Times (minutes) | Proportions inverted, $x/a$ | $K_3 \cdot 10^5$ |
|---|---|---|
| | I. 0.5M sucrose, $a = 0.5$ | |
| 75 | 0.037 | (796) |
| 186 | 0.103 | 904 |
| 399 | 0.228 | 958 |
| 505 | 0.292 | 987 |
| 557 | 0.322 | 997 |
| 1120 | 0.589 | 1002 |
| 1172 | 0.611 | 1005 |
| | | Mean: 950 |
| | II. 0.5M sucrose, $a = 0.5$ | |
| 69 | 0.042 | 981 |
| 183 | 0.112 | 1000 |
| 392 | 0.240 | 1031 |
| 504 | 0.305 | 1038 |
| 1136 | 0.630 | 1079 |
| | | Mean: 1026 |
| | III. 0.3M sucrose + 0.2M invert sugar, $a_1 = 0.3, i = 0.2$ | |
| 77 | 0.054 | 853 |
| 187 | 0.137 | 912 |
| 398 | 0.311 | 1030 |
| 566 | 0.383 | 1023 |
| 1128 | 0.678 | 982 |
| | | Mean: 960 |
| | IV. 0.2M sucrose + 0.3M invert sugar, $a_1 = 0.2, i = 0.3$ | |
| 71 | 0.057 | 817 |
| 183 | 0.157 | 902 |
| 393 | 0.355 | 960 |
| 502 | 0.428 | 1007 |
| 1128 | 0.719 | 929 |
| | | Mean: 923 |
| | V. 0.3M sucrose, $a = 0.3$ | |
| 77 | 0.051 | (668) |
| 187 | 0.153 | 845 |
| 398 | 0.349 | 911 |
| 506 | 0.430 | 953 |
| 557 | 0.463 | 944 |
| 1126 | 0.801 | 1003 |
| | | Mean: 931 |

The value is badly printed in the original, but it is clear that 0.042 is intended.

| Times (minutes) | Proportions inverted, $x/a$ | $K_3 \cdot 10^5$ | |
|---|---|---|---|
| | VI. 0.2M sucrose, $a = 0.2$ | | |
| 71 | 0.080 | 803 | |
| 182 | 0.230 | 936 | |
| 393 | 0.501 | 1040 | |
| 502 | 0.614 | 1058 | |
| 1126 | 0.918 | 992 | |
| | | Mean: 966 | |
| | VII. 0.1M sucrose + 0.1M invert sugar, $a_1 = 0.1, i = 0.1$ | | |
| 69 | 0.132 | 997 | |
| 183 | 0.337 | 1041 | |
| 393 | 0.618 | 1047 | |
| 502 | 0.720 | 1046 | |
| 1131 | 0.919 | 829 | |
| | | Mean: 992 | |
| | VIII. 0.1M sucrose, $a = 0.1$ | | |
| 70 | 0.148 | 882 | |
| 183 | 0.418 | 1046 | |
| 393 | 0.738 | 1056 | |
| 504 | 0.818 | 1000 | |
| 1135 | 0.965 | 760 | |
| | | Mean: 948 | |
| 1st *May* 1902 | | | |
| | I. 0.01M sucrose, $a = 0.01$ | | |
| 232 | 0.800 | 830 | 852 |
| 459 | 0.969 | 875 | |
| | II. 0.025M sucrose, $a = 0.025$ | | |
| 233 | 0.77 | 910 | |
| | III. 0.05M sucrose, $a = 0.05$ | | |
| 233 | 0.652 | 958 | 955 |
| 459 | 0.901 | 952 | |
| | IV. 0.1M sucrose, $a = 0.1$ | | |
| 457 | 0.790 | 1026 | |
| | V. 0.25M sucrose, $a = 0.25$ | | |
| 455 | 0.497 | 1073 | |
| | VI. 0.25M sucrose, $a = 0.05$ | | |
| 455 | 0.498 | 1073 | 1076 |
| 1055 | 0.867 | 1080 | |

| Times (minutes) | Proportions inverted, $x/a$ | $K_3 \cdot 10^5$ | |
|---|---|---|---|
| | VII. 0.5M sucrose, $a = 0.5$ | | |
| 450 | 0.267 | 1004 | 1001 |
| 1098 | 0.580 | 998 | |
| | VIII. 0.5M sucrose, $a = 0.5$ | | |
| 449 | 0.269 | 1010 | 1004 |
| 1106 | 0.584 | 998 | |
| | IX. 1M sucrose, $a = 1$ | | |
| 445 | 0.118 | 828 | 829 |
| 1104 | 0.280 | 830 | |
| | X. 1M sucrose, $a = 1$ | | |
| 444 | 0.118 | 828 | 829 |
| 1103 | 0.279 | 830 | |

It can be seen that the experiments of the 1st May gave $K_3$ values sufficiently constant with wide limits, spread between $a = 0.01$M and $a = 1$M: these are experiments that could not be represented by Bodenstein's formula.

Here again are some mean values of $K_3$ for the experiments of 8 May 1902 and 25 February 1901:

8 *May* 1902

| Sucrose | Invert sugar | $K_3$ | $K_2$ (Bodenstein) |
|---|---|---|---|
| 0.025M | | 107 | 279 |
| 0.05M | | 119 | 454 |
| 0.1M | | 111 | 565 |
| 0.2M | | 101 | 600 |
| 0.5M | | 95 | 575 |
| 1M | | 77 | 500 |

25 *February* 1901

| | | | |
|---|---|---|---|
| 0.05M | | 559 | 201 |
| 0.05M | 0.05M | 505 | 253 |
| 0.05M | 0.1M | 505 | 295 |
| 0.2M | | 564 | 338 |
| 0.2M | 0.1M | 479 | 315 |
| 0.2M | 0.2M | 463 | 325 |
| 0.2M | 0.3M | 446 | 330 |
| 0.5M | | 454 | 309 |
| 0.5M | 0.1M | 439 | 305 |
| 0.2M | 0.4M | 419 | 320 |

In summary, it can be seen that setting $m$ and $n$ to the values indicated earlier leads to values for $K_3$ that, on the one hand, remain constant during the whole period of the reaction and, on the other hand, are the same when the different series are done with different concentrations of sucrose and invert sugar.

It follows that the rate of inversion of sucrose produced by invertin can be

represented by the law

$$\frac{dx}{dt} = \frac{K_3(a-x)}{1+m(a-x)+nx}$$

At the temperature of 25° and for solutions made in distilled water one has $m = 30$ and $n = 10$, with $a$ expressed in M.

It is very likely that when the temperature is varied, or the mixture is varied by the addition different substances, the values of the coefficients $m$ and $n$ should be different. This is a point that I have not yet studied.

**27. Effects of acids and bases on invertin.** Many authors since Kjeldahl have been concerned with the effects of acids and bases on invertin. The results obtained by the different authors have been in good agreement, and I shall not take up this question again. I shall only draw attention to a result interesting for the study of invertin, as it shows the constancy of this ferment, and can serve in the interpretation of the effects of acids and bases.

Johan Gustav Thorsager Kjeldahl (1849–1900) was a Danish chemist who developed a method for determining the amount of nitrogen in organic compounds. The reference may be to J. Kjeldahl (1881) "Untersøgelser over Kulhydrater i Byg og Malt med særligt Heusyn til Forkomsten af Rørsukker" *Meddelelser (Carlsberg Laboratory)*, 331–379.

It is known that adding a small amount of alkali to a mixture of sucrose and invertin stops the reaction completely, and if subsequently the neutrailty is restored the reaction often starts again. But the authors state that the activity of the ferment is found to be weakened. This is the particular point that I shall examine here.

If the action of invertin is stopped with an extremely small amount of soda, and if later the neutrality is retored with some acid after the mixture has been left for several hours at 25°, it is seen that the reaction occurs with the same rate as in the beginning with a control reaction that has not been treated with soda. The soda thus produces only a temporary inactivation without permanently changing the ferment. Here are some numerical results to illustrate this proposition.

*7 June* 1901. I. 0.5M sucrose; 50 cm$^3$ of sugar + 0.5 cm$^3$ 0.02M NaOH + 1 cm$^3$ acetic acid. This mixture is made at the beginning and constitutes the control flask.

No mention is made here of the addition of ferment. However, as the inversion occurred, and by comparison with experiment II, one may suppose that invertin was in fact present.

| Times (min) | Proportions inverted | $K_2$ |
|---|---|---|
| 63 | 0.192 | 643 |
| 142 | 0.430 | 698 |
| 190 | 0.548 | 705 |
| 254 | 0.678 | 713 |
| 365 | 0.822 | 697 |
| 522 | 0.919 | 657 |

Mean: 685

*7 June* 1901. II. 0.5M sucrose; 0.5 cm$^3$ 0.02M NaOH + ferment. After 75 min at 25° 1 cm$^3$ of acetic acid was added, and the reaction was followed from that moment.

It is rather surprising that Henri tabulates $K_2$ (Bodenstein) here rather than $K_3$, but probably at 0.5M sucrose the difference is not important.

Although it is not specified in the original, 50 cm$^3$ of sugar was probably present.

| Times (min) | Proportions inverted | $K_2$ |
|---|---|---|
| 80 | 0.227 | 606 |
| 127 | 0.362 | 639 |
| 191 | 0.528 | 669 |
| 303 | 0.733 | 677 |
| 460 | 0.883 | 658 |

Mean: 650

*7 June* 1901. III. 0.5M sucrose; 0.5 $cm^3$ 0.02M NaOH + ferment. After 150 min 1 $cm^3$ of acetic acid was added.

Although it is not specified in the original, 50 $cm^3$ of sugar was probably present.

| Times (min) | Proportions inverted | $K_2$ |
|---|---|---|
| 47 | 0.126 | 554 |
| 110 | 0.323 | 648 |
| 224 | 0.599 | 675 |
| 380 | 0.826 | 677 |

Mean: 639

*7 June* 1901. IV. 0.5M sucrose; 50 $cm^3$ of sugar + 0.5 $cm^3$ 0.02M NaOH + ferment. After 260 min 1 $cm^3$ of acetic acid was added.

| Times (min) | Proportions inverted | $K_2$ |
|---|---|---|
| 114 | 0.349 | 688 |
| 270 | 0.707 | 716 |

Mean: 702

It may be seen that the discrepancies between the means are within the limits of experimental error.

# Chapter IV

## Action of emulsin on salicin

**28. Tammann's research. Method.** The laws according to which emulsin acts were studied by Tammann: this author studied the effect of this diastase on salicin and amygdalin. He also made some separate experiments with urea, aesculin and arbutin. The results were as follows:

1. The action of emulsin does not go to completion;
2. addition of reaction products decreases the rate;
3. emulsin becomes progressively altered with time, both when it is solution alone in water and when it is in the presence of its substrates;
4. the law that the activity follows is very complex, the rate being slower than the logarithmic law for acids would predict.

I have taken up the study of the law of action of emulsin again, choosing above all its effect on salicin, as the experiments with amygdalin and arbutin are more complicated to analyse on account of secondary reactions. These experiments were done in the laboratory of physical chemistry of Professor Ostwald in Leipzig during the months of August, September and October 1902 in Leipzig.

The method used was the same that for invertin. The ferment (from Merck) was dissolved in distilled water, around 0.4 g in 50 $cm^3$ of water, then filtered five or six times, left at 25° for several hours and then filtered again several times. In general I used 4 $cm^3$ for 100 $cm^3$ of salicin solution.

The salicin solution was likewise prepared several hours before the beginning of the experiments, boiled, filtered and brought to 25°. The same precautions were taken with the solutions of saligenin and glucose, to avoid the effects of birotation.

Birotation is an obsolete term for mutarotation. It is remarkable that Henri mentions it here, as there is no indication that he took account of it in the experiments on invertin. In their paper Michaelis and Menten criticized Henri for this omission, as had Hudson before them [C. S. Hudson (1908) "The inversion of cane sugar by invertase" *J. Amer. Chem. Soc.* **30**, 1160–1166].

The measurements were made either in the polarimeter of with Fehling's reagent. A complete series was carried out by the two methods, with results in complete agreement, giving discrepancies that in general did not exceed 2%, which is quite sufficient for this research.

**29. The rate of hydrolysis of salicin is slower than expected from the logarithmic law of acids.** When the results made during the course of reaction for a series of experiments are examined it can be seen that the value of the expression $K = \frac{1}{t} \log \frac{a}{a-x}$ decreases from the beginning of the reaction to the end. Here are some examples:

| Times (min) | Proportions inverted | $K = \frac{1}{t} \log \frac{a}{a-x} \cdot 10^5$ |
|---|---|---|
| | 3 *October* 1902: 0.14M salicin | |
| 25 | 0.132 | 246 |
| 55 | 0.209 | 185 |
| 87 | 0.306 | 182 |
| 211 | 0.534 | 157 |
| 271 | 0.603 | 148 |
| 373 | 0.686 | 135 |
| 1323 | 0.950 | 100 |
| | 3 *October* 1902: 0.07M salicin | |
| 24 | 0.174 | 345 |
| 54 | 0.351 | 348 |
| 86 | 0.450 | 302 |
| 210 | 0.691 | 243 |
| 270 | 0.775 | 240 |
| 371 | 0.847 | 220 |
| 1322 | 0.997 | 190 |
| | 8 *October* 1902: 0.14M salicin | |
| 31 | 0.110 | 163 |
| 123 | 0.305 | 128 |
| 211 | 0.447 | 122 |
| 276 | 0.516 | 114 |
| 343 | 0.583 | 111 |

The numbers in the right-hand column clearly decrease progressively from the beginning of the reaction to the end. Thus this result shows that the action of emulsin on salicin follows a different law from what we have found for the action of invertin on sucrose. The expression

$$K_1 = \frac{1}{t} \log \frac{a+x}{a-x}$$

remained constant in the case of invertin and strongly decreases in the case of emulsin.

**30. Effect of the salicin concentration.** Comparison of the reactions at different salicin concentrations shows that the rate varies with the concentration, but this variation is not proportional to the concentration as is the case with acids; the relationship between the rate of hydrolysis and the amount of salicin resembles very much what we found with invertin and sucrose. Thus we found after an hour that the following proportions of salicin had been hydrolysed by the same amount of emulsin:

| Solutions | Proportions hydrolysed |
|---|---|
| 0.14M salicin | 0.179 |
| 0.105M salicin | 0.216 |
| 0.07M salicin | 0.221 |
| 0.035M salicin | 0.394 |

**31. Effect of reaction products. Constancy of the ferment.** As for invertin we find in the case of emulsin that addition of saligenin + glucose slows down the

reaction, this inhibition being stronger at higher amounts of added products and at lower amounts of salicin. There is thus a complete parallel with the results obtained for invertin. Here are some numerical examples:

| Salicin | Hydrolysis products | Proportions hydrolysed after 60 min |
|---|---|---|
| 0.07M | | 0.221 |
| 0.07M | 0.0M | 0.157 |
| 0.07M | 0.07M | 0.146 |
| 0.035M | 0.035M | 0.394 |
| 0.035M | 0.035M | 0.194 |
| 0.035M | 0.07M | 0.182 |
| 0.035M | 0.105M | 0.151 |

Henri left the middle column of row 1 blank. Probably 0 was intended.

It can be seen that the same amount of glucose equal to 0.035M has a greater effect on the rate of hydrolysis for a 0.035M solution of salicin than for the 0.07M solution. In the first case the mean rate decreases in the ratio 394/194 and in the second in the ratio 221/157.

*Tammann* has tried to explain the law of action of emulsin in terms of a progressive change in the ferment with time. It was necessary to examine whether, in the experiments done in my conditions, the ferment lost activity during the course of reaction. I satisfied myself that if a new amount of salicin was added after the ferment had produced some hydrolysis, the reaction occurred at a rate very similar to that at the beginning. In addition, several parallel series were done in such a way as to see whether the emulsin in solution in water at 25° changed over several hours. The same amount of ferment was added to identical solutions of salicin, only the times of addition being spaced out. Let us give some examples:

8 *October* 1902

I. 0.14M salicin.

| Times (min) | Proportions hydrolysed |
|---|---|
| 31 | 0.110 |
| 123 | 0.305 |
| 211 | 0.447 |
| 276 | 0.516 |
| 343 | 0.583 |

II. 0.14M salicin, started 20 h after the first series, the solution of emulsin being kept at 25° during these 20 h.

| Times (min) | Proportions hydrolysed |
|---|---|
| 65 | 0.203 |
| 260 | 0.490 |
| 390 | 0.562 |

It can be seen that the ferment was a little less active in the second series than in the first, but the difference is small.

10 *October* 1902

First series. 0.14M salicin. Addition of 5 cm$^3$ of emulsin after 11 h 20 min.

| Times (min) | Proportions hydrolysed |
|---|---|
| 60 | 0.179 |
| 177 | 0.371 |
| 294 | 0.505 |
| 355 | 0.550 |
| 415 | 0.579 |

Second series. 0.14M salicin, 5 cm$^3$ emulsin added at 4 h 45 min, which had remained all the time at 25°.

| Times (min) | Proportions hydrolysed |
|---|---|
| 60 | 0.171 |
| 92 | 0.224 |

The rate of hydrolysis can be seen to be the same in the two series, so maintaining the ferment in distilled water for more than five hours did not appreciably change its activity.

One can therefore conclude from these results that for the experiments that I have carried out on salicin, which in general did not last more than 7 h, the progress of the hydrolysis was not modified by secondary effects other than those related to the composition of the mixture.

**32. Theoretical discussion of the results. The rate law is the same as for invertin.** The collection of results given above shows that the activity of emulsin qualitatively closely resembles the activity of invertin with sucrose. It is therefore appropriate to try to apply the same quantitative law for this diastase.

First of all we notice that the rate is slower than expected from the logarithmic curve. Taking account of this, suppose that the effect of products on the reaction is stronger than that of salicin, that is to say that in the general equation

$$K_3 = \frac{a}{t}\left[(m-n)\frac{x}{a} + n\ln\frac{a}{a-x}\right] + \frac{1}{t}\ln\frac{a}{a-x}$$

the coefficient $n$ ought to be larger than $m$ (recall that for invertin we had $m = 30, n = 10$).

In trying out a series of different values I stopped at values that express the progress of the reaction satisfactorily enough : these values were $m = 40, n = 120$. The constant of the reaction then has for expression

$$K_3 = \frac{40\cdot a}{t}\left[-2\frac{x}{a} + 3\ln\frac{a}{a-x}\right] + \frac{1}{t}\ln\frac{a}{a-x} \qquad (36)$$

Originally equation I

When there are amounts $a_1$ of salicin and $i$ of hydrolysis products (saligenin + glucose) at the beginning the value of $K_3$ becomes

$$K_3 = \frac{40\cdot a_1}{t}\left[-2\frac{x}{a_1} + 3\left(1+\frac{i}{a_1}\right)\ln\frac{a}{a-x}\right] + \frac{1}{t}\ln\frac{a}{a-x} \qquad (37)$$

Originally equation II

Henri had $40\cdot a/t$ instead of $40\cdot a_1/t$, but it seems clear that that was an error.

These equations 36 and 37 are to be used to calculate the experimental results.

Let us now give some numerical results to justify the choice of constants.

| Times (min) | Proportions hydrolysed | $10^4 \cdot K_3$ |
|---|---|---|
| 30 *October* 1902: I. 0.14M salicin | | |
| 25 | 0.132 | 414 |
| 55 | 0.209 | 327 |
| 87 | 0.306 | 350 |
| 211 | 0.534 | 360 |
| 271 | 0.603 | 358 |
| 375 | 0.685 | 345 |
| 1325 | 0.950 | 331 |
| | | Mean: 355 |
| II. 0.07M salicin | | |
| 24 | 0.174 | 340 |
| 54 | 0.351 | 388 |
| 86 | 0.450 | 360 |
| 210 | 0.691 | 341 |
| 270 | 0.775 | 357 |
| 371 | 0.847 | 348 |
| | | Mean: 356 |
| 8 *October* 1902: I. 0.14M salicin | | |
| 31 | 0.110 | 281 |
| 123 | 0.305 | 248 |
| 211 | 0.447 | 263 |
| 276 | 0.516 | 256 |
| 343 | 0.583 | 260 |
| | | Mean: 262 |
| II. 0.07M salicin | | |
| 122 | 0.476 | 279 |
| 209 | 0.651 | 288 |
| 275 | 0.691 | 260 |
| 342 | 0.767 | 275 |
| | | Mean: 275 |
| III. 0.035M salicin | | |
| 28 | 0.182 | 190 |
| 121 | 0.564 | 228 |
| 208 | 0.685 | 195 |
| 275 | 0.818 | 238 |
| 341 | 0.879 | 249 |
| | | Mean: 220 |

| Times (min) | Proportion hydrolysed | Saligenin + glucose added | $10^4 \cdot K_3$ |
|---|---|---|---|
| 10 *October* 1902 | I. 0.14M salicin | | |
| 60 | 0.216 | | 252 |
| 177 | 0.371 | | 231 |
| 294 | 0.505 | | 233 |
| 355 | 0.550 | | 227 |
| 415 | 0.579 | | 212 |
| | | | Mean: 231 |
| | II. 0.105M salicin | | |
| 60 | 0.179 | | 252 |
| 176 | 0.462 | | 239 |
| 293 | 0.606 | | 257 |
| 357 | 0.636 | | 235 |
| | | | Mean: 235 |
| | III. 0.075M salicin | 0.035M | |
| 57 | 0.157 | | 252 |
| 172 | 0.400 | | 275 |
| 291 | 0.539 | | 257 |
| 355 | 0.597 | | 253 |
| | | | Mean: 259 |
| | IV. 0.105M salicin | 0.035M | |
| 59 | 0.128 | 231 | |
| 176 | 0.344 | 261 | |
| 293 | 0.459 | 243 | |
| 357 | 0.525 | 245 | |
| | | | Mean: 245 |
| | V. 0.07M salicin | 0.07M | |
| 57 | 0.146 | 348 | |
| 173 | 0.327 | 303 | |
| 292 | 0.376 | 215 | |
| 355 | 0.536 | 278 | |
| | | | Mean:286 |
| | VI. 0.07M salicin | | |
| 58 | 0.221 | 191 | |
| 172 | 0.524 | 233 | |
| 291 | 0.688 | 243 | |
| 355 | 0.612 | 313 | |
| | | | Mean: 245 |

The original had 6.539 rather than 0.539: this is assumed to be an error.

10 *October* 1902 continued ...

| Times (min) | Proportion hydrolysed | Saligenin + glucose added | $10^4 \cdot K_3$ |
|---|---|---|---|
| | VII. 0.035M salicin | 0.035M | |
| 57 | 0.194 | | 262 |
| 170 | 0.469 | | 273 |
| 289 | 0.618 | | 251 |
| | | | Mean: 262 |
| | VIII 0.035M salicin | | |
| 56 | 0.394 | 269 | |
| 170 | 0.685 | 240 | |
| 288 | 0.880 | 297 | |
| | | | Mean: 269 |

Let us summarize the eight series of the 10th October, which gave the following results:

| | | |
|---|---|---|
| 0.14M salicin | | 231 |
| 0.105M salicin | | 245 |
| 0.105M salicin | 0.035M saligenin + glucose | 245 |
| 0.07M salicin | 0.07M saligenin + glucose | 286 |
| 0.07M salicin | 0.035M saligenin + glucose | 259 |
| 0.07M salicin | | 245 |
| 0.035M salicin | 0.035M saligenin + glucose | 262 |
| 0.035M salicin | | 269 |

It can be seen that the values of $K_3$ calculated according equations 36 or 37 are sufficiently constant, as the discrepancies are with the limits of experimental error. It is even possible that in taking somewhat different values for $m$ and $n$ one might obtain better constancy of $K_3$.

In summary, we see that the laws of action of emulsin on salicin are the same as those we have found for invertin: the same equation applies; only the values of the constants are different.

# Chapter V

## Action of amylase on starch

**33. Method. H. Brown and Glendinning's research.** Study of the laws of action of amylase on starch have concerned various authors, in particular the influence of the concentrations of the ferment and of starch, the extent of hydrolysis that could be obtained, and the effects of temperature and the different chemical substances. Very little research on the law that determines the progress of the reaction has been done until now. Let us mention those of Wroblewsky, but the conditions were not sufficiently precise to allow any conclusions to be drawn.

*H. Brown and Glendinning* published, in April 1902, a report on the law of action of malt amylase on soluble starch. They found that the rate of formation of maltose follows a law that is in general faster than the logarithmic law of the acids. The expression $K = \log \frac{a}{a-x}$, in which $a$ represents the amount of maltose obtained at the end of the reaction and $x$ that one finds at time $t$, increases in general from the beginning of the hydrolysis to the end. But this variation is not always observed: thus we see in four of the authors' publications that in two cases the increase is very clear, and, on the other hand, in the other two there is not an equally notable variation.

I have taken up again the study of the law of action of amylase on soluble starch with the use of different diastases:

1. Malt amylase from Merck;
2. Malt amylase from Grübler;
3. Amylase from dog pancreatic juice obtained by temporary fistula after injection of secretin, this juice having been desiccated and conserved two months in a dry state;
4. Amylase from dog pancreatic juice used in a fresh state.

These experiments were done in Professor Ostwald's laboratory in Leipzig.

The soluble starch used in these experiments gave a stable solution containing 3% starch. This starch reduced Fehling's reagent somewhat, and this initial reducing capacity was determined before each experiment.

The measurements were made with Fehling's reagent, all experiments being done at 25°. The precautions for preparing the solutions were the same as those we have desribed for invertin and emulsin.

**34. Law for the progress of the hydrolysis of starch by amylase.** One must before everything stress an essential point that has not been noticed by several authors when discussing the laws of action of amylase. In the formula $K = \frac{1}{t} \log \frac{a}{a-x}$ that defines the action of acid on sucrose, $a$ designates the amount of sucrose existing at the beginning and $x$ the amount of sucrose transformed at

time $t$, and this last quantity is here equal to the amount of invert sucrose there is in the solution.

If one wanted to apply the same law to the hydrolysis of starch one would have to define $a$ as the amount of starch existing at the beginning and $x$ as the amount hydrolysed at time $t$. Now, what is measured is the amount of the product maltose, and it is known that various intermediate substances are present in the solution between the starch stage and the maltose stage, so that one cannot at all measure the value of $x$. One is therefore in the impossibility of being able to directly verify whether the law for acids is applicable to the hydrolysis of starch by the diastase. One is therefore obliged to study this question by a detour.

I shall not pause for a theoretical discussion of the problem; it is complicated enough, even if we suppose just a single intermediate between starch and maltose, and the mathematical discussion will lead to complicated formulae containing as many constants as there are intermediate stages. Now, as we do not know how many there are, we cannot use the results that such a mathematical study would yield. Let us say only that if we suppose the existence of just one intermediate, one sole dextrin, and if one found that the rate of appearance of maltose followed the logarithmic law of acids, the rate of transformation of starch to dextrin and of dextrin into maltose would necessarily occur according to the logarithmic law of the acids.

Study of the laws that define starch hydrolysis cannot therefore be equated directly with those of general chemistry. One would have to make very complicated chemical analyses to have the elements necessary for such a discussion.

Also one is led to study not the rate of transformation of starch but the rate of appearance of maltose, which is not the same thing, as several authors have supposed. It is therefore this result that must be called empirical that will apply in the experiments that follow.

If we define $a$ as the amount of maltose obtained when the hydrolysis is finished, $x$ as the amount of maltose at time $t$, one finds that the expression $K = \frac{1}{t} \log \frac{a}{a-x}$ remains constant from the beginning of the reaction to the end. Here (on the next page) are several numerical examples that illustrate the degree of constancy of this value of $K$.

It is seen that the values in the right-hand column vary around a mean value: the law of formation of maltose is definitely therefore a logarithmic law.

I find myself on this point in disagreement with H. Brown and Glendinning. It is difficult to say what can be the origin of this disagreement. Perhaps it is due to the way in which these authors determined the extent of hydrolysis, or maybe there is a difference in the ferment, or finally a difference of temperature.

**35. Effect of the amount of starch. Difference between malt amylase and pancreatic juice amylase.** In relation to the amount of starch, several authors have found that a certain amount of amylase transformed the same number of grams of starch in the same time, regardless of the starch concentration in the solution.

This result needs to be corrected. It applies to malt amylase when the concentration in the solution exceeds 0.75 g soluble starch in 100 $cm^3$ of water, but for weaker solutions the amount of starch influences the rate of hydrolysis.

The result is different for the amylase of pancreatic juice: in that case the starch concentration has a clear effect up to a concentration of 2 g %, and the preceding rule only applies to stronger concentrations.

This difference between the activity of malt amylase and amylase from pancreatic juice shows clearly that these are two different ferments that follow different laws. I shall give several numerical examples of these results, which have some interest.

11 *September* 1902

| Times (min) | Amount of maltose obtained | Value of the ratio $x/a$ | $K \cdot 10^6$ |
|---|---|---|---|
| | I. Soluble starch at 3%, plus malt amylase | | |
| 29 | 0.090 | 0.046 | 707 |
| 53 | 0.179 | 0.092 | 790 |
| 89 | 0.283 | 0.146 | 769 |
| 113 | 0.358 | 0.184 | 781 |
| 150 | 0.459 | 0.236 | 779 |
| 184 | 0.552 | 0.284 | 788 |
| 227 | 0.641 | 0.330 | 766 |
| 326 | 0.865 | 0.446 | 787 |
| 402 | 0.977 | 0.504 | 757 |
| 467 | 1.121 | 0.578 | 802 |
| | II. Soluble starch at 1.5%, plus malt amylase | | |
| 39 | 0.111 | 0.114 | 1350 |
| 98 | 0.305 | 0.314 | 1670 |
| 179 | 0.488 | 0.503 | 1690 |
| 331 | 0.713 | 0.735 | 1740 |
| 452 | 0.804 | 0.828 | 1690 |
| | III. Soluble starch at 0.75%, plus malt amylase | | |
| 43 | 0.121 | 0.250 | 2900 |
| 58 | 0.175 | 0.361 | 3350 |
| 95 | 0.252 | 0.519 | 3340 |
| 212 | 0.392 | 0.808 | 3380 |
| 358 | 0.435 | 0.897 | 2750 |
| | IV. Soluble starch at 0.38% | | |
| 48 | 0.098 | 0.454 | 5480 |
| 102 | 0.165 | 0.764 | 6150 |

17 *September* 1902

| Times (min) | Amount of maltose obtained | Value of the ratio $x/a$ | $K \cdot 10^5$ |
|---|---|---|---|
| | I. Starch at 3%, pancreatic juice | | |
| 24 | 0.079 | 0.041 | 76 |
| 50 | 0.158 | 0.082 | 74 |
| 90 | 0.264 | 0.137 | 71 |
| 122 | 0.351 | 0.182 | 71 |
| 150 | 0.429 | 0.223 | 73 |
| 265 | 0.718 | 0.373 | 76 |
| 346 | 0.850 | 0.447 | 71 |
| | II. Starch at 1.5% , pancreatic juice | | |
| 29 | 0.101 | 0.105 | 166 |
| 85 | 0.250 | 0.260 | 154 |
| 128 | 0.342 | 0.355 | 148 |
| 269 | 0.558 | 0.580 | 140 |

17 *September* 1902 continued...

| Times (min) | Amount of maltose obtained | Value of the ratio $x/a$ | $K \cdot 10^5$ |
|---|---|---|---|
| | III. Starch at 0.75%, pancreatic juice | | |
| 34 | 0.082 | 0.170 | 238 |
| 55 | 0.118 | 0.245 | 222 |
| 94 | 0.187 | 0.389 | 227 |
| 133 | 0.230 | 0.478 | 212 |
| 273 | 0.344 | 0.715 | 200 |

20 *September* 1902

| | I. Starch at 1.5%, fresh pancreatic juice | | |
|---|---|---|---|
| 44 | 0.130 | 0.141 | 150 |
| 78 | 0.206 | 0.224 | 141 |
| 119 | 0.307 | 0.334 | 148 |
| 186 | 0.420 | 0.456 | 142 |
| 218 | 0.483 | 0.525 | 148 |
| 385 | 0.692 | 0.752 | 157 |
| 484 | 0.740 | 0.804 | 146 |

**Experiments with dried pancreatic juice**

In each line the amount of maltose after the specified time is tabulated, and the values shown as % values are the percentages of starch present.

| Date | Time | 2.5% | 1.25% | 0.5% | | |
|---|---|---|---|---|---|---|
| 22 *August* 1902 | 180 min | 0.64 | 0.44 | 0.18 | | |
| | | 3% | 1.5% | 0.75% | | |
| 6 *September* 1902 | 90 | 0.78 | 0.75 | 0.53 | | |
| 17 *September* 1902 | 2ml of redissolved pancreatic juice in each series | | | | | |
| | 30 | 0.095 | 0.101 | 0.073 | | |
| 17 *September* 1902 | 4ml of redissolved pancreatic juice in each series | | | | | |
| | 30 | 0.257 | | 0.116 | | |
| 20 *September* 1902 | 30 | 0.240 | 0.196 | 0.090 | | |
| 7 *October* 1902 | | 4% | 3% | 2% | 1% | 0.5% |
| | 40 | 0.370 | 0.364 | 0.337 | 0.219 | 0.105 |

**Experiments with fresh pancreatic juice**

In each line the amount of maltose after the specified time is tabulated.

| Date | Time (min) | 3% starch | 1.5% starch | 0.75% starch |
|---|---|---|---|---|
| 28 *August* 1902 | 140 | 2.04 | 1.01 | 0.46 |
| 29 *August* 1902 | 60 | 0.145 | 0.085 | 0.066 |
| 1 *September* 1902 | 140 | 0.085 | 0.046 | 0.028 |
| 3 *September* 1902 | 80 | 0.078 | 0.053 | 0.038 |
| 6 *September* 1902 | 75 | 0.142 | 0.112 | 0.081 |
| 9 *September* 1902 | 60 | 0.114 | 0.112 | 0.055 |
| 11 *September* 1902 | 45 | 0.101 | 0.112 | 0.056 |

The value of 0.081 in the right-hand column was shown as 0.81. That is presumably an error.

One can see that the results are the same for fresh pancreatic juice and for dried pancreatic juice. The effect of the starch concentration here resembles very much the results we have obtained for the effect of the sucrose concentration in the case of invertin and of that of salicin in terms of emulsin.

Maybe Henri could see that, but I cannot!

Finally here are the results obtained with malt amylase, which agree very well with the classic results of different authors.

**Experiments with malt amylase**

| Date | Time (min) | 3% starch | 1.5% starch | 0.75% starch | 0.375% starch |
|---|---|---|---|---|---|
| 5 *September* 1902 | 60 | 0.192 | 0.174 | 0.145 | |
| 6 *September* 1902 | 40 | 0.180 | 0.154 | 0.124 | |
| 11 *September* 1902 | 40 | 0.114 | 0.114 | 0.112 | 0.082 |
| 20 *September* 1902 | 30 | 0.098 | 0.104 | 0.100 | |

**36. Theory of the law of action of amylase.** This analogy between the results obtained for amylase and for the two previous ferments, invertin and emulsin, allows us to suppose that the law of action of amylase is the same as that of the other two ferments. We suppose, therefore, that amylase forms complexes with starch and with one or more of the hydrolysis products, in such a way that the expression that must remain constant as the following form:

$$K_3 = \frac{a}{t}\left[(m-n)\frac{x}{a} + n\ln\frac{a}{a-x}\right] + \frac{1}{t}\ln\frac{a}{a-x}$$

except that, in the case of amylase, as the curve of formation of maltose is logarithmic, one can suppose that the constants $m$ and $n$ are equal to one another. In that case the formula takes the following simpler form:

$$K_3 = \frac{1}{t}(1+ma)\ln\frac{a}{a-x} \tag{38}$$

It remains to determine the constant $m$, but we cannot do that yet on account of insufficient data, as we have seen earlier.

Equation 38, and better the expression for the rate,

$$\frac{dx}{dt} = \frac{K_3(a-x)}{1+ma} \tag{39}$$

allow a qualitative discussion of the general form of the curve and of the effect exerted by the starch concentration. This discussion was made in §25, but we do not stop there. Recall only that for the effect of the amount of starch on the rate of hydrolysis it gives a result in agreement with the experimental data that we reported in the preceding section.

**37. General conclusion.** The general conclusion that emerges from the whole set of experiments described in this work is that the activities of diastases can be

explained in terms of the laws of general chemistry. To do that it is necessary to suppose that intermediate complexes are formed, on the one hand between the diastase and the substrate, and on the other hand between the diastase and one or more products of the reaction. These complexes are considered incomplete, and obey the law of mass action of Berthollet, Guldberg and Waage.

The hypothesis of the formation of intermediate complexes is justified by a series of arguments, as follows:

1. E. Fischer's research on the diastases that showed that there is a constant relationship between a diastase and the chemical constitution of the substrate transformed by this diastase.

2. The inhibitory effect exerted by the products of the reaction: this inhibitory effect depends not only on the amount of these products but also on the amount of substrate present in the solution.

3. Kinetic study of the diastatic reactions shows that these reactions form part of the group of catalytic reactions in which there is incomplete formation of intermediate complexes, that are formed rapidly and decompose slowly.

4. The theoretical rate law for a diastatic reaction obtained by writing the equations characteristic of the equilibria between the diastase and the substances that participate in the reaction gives the following expression for this rate:
$$\frac{dx}{dt} = \frac{K_3(a-x)}{1+m(a-x)+nx}$$
and very many experiments made with invertin and emulsin have shown that values of $m$ and $n$ could be found such that this law could be verified very satisfactorily over wide ranges of concentration.

The law of activity of the diastases thus contains two constants $m$ and $n$ that are characteristic of the diastase and which, for a given diastase, could vary with the nature of the mixture and above all with the temperature.

It is above all this variation of $m$ and $n$ with the temperature that will be interesting to study. In effect, $m$ and $n$ are the constants of equilibrium between the ferment and the substrate or the reaction products. Now we know, from van 't Hoff's research, that in an equilibrium between several substances

$$\mathrm{A} + \mathrm{B} \rightleftarrows \mathrm{C} + \mathrm{D}$$

that is represented by the following relationship between the four concentrations

$$c_1 c_2 = K c_3 c_4$$

the constant $K$ varies with the temperature, and this variation is linked to the heat of the chemical reaction that gives rise to the equilibrium. In fact, according to van 't Hoff,

$$\frac{d \ln K}{dT} = \frac{q}{2 \cdot T^2}$$

where $q$ is the heat of reaction and $T$ the absolute temperature.

In consequence, if the variation of the constant $K$ is measured over a specified range of temperature one can deduce from it the value of $q$ by calculation. Therefore studying the effect of temperature on the law of action of diastases would be able to provide a more precise knowledge of each of the two proposed complexes, first between the diastase and the substrate, and second between the diastase and the reaction products. It is in this direction that we plan to continue this research.

# Conclusions

## Introduction

I. Study of the catalytic activities shows that there is a whole series of different groups of these reactions that need to be considered. The classification proposed in §5 includes five different cases:

   a) Pure catalysis by simple presence;
   b) Autocatalysis;
   c) Very fast formation of intermediate complexes, these complexes being either complete or not;
   d) Intermediate complexes produced slowly;
   e) Action of a catalyst on a series of reactions in succession.

II. Study of the rate law of a catalytic reaction allows a decision as to which group this activity belongs.

## Studies on invertin

III. the rate of inversion of sucrose produced by invertin is greater than indicate by the logarithmic law of acids.

IV. The remains comparable with itself during the whole duration of the reaction, its activity depending only on the composition of the mixture in which it finds itself.

V. Invert sugar inhibits the reaction produced by invertin. This inhibition is stronger when the amount of invert sugar is higher.

VI. A given amount of invert sugar inhibits the inversion more strongly as the amount of sucrose in the solution becomes smaller.

VII. The inhibitory effect of the reaction products is due almost solely to the laevulose.

VIII. When the rate of inversion is studied for different concentrations of sucrose in the solution it is found that for dilute solutions (below 0.1M) the rate increases with the concentration; for intermediate solutions (between 0.1 and 0.5M) the rate is independent of the amount of sucrose; and in concentrated solutions the rate decreases as the concentration increases.

IX. The rate of inversion is proportional to the amount of invertin.

X. If we suppose that the diastase forms two different complexes, with sucrose and with invert sugar, giving rise to equilibria, and we apply the law of mass action to these two equilibria, we can deduce from that a law of action of the diastase a formula that completely satisfies all of the experiments on invertin. According to this law the rate of the diastatic action is given by the following expression:

That is an exaggeration. The inhibition at high sucrose concentrations (VIII) is not explained.

$$\frac{dx}{dt} = \frac{K(a-x)}{1+m(a-x)+nx}$$

The constants $m$ and $n$ being characteristic of the diastase, the temperature and the composition of the mixture.

It is surprising that Henri showed this equation rather than equation 33, showing the effect of invert sugar:

$$\frac{dx}{dt} = \frac{K_3 \cdot (a_1 - x)}{1+m(a_1-x)+n(x+i)}$$

## Action of emulsin on salicin

XI. The rate of hydrolysis of salicin by emulsin is lower than indicated by the logarithmic law of acids.

XII. The relationship between the concentration of salicin and the rate of hydrolysis is the same as in the case of the inversion of sucrose by invertin.

XIII. Emulsin remains comparable with itself during the whole duration of the hydrolysis. Its activity depends only on the composition of the mixture.

XIV. The products of the hydrolysis inhibit the activity of emulsin in the same as in the case of invertin.

XV. The theoretical law established for invertin explains satisfactorily the experimental results obtained with emulsin. Only the values of the constants $m$ and $n$ are different.

## Action of amylase on starch

XVI. The rate of formation of maltose in the hydrolysis of starch by amylase follows a logarithmic curve, the same that observed with acids.

XVII. The amount of starch affects malt amylase and amylase from pancreatic juice differently.

XVIII. A theory for the activity of amylase is impossible to give completely, in view of the lack of data on the different successive stages of the hydrolysis. Only a qualitative study can be made in terms of the same law as for invertin and emulsin. One can suppose that $m$ and $n$ are equal to one another.